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BIOPHYSICAL CONTROLS AND MITIGATION OF CADMIUM CONTENT IN COCOA BEANS IN SANTANDER, COLOMBIA

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This dissertation is dedicated to the memory of my father, **Eld. William Kofi Zorve.**

“In my life I have found two things of priceless worth - learning and loving. Nothing else - not fame, not power, not achievement for its own sake - can possible have the same lasting value. For when your life is over, if you can say 'I have learned' and 'I have loved,' you will also be able to say 'I have been happy.”

― Arthur C. Clarke

SUMMARY

Cocoa (*Theobroma cacao* L.) is a significant agricultural export commodity in the world and a vital raw material for chocolate manufacturing and other cocoa-derived products. Recent EU regulation on cadmium (Cd) in cocoa-derived products threatens the sustainability of cocoa production in the province of Santander, Colombia. A comprehensive analysis of available soil Cd concentration, cocoa leaves and cocoa beans Cd levels was carried out as well as pH adjustment to reduce the phyto-availability of soil Cd. Metal analyses in all the samples were done using ICP-MS. The available Cd concentrations in soil samples from the various investigated farms differed significantly. However, the available Cd concentrations in soils under the three (3) examined clones were similar. Mean Cd concentrations in cocoa leaves and beans were 10.2±15.4 mg kg^{-1} and 6.5±8.2 mg kg^{-1} respectively. For the available Cd concentrations in soils, a mean of 79.2±260.5 µg kg^{-1} was recorded. The findings indicated that there is high spatial variability apparently present. Mean Cd concentrations in cocoa beans were markedly higher, where all the bean samples exceeded the adopted threshold (0.60 mg kg^{-1}) for import to the EU. The distribution of Cd in cocoa leaves and beans on plantation soils in Santander revealed the following trend: $Cd_{soil} < Cd_{beans} < Cd_{leaves.}$ In terms of Cd contents in leaves and beans, there was no statistical difference between the clones investigated. Nevertheless, statistically different leaves and beans Cd levels were observed among the different studied farms, demonstrating considerable variations in Cd contents over short distances. A significant correlation between available soil Cd and the Cd contents in leaves and beans was observed. This correlation decreased in the order: leaves (R^2 = 0.7) ≈ beans (R^2 = 0.4). Liming as a mitigation strategy was employed by adjusting the pH of the soil. A soil pH-increase of 2.4 units on the average resulted in a decrease in available soil phosphorus (P) and available soil Cd by 1.9 mg kg^{-1} and 90.3 µg kg^{-1} respectively. The mean available P concentrations in the soil before and after pH-H_2O adjustment were significantly different. The same was observed between mean available Cd concentrations in the soil before and after pH-H_2O increase. Based on the lime requirement computations, the proposed recommended rate of lime ($CaCO_3$) to be used as agricultural liming material to increase the soil pH by 1 unit is ca. 50 tonnes $CaCO_3$ ha^{-1}. More cocoa farmers in this province are to embrace the use of fertilizers as well as liming on their farms to augment the production of cocoa. This will enable them to meet the proposed EU standard for Cd in cocoa beans and enhance productivity.

Key words: *Theobroma cacao* L., cadmium, ICP-MS, liming, phosphorus, soil pH, $CaCO_3$.

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LIST OF ABBREVIATIONS

ADAM	Areas for Municipal-Level Alternative Development
AEM	Anion Exchange Membrane
Ag	Silver
As	Arsenic
Ca	Calcium
CAGR	Compound Annual Growth Rate
CAOBISCO-ECA-FCC	Chocolate, Biscuits & Confectionery of Europe-European Cocoa Association-Federation of Cocoa Commerce
CAU39	Caucasia 39 Clone
CCN51	CCN51 Hybrid
CEC	Cation Exchange Capacity
Cd	Cadmium
CONTAM	Scientific Panel on Contaminants in the Food Chain
COVID-19	Coronavirus Disease
Co	Cobalt
Cr	Chromium
Cu	Copper
DeSIRA	Development-Smart Innovation through Research in Agriculture
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
F1, CAU39, A2	Sample Labelling; Farm Number, Clone, Number of Sampled Tree
FEAR5	Fedecacao Arauquita 5 Clone
FEC2	Fedecacao 2 Clone
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FEDECACAO	Federación Nacional de Cacaoteros
FFC	Fine Flavour Cocoa
GAIN	Global Agricultural Information Network

ha	Hectare
HCl	Hydrochloric Acid
Hg	Mercury
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICCO	International Cocoa Organization
k	Kilo
K	Potassium
KH_2PO_4	Potassium Dihydrogen Phosphate
LAC	Latin America and the Caribbean
LOD	Limit of Detection
LOQ	Limit of Quantification
m.a.s.l	Metres Above Sea Level
Mg	Magnesium
MIDAS	More Investment in Sustainable Alternative Development
MQ water	Milli-Q water
NaOH	Sodium Hydroxide
$NaHCO_3$	Sodium Hydrogen Carbonate
Ni	Nickel
P	Phosphorus
Pb	Lead
pH	Power of Hydrogen
RASFF	Rapid Alert System for Food and Feed
Sb	Antimony
SD	Standard Deviation
SRM	Standard Reference Material
SV012	Soil from San Vicente at a Medium Range Altitude (600-900 m.a.s.l)
UK	United Kingdom
USAID	United States Agency for International Development
WRB	World Reference Base
WCF	World Cocoa Foundation

WHO	World Health Organization
Zn	Zinc

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1.0 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a significant agricultural export commodity in the world particularly for some developing countries. It is the vital raw material for chocolate manufacturing and other cocoa products (Afoakwa, 2016). The seeds, commonly known as cocoa beans, are obtained from the pods. The pods are oval, measures between 12 and 30 cm long, and contain 30 to 40 beans embedded in a mucilaginous pulp, which comprises approximately 40% of the bean fresh weight (Schwan & Wheals, 2004). The current global production consists of ca. 4.7 million tonnes with Cote d'Ivoire, Ghana, Ecuador, Cameroon, Nigeria, Brazil and Indonesia as the major producers but collectively, Latin America contributes 18.4% (International Cocoa Organization [ICCO], 2020a)

The global demand for high quality cocoa is increasing (de Walque, 2018). Cocoa production in Colombia has increased by more than 30% between 2010 and 2019, reaching an output of ca. 102 k tonnes in the latter year (Food and Agriculture Organization Corporate Statistical Database [FAOSTAT] 2019). In 2010, the (ICCO) qualified Colombia as a producer of high-quality 'fine flavour' cocoa and has the potential to meet the growing demand for single-origin 'fine flavour' cocoa though it recorded just 1.5% of the global cocoa market. Colombia is the fifth biggest cocoa producing country in Latin America, after Brazil, Ecuador and Peru and the tenth worldwide (FEDECACAO, 2017; ICCO, 2020a).

Cocoa beans and chocolates have several health benefits, including high antioxidants, which reduce the number of free radicals, help prevent infectious and autoimmune diseases, and reduce the risk of heart attack (Kelishadi, 2005) but they sometimes contain high levels of cadmium (Cd). Cadmium is a toxic heavy metal, present in the soils of some cocoa plantations. It can bioaccumulate and persist but not biodegrade, and a precursor of various cancers, oxidative stress, inflammation, and tissue injury in humans (Das & Al-Naemi, 2019; Londoño-Franco, Londoño-Muñoz & Muñoz-García, 2016). Salama (2018), and Jiménez (2015) revealed the risk of human contamination from cocoa-derived products intake. It accumulates over time in the human body and has detrimental effects on kidneys, lungs and bones.

To address this health issue, maximum allowed Cd limits have been defined by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), (2016) for the different products, ranging from 0.2 to 2.0 mg kg^{-1}. Based on further studies that demonstrated even higher sensitivity of humans to Cd, the European Food Safety Authority (EFSA) and the European Commission (EC) came up with a regulation to protect consumers and particularly the most vulnerable consumers such as children by implementing a more stringent Cd limits for cocoa-derived products (EFSA, 2012; EC, 2014). Limits between 0.10 and 0.80 mg Cd kg^{-1} have been imposed by the EC on chocolate products (milk chocolate, chocolate and cocoa powder). These maximum limits were based on recommendations of the Scientific Panel on Contaminants in the Food Chain (CONTAM) of the EFSA. In the assessment by the CONTAM panel, the highest Cd concentrations were detected in food commodities such as chocolate (EFSA, 2009). Three (3) maximum standards have been defined for chocolate, based on the contents of the chocolate varieties. The darker the chocolate, the higher the maximum levels are. For cocoa powder intended for direct use, a fourth maximum level has been set. The EC's regulation, which took effect on January 1, 2019, has sparked interest in Cd in cocoa.

According to Meter *et al.* (2019), Latin America is known for having high levels of natural and anthropogenic Cd in soils due to volcanic eruptions, fertilization and irrigation. It can accumulate in cocoa beans and as a consequence, cocoa from Latin America is often too high in Cd which leads to export problems to the European Union (EU). In these regions, the Cd concentrations of cocoa beans commonly exceed 0.6 to 0.8 mg kg^{-1} which is the approximate upper range for manufacturing cocoa products that are in accord with the recent EU regulations. For example, a study of more than 500 farms in Ecuador revealed that nearly 50% of the cocoa beans had Cd concentrations that exceeded 0.6 mg kg^{-1} (Barraza *et al*., 2019). According to the Rapid Alert System for Food and Feed (RASFF), four cases of cocoa-based products being denied entry or removed from the EU market have been recorded. In all cases, elevated Cd levels (0.9 to 2.5 mg kg^{-1}) were found in cocoa products from Latin American countries (Ecuador, Colombia and Peru) (RASFF Portal). These thresholds may have grave economic ramifications for Colombia and other affected countries, especially for the many small-scale farmers and their families who grow and harvest cocoa beans. Besides, higher costs for laboratory tests as well as the implementation of mitigation measures cannot be overlooked. The biggest impact is felt by smallholder farmers, co-operatives, and exporting companies of specialty beans and powder in Latin America where cocoa is a native plant and is renowned for fine flavour beans (World Cocoa Foundation [WCF], 2019). Despite the Cd threshold regulation, the EU is already implementing a specific development programme under DeSIRA (Development-Smart Innovation through Research in Agriculture) initiative, a 6 million Euros intervention on low Cd and climate-relevant innovation to promote sustainable cocoa production in Colombia, Ecuador and Peru (https://europa.eu/capacity4dev/desira).

Aguirre-Forero *et al.* (2020) concluded on a study conducted in the province of Magdalena, Colombia, that the Cd concentration in cocoa beans exceeded the permissible limit however, the soil Cd concentration showed that the soil is optimal for cocoa cultivation. Furthermore, the correlation with other plant nutrients suggests that soil carbonates or organic amendments can reduce Cd uptake.

San Vicente de Chucurí and Rionegro are two municipalities in the province of Santander in Colombia which are noted for cocoa production. Both are located to the north eastern of the country and Agronet (2014) mentioned Santander province to be the largest producer concentrating 28.5% of national production. In addition, Santander is the great producer of fine flavour cocoa (FFC) in the country (Sepúlveda *et al.*, 2019). Cocoa accounts for 60% of the total agricultural production that is achieved in San Vicente de Chucurí (de Walque, 2018). In a study to investigate biophysical control on cocoa quality by de Walque (2018) revealed the presence of Cd in the soils of both San Vicente and Rionegro with an average of 3.3±0.7 mg kg^{-1} and 2.3±0.7 mg kg^{-1} respectively. Thus the presence of Cd is the main barrier preventing the global demand to be fulfilled. Soils in these regions are characterized by acidic properties hence suggested possible solutions included increasing the pH above 5.5 through liming to hinder Cd from becoming available for cocoa plants uptake (de Walque, 2018). In a different investigation conducted by Hoogerwerf (2020) in the village of San Vicente de Chucurí, Colombia, reported 0.6±8.2 to 32.8±8.2 mg kg^{-1} as the concentration of Cd in dried cocoa beans which also exceeded the maximum permissible limit according to the recent EU regulation. Among the objectives of the study were to investigate potential differences in Cd content among beans and leaves from the same cocoa tree and ascertain the effect of soil Cd on Cd concentration in different tissues of the cocoa tree but due to the COVID-19, only 50% of the data was available for further analysis (Hoogerwerf, 2020).

This study aims to propose strategies to mitigate Cd content in cocoa beans in Santander, Colombia. There has been an increase in research efforts in the province of Santander to address the problem of Cd accumulation in cocoa, with the aim of finding solutions as the province of Santander contributes ca. 42.1% of the national production in Colombia. This research focuses on performing a comprehensive analysis of total soil Cd, available soil Cd, cocoa bean Cd and cocoa leaf Cd data from San Vicente, Colombia. Also, to treat soil by the addition of soil amendments that alter soil pH to an agronomic desirable pH and maximizes P-availability in the soil which can reduce Cd uptake.

2.0 LITERATURE REVIEW

2.1 Brief History of Cocoa

The cocoa tree (*Theobroma cacao L.*) originates from the tropical rainforests of the Americas and it has been known to the Mayas since pre-Colombian times and is grown in agroforestry systems (Almeida & Valle, 2007). Cocoa, a native to the Americans, was a valuable crop in the earliest South American cultures. The term cocoa originated from the Nahuatl word "cacahuatl". Many believe that the plant first grew in the Amazon and upper Orinoco basin, but the Maya's and the Aztecs eventually developed techniques to cultivate cocoa successfully.

The plant was considered as a symbol of wealth for these civilizations and its beans were used as currency (Dillinger *et al*., 2000). Cocoa was considered divine in origin (Prabhakaran Nair, 2010) and in 1737, the Swedish botanist Carolus Linneaus named the cocoa tree *Theobroma cacao*, now its official botanical name from the Greek word "ambrosia," which refers to the mythical history of the tree literally meaning "cocoa, food of the gods" (Alvim, 1984).

At the beginning of the 19^{th} century, nearly all of the world's cocoa was produced in tropical Latin America and Trinidad (Cunningham & Arnold, 1962) but in recent times, most production has occurred in West Africa (mainly Côte d'Ivoire and Ghana), followed by South East Asia (mainly Indonesia) (FAOSTAT, 2015).

2.2 Varieties of Cocoa

There are many different varieties of cocoa (Figure 1.1) and are often classified into three groups: Criollo, Trinitario and Forastero, which, although the distinctions are inadequately defined, are supposed to vary according to morphology and genetic and geographical origins (Bartley, 2005; Almeida & Valle, 2007; Afoakwa *et al.,* 2011; Aprotosoaie *et al*., 2016). The Criollo varieties, which are thought to have originated in Venezuela and were the first to be cultivated in Trinidad (Bartley, 2005) have been praised for their fine flavour. However, they lack vigour, yield poorly and are reported to be extremely susceptible to pests and diseases (Toxopeus, 1985; Almeida & Valle, 2007). It is commonly thought that Trinitario developed as a cross between Criollo and Forastero and that many of its features are intermediate between the two (Toxopeus, 1985). Forastero varieties now account for about 80% of global production due to their preferred crop characteristics leading to high productivity and include both the Amazon and the Amelonado varieties (Toxopeus, 1985; Almeida & Valle, 2007; van Vliet & Giller, 2017). According to Aneani and Ofori-Frimpong (2013), the Amazon cocoa produces pods throughout the year while Wood and Lass (1985) emphasized that Amelonado's high level of homogeneity is much appreciated by manufacturers. Amelonado was first introduced from Brazil to Príncipe in 1822, expanding from there to most of West Africa where, as in Brazil, it is still widely cultivated (Toxopeus, 1985; Bartley, 2005). Forastero varieties are much more vigorous and harder than Criollos. They are often referred to as 'bulk' cocoa as opposed to 'fine' cocoa, although the Ecuadorian 'Cacao Nacional' with its distinctive fine flavour is an Amelonado variety (Toxopeus, 1985).

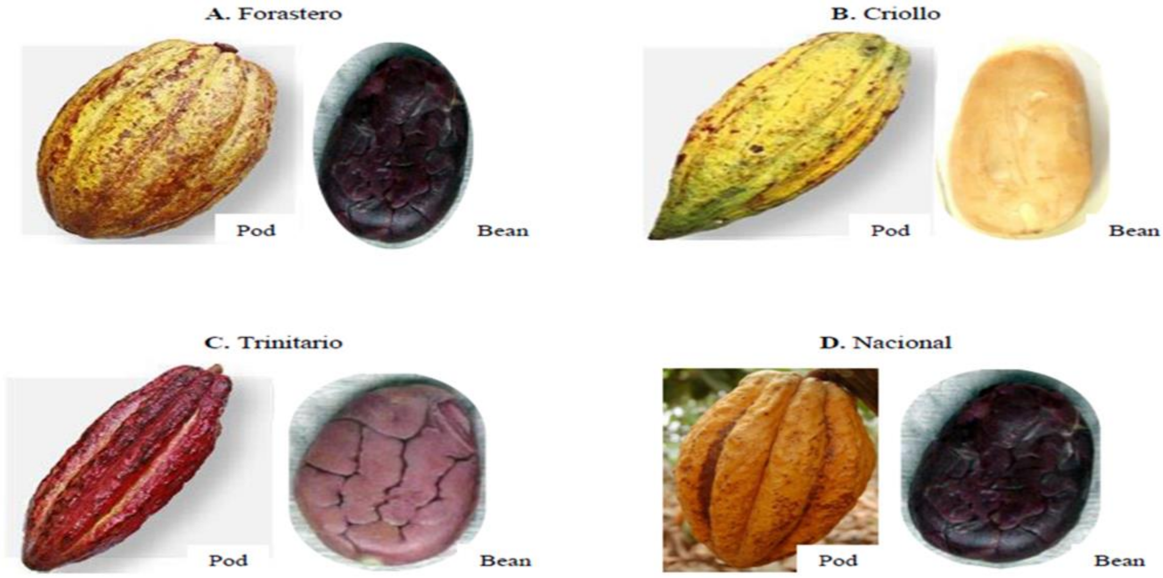

Figure 1.1: Pod and bean of the major varieties of cocoa cultivated. Source: CasaLuker, (2018)

2.3 Global Cocoa Production

According to Daymond and Hadley (2004), West Africa, Central and South America and Southeast Asia are the primary cocoa cultivation areas. For the efficient growth of cocoa plants, certain environmental and edaphic conditions must be at their optimum. These conditions include temperature, precipitation, humidity, soil type, soil pH and nutrition of the soil. The cultivation of cocoa requires a suitable environment that is mostly found within the region bounded by the Tropics of Cancer and Capricorn. Most of the cocoa in the world is grown on small or large plantations within 10^0 North and South of the Equator and is best suited to altitudes from sea level up to about 1000 m even though most of the cocoa in the world grows at an altitude of less than 300 m (Afoakwa, 2016). According to Kroeger *et al*. (2017), global production relies almost entirely on 5 – 6 million smallholder farmers and more than 90% of production comes from smallholder farms, with an average size of 2 – 3 hectares.

Global annual cocoa production for the 2019/2020 season is reported to be ca. 4.7 million tonnes (ICCO, 2020a). The major cocoa producing countries in terms of 2019/2020 outputs are Côte d'Ivoire, Ghana, Ecuador, Cameroon, Nigeria, Brazil and Indonesia as depicted in Figure 1.2. Together, these countries produced ca. 4.2 million tonnes representing 88.5% of total global production, with Côte d'Ivoire being the leading producing country contributing ca. 45% of the global production. In addition, 18.4% of the world's cocoa is produced by smallholder farmers in Latin America (ICCO, 2020b).

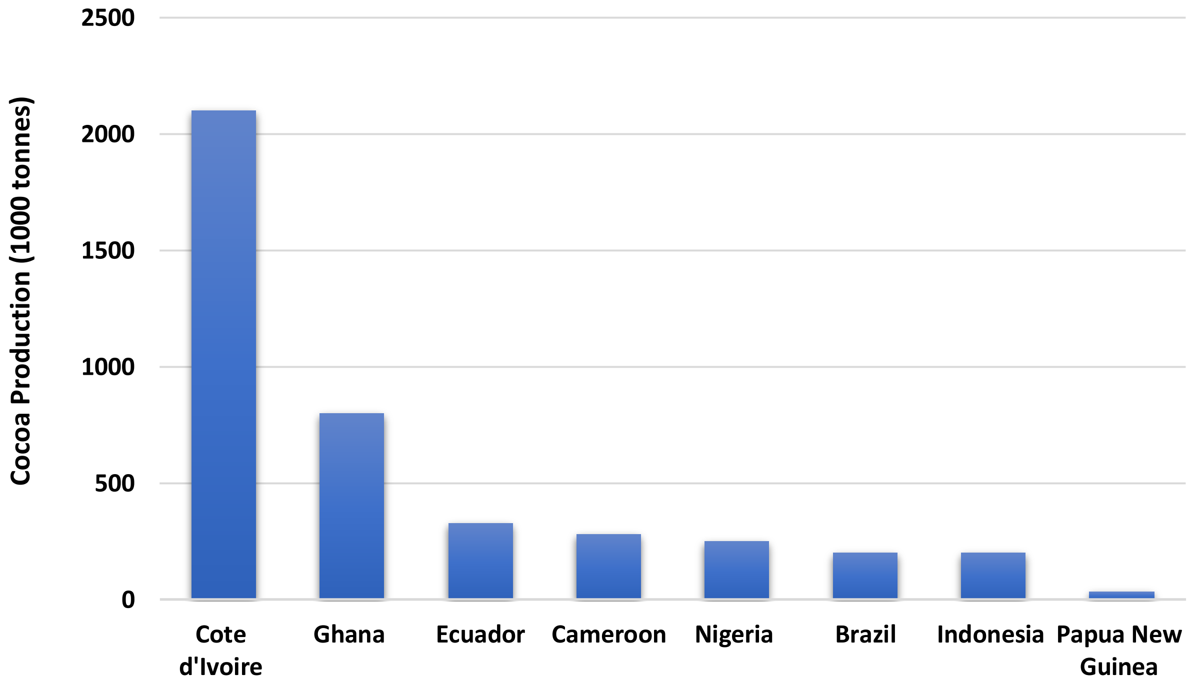

Figure 1.2: Production of cocoa beans (1000 tonnes) by the major cocoa producing countries for the 2019/2020 season. Source: Based on data from ICCO, (2020a)

2.4 Importance of Cocoa

Cocoa (*Theobroma cacao L.*) is one of the most important agricultural export commodities in the world and forms the backbone of the economies of some countries in West Africa, South America and South-East Asia. It is the leading foreign exchange earner and a great source of income for many families in most of the world's developing countries (Afoakwa, 2016). Exported cocoa beans, whether whole or broken, raw or roasted, had a combined value of $8.6 billion in 2017. The global cocoa beans market is expected to grow at a compound annual growth rate (CAGR) of 7.3% from 2019 to 2025 to reach $16.3 billion. The chocolate industry, which consumed 43% of all cocoa in 2017, had a retail market value of $106.2 billion in 2017 and is expected to grow to $189.9 billion by 2026 (Vivek *et al.*, 2019).

Cocoa also supports substantial economic growth in the confectionery sector, in particular in Europe and the United States, as well as promoting the economies of developing countries and smallholder farm families. The Netherlands is reported to import almost $2 billion of cocoa beans, followed by the United States with an estimated $1.2 billion of beans. Also, it has been reported that approximately 650 companies make up the cocoa and chocolate industry in the United States and directly employ nearly 70,000 Americans, whiles more than 2,000 companies in the EU are part of the chocolate and confectionery products industry (Houston & Wyer, 2012). Accordingly, Vivek *et al.* (2019) posited that the largest exporter of cocoa beans was Côte d'Ivoire ($3.9 billion), followed by Ghana ($2.5 billion) and Nigeria ($0.8 billion) in 2016. The largest importers were the Netherlands ($2.6 billion), Germany ($1.5 billion) and the United States ($1.3 billion).

Other than the economic importance of cocoa, chocolate and other cocoa products, consumption is noted to contribute positively to health due to the presence of polyphenols in cocoa beans which exhibit anti-carcinogenic, anti-atherogenic and vasodilatory effects, and they exert them mainly as antioxidants (Afoakwa, 2016; Anklam & Wollgast, 2000; de Oliveira *et al*., 2018). According to the ICCO (2020c), chocolate has been appreciated as a high-calorie food to boost energy in the past, for example for athletes and soldiers.

2.5 Cocoa in Colombia

In Colombia where cocoa is commonly consumed as a beverage, the production of cocoa (*Theobroma cacao L*.) has a long history. According to Abbott *et al*. (2018), it has become a priority of the Colombian government, as it is one of the crops promoted in the development of programs aimed at fostering stability in post-conflict regions and replacing crops traditionally used for illegal purposes. Colombia's earliest known cocoa plantation dates from 1622, near the city of Cali, in the Valle del Cauca Province (Patino, 2002). The main type of cocoa grown in Colombia until 1885 was Criollo.

About 95% of Colombian exported beans were categorized as FFC, and the country was ranked as the 5th largest exporter of FFC cocoa with 13,056 tonnes or 3.9% of the total market volume. In spite of being ranked as the fifth largest exporter of FFC, the country has not taken advantage of its full potential as it contributes to ca. 1.5% of global cocoa production (Escobar *et al*., 2020). According to FEDECACAO (2020), chocolates made with Colombian cocoa, were awarded with three stars in the international Great Taste Awards competition in the United Kingdom. The international Great Taste Awards competition is the UK's most prestigious food and beverage accreditation system. Additionally, Luisa's Vegan Chocolates (UK chocolate bars), made with cocoa produced by female cocoa farmers from the provinces of Huila and Antioquia was also awarded during the same event, for promoting artisans dedicated to the sale of food select in the world. Luker Chocolate (2020) noted that there are approximately 176,000 hectares of cocoa plantations in Colombia, in 25 of its provinces and in 2019, 59,665 tonnes of cocoa were produced. Cocoa is planted between 0 and 1300 m above sea level, in four agro-ecological regions: tropical rainforest, dry inter-Andean valleys, Andean region and the mountains of Santander. In terms of genetics, Colombian crops are planted with cocoa hybrids of universal and regional Trinitarian clones (Luker Chocolate, 2020).

In Colombia, the annual production of cocoa beans is obtained from the exploitation of ca. 0.12 million hectares planted in 24,500 farms and for the 2019 production season, the average yield per harvested hectare was 867 kg of cocoa beans as shown in Figure 1.3 (A) and (B) (FAOSTAT, 2020). There was a decline as compared to the previous production season and FEDECACAO (2019) reported that, the causes of the low yield obtained per hectare were related to four aspects that affected cultivation: the advanced age of the established plantations; the type of propagating material used (hybrid and common cocoas with low levels of tolerance to pests and diseases); the low density of trees in production per hectare and; the difficulties for the farmer to put into practice the recommendations for the integral management of the crop.

Abbott *et al*. (2018) highlighted that since at least 2000, USAID and other international donors have supported cocoa production in Latin America as a substitute for illicit crops. A significant boost in Colombia's cocoa production came when two alternative development projects were launched: More Investment in Sustainable Alternative Development (MIDAS) in 2006 and Areas for Municipal-Level Alternative Development (ADAM) in 2005. Cocoa was a small but significant part of USAID's alternative growth plan, though it was not the only crop promoted as a substitute for illicit crops. Cocoa initiatives included: farmers given free trees to plant, technical assistance to get farmers better prepared to grow new crops in areas where they were uncommon, research and institutional support to identify areas where cocoa production was appropriate, planting materials likely to produce higher yields and better-quality beans, and training on production methods and post-harvest practices. According to FAOSTAT (2016) and FEDECACAO (2015), the cocoa initiatives of these projects were quite successful as ADAM and MIDAS reported planting over 50,000 new hectares of cocoa trees with an emphasis on high-yielding bulk varieties.

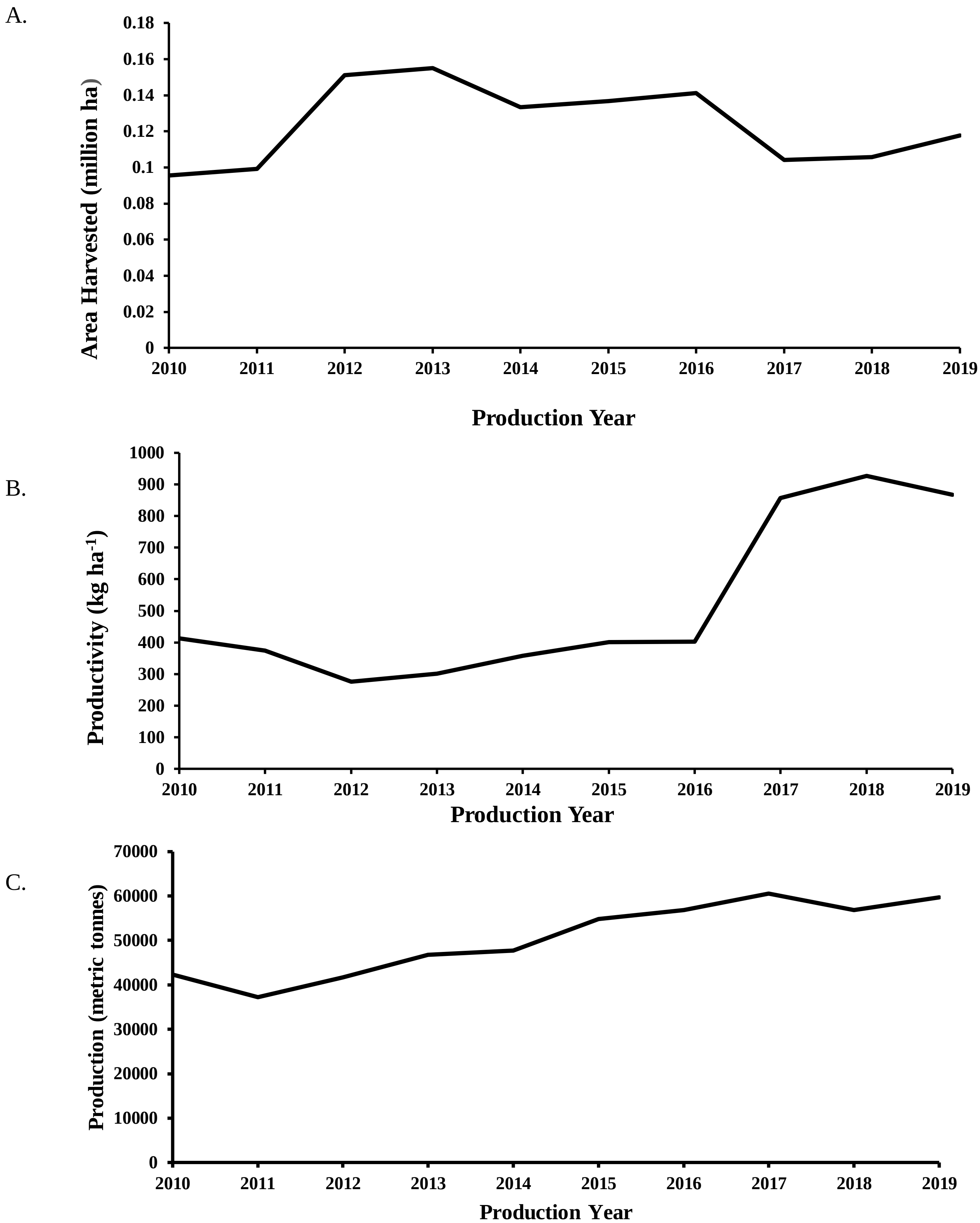

Figure 1.3: Area under cocoa cultivation (A), productivity of farmers (B) and annual cocoa production (C) in Colombia from 2010 to 2019. Based on data from FAOSTAT (2020) and Statista (2021).

As depicted in Figure 1.3 (B), the outcome was observed in an increase in productivity in the 2015/2016 production season. The global outbreak of the COVID-19 is having a major impact on cocoa marketing and the supply chain nevertheless, current report by FEDECACAO (2021) revealed that despite the pandemic, the Colombian cocoa subsector registers a new record in cocoa production, going from 59,665 in 2019 to 63,416 metric tonnes in 2020, showing a significant growth of 6%. Additional data on the production of Colombian cocoa are depicted in Figure 1.3 (C). According to ICCO 2019-2020 cocoa year report, Colombia is ranked tenth in the world as a cocoa producer and fifth in Latin America, showing the determination to increase productivity, which will continue to improve due to the advancement of the cocoa plantation renovation programme, which is expected to impact 10,000 hectares of unproductive crops (FEDECACAO, 2020).

The mountains of Santander (Santander and Norte de Santander) have average temperatures between 23 and 28°C, annual precipitation between 1500-2500 mm with mostly clay to clay loam soils (FEDECACAO, 2016), altogether with other factors, defines Santander's cocoa, with its balanced bitterness, low astringency, slight acidity and a delicate fruity tone resembling dark fruits such as plums (Luker Chocolate, 2020). Additionally, its cocoa content is of 65% FFC. With ca. 42.1% of Colombia's cocoa production, Santander is the most productive province, followed by ca. 8.8% in Antioquia and ca. 7.6 % in Arauca as illustrated in Figure 1.4 (FEDECACAO, 2019).

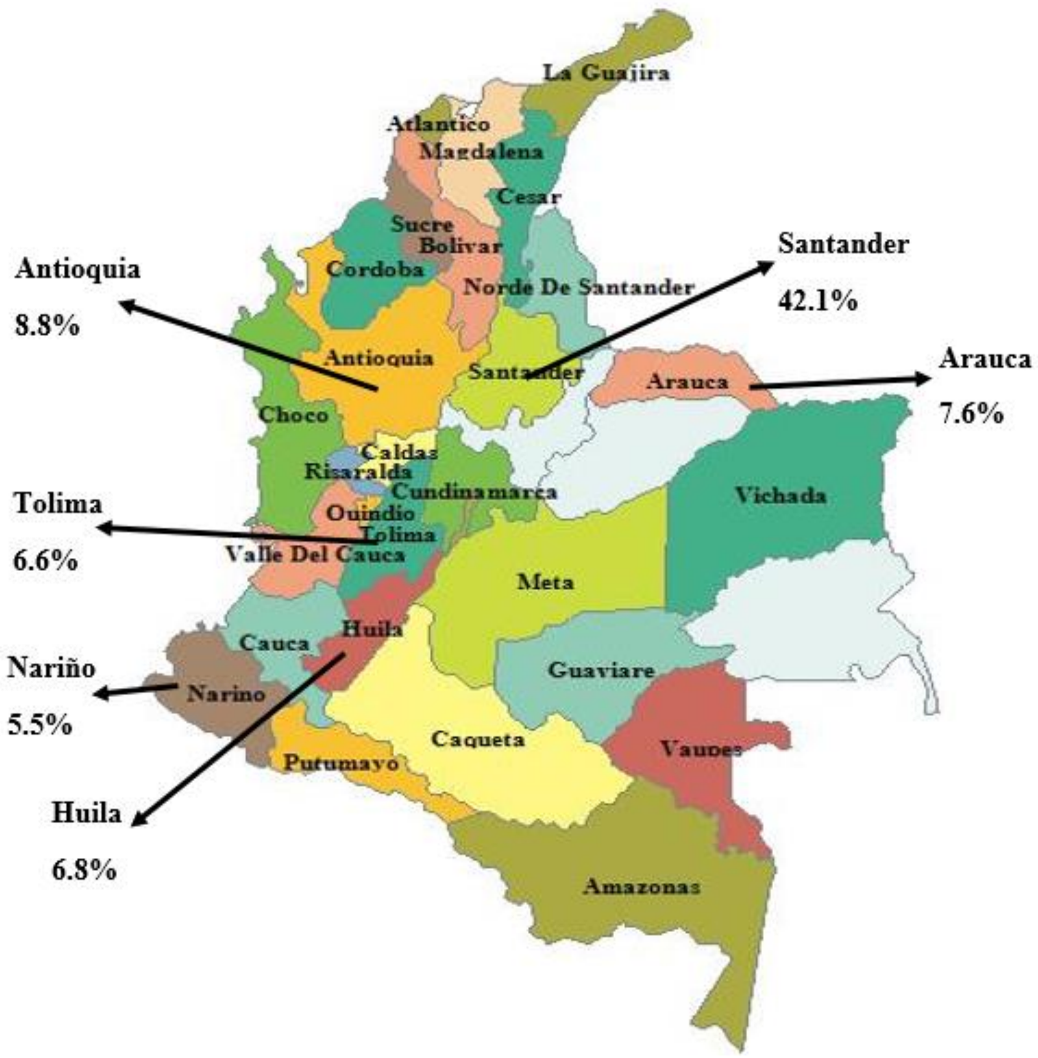

Figure 1.4: National cocoa production by provinces in Colombia. Source: FEDECACAO (2019)

2.6 Factors Affecting Cocoa Bean Quality

The international cocoa standards requirements of tradable cocoa quality include fermentation, thorough drying, free of smoky beans, unnatural or foreign odours and free of any signs of adulteration (Global Agricultural Information Network [GAIN], 2012). Cadmium is considered among the principal food safety concerns for the cocoa industry and its associated problem relates to beans from certain regions of some producing countries, especially in the Latin America and Caribbean (LAC) area (CAOBISCO-ECA-FCC, 2015). According to Lima *et al.* (2011), processors of cocoa beans and chocolate producers look for cocoa beans with a consistent ability to develop a strong cocoa flavour when processed. In addition to the possible qualities of taste, content yield and soundness are main requirements for the marketing stage. The emergence of increased demand for chocolates has had a major effect on the market for cocoa beans, both in terms of quantity and quality. The quality of cocoa beans is an important trade parameter since the quality of chocolate is largely dependent on the quality of the cocoa beans used (Afoakwa, 2016). Cocoa beans from LAC regions are high in Cd however, the high levels are generally associated with naturally high levels of Cd in the soil which impacts the quality of cocoa beans produced in addition to the chocolate quality (CABISCO-ECA-FCC, 2015). Diverse factors impact the production of high-quality cocoa beans which include genotype and origin of cocoa, bean chemical composition, environmental conditions, age of cocoa tree, farm management practices and post-harvest treatments such as pulp pre-conditioning, fermentation and drying (CAOBISCO-ECA-FCC, 2015; Oberrauter *et al.*, 2018).

2.6.1 Genotype and origin of cocoa beans

Chocolate has a distinctive flavour character, with specific notes related to cocoa bean genotype, growing conditions and processing factors. The amount and type of bean storage proteins, carbohydrates and polyphenols is determined by the genotype (genetic make-up) and origin of the cocoa tree that produced the cocoa beans (Clapperton, 1994; Beckett, 2003; Whitefield, 2005; Afoakwa *et al*., 2008). Afoakwa (2016) noted that the genetic make-up of the bean is also certainly crucial to this process as this determines the amounts and type of flavour precursors produced during the processes of fermentation and drying that lead to the creation of flavour, thereby affecting both the type of flavour and the intensity of the beans.

2.6.2 Environmental conditions

The cocoa tree, *Theobroma cacao L*., originated from the wet forests of South America and in its natural habitat, rainfall is heavy, and the temperature is relatively uniform. Environmental conditions, comprising of climate, sun exposure, rainfall and soil conditions in the region of origin influence the chemical composition of raw cocoa beans during the pod development, ripening, harvesting as well as bean fermentation (Oberrauter *et al*., 2018). According to Bunn *et al*. (2019), high precipitation of about 1500 – 2000 mm well distributed throughout the entire year is considered optimal for the cocoa crop. In general, for optimal conditions, temperatures should be within 18°C and 32°C for high assimilation rates (Almeida & Valle, 2007). Temperatures below 10°C can be lethal (Food and Agriculture Organization [FAO], 2007).

2.6.3 Farm management and soil fertility management practices

In the growth of the cocoa tree and the subsequent yield obtained, farm management activities involving pest and disease control, fertiliser application and pruning, weed control, among others, and soil fertility management play a crucial role (Kongor, 2018). These practices are frequently carried out by the farmer to ensure good yield. Accordingly, Wessel and Quist-Wessel (2015) posited that low levels of adoption of good cocoa farming practices, pest and disease attacks, ageing plantations, and poor and decreasing soil fertility contribute to poor average yields. While the type of cocoa, environmental factors and post-harvest treatments decide the characteristics of the flavour of beans, physical and chemical quality, farm management practices and soil fertility can affect the quality of cocoa beans (Kongor, 2018). The reduction of cocoa yields contributes to deforestation due to expansion of cocoa farms (Ruf & Zadi, 1998). In order to increase cocoa production with a minimum negative environmental impact, conservation and improvement of soil fertility are necessary (Liniger *et al*., 2011; Vanlauwe *et al*., 2010, 2015). Loss of soil fertility is usually triggered by the removal of nutrients from the soil through plant uptake, coupled with low or no application of fertilizer (Hartemink, 2006). There is the need to apply fertilizers and other amendments to replenish the lost nutrients. According to de Walque (2018), 83% of the farmers in San Vicente de Chucurí and Rionegro fertilized their cocoa plantation. It is imperative for farmers applying phosphate and zinc-containing fertilizers to ensure that they do not contain high levels of Cd especially in many regions of LAC where soil Cd levels are known to be high (Meter *et al*., 2019). Adequate plant nutrition is essential as nutrients and elements of the soil can influence Cd bioavailability and uptake by cocoa plants.

Pimiento and Vega (2006) revealed that FEDECACAO, the national federation of cocoa growers in Colombia, was working towards the objective of ensuring the safe and consistent quality of all Colombian cocoa beans by standardizing all agricultural practices. The FEDECACAO technical unit in Granada conducts training of interest to cocoa farmers, where technology is transferred to them through the improvement of the quality of cocoa and its different cultural practices. In the municipality of La Belleza Santander, a technical course was given on the integrated management of cocoa cultivation, with emphasis on the topics of clone recognition, good farm management practices, benefit and quality of the bean (FEDECACAO, 2020).

2.6.4 Post-harvest management practices

Postharvest treatment of cocoa which includes pulp preconditioning (such as pod storage and mechanical depulping), fermentation and drying of cocoa beans entails all the fundamental processes the harvested cocoa pods goes through before the final dried beans are obtained (Kongor, 2018). These processes are usually carried out by farmers in the country of origin and play a critical role in the final colour and flavour profile of the dried cocoa beans, as well as the shelf stability of the beans during transport and storage (Afoakwa, 2014; Krähmer *et al*., 2015). Accordingly, David (2005) mentioned that harvesting is the beginning of the post-harvest process that determines the quality of the cocoa beans to be sold to the cocoa and chocolate industry. Kadow *et al*. (2013) indicated that fermentation results in chocolate flavour precursor formation in addition to the reduction of bitterness and astringency as illustrated in Figure 1.5. These changes in the flavour profile correspond with a change in colour from pale purple (unfermented) to brown (fully fermented). The fermentation stage is a particularly important part of quality formation that, if not carried out adequately, makes it impossible to recover the attribute afterwards (Afoakwa, 2016; Escobar *et al*., 2020).

Figure 1.5: Fermentation in wooden boxes and drying of fermented cocoa beans using roof (on wheels) in Santander, Colombia. Source: de Walque (2018)

2.7 Cadmium in Cocoa

2.7.1 Introduction

Cadmium, as well as zinc (Zn) and mercury (Hg), is a second-row transition metal belonging to group 12 of the periodic table. The element has an atomic number of 48, an atomic mass of 112, one primary oxidation state (+2) and eight isotopes that exist naturally (106Cd, 108Cd and 110Cd to 116Cd). Isotopes 114Cd, 112Cd, 111Cd, 110Cd and 113Cd have abundances of 28.73%, 24.13%, 12.80%, 12.49% and 12.22%, respectively (Smith, 1999). A significant route for potentially hazardous heavy metals from soils to humans is cultivated plants, because these elements can accumulate in plant organs and affect the quality of diets that contain them (Mite *et al.*, 2010). There is a well-known toxicity of Cd to animals and plants. Even at relatively low concentrations, Cd is highly toxic to humans as it accumulates in the body over decades and is carcinogenic and harmful to the kidneys and bones after prolonged exposure (Barraza *et al*., 2019). Cadmium contamination represents one of the highest risks for food safety and human health (Chavez *et al*., 2015; Baligar *et al*., 1998; Benavides *et al*., 2005; Marschner, 2012); and the risk is exacerbated by the high mobility of Cd towards the edible parts of crops (McLaughlin *et al*., 1996). Cadmium can also cycle between soils and cocoa trees through the leaf litter (Gramlich *et al*., 2018). Farm management practices such as leaving leaf litter and pod husks on the ground can recycle Cd within the system (Gramlich *et al*., 2018; Meter *et al*., 2019). Hoogerwerf (2020) noted that half of the farms in the village of San Vicente de Chucurí, Colombia used cocoa shells as fertilizer while the other half discarded them. Even though this element is not essential, given that it does not fulfil a metabolic purpose in plants, it is absorbed as a divalent cation (Coco *et al*., 2003; Chavez *et al*., 2015), and accumulates in roots, buds and cocoa beans (Zarcinas *et al*., 2004; Rascio & Navari-Izzo, 2011) which defines cocoa plants as Cd accumulators as compared to other food crops (Barraza *et al*., 2019).

2.7.2 Origin and risks

A combination of natural processes and anthropogenic factors is the product of the presence of Cd in cocoa-growing soils as illustrated in Figure 1.6. Natural processes include rock weathering, volcanic eruptions, forest fires, erosion and deposition in river sediments, while anthropogenic factors include mining and industrial pollution, as well as agricultural activities, such as irrigation and fertilization, which can contribute to the introduction of Cd into the soil (Meter *et al*., 2019). Natural activities contribute to the release of variable amounts of Cd into the soil. Volcanic eruptions, forest fires, windblown dust, and sea spray are among the natural sources of Cd to the atmosphere (Khan *et al*., 2017). Weathering of parent rocks also contribute to the release of Cd to the environment (Khan *et al*., 2010; Liu *et al*., 2013).

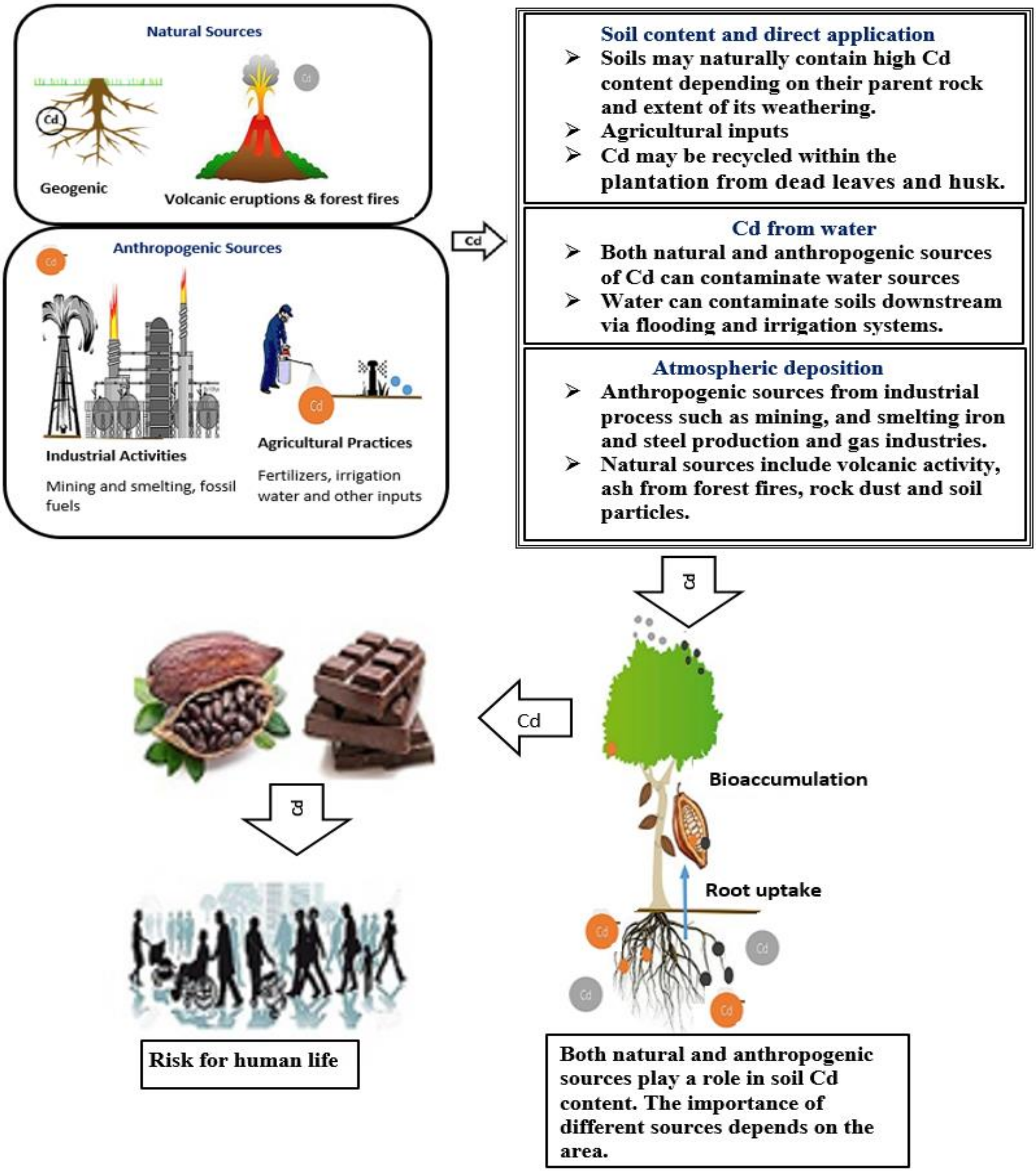

Figure 1.6: Sources of Cd input to cocoa growing soils. Source: Adapted from Ruales *et al.* (2017); Meter *et al.* (2019)

Atmospheric circulation models indicated that global climate is closely linked to the atmospheric CO_2 content; increased CO_2 results in warmer temperatures, changing precipitation patterns, and an overall increase in runoff (Labat *et al*., 2004; Gedney *et al*., 2006; Alley *et al*., 2007). The changes in these factors overtime affect the weathering of rocks. Mafic and ultramafic rocks contain high amounts of Cd, and thus upon weathering, these rocks release significant quantities to soil (Shah *et al*., 2010). Deheuvels *et al*. (2014) noted that these factors can also affect soil characteristics, such as soil water balance, organic matter, and nutrient cycling and availability as well as temperature fluctuations and all can influence Cd availability. In an investigation by He *et al*. (2015) at Sustainable Perennial Crops Laboratory in Beltsville revealed that the contribution of natural processes to soil Cd contamination is 3- to 10-fold lower than that of anthropogenic sources. Additionally, comparing the weathering of different soil types, those derived from igneous rocks usually contain low amounts of Cd, soils obtained from metamorphic rocks are intermediate, and soils weathered from sedimentary rocks (especially shales) contain high amounts. Studies by Gramlich *et al*. (2018) highlighted that Cd levels in cocoa-growing soils varied significantly across various geological substrates in Honduras and were highest in alluvial soils resulting from sedimentary materials. In another study of 159 farms in Ecuador, a similar trend was found (Argüello *et al*., 2019). Other natural sources of soil Cd include volcanic activity, forest fires, wind-blown soil particles and rock dust.

By applying phosphate fertilisers extracted from sedimentary materials and irrigation water from areas with high Cd levels, anthropogenic activity may increase the concentration of Cd in cocoa-growing soils. Localized Cd exposure can also result from the mining and smelting of minerals, fossil fuel burning, and other industrial activities. Actions such as mining and land erosion on upstream metal-rich soils can be a major source of Cd in downstream agricultural areas (Meter *et al*., 2019). The Cd input resulting from the use of contaminated fertilisers depends not only on the source rock concentration, but also on the fertilization programme practiced by the farmer. Previous land use can also be relevant as it may have contributed to Cd accumulation in the soil (Alloway *et al*., 1999; Gramlich *et al*., 2018), although this would be expected to be eliminated over time by leaching (Smolders, 2017).

According to the EFSA (2012), it should be noted that 90% of human exposure to Cd for the non-smoking population is related to food products and an estimate of Cd dietary exposure is ca. 2.5 µg kg^{-1} of body weight per week over a lifetime assuming an average life span of 77 years. Within the 20 main groups described in the food classification system (Foodex), cocoa beans and chocolate contribute from 4 to 26% and from 15 to 92% respectively depending on the consumer's age (EFSA, 2012). Children and adolescents have been identified as the most vulnerable (Yanus *et al*., 2014) thus, the susceptibility of adverse effects in these groups is much higher compared to adults. Chronic Cd ingestion causes kidney, liver, and skeletal damage together with an increased risk of cancer (Zug *et al*., 2019), therefore, it is classified as a human carcinogen by the International Agency for Research on Cancer of the World Health Organization (Rosales-Huamani *et al*., 2020).

2.7.3 Legislation

EU represent only 6% of the world's population but consumes half of the world's chocolate (https://europa.eu/capacity4dev/desira). The EU needs to import all of its cocoa beans as there is no domestic cocoa production in Europe. Considering the dangers to humans of excess consumption of Cd and that the levels of it differ in different cocoa products, maximum allowed limits have been defined according to EFSA. The EC Regulation (EC) No 1881/2006 establishes maximum levels for Cd in a range of foodstuffs as depicted in Table 1.1

Table 1.1: EC maximum permissible levels of Cd in cocoa and chocolate products; adapted from European Commission regulation (EC) 1881/2006.

Specific Cocoa and Chocolate Products	**Maximum Permissible Cadmium Level (mg kg^{-1})**
• Milk chocolate with < 30% total dry cocoa solids	0.10
• Chocolate with < 50% total dry cocoa solids; milk chocolate with ≥ 30 % total dry cocoa solids	0.30
• Chocolate with ≥ 50% total dry cocoa solids	0.80
• Cocoa powder sold to the final consumer or as an ingredient in sweetened cocoa powder sold to the final consumer (drinking chocolate).	0.60

These stricter limits set for cocoa products were implemented 1st January, 2019 (EC, 2019). However, the EU regulation does not highlight permissible limits for cocoa beans (Meter *et al*., 2019). In addition to generating risk to human and animal health, surveys conducted by Argüello *et al*. (2019) indicated that the implemented EC regulation will affect a large fraction of the cocoa production industry in Latin America as Europe is one of its main destination markets. The Cd accumulation process is of particular interest in cocoa farms in Latin America especially Colombia because these producing areas are characterized by high Cd content that dangerously join the food chain and thus, affect human health (Aguirre-Forero *et al.*, 2020). de Walque (2018) established that while the quality of cocoa beans in San Vicente de Chucuri seems optimal to meet the global demand, the Cd content might constrain export.

2.7.4 Cadmium in Latin America

The LAC region is known as the main producer of FFC nevertheless, the cocoa sector in the area is faced with numerous challenges such as high levels of Cd concentration in soil. In comparison to other cocoa growing regions of the world, the levels of Cd in cocoa regularly exceed the implemented limits in certain areas of LAC (Meter *et al.*, 2019). Cadmium accumulates in cocoa beans and has adverse repercussions on human health. Surveys across the world's cocoa-producing regions show that the content of Cd in cocoa beans is especially a problem in LAC (Figure 1.7).

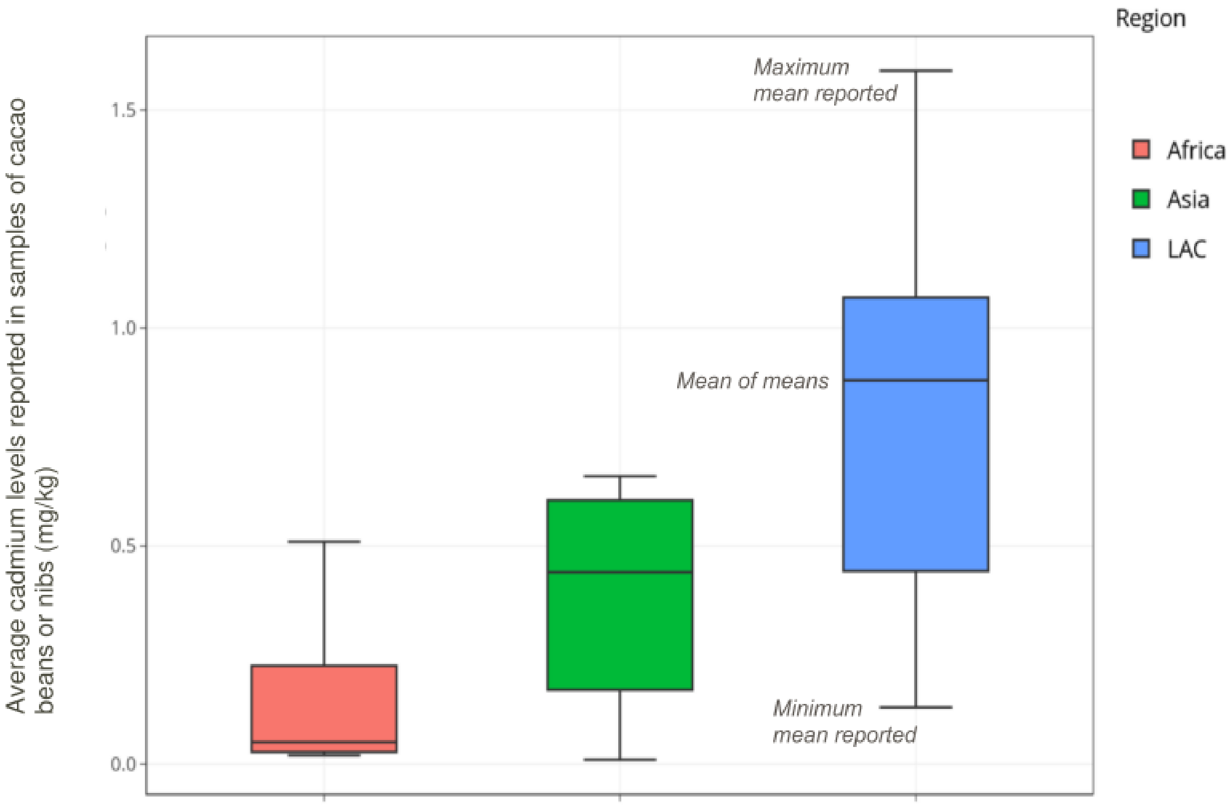

Figure 1.7: Distribution of reported average Cd levels in cocoa beans or nibs from Africa, Asia and LAC. Source: Meter *et al*. (2019)

The presence of Cd in cocoa fruit has been reported in Peru, Ecuador and Colombia, but only some of the territory is believed to be problematic (Aguirre-Forero *et al.*, 2020). Accordingly, Meter *et al*. (2019) emphasized that smallholder producers from Latin America and the Caribbean (LAC) countries will be most affected, although these regulations have a global impact on the entire cocoa supply chain. As cocoa plants are known to be Cd accumulators as compared to other food crops, the established regulations for the maximum levels of Cd allowed in (imported) cocoa products enforced by the EU will affect cocoa producers in the region. This new standard poses a potential threat to the livelihoods of many smallholder farmers as large part of cocoa exports (especially fine aroma) from the Andean region is blocked. This jeopardizes the niche market of Colombia, Ecuador and Peru (Barraza *et al*., 2019; Meter *et al*., 2019; Zug *et al*., 2019), as well as economic and social repercussions.

Studies conducted by Aguirre-Forero *et al*. (2020) in the province of Magdalena, Colombia indicated that the Cd concentration in the cocoa beans fluctuated between zones and plots, but the values all exceeded 0.5 mg Cd kg^{-1}, indicating that the grains were contaminated. In the major cocoa-growing regions of Ecuador, Argüello *et al*. (2019) conducted a nationwide survey, collecting 560 samples from 159 cocoa farms, and found a mean Cd bean concentration of 0.90 mg kg^{-1} with 45% of the samples exceeding 0.60 mg kg^{-1}. This threshold is used because the EC regulation defines upper limit of 0.60 mg kg^{-1} for Cd concentration in cocoa powder which is used as a Cd concentration threshold in cocoa beans.

Barraza *et al*. (2017) and Chavez *et al*. (2015) found similar results. In a study in Peru with a sample size of 70, the concentration of bean Cd from nearly 57% of sample sites exceeded the defined threshold (Arévalo-Gardini *et al*., 2017). With a sample of 40 cocoa beans from the Huánuco region of Peru, Zug *et al*. (2019) recorded an average Cd content of 2.46 mg kg^{-1}. An investigation carried out by (Acuña *et al*., 2017) to evaluate the content of Cd in table chocolate produced in the municipality of Chiquinquira- Boyaca, Colombia, the values between 0.23 - 0.26 mg kg^{-1} were found in powdered chocolate samples and 0.21 - 0.23 mg kg^{-1} in samples of granulated chocolate which were below the threshold. Farm-level surveys indicated substantial variation across sites, with some areas or 'hotspots' showing significantly higher levels of Cd than others (Gramlich *et al*. 2018; Bravo *et al*. 2018; Barraza *et al*. 2017; Argüello *et al*. 2019; Arévalo-Gardini *et al*. 2017; Tantalean Pedraza *et al*. 2017; Mite *et al*. 2010). Recent findings from Ecuador showed variation in the content of cocoa bean and soil Cd at multiple levels, suggesting a high degree of heterogeneity on several scales between provinces, cantons and even within the fields of farmers (Argüello *et al*., 2019).

Fermentation accelerates the migration of Cd from the cocoa beans to the testa, leading to a reduction of the Cd content in cocoa beans. Nevertheless, this Cd migration only occurred when the fermentation was sufficiently intense to lower the pH to < 5.0 in the beans (Vanderschueren *et al*., 2020). Hoogerwerf (2020) found that fermentation may not have a significant impact on decreasing the Cd content in different clones as only 2 out of 44 samples recorded Cd concentrations ≤ 0.78 mg kg^{-1}.

2.7.5 Soil properties and cadmium availability

Cocoa plants absorb Cd from available forms in the soil therefore the amount and availability of Cd present in the soil determines the accumulation of Cd by cocoa plants. The higher the levels of total soil Cd content, the greater the potential for Cd adsorption (Meter *et al*., 2019). According to Gramlich *et al*. (2018), Cd uptake by cocoa plants is intimately related to its mobility in soils which is high as compared to other trace metals. Several investigations have noted positive and statistically significant low to moderate correlations between total soil and cocoa bean Cd concentrations (Ramtahal *et al*., 2016; Fauziah *et al*., 2001; Gramlich *et al*., 2018; Zug *et al*., 2019). Since not all soil Cd is directly available to plants, the ease with which it can be transferred from the soil to plant tissues relies on the amount of it available, soil type, mineralogy, pH content, quality of organic matter, and presence of other micro-elements such as zinc (He *et al*., 2005; Kirkham, 2006; PNUMA, 2008; Alloway, 2013). Therefore, it is imperative to understand how these soil properties affect the bioavailability of metals and specifically Cd in cocoa-growing soils as manipulating them is key to develop effective soil mitigation measures (Hamid *et al*. 2019) (Figure 1.8).

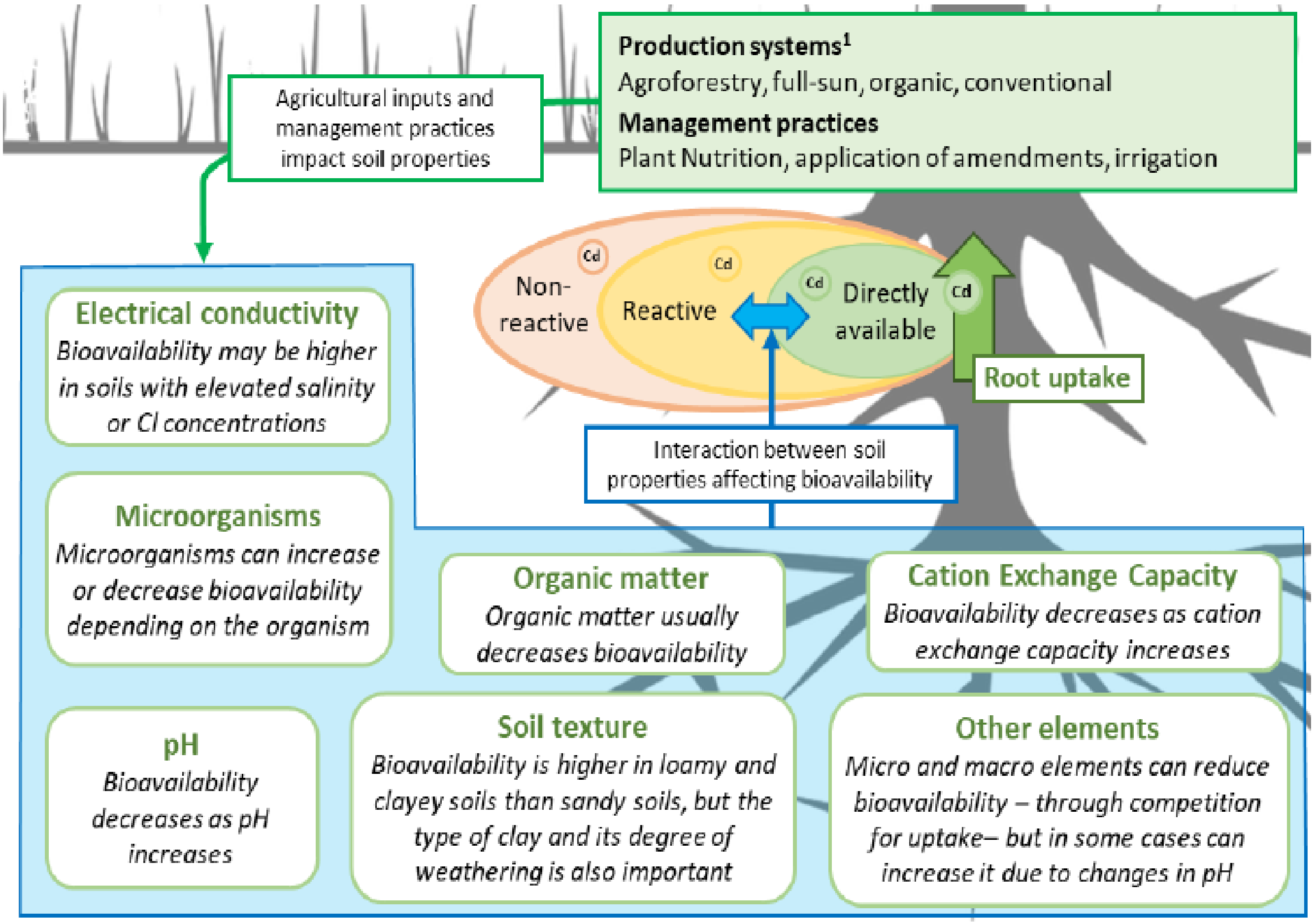

Figure 1.8: Soil properties affecting Cd availability to cocoa plants. Source: Meter *et al.* (2019)

2.7.5.1 Cation exchange capacity (CEC)

Cation exchange capacity (CEC) is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton & Murphy 2007). The main ions associated with CEC in soils are the exchangeable cations calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) (Rayment & Higginson, 1992), and are generally referred to as the base cations. A higher soil CEC implies a higher capacity for soil particle surfaces to retain base cations and can lead to a decrease in Cd bioavailability. As CEC decreases there is an increased competition between H^{+} and Cd^{2+} ions for binding sites which results in Cd desorption from soil particles into the soil solution (Meter *et al*., 2019). The ability of sediment to bind with cations is often measured by CEC which could relate to migration of metals in soil. The CEC of soil is affected by several factors such as pH, particle size and organic matter content, clay content and mineralogy. While clay content is key in cation exchange, not all clay mineralogy has the same capacity: whereas 1:1 clay are common in highly weathered tropical soils and have a low CEC, 2:1 clay have a high CEC. An increase in pH and soil organic matter content is also usually associated with a higher CEC, especially in tropical soils (Adriano 2001).

2.7.5.2 Soil pH

The soil pH is a measure of soil acidity or alkalinity and it directly affects the availability of nutrients in the soil. According to Adriano (2001), it is one of the most vital parameters influencing Cd speciation, mobility, solubility and thus, its bioavailability. An increase in soil pH results in a corresponding increase in soil CEC. The pH levels that best suit the growth and development of cocoa trees ranges from 5.0 to 7.5 (Zug *et al*., 2019; Gramlich *et al*., 2018; Meter *et al*., 2019). In alkaline soils, Cd tends to be less bioavailable as it is less soluble. Ramtahal *et al*. (2018) noted that the application of certain treatments to acidic soils that raise the pH can decrease the percentage of Cd that is bioavailable and thus reduce its uptake by plants. For soil amendments, ensuring that they are integrated into the soil is one important factors in the success of soil pH-modification. This can be a problem for existing cocoa plantations because of the possibility of damaging the surface roots (Meter *et al*., 2019). However, it has been reported that if mixed with organic matter (composts, manures, bio-solids, or green manures), and applied to the surface, biodegradation of the organic matter causes the formation of Ca salts which are soluble and will leach into the soil (Hue, 1999; Liu *et al*., 2001). Except work done by Fauziah *et al*. (2001) in Peninsular Malaysia, the majority of studies on Cd in cocoa established significant and negative correlations between soil pH and bioavailable Cd (Low *et al*., 1994; Bravo *et al*., 2018; Barraza *et al*., 2017; Gramlich *et al*., 2018, Argüello *et al*., 2019). An investigation conducted by Chavez *et al*. (2016) in a laboratory experiment to ascertain the effect of zeolite on Cd bioavailability, 0.5 and 2% of total weight with different doses of Cd was applied on three soil types. The study revealed no increase in soil pH, and neither 0.01 M $CaCl_2$ nor Mehlich extractable Cd levels were lowered after 28 days in any of the treatments.

2.7.5.3 Organic matter content

In soil, a fully decomposed organic matter is called humus and it is important for its structure and fertility because it holds individual mineral particles together in clusters. The organic matter content of soils plays an important role in Cd bioavailability due to its ability to adsorb Cd. The capacity of organic matter to bind with Cd is due to its high CEC as well as its chelating ability (Adriano 2001; He *et al*., 2015). In the cultivation of cocoa, the main source of organic matter is the decomposition of leaf litter as well as crop residues (Hartemink, 2005) and can reduce Cd bioavailability indirectly by affecting other soil properties (Shahid *et al*., 2016), mainly by increasing soil pH (Khan *et al*., 2017). However, humic substances sometimes form soluble complexes with Cd and increase its mobility (He & Singh, 1993; Khan *et al*., 2017). Arévalo *et al*. (2016) found a positive correlation between Cd, pH and organic matter. In general, pH controls availability and mobility of Cd in soils (Christensen, 1984; Alloway, 2013) and organic matter has significant effects on its availability (Degryse *et al*., 2009). Organic matter absorbs Cd forming organometallic complexes and inhibiting its solubility and mobility (Eriksson, 1988; Kabata-Pendias & Pendias 2000). An investigation conducted by Rodríguez Albarrcín *et al*. (2019) in the cocoa-producing area of central Colombia concluded that liming and application of organic matter are the most promising mitigating strategies, with liming potentially the most cost-effective.

2.7.5.4 Soil texture

Soil texture represents the proportion of sand, silt and clay sized particles that make up the mineral fraction of the soil and impacts both Cd content and its bioavailability in soils due to different cation exchange capacities of sand, silt and clay (Kabata-Pendias, 2010). Concentration of Cd in the soil vary according to the type of rock present in soils, e.g. soils with sedimentary rocks show higher Cd levels compared to soils with igneous and metamorphic rocks (Vega *et al*., 2010; Rodríguez Albarrcín *et al*., 2019). In general, fine textured soils (clays) have a higher capacity of adsorption than coarser textured soils (sands), while in loamy soils (a mixture of clay, sand and silt), the total Cd content and bioavailability tend to be higher than in sandy soils (Adriano, 2001; Kabata-Pendias, 2010). An investigation conducted by Gramlich *et al.* (2017) revealed a positive influence of clay content on the available Cd in the soil.

2.7.5.5 Macro and micro-nutrients

Meter *et al*. (2019) noted that some ions can directly affect Cd uptake through competition for soil exchange sites, and Cd compound chelation or complexation. However, it is seldom straightforward to predict the effect as it also relies on the compound applied and the mode of application that can lead to a change in pH or CEC and thus affect the bioavailability of Cd. As highlighted by Zarcinas *et al*. (2004), the uncontrolled application of cheap and contaminated phosphate fertilizers may have contributed to elevated levels of Cd in soil and cocoa crops. The total fertilizer consumption of Ecuador, a country in the LAC region reached 368,370 tonnes in 2014 (FAO, 2017). It has been reported that the use of phosphate fertilizers uncontaminated by Cd either decreases the bioavailability of it by immobilizing it in the soil or increases it by reducing soil pH (He *et al*., 2015; Mahar *et al*., 2015). Fauziah *et al*. (2001) and Zug *et al*. (2019) also found that bioavailable P soil content was positively correlated with bioavailable Cd soil content, but they assumed it was due to the use of heavy metal contaminated phosphate fertilizers. The chemical properties of Cd and Zn are very similar, leading to the conclusion that a relative lack of Zn in the soil may lead to increased Cd uptake as they compete for the same transport membranes (Sarwar *et al*., 2010; Adriano 2001).

2.7.6 Cadmium uptake in cocoa plants

The cocoa plant is a perennial tree, 4 to 8 m in height (Afoakwa, 2014) and there are mechanisms that control Cd uptake by it from soils as well as the processes that take place within it. These processes are responsible for the translocation of Cd from the roots to above-ground parts and the internal sequestration of the element (Barraza *et al*., 2019). Cadmium cation (Cd^{2+}) is transported to the xylem after uptake by the root system, and travels to the leaves where it is actively transported into the phloem and subsequently enters the fruit (Shahid *et al*., 2016; Clemens *et al*., 2013). Additionally, some Cd can enter the fruits directly from the xylem without passing through the leaves and it is relocated into developing beans from pod husks. These processes appear to play a key role in Cd accumulation and distribution within cocoa as illustrated in Figure 1.9. Argüello *et al*. (2019) posited that young cocoa plants have the capacity to take in more Cd as compared to older ones and this shows that the age of the cocoa plant may influence its Cd absorption.

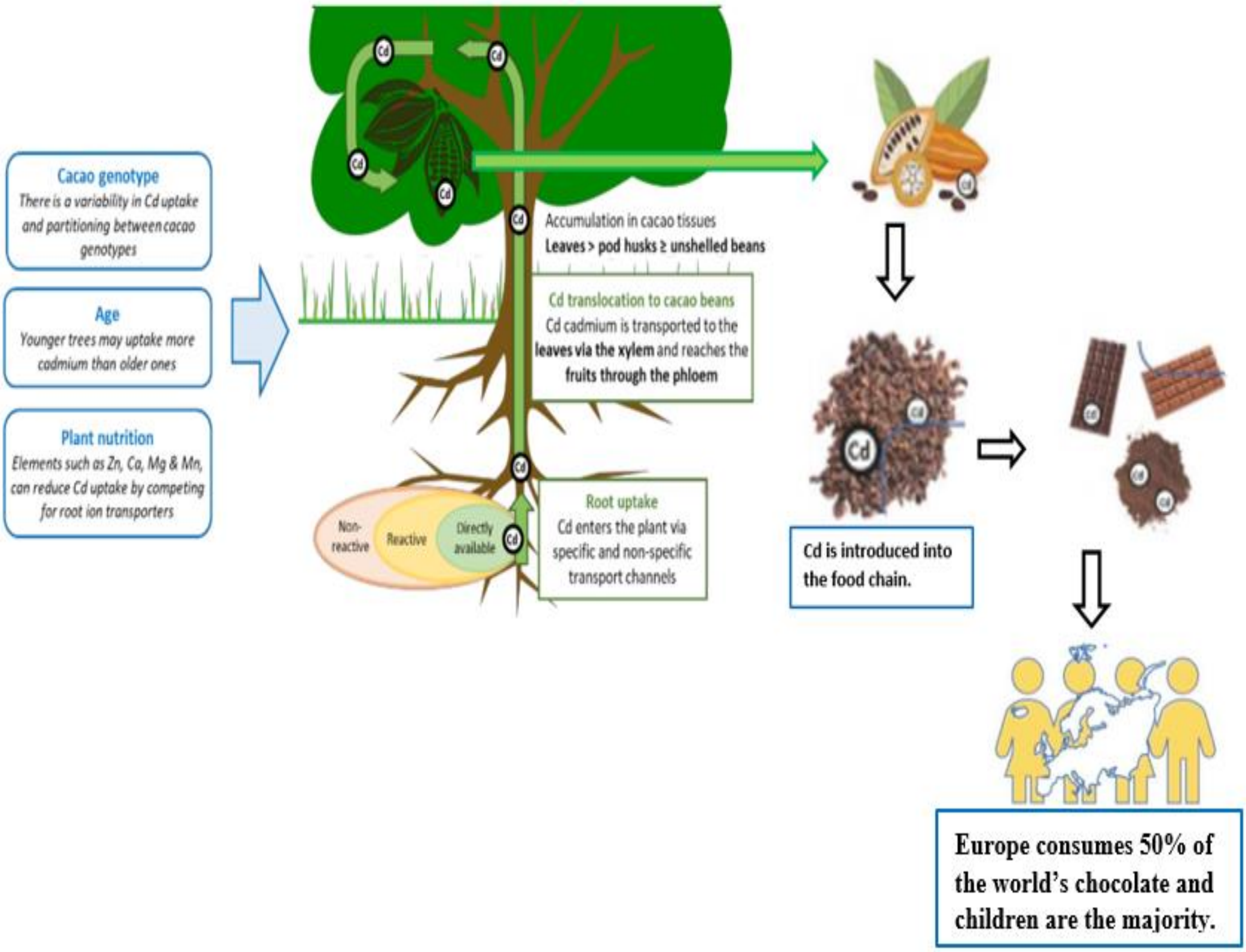

Figure 1.9: Uptake and partitioning of Cd within the cocoa plant. Source: Adapted from Meter *et al*. (2019)

The cocoa tree, as a plant species, includes a wide range of vastly different varieties that accumulate pronouncedly different quantities of Cd (Ji *et al*., 2011; Zug *et al*., 2019). Cadmium is accumulated in different tissues depending on the species (Ji *et al*., 2011; Li *et al*., 2017, Zug *et al*., 2019). Among the trace elements, Cd, a non-essential trace metal, seems to accumulate mainly in the edible parts of cocoa, which entails potential risks for human health by ingestion of contaminated products. Generally, Cd concentration in plant tissue decreases from roots > stems > leaves > pod husks > seeds (Benavides *et al*. 2005).

Several studies have reported genotypic variability in Cd accumulation among cocoa varieties (Cryer *et al*., 2012; Arévalo-Gardini *et al*., 2017; Gramlich *et al*., 2017; Barraza *et al*., 2017; Lewis *et al*., 2018) and not only on the variety but also on the geographical site (Bertoldi *et al*., 2016). Barraza *et al*. (2019) found a significant difference in Cd transfer from soil to plant tissues between the Ecuadorian national FFC and the CCN51 hybrid. Hoogerwerf (2020) concluded that Caucasia 39 clones were highly significant in Cd concentration as compared to Fedecacao Arauquita 5 and Fedecacao 2 clones. Also, less correlation between Cd concentration in cocoa beans and quantitative morphological characteristics was noticed. An investigation conducted in the northern part of Honduras which involved 11 grafted cocoa cultivars revealed significant differences in bean Cd content between cultivars and no correlation between bean and soil Cd content (Engbersen *et al*., 2019). The variations in bean Cd content could be attributed to genotype difference in Cd loading during bean maturation.

2.7.7 The effects of lime addition or other amendments on cadmium uptake

Cadmium in cocoa beans results from direct uptake of the heavy metal by the cocoa tree (*Theobroma cacao L*.) from soils containing Cd (Chavez *et al*., 2016; Barraza *et al*., 2017; Arévalo-Gardini *et al*., 2017; Argüello *et al*., 2019; Zug *et al*., 2019). Thus the levels of Cd found in the soils of cocoa plantations and their availability to the cocoa plant is of great importance in formulating mitigation strategies. With favourable soil conditions, Cd is readily taken up by the roots and distributed throughout plant tissues (Adriano, 2001). Some soil properties affect the phyto-availability of Cd in soils which include pH and is one of the most important soil chemical properties affecting solubility of Cd (Basta *et al*., 2005).

Several studies carried out on cocoa in Colombia (Bravo *et al*., 2018), Ecuador (Chavez *et al*., 2015; Argüello *et al*., 2019), Honduras (Gramlich *et al*., 2017, 2018), Peru (Zug *et al*., 2019) and Trinidad and Tobago (Ramtahal *et al*., 2018) investigating the relationship between soil and cocoa bean Cd have revealed significant effect of acidic low pH soils on Cd phyto-availability. According to Lambert *et al.* (2000), suitable application of amendments to contaminated soils can reduce the phyto-availability of Cd, hence reducing their uptake by plants. Additionally, a number of studies have shown the effectiveness of organic matter or biochar on immobilizing Cd by increasing CEC (Adriano, 2001; Laird *et al*., 2010; Beesley & Marmiroli, 2011; Chavez *et al*., 2016). Furthermore, biochar also known as activated wood charcoal was found to be more effective than organic matter in reducing uptake (Liang *et al*., 2006). The formation of surface functional groups and adsorption sites on biochar could influence its CEC (Zeng *et al*., 2008; Liang *et al*., 2006) and consequently the capacity of biochar amended soils to form complexes with metal ions. Ramtahal *et al*. (2018) demonstrated lime as an effective soil remedial measure to decrease Cd in cocoa. Both lime and biochar as ameliorants can be used to reduce the solubility and uptake of Cd by cocoa as depicted in Figure 2.0. Ramtahal *et al*. (2019) concluded in a pot study conducted that the effectiveness of lime in reducing leaf Cd levels was greater than biochar; whereas Egene *et al*. (2018) highlighted the superiority of biochar over other organic amendments for metal immobilization. It is also important to ensure that surface application of liming materials can penetrate into the root zone in the soil, achieved through combination with organic matter and in areas such as Colombia, where some sources of liming materials are forbidden due to its use in making cocaine, alternative amendments such as biochar need to be used (Meter *et al*., 2019).

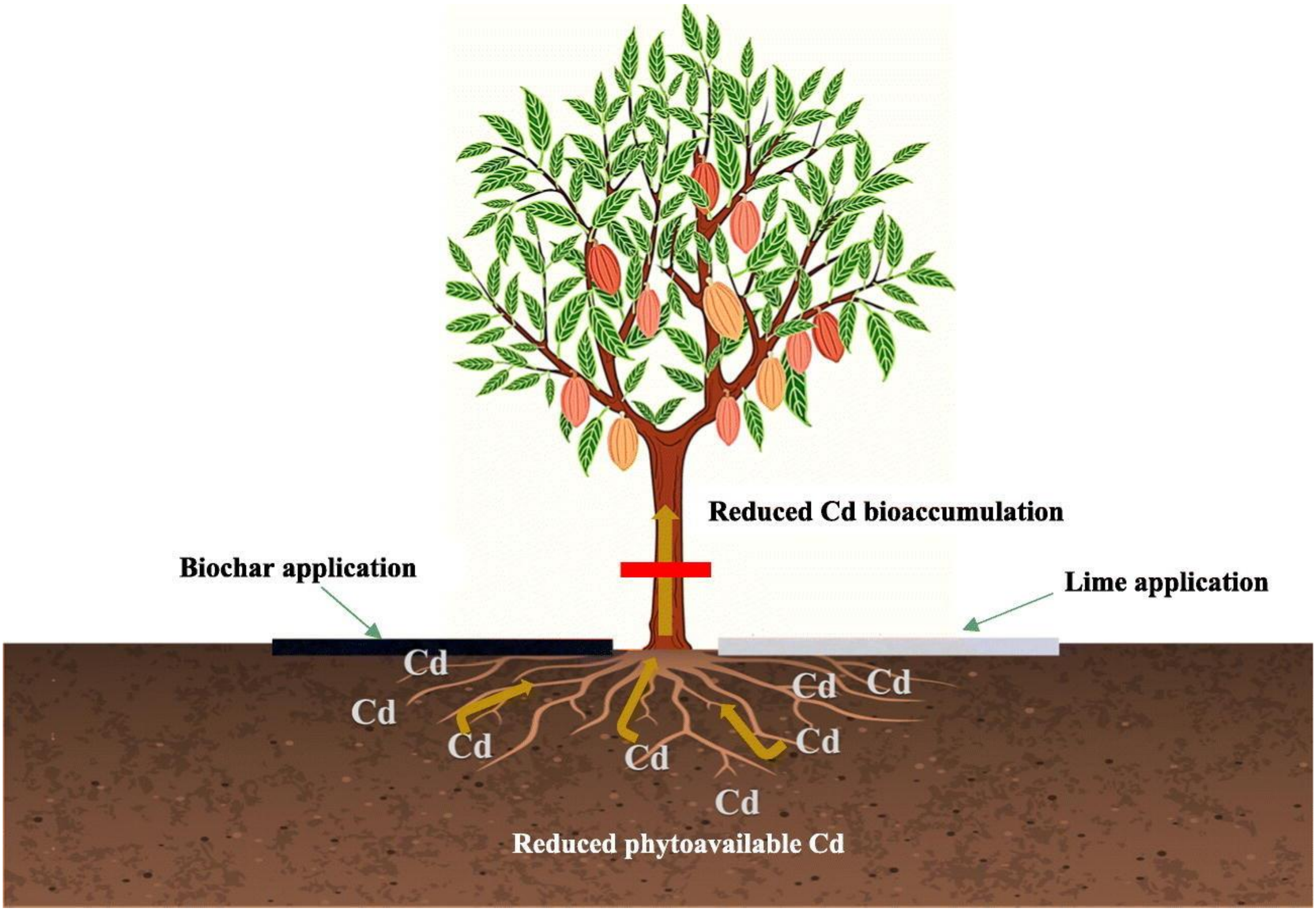

Figure 2.0: Reduction in Cd uptake by the application of ameliorants (lime and biochar) Source: Ramtahal *et al*. (2019)

2.7.8 Other heavy metals in cocoa

Natural processes such as volcanic activity or rock erosion, together with anthropogenic factors such as industrial waste, mining, smelting and rock fertilizers, introduce high-density chemical elements into the environment which have led to a destabilization in the biological balance (Rosales-Huamani *et al*., 2020). All these factors have increased the presence of compounds and xenobiotic elements with bio-accumulative and toxic properties, among which are heavy metals, which pass directly or indirectly to different foods, finally affecting the human being (Huang *et al*., 2014). Apart from Cd, other heavy metals with potential hazards in soils such as Cr, Pb, Zn and Cu have been reported in the inland areas of Peru. Zug *et al*. (2019) noted that heavy metals differ in their frequency (Pb > Zn > Cd > Cu) and in their availability for living cells. Some, such as Co, Cr, Cu, Ni, and Zn, are essential for plants and therefore non-toxic at low concentrations, whereas others, such as Ag, As, Cd, Hg, Pb, and Sb, are toxic at any level. Some heavy metals possess chemical properties similar to those of nutrients such as calcium or magnesium and, if available in water or soil, can be absorbed by cocoa plants (Kabata-Pendias, 2000). Low levels of Zn availability in the soil increases Cd uptake due to Zn-Cd interaction during uptake and translocation (Mitra, 2015; Arévalo-Gardini *et al*., 2017). Gramlich *et al*. (2018) highlighted a potential explanation for this observation is a competition for the same carriers or transporters in the root cell membranes.

2.8 ICP-MS

ICP-MS is the fastest growing multi-element trace element analysis technique with more than 8000 ICP-MS instruments installed worldwide (Thomas, 2008). The gaseous ions are separated in the mass spectrometer according to their mass-to-charge (m/z) ratio and are detected in proportion to their abundance. The recommended unit is $\mu g\ L^{-1}$. The detection limit is the smallest sample quantity that yields a signal that can be distinguished from the background noise. Generally it is a signal equal to 3 times the background noise and 10 times for the limit of quantification (de Hoffman & Stroobant, 2007). The technique was commercially developed in the early 1980s and because of superior detection capabilities has superseded many other analytical methods including: atomic absorption, optical emission spectroscopy, and ICP atomic emission spectroscopy (Wolf, 2005). Zoorob *et al*. (1998) posited that ICP-MS is considered the method of choice for elemental analysis for several reasons such as attaining low detection limits, achieving multi-element capability, and giving of isotope information. Digestion of biological sample using acid digestion reagent combination such as nitric acid is a normally used method in sample preparation for metal analysis (Chavez *et al*., 2015; Oliva *et al*., 2019). Commonly used techniques in the determination of Cd concentration include ICP-MS (Behrooz *et al*., 2009; Meter *et al*., 2019). The most appropriate choice depends on level sensitivity required, whether one or more trace metals are being measured, as well as budgetary limitation (Thermo Elemental, 2002).

2.9 Resin P Method

Resin P analysis requires shaking soil, MQ-water and resin membrane square overnight. During this process, phosphate in the soil moves into the water phase where it is efficiently taken up by the resin membrane square. The amount of P adsorbed onto the membrane is proportional to the soils ability to replenish P into the water phase as depicted in Figure 2.1. In the laboratory, the resin P test uses a mechanism that closely mimics the soil/soil solution/plant root model. Thus, P in the soil are in equilibrium with those in the soil solution, and plant roots use an ion exchange mechanism to take up available P from the soil solution as required (www.hill-laboratories.com).

Several studies comparing soil P determination methods have been carried out. Saggar *et al*. (1992) concluded the resin P test extracted more than twice as much P as compared to the Olsen test in a glasshouse experiment conducted on four soils contrasting in P sorption capacity and exchangeable Ca content with perennial ryegrass. Also, a literature review by Silva and Raij (1999) comprised of papers published with soils of different countries, in which P determined by various methods was correlated with plant P uptake. The data revealed that the resin membrane method is better correlated with P uptake than the other methods and that it is suitable for all types of soils, both acidic and alkaline.

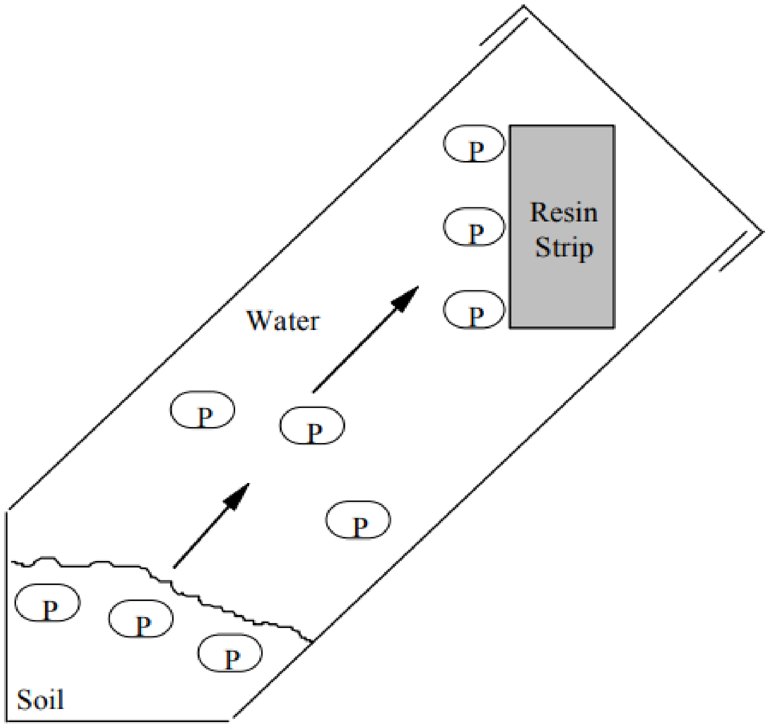

Figure 2.1: Resin P extraction in the laboratory; P in the soil moves into the water phase for uptake by the resin strips. Source: www.hill-laboratories.com

3.0 MATERIALS AND METHODS

3.1 Study Area

The samples for this study were obtained in the Colombian villages of San Vicente de Chucurí and Rionegro as depicted in Figure 2.2 and 2.3 respectively. According to de Walque (2018), Colombia, like other bimodal climate nations, has two major harvest seasons. The first occurs in April to June while the second from November December.

In the province of Santander, the municipalities of San Vicente de Chucurí and Rionegro are known for their cocoa production. San Vicente de Chucurí is the province's largest cocoa producer which is why it bears the unofficial title of "Capital Cacaotera de Colombia" (Cocoa Capital of Colombia). About 55% of the total population (34, 640 inhabitants) are farmers living outside the village (de Walque, 2018). It is located in the north-eastern part of Colombia at 6°52´55" N and 73°24´43" O and 643 m.a.s.l. It is characterized by an average annual temperature range between 20.7°C and 30.0°C and an average precipitation of 1,820 mm/year (Climate Data, n.d.). According to the Köppen-Geiger climate classification, the climate in San Vicente de Chucurí is classified as Af, or tropical rainforest climate (Appendix 1). The dominant soil types are humic Cambisols (CMu) and umbric Leptosols (LPu) at high altitudes (de Walque, 2018).

According to de Walque (2018), Rionegro has 27, 114 residents and generates half of cocoa produced by San Vicente de Chucurí. It is also located in the north-eastern part of Colombia at 7° 15' 51" N and 73° 08' 58" O and 590 m.a.s.l. The municipality has an average annual rainfall of 1,620 mm, mean annual temperature of 25°C (climate-data.org, 2018) with the same climate classification as that of San Vicente de Chucurí (Köppen-Geiger classification). It is characterized by Gleyic Arenosols (ARg) as the main soil types. These terrains are distinguished by having a steep relief with slopes higher than 50% that are characteristic of Colombia`s eastern mountain range (ICA-IGAC, 1985). According to Instituto de Geografico Agustin Cadozzi (1983), both San Vicente de Chucurí and Rionegro are characterized by soils with a warm, humid climate, with a relief that is steep to very steep, little to moderately developed and generally unsaturated.

For the three (3) clones investigated, CAU39 is associated with a tropical monsoon climate, while the regional origin of FEAR5 constitute a climate with warm annual temperatures and no dry season. FEC2 clones originate from a tropical rainforest climate (USAID, 2017) (Appendix 2).

Figure 2.2: San Vicente de Chucurí (6°52´55” N and 73°24´43” O), Santander, Colombia

Source: de Walque (2018)

Figure 2.3: Rionegro (7° 15' 51" N and 73° 08' 58" O) Santander, Colombia

Source: de Walque (2018)

3.2 Sampling

3.2.1 Cocoa beans and leaves sampling

Three (3) different regional cocoa clones were sampled from ten farms: CAU39, FEAR5 and FEC2. Based on the availability of sufficient mature cocoa fruits, ten (10) farms were selected as shown in the Figure 2.4. Three (3) different trees of each cocoa clone were randomly selected and sampled in each farm. At least a cocoa pod per each tree was harvested. A total of ninety (90) bean samples were collected which implies thirty (30) bean samples were collected per clone. All samples were labelled as follows: farm number, clone, sampled tree (e.g. F1, CAU39, A2 represents the sample from the second sampled tree of clone CAU39 collected in the first farm). After harvesting, the beans were separated and dried until the humidity was reduced to 6-7%. The dried cocoa beans were packed in paper bags to enable the beans to respire and to avoid fungal growth. Additionally, two (2) leaves were sampled from every harvested cocoa tree. The leaves were dried using silica gel and stored at -20°C. The leaf samples were lyophilized (CHRIST, model Alpha 1-2 LDplus, process conditions; 0.12 mbar, -40°C, 24h) upon arrival in Belgium. A total of ninety (90) leaf samples were collected.

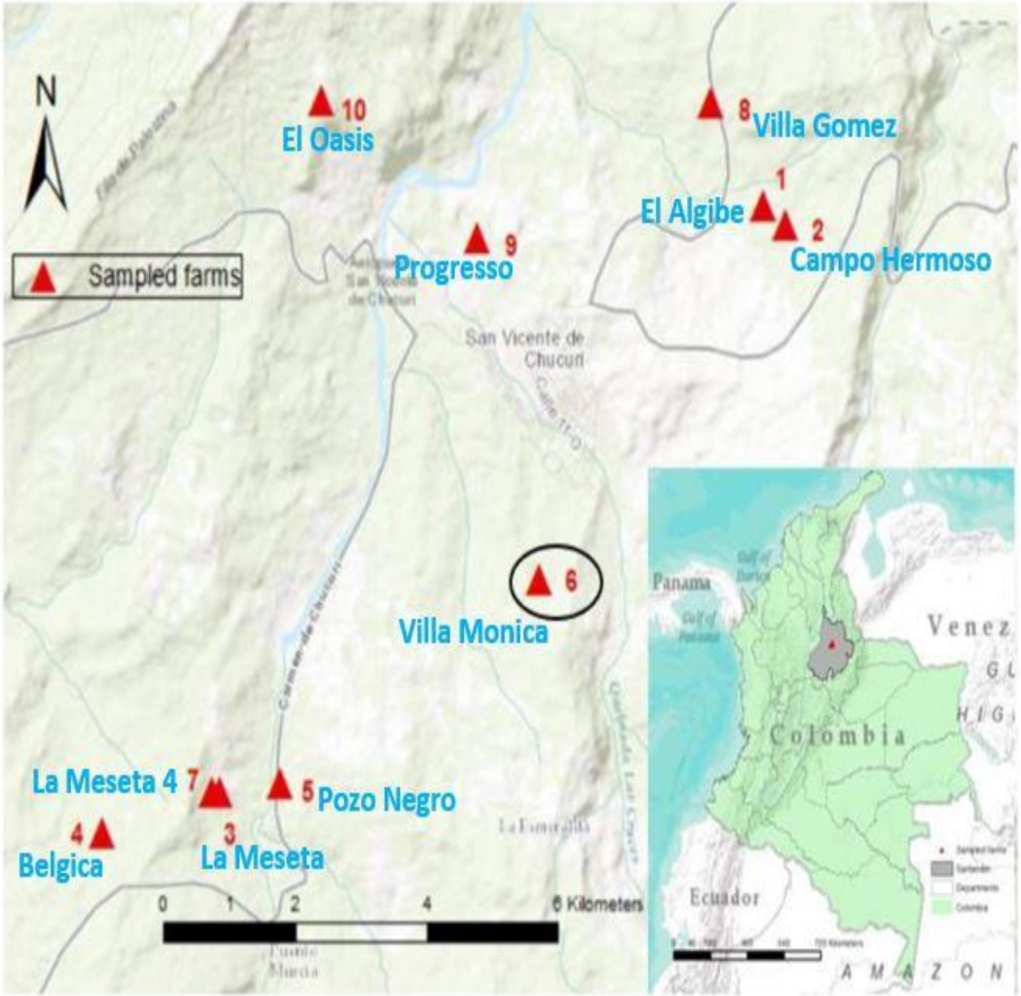

Figure 2.4: Location of the investigated farms in San Vicente de Chucurí, Colombia. In circle is the reference farm (sixth farm) "Villa Monica". Source: Hoogerwerf (2020)

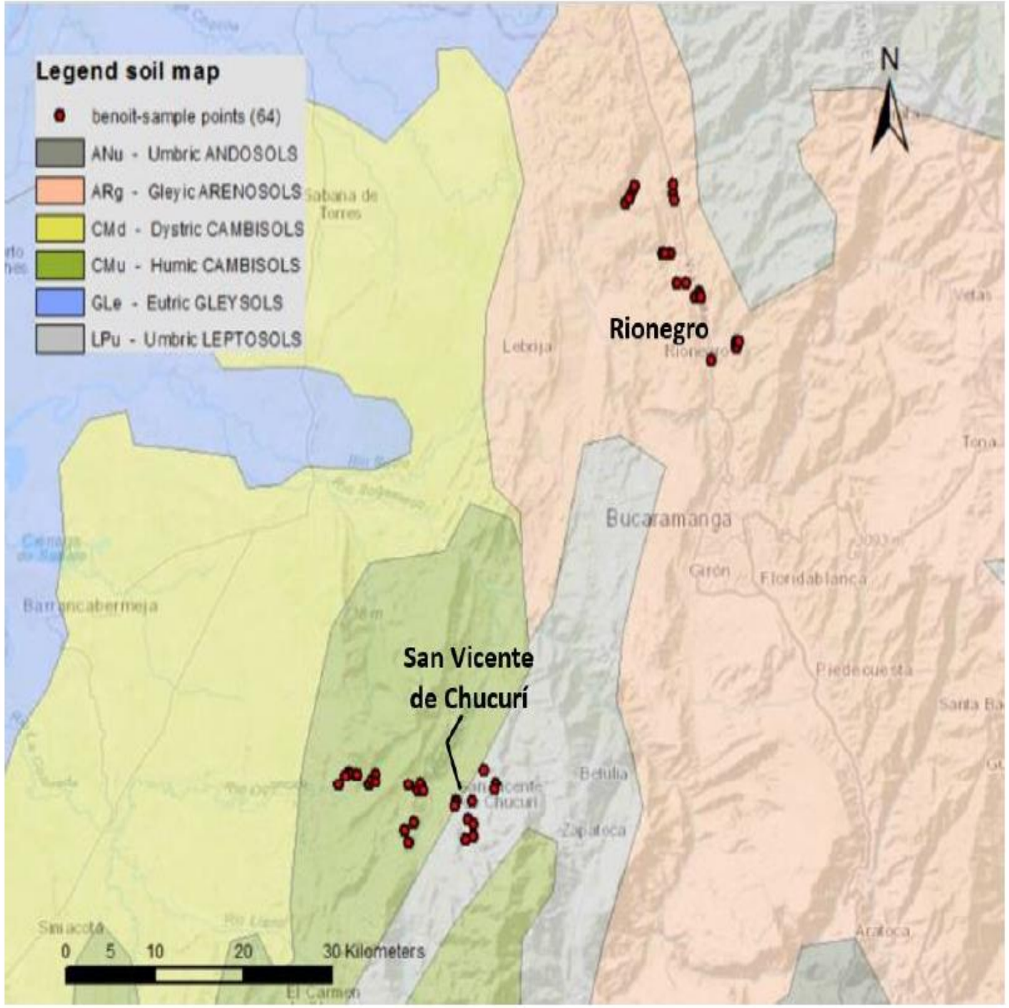

Figure 2.5: Soil types (WRB) in San Vicente de Chucurí and Rionegro, Colombia as well as the sampling points. Source: https://www.isric.org as cited in de Walque (2018)

3.2.2 Soil sampling

Soil samples were collected in each selected farm for analysis as illustrated in Figure 2.5. After removing the upper organic and fermented layer, the soils were sampled with an auger at 0-30 cm depth around the harvested trees. Samples were taken in a triangle shape at a distance of one (1) meter around each tree. At the end, all samples were mixed to form a composite sample per farm. The composite soil was air dried, sieved to 2 mm, stored in a plastic zip-bag and labelled accordingly. A total of ninety (90) soil samples collected. The soil samples together with the cocoa bean and leaf samples were sent to Ghent University for analysis.

3.3 Available Cadmium Metal Analysis

3.3.1 Sample preparation

The materials and reagents used in the preparation of samples are shown in Table 1.2. The cocoa leaf samples were milled using the Ultra Centrifugal Mill ZM 200 to obtain a sample of final fineness of < 40 µm. About 0.2 g of each of the samples were weighed in triplicate into a 50 mL beaker. The Cd trace metal analysis contained nine (9) process blanks. These blanks were treated in the same way as the cocoa leaf samples in the course of sample preparation. They were taken into account to determine the limits of detection and quantification. Spinach leaves (SRM 1570a) reference material in nine (9) replicates were included in the four (4) hot plate batches. A 0.25 µg Cd spiked samples were prepared by randomly selecting four (4) of the cocoa leaf samples and measured 0.2 g of each sample into a beaker. After weighing, 10 mL of Pico-Pure HNO_3 67-69% (Chem-Lab Analytical) was added with the aid of an automatic pipette to each of the samples in the beakers. They were swirled gently to obtain a uniform mixture and left overnight.

The beakers were placed on LHG TYPE HT21 heating plate at boiling point for two (2) hours for complete destruction of the samples. In the course of boiling, samples were checked frequently and an appreciable amount of Milli-Q water added to prevent complete evaporation of the mixture. Samples were allowed to stand for ca. 45 minutes after boiling to cool down. With the aid of a funnel stand, funnels and filter papers, extracts were completely filtered into a 50 mL ultrapure plastic cup to obtain a clear solution. All glass wares were rinsed three (3) times with Milli-Q water to ensure all the components of the sample were transferred into the ultrapure plastic cup. Lastly, the samples were diluted with Milli-Q water to the 50 mL mark of the ultrapure plastic cup and shaken.

Table 1.2: Materials and reagents used during the cocoa leaf samples preparation for Cd analysis

Reagents	Materials
➢ Spinach leaves (reference material) * ➢ Milli-Q water ** ➢ Pico-Pure HNO_3*** ➢ Cd element standard ****	➢ Ultra Centrifugal Mill ZM 200 ➢ LHG TYPE HT21 heating plate ➢ Funnels ➢ Funnel stand ➢ Ultrapure plastic cups (50 mL) ➢ Beakers (50 mL) ➢ Pipette ➢ Filter papers ➢ Measuring balance

3.3.2 Determination of cadmium concentration using ICP-MS

Cadmium metal analysis was performed with the aid of Inductively Coupled Plasma Mass Spectrometer (ICP-MS). External standards with varying concentrations from 0 ppb to 500 ppb were prepared from Cd element standard (VWR Chemicals, 1000 mg L^{-1}) for ICP-MS prior to the analysis. Unknown Cd concentrations in the samples were determined based on the obtained calibration curves. The ICP-MS analysis was performed in a standard mode using the ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{114}Cd and ^{116}Cd isotopes. Inaccuracies caused by a varying sample quantity or varying detecting response factor were corrected with ^{103}Rh as the internal standard. The extraction protocol, choice of analytical instrument, its calibration and the quality control protocols adopted all played an important role in the reliable analysis of Cd concentrations in the cocoa leaf samples. To check these, spinach leaves were included as a reference material.

3.4 Available Phosphorus (P) in soil – Resin Method

3.4.1 Sample preparation

3.4.1.1 Chemicals

A litre of 0.5 M $NaHCO_3$ was prepared by weighing ca. 42 g $NaHCO_3$ into a 1 L Schott Duran bottle and MQ water added to 1 L. The pH was adjusted to 8.5 by adding a few drops of 2.5 M NaOH, mixed very well and measured the pH.

For the preparation of 0.5 M HCl in 1 L, ca. 500 mL of MQ water was transferred into a 1 L Schott Duran bottle and ca. 41.66 mL of 37% HCl added. Additional MQ water was added to reach the 1 L mark and mixed very well.

A phosphate standard was prepared by weighing ca. 43.93 mg KH_2PO_4 into a 500 mL volumetric flask. MQ water was added to the mark and mixed very well.

3.4.1.2 Resin strips

Used Anion Exchange Membrane (AEM) strips were washed thoroughly three (3) times with MQ water for 0.5 hour. The strips were then placed in 0.5 M NaHCO3 (pH 8.5) four (4) times for regeneration into the bicarbonate form. This was done for 0.5 hour followed by 1 washing step with MQ water for 0.5 hour. Lastly, one time washing in 0.5 M NaHCO3 for 0.5 hour.

3.4.1.3 Soil samples

The materials and reagents used in the preparation of the soil samples are illustrated in Table 1.3. About 1.0 g of each of the soil samples were weighed in triplicate into 60 mL plastic tubes. Blank sample and phosphate standard in triplicates were also included in the sample list. In preparing the phosphate standard samples, 1 mL of phosphate standard was added to 29 mL of MQ water in 60 mL plastic tubes. Phosphorus was extracted from soils by shaking the soil sample and two (2) pre-activated resin membrane square in 30 mL of MQ water end-over-end for 16 hours (overnight) at 25° C (room temperature). After shaking, the resin membranes were removed and cleaned with little MQ water until all soil particles were removed. Any residual soil was placed back into the plastic tube with soil samples. Phosphorus retained on the resin membrane square was removed by shaking the square end-over-end with 20 mL of 0.5 M HCl for 16 hours (overnight). The resin membranes were removed and rinsed with MQ water after shaking. The HCl desorption solution was kept for analysis.

The working phosphate standard solution was prepared by diluting 60 µL of the 10 mM standard in 5940 µL 0.5 M HCl (using the same matrix as the soil samples) in a 6 mL Eppendorf vial to prepare a 10 mM working solution. A vortex was used to mix the solution. Standard solutions were prepared by adding 0 (blank), 50, 100, 150, 200, 250, 500 µL working standard solution to a 2 mL Eppendorf vial and topped up to 1000 µL by MQ water. Prior to the measurement of the absorbance, the spectrophotometer was switched on for at least 20 minutes. In order to measure the available P concentration using the phosphate colorimetric kit, the semi micro cuvettes (1 mL cuvette VWR 634-0676 semi-micro PS cuvettes) were prepared. This was done by adding 250 µl standard dilution + 150 µl phosphate reagent and topping up by

MQ water to 1 mL. The blank was used to zero the spectrophotometer and absorbance measured at wavelength 650 nm. The soil samples were measured using 250 µL of 0.5 M HCl extract + 150 µL phosphate reagent + 600 µL of MQ water to 1 mL.

Table 1.3: Materials and reagents used during the soil samples preparation for Resin P analysis

Reagents	Materials
➢ Milli-Q water	➢ Resin strips
➢ 0.5 M $NaHCO_3$	➢ Mechanical shaker
➢ 2.5 M NaOH	➢ pH meter
➢ 0.5 M HCl	➢ Spectrophotometer
➢ 10 mM standard	➢ 60 mL plastic tubes
➢ Phosphate Reagent	➢ Vortex
➢ KH_2PO_4	➢ 500 mL volumetric flask
	➢ Automatic pipette
	➢ 1 mL cuvette (VWR 634-0676 semi-micro PS cuvettes)
	➢ 2 mL and 6mL Eppendorf vials
	➢ Measuring balance

3.5 Soil pH-H_2O Adjustment

About 2.0 g of air-dried soil samples were measured into 12 mL plastic vials. Milli-Q water was added at 1:5 soil water suspension ratio. With the aid of a mechanical shaker, the mixture was shaken for ca. 30 minutes**.** The suspension was allowed to stand for about an hour. The initial pH was measured with the help of a pH meter and glass electrode. Before taking pH readings, the pH meter was calibrated using standard buffer solutions at pH 4 and pH 7. $NaHCO_3$ solution of 50 mg/mL was prepared and added in increments of 50 µL to the mixture and allowed to stand for about an hour. The final pH was measured. (Readings were taken from time to time until they stabilized). The previous steps were repeated until a pH range of 6.6 – 6.7 was obtained. The volume of $NaHCO_3$ solution to be added to the soil for incubation was computed. About 6.0 g of the air-dried soil samples were measured into 50 mL ultrapure plastic cups. The exact volume of $NaHCO_3$ solution per soil was added in smaller quantities and stirred thoroughly upon each addition. About 20% of water (of weight of the soil sample) was subsequently added. The sample in the ultrapure plastic cup was covered with parafilm, the weight was recorded and incubated for a week. After incubation, the weight was recorded to determine the water content.

3.6 Available P (Resin Method-Soil) and Available Cadmium Metal Analysis (ICP-MS) after Incubation

After incubation, the parafilm was removed and equivalent 1.0 g dry soil transferred into 60 mL plastic cup for resin P analysis as detailed in section 3.4. Milli-Q water (24.9 mL) was added to the remaining 5 g soil sample, shaken for about 30 minutes and pH measured. A volume of 100 µL 2.5M $CaCl_2$ solution was added to the mixture after the pH readings and shaken overnight. After shaking, samples were filtered and the filtrates were acidified with three (3) drops of nitric acid. The bio-available Cd was measured using the ICP-MS as described in section 3.3.2.

3.7 Statistical Analysis

Statistical analyses were performed using IBM's SPSS 25.0 and Microsoft Excel software. Descriptive statistics such as mean, standard deviation, median, minimum and maximum were identified for cocoa leaves, beans and soils. The difference in Cd concentrations in cocoa leaves, beans and soils were compared between the different clones and farms. The farms and clones were defined as the factors (main effects or independent variables) while the Cd concentration in cocoa leaves, beans and soils were used as dependent variables. As the normality condition was not fulfilled at 5% significance level ($p < 0.05$), a non-parametric Kruskal-Wallis test was conducted. Prior to the analysis, Shapiro-Wilks test for normality and Levene's test for equality of variance were ran.

Differences in Cd concentrations between the cocoa leaves, beans and soils were assessed using Tukey's test for the multiple comparisons of means at a significance level of 0.05, performed after a one-way ANOVA (analysis of variance) with cocoa leaves, beans and soils as the factor. Pearson correlation was performed to determine the relationship between Cd concentrations in cocoa leaves, beans and soils.

The paired sample t-test (dependent sample *t*-test) was used to determine whether the adjustment of soil pH-H_2O influenced the concentration of available Cd and available P.

4.0 RESULTS AND DISCUSSION

4.1 Metal Analysis

4.1.1 Introduction

Cadmium concentrations in cocoa leaves were obtained from ICP-MS analysis with the aid Syngestic software. Results were obtained for Cd concentrations from three (3) isotopes namely; Cd 111, Cd 112 and Cd 114. Isotope 111 was selected for further analysis because it recorded the lowest LOD. The LOD (±SD) which is an analytical threshold defining the smallest true value that can be distinguished from an analytical blank sample was 0.5±0.2 µg Cd L^{-1}. Concentrations of Cd were reported for all cocoa leaf samples because the values were greater than the method's limit of quantification (> LOQ). The Spinach leaves (SRM 1570a) recoveries of Cd concentrations calculated relative to the certified concentration (in %) varied between 99.6 – 114.4 for the selected isotope. Cocoa beans dataset obtained by Hoogerwerf (2020) together with soil Cd data were also useful in this comprehensive analysis.

4.1.2 Comparison of cadmium contents in cocoa leaves between clones

The mean Cd content in the cocoa leaves did not differ significantly (Kruskal-Wallis test, $p < 0.05$) across the clones with means (±SD) of 9.8±13.3 mg kg^{-1} in CAU39, 14.8±21.7 mg kg^{-1} in FEAR5 and 6.2±6.2 mg kg^{-1} in FEC2. Nevertheless, there is a tendency of 6.0 < 10.0 > 15.0 mg Cd kg^{-1} recorded among the three clones. The distribution of Cd contents in the cocoa leaves between the clones were revealed by descriptive statistics as illustrated in Table 1.4. This is also visualised by means of box plots as depicted in Figure 2.6 for each of the clones. Box plots show lower quartile, median and upper quartile values. The whiskers are lines extending from each end of the box to show the extent of the rest of the data. Genotypic variability in Cd accumulation between cocoa varieties has been reported in several studies (Cryer *et al*. 2012; Arévalo-Gardini *et al*. 2017; Gramlich *et al*. 2017; Barraza *et al*. 2017; Lewis *et al*. 2018**).** This research revealed no statistically difference between the clones in their accumulation of Cd in leaves. However, different concentrations of Cd were observed between the leaves of the different clones with some values beyond the ends of the whiskers. This indicates substantial variation in Cd accumulation across the clones. The highest mean Cd content was observed in FEAR5 than those of clones CAU39 and FEC2. The variation could be as a result of genotype effect which influenced the accumulation of Cd concentration in the leaves of the different clones. Engbersen *et al*. (2019) also observed various Cd concentrations among different cocoa cultivars in Northern Honduras.

Table 1.4: Descriptive statistics of Cd concentrations (mg kg^{-1}) in cocoa leaves among the three (3) clones.

Clone	Mean ± Standard deviation	Median	Min.	Max.
CAU39	9.8±13.3[a]	5.1	1.2	69.6
FEAR5	14.8±21.7[a]	3.3	0.6	77.6
FEC2	6.2±6.2[a]	3.9	0.6	27.7

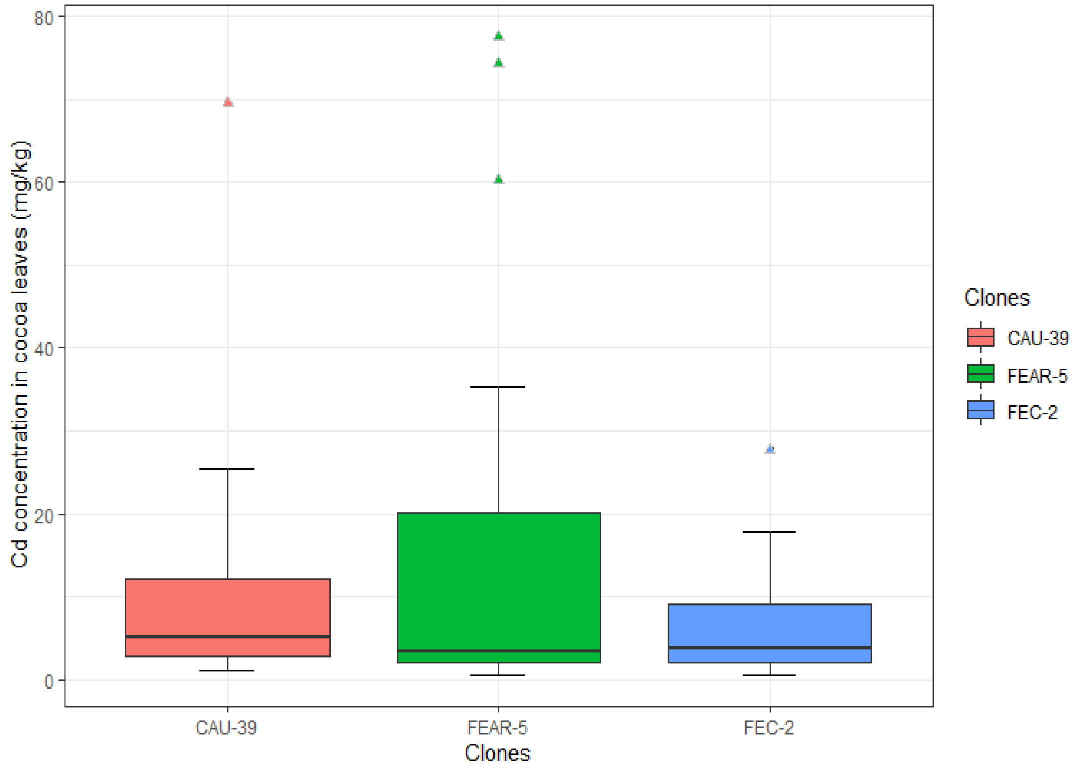

Figure 2.6: Boxplots showing the distribution of Cd concentrations (mg kg^{-1}) in cocoa leaves for each of the clones (CAU39, FEAR5 and FEC2).

4.1.3 Comparison of cadmium contents in cocoa leaves between farms

The concentrations of Cd in cocoa leaves showed wide variations between farms which ranged from 2.2±1.5 to 39.6±30.2 mg kg^{-1} and produced a mean of 10.3±15.4 mg kg^{-1}. However, it was found that farm El Oasis recorded the highest Cd concentration as illustrated in Figure 2.7. Six (6) farms thus Villa Monica, Campo Hermoso, Pozo Negro, Progresso, El Algibe and Villa Gomez recorded the lowest range of Cd concentrations (2.2±1.5 to 4.7±2.1 mg kg^{-1}) as shown in Table 1.5. A Kruskal-Wallis test ($p < 0.05$) revealed a highly significant difference between farm El Oasis and five other farms (Campo Hermoso, El Algibe, Progresso, Pozo Negro and Villa Monica) as compared to the rest. Additionally, significant difference was observed between farm Belgica and five other farms thus El Algibe, Villa Monica, Campo Hermoso, Progresso and Pozo Negro. Accordingly, Perea Villamil *et al*. (2013) posited that cocoa farms in Colombia (mostly 3 to 5 ha) are typically located at an altitude ranging from 0 to 1200 m.a.s.l. The altitudinal transect chosen for this study varied from 1 to 900 m.a.s.l. The outliers suggest a high degree of heterogeneity over short distances on several scales between fields of farmers in this region of Santander. This anomaly could be attributed to the altitudinal differences between the ten (10) farms with some areas showing significantly higher levels of Cd than others.

Table 1.5: Descriptive statistics of Cd levels (mg kg^{-1}) in cocoa leaves from the different farms

Farm	Mean ± Standard deviation	Median	Min.	Max.
El Algibe	3.4 ± 1.7^{a}	2.5	1.4	6.8
Campo Hermoso	2.5 ± 1.1^{a}	2.0	1.7	5.1
La Meseta	13.5 ± 11.9^{ac}	10.5	0.6	35.3
Belgica	20.1 ± 9.8^{ab}	17.9	3.6	34.4
Pozo Negro	2.9 ± 1.1^{a}	2.4	1.3	4.9
Villa Monica	2.2 ± 1.5^{a}	1.6	0.7	5.4
La Meseta 4	10.3 ± 5.1^{ab}	11.4	4.2	18.1
Villa Gomez	4.7 ± 2.1^{ab}	4.3	1.6	9.1
Progresso	3.3 ± 2.7^{a}	2.8	0.6	9.7
El Oasis	39.6 ± 30.2^{bc}	25.5	3.0	77.6

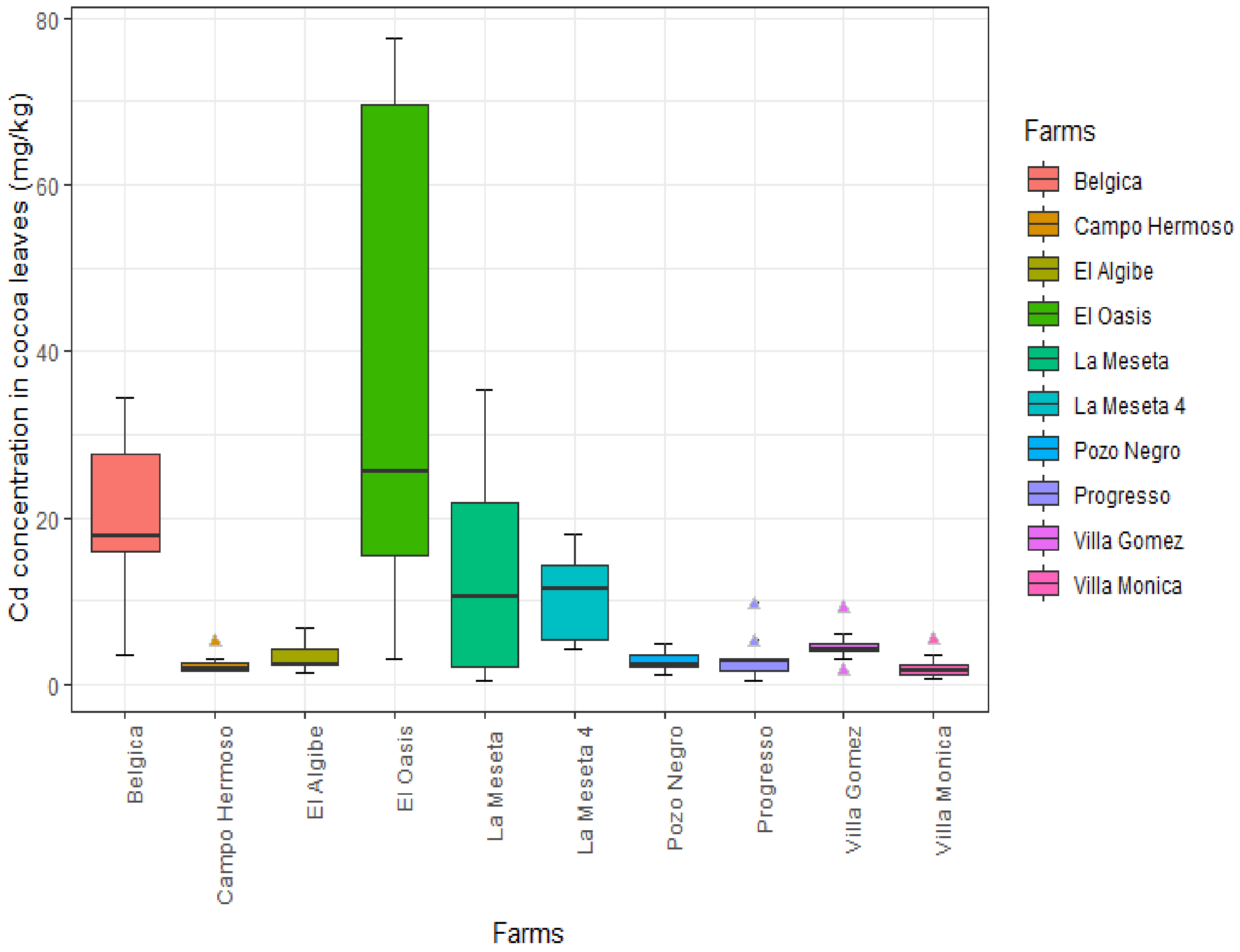

Figure 2.7: Boxplots showing distribution of Cd concentrations (mg kg^{-1}) in cocoa leaves for each of the ten (10) farms in San Vicente de Chucurí, Colombia.

4.1.4 Comparison of cadmium contents in cocoa beans between clones

The three (3) clones had a mean (±SD) of 6.5±8.2 mg Cd kg^{-1} content. The descriptive statistics of cocoa beans grown in the study area are shown in Table 1.6. The beans from FEAR5 had a mean (±SD) of 9.0±10.9 mg Cd kg^{-1} content which was the highest. Clones CAU39 and FEC2 had an average (±SD) Cd contents of 6.1±7.0 and 4.5±5.1 respectively. However, there is a tendency of $4.0 < 6.0 > 10.0$ mg Cd kg^{-1} observed between the three (3) clones. The distribution of Cd among the clones showed the following trend: FEC2 < CAU39 < FEAR5 which is the same as observed in the cocoa leaves Cd levels. Rodríguez Albarrcín *et al*. (2019) found spatial distribution of Cd contents in cocoa leaves and beans to be similar in samples from Yacopí (Cundinamarca, Colombia) (Appendix 4 and 5).

Cadmium levels obtained exceeded the maximum permissible limit (0.6 mg kg^{-1}) as depicted in Figure 2.8. As of January 2019, the EC established the maximum level (0.6 mg kg^{-1}) of this contaminant in cocoa powder (EC, 2019). Since the content of Cd in cocoa beans has not been regulated, a threshold level of 0.6 mg kg^{-1} is adopted. No significant difference in Cd concentration in cocoa beans was observed across the categories of clones (Kruskal-Wallis test, $p>0.05$). Though a study conducted by Hoogerwerf (2020) revealed a significant higher Cd concentration in CAU39 clone compared to FEAR5 and FEC2. In another investigation by Martínez and Palacio (2010) in San Vicente de Chucurí, Cd contents in cocoa beans varying from 4.0 to 7.0 mg kg^{-1} were reported. These concentrations were lower than those found in this research. Argüello *et al.* (2019) reported that cocoa beans Cd concentration did not differ between genotypes. Accumulation of Cd concentration in cocoa beans depends on cocoa genotype. This affects uptake and translocation of Cd which is revealed by the variation of Cd contents in the cocoa beans. In a study in San Vicente de Chucuri, de Walque (2018) discovered a high variability of Cd content in cocoa beans over short distances.

Meter *et al.* (2019) reported that Cd content in cocoa beans is particularly an issue in LAC, based on studies conducted in cocoa-producing countries around the world. In comparison to Africa and Asia, the LAC region had the highest mean Cd content in cocoa beans, ranging from 0.13±0.03 to 1.59±0.15 mg kg^{-1}. This indicates that the average Cd content in cocoa beans measured across the three (3) clones in this research is extremely high.

Table 1.6: Descriptive statistics of Cd concentrations (mg kg^{-1}) in cocoa beans among the three (3) clones.

Clone	**Mean ± Standard deviation**	**Median**	**Min.**	**Max.**
CAU39	6.1±7.0[a]	3.2	0.4	30.5
FEAR5	9.0±10.9[a]	2.3	0.5	34.0
FEC2	4.5±5.1[a]	2.7	0.6	23.5

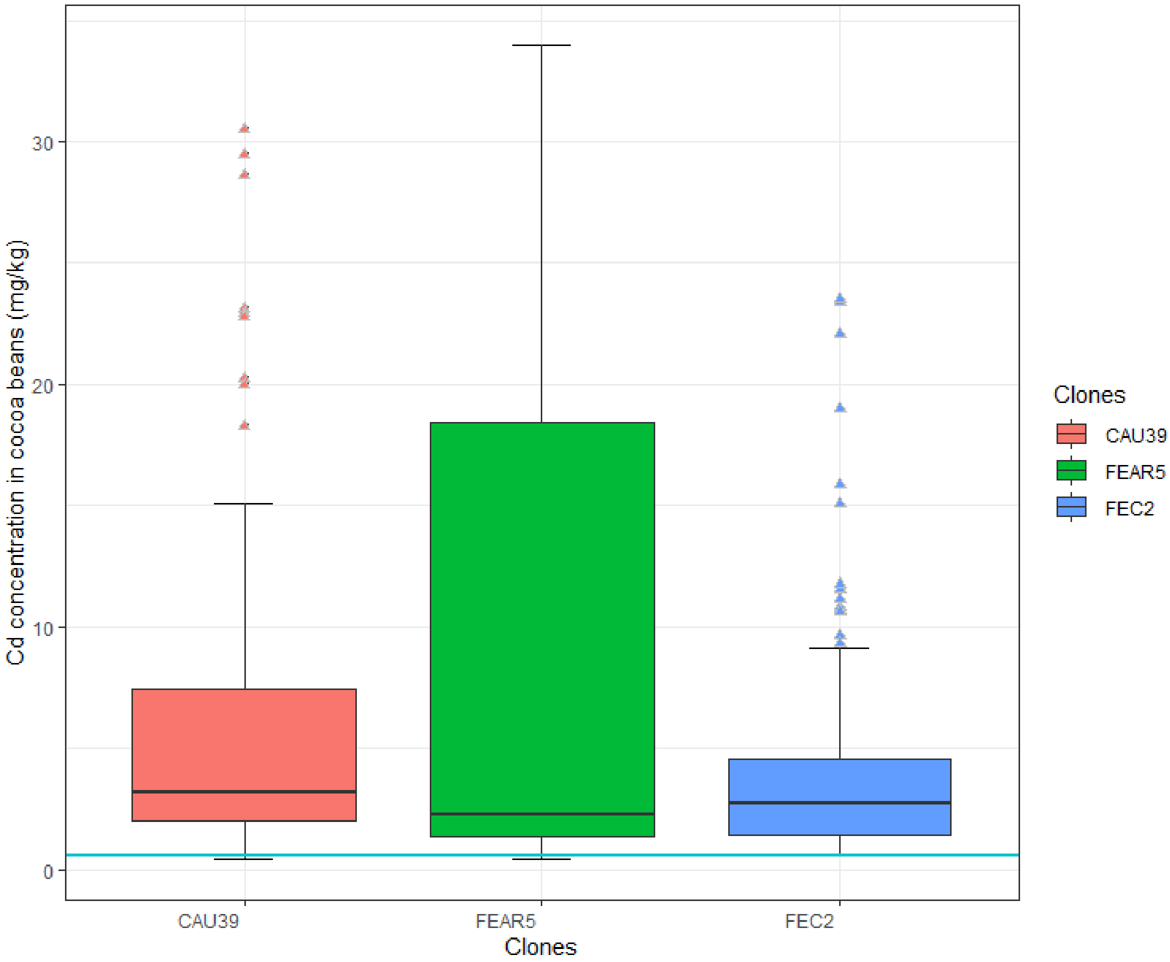

Figure 2.8: Boxplots showing distribution of Cd contents (mg kg^{-1}) in cocoa beans for clone CAU39, FEAR5 and FEC2 in San Vicente de Chucurí, Colombia. The turquoise horizontal line is the maximum permitted level (0.6 mg kg^{-1}) adopted for cocoa beans.

4.1.5 Comparison of cadmium contents in cocoa beans between farms

The process of Cd accumulation is of particular interest in Colombian cocoa farms because there are some producing areas with high Cd levels. The contents of Cd in cocoa beans from the ten (10) farms varied between 1.0±0.5 to 18.7±11.9 mg kg^{-1} with a mean of 6.5±8.2 mg Cd kg^{-1}. Villa Monica recorded the lowest value of 1.0±0.5 mg Cd kg^{-1} content as compared to El Oasis which had the highest 18.7±11.9 mg Cd kg^{-1} content as presented in Table 1.7. Except for farm Belgica and La Meseta, cocoa beans from farm El Oasis had significantly higher Cd concentrations (Kruskal-Wallis test, $p < 0.05$) than those from the other farms. In the case of Cd content in cocoa leaves, a similar pattern was observed, with the lowest and highest Cd accumulations reported in farms Villa Monica and El Oasis, respectively. However, there was a significant difference in Cd content in cocoa leaves between farm El Oasis and five (5) other farms, while in cocoa bean, farm El Oasis and seven (7) other farms differed significantly suggesting substantial variation in cocoa bean Cd levels. Six (6) farms had low (1.0±0.5 to 3.6±1.5 mg Cd kg^{-1}) contents nevertheless, all recorded Cd concentrations in this study exceeded the maximum permitted level adopted for cocoa beans as visualized by the box plot in Figure 2.9. Hoogerwerf (2020) reported similar significant higher bean Cd content in farm El Oasis compared to the other farms except for farm Belgica and La Meseta. Barraza *et al.* (2019) investigated more than 500 farms in Ecuador and found 50% of cocoa beans had Cd contents that surpassed 0.6 mg kg^{-1}. In another investigation conducted by de Walque (2018), significant higher Cd levels which varied from 0.9 to 9.3 mg kg^{-1} with a mean of 3.0 ± 2.2 mg kg^{-1} were recorded for beans from farms in San Vicente de Chucuri. Meter *et al*. (2019) stated that there is an increasing understanding that fine scale variation in soil Cd levels and bioavailability is reflected in bean Cd levels, resulting in very different Cd concentrations in harvested beans from neighbouring farms. Hence the high variability in beans Cd contents observed between the ten (10) farms.

Table 1.7: Descriptive statistics of Cd concentrations (mg kg^{-1}) in cocoa beans from the ten (10) different farms.

Farm	**Mean ± Standard deviation**	**Median**	**Min.**	**Max.**
El Algibe	2.4 ± 0.9^{b}	2.0	1.3	4.1
Campo Hermoso	1.3 ± 0.7^{ab}	1.1	0.7	3.4
La Meseta	11.2 ± 10.1^{c}	7.5	1.0	31.2
Belgica	15.9 ± 6.2^{c}	15.9	4.4	23.5
Pozo Negro	3.6 ± 1.5^{b}	2.8	2.0	6.5
Villa Monica	1.0 ± 0.5^{a}	0.9	0.4	2.1
La Meseta 4	5.0 ± 4.1^{b}	3.3	0.6	15.1
Villa Gomez	2.6 ± 1.5^{b}	2.1	1.1	6.5
Progresso	3.5 ± 3.0^{b}	2.9	0.9	11.8
El Oasis	18.7 ± 11.9^{c}	20.0	2.5	34.0

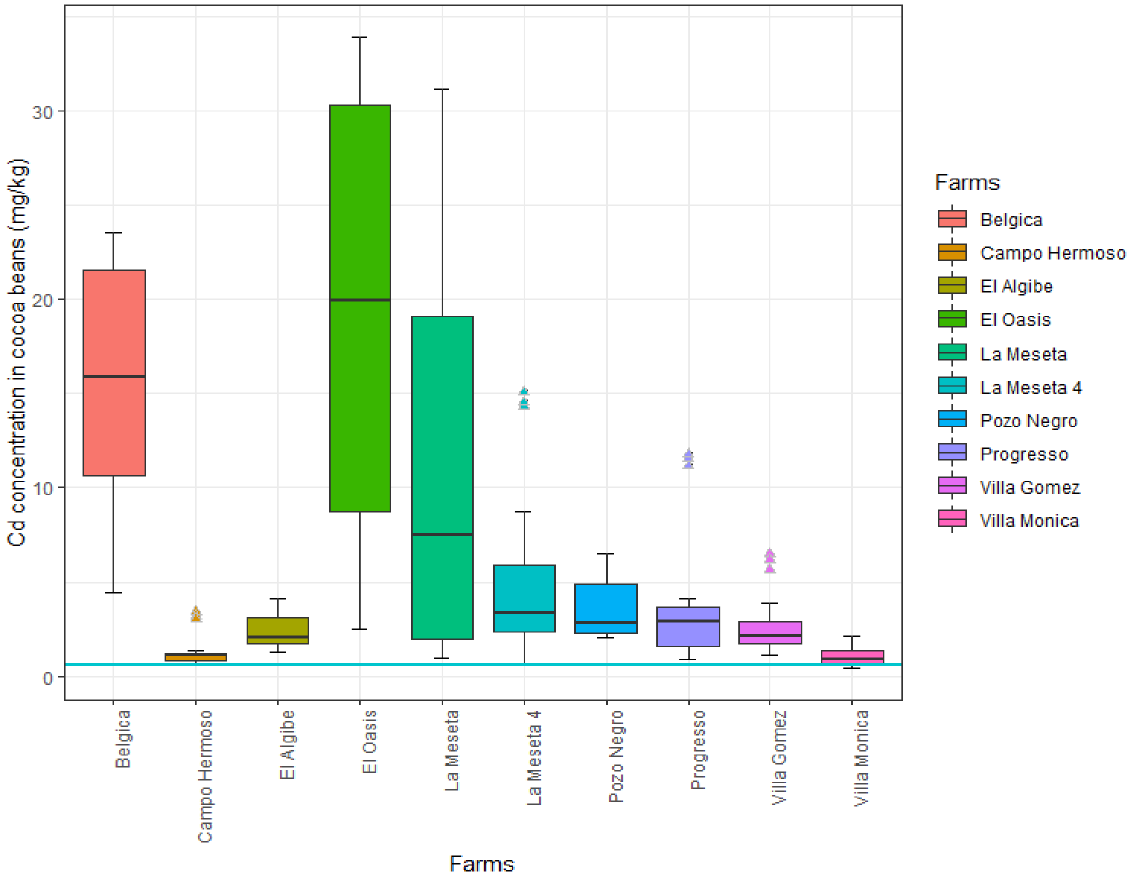

Figure 2.9: Boxplots showing distribution of Cd contents (mg kg^{-1}) for bean Cd from the ten (10) farms in San Vicente de Chucurí. The turquoise horizontal line is the maximum permitted level (0.6 mg kg^{-1}) adopted for cocoa beans.

4.1.6 Available cadmium contents in soils under the three (3) clones

Clones CAU39, FEAR5 and FEC2 had a mean (±SD) of 79.2±260.5 µg Cd kg^{-1} content. The descriptive statistics of available Cd contents related to the soils in the study area are depicted in Table 1.8. The mean available Cd concentrations in soils sampled from the nine (9) farms did not vary significantly (Kruskal-Wallis test, $p < 0.05$) across the clones with means (±SD) of 51.1±76.2 µg kg^{-1} in CAU39, 154.2±407.3 µg kg^{-1} in FEAR5 and 20.4±26.0 µg kg^{-1} in FEC2. Concentrations of available soil Cd recorded for each of the clones were lower except for FEAR5 which had extremely high outliers as visualized in Figure 3.0. The highest available Cd concentration in soil was found in FEAR5, which explains its high Cd levels in cocoa leaves and beans. Similar trend found in CAU39 and FEC2 for cocoa leaves and beans are reflected in the available soil Cd as well. An investigation conducted by de Walque (2018) highlighted the presence of available Cd in the soils of both San Vicente de Chucurí and Rionegro. The Cd contents recorded were 3.3±0.7 and 2.3±0.7 mg Cd kg^{-1} respectively. These values are higher as compared to concentrations of Cd recorded in this research. The outliers indicated that Cd content varied widely across soils in San Vicente de Chucuri over close ranges.

Table 1.8: Descriptive statistics of available Cd concentrations (µg kg^{-1}) in soils under the three (3) clones.

Clone	Mean ± Standard deviation	Median	Min.	Max.
CAU39	51.1±76.2[a]	21.0	4.3	280.3
FEAR5	154.2±407.3[a]	12.3	0.5	1656.0
FEC2	20.4±26.0[a]	10.7	0.6	113.1

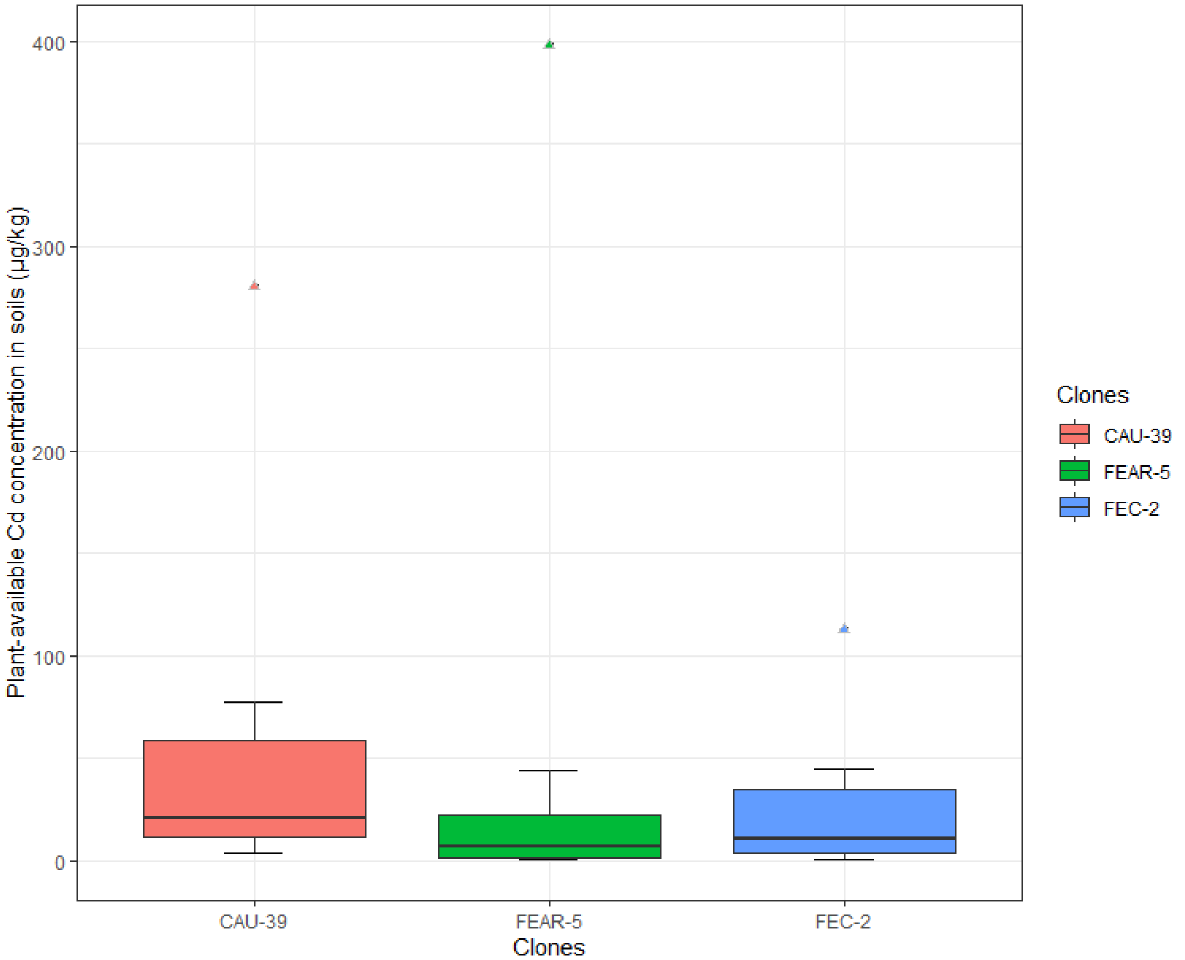

Figure 3.0: Boxplots showing distribution of plant-available Cd contents (µg kg^{-1}) in soils under the three (3) clones (CAU39, FEAR5 and FEC2).

4.1.7 Available cadmium contents in soils under the different farms

Soil data was not available for farm La Meseta 4 hence analysis was carried out without it. The contents of available Cd in soils from the nine (9) farms ranged between 1.4±1.2 to 395.1±559.6 µg kg^{-1} with a mean of 79.2±260.5 µg Cd kg^{-1} concentration as shown in Table 1.9. Pozo Negro recorded extremely low value of 1.4±1.2 µg Cd kg^{-1} content as compared to El Oasis which had the highest (395.1±559.6 µg Cd kg^{-1}) content. The other seven (7) farms recorded low (6.9±5.4 to 36.6±30.1 µg Cd kg^{-1}) contents as depicted in Figure 3.1. Farm El Oasis had significantly higher available Cd concentrations (Kruskal-Wallis test, $p < 0.05$) than farms El Algibe and Pozo Negro. Also, two (2) farms (Villa Gomez and La Meseta 4) were statistically different from farms El Algibe and Pozo Negro. Farm El Oasis had the highest available Cd concentration in soil, which is reflected in its high Cd levels in cocoa leaves and beans. Nevertheless, there is less variation in the available soil Cd as the significant difference was observed between this farm and other two farms. For Cd content in cocoa leaves, it was between this farm and five (5) other farms while in cocoa beans, it was between seven (7) farms. Due to the variable response of cocoa trees to available soil Cd levels, site identification (farm) is not straightforward (Meter *et al.*, 2019). This means that certain farm areas are capable of producing cocoa with high levels of Cd as compared to others. In a current national survey of Cd in cocoa-growing farm soils in Colombia, Bravo *et al*. (2021) reported Santander province showed the highest variability as illustrated in Figure 3.2. A high variability of available Cd concentration in cocoa-growing soils at the national level was revealed.

Table 1.9: Descriptive statistics of available Cd concentrations (µg kg^{-1}) in soils from the different farms.

Farm	Mean ± Standard deviation	Median	Min.	Max.
El Algibe	6.9±5.4[a]	10.6	0.9	12.6
Campo Hermoso	8.4±8.6[ab]	3.6	3.1	18.3
Belgica	17.0±13.7[ac]	10.0	6.8	40.5
Pozo Negro	1.4±1.2[a]	0.7	0.5	3.1
Villa Monica	10.9±9.7[ab]	12.3	0.6	19.9
La Meseta 4	36.6±30.1[abc]	34.0	4.9	77.6
Villa Gomez	29.5±10.5[abc]	30.0	18.2	41.4
Progresso	15.8±13.7[ab]	13.6	3.8	40.1
El Oasis	395.1±559.6[bc]	113.1	8.3	1656.0

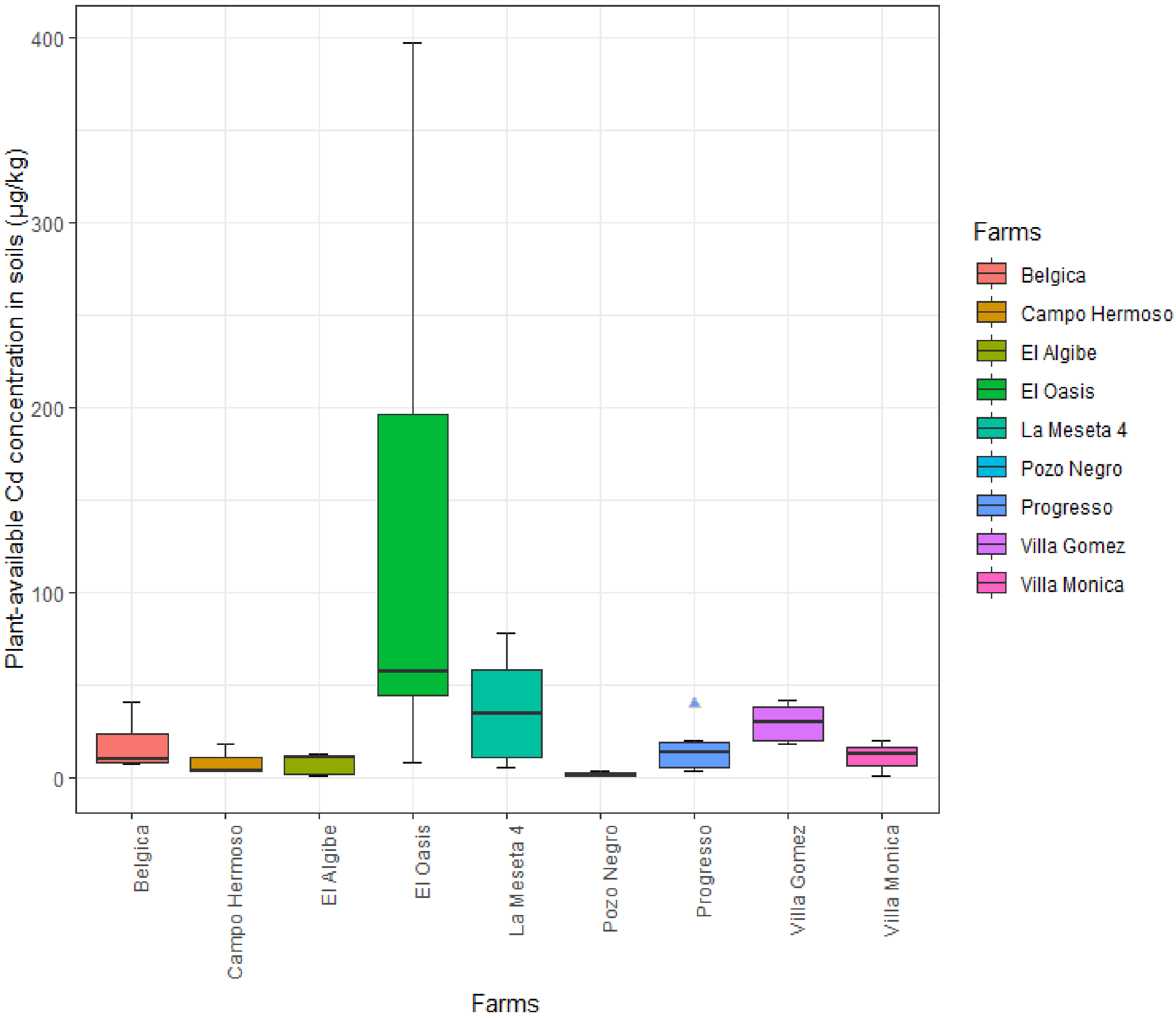

Figure 3.1: Boxplots showing distribution of plant-available Cd contents (µg kg^{-1}) in soils from the nine (9) farms in San Vicente de Chucurí.

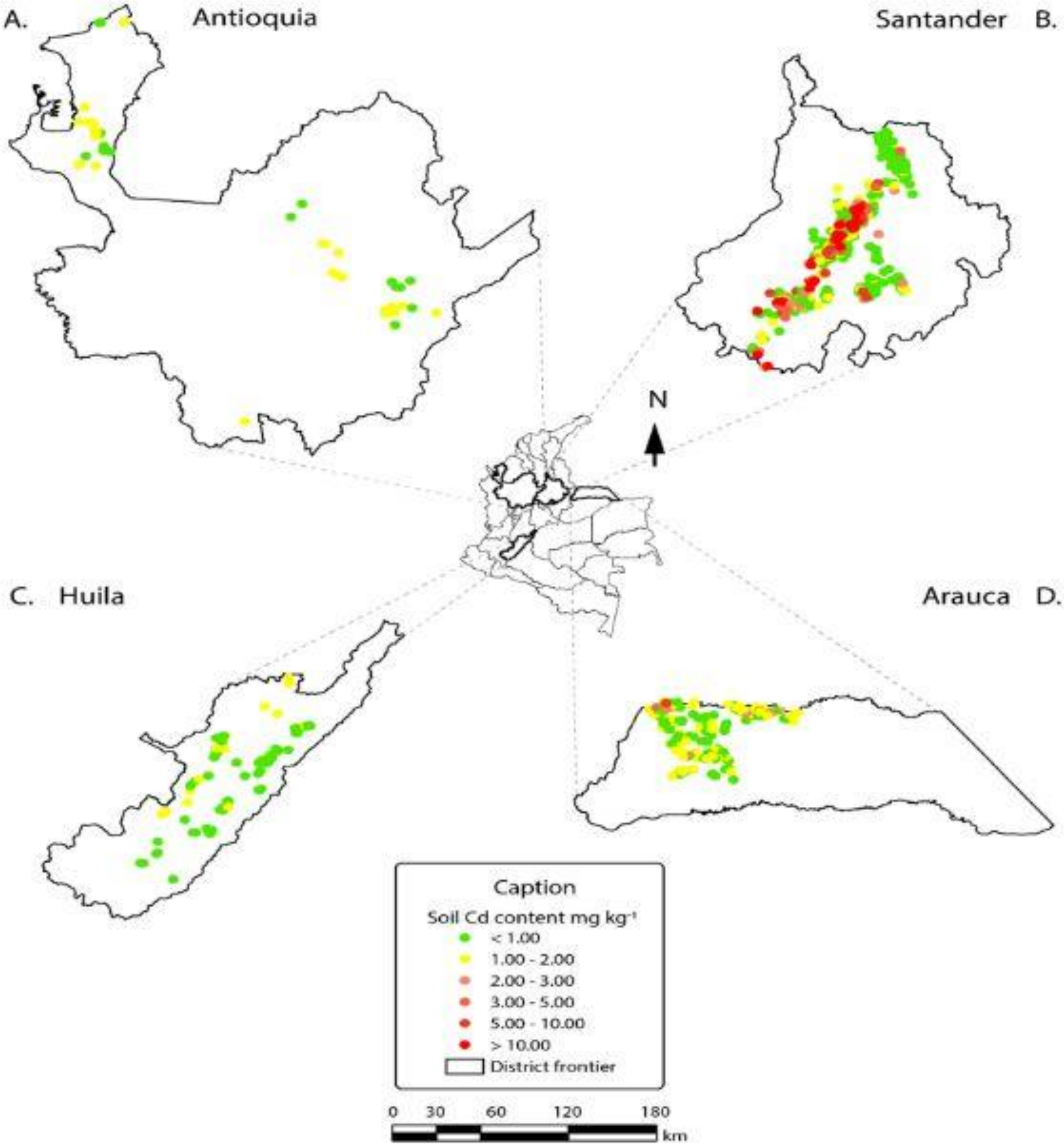

Figure 3.2: The relationship between available Cd content in the soil (mg kg^{-1}) and the number of farms assessed in four out of nineteen districts selected for comparison. It is highlighted the bigger number of samples in Santander, regarding other districts. (A). Antioquia district, (B). Santander district, (C). Huila district, (D). Arauca district.

Source: Bravo *et al*. (2021)

4.1.8 Concentration of available soil cadmium and cadmium levels in cocoa leaves and beans

Accumulation of Cd in cocoa plants is of great concern due to the potential for food chain contamination through the soil-plant interface. Due to the unavailable soil data for farm La Meseta, Cd contents in cocoa leaves and beans data for this farm were eliminated before comparing the means. The mean concentration of Cd in cocoa leaves was the highest (12.2±18.3 mg kg^{-1}) followed by beans (7.0±8.5 mg kg^{-1}) and soils (0.1±0.3 mg kg^{-1}). However, among the soils, cocoa leaves and beans, a trend of $0.1 < 7.0 > 12.0$ mg Cd kg^{-1} was observed as visualized in Table 2.0 and by the box plot in Figure 3.3. Tukey's test for the multiple comparisons of means ($p < 0.05$) revealed significant difference between Cd contents in soil and cocoa leaves. Also significant difference in Cd levels was observed between beans and soils. No significant difference was observed between Cd contents in cocoa leaves and beans. The outliers suggest that Cd contents in cocoa leaves and beans varied substantially as compared to that of the soils in this region showing significantly higher contents of Cd in cocoa leaves and beans than the soil.

Table 2.0: Descriptive statistics of available soil Cd concentration (mg kg^{-1}), cocoa leaves and beans Cd contents (mg kg^{-1}) from the farms in San Vicente de Chucurí.

	Mean ± Standard deviation	**Median**	**Min.**	**Max.**
Cocoa leaves	12.2±18.3^{b}	4.2	1.4	77.6
Cocoa beans	7.0±8.5^{b}	3.2	1.0	32.5
Soils	0.1±0.3^{a}	0.0	0.0	1.7

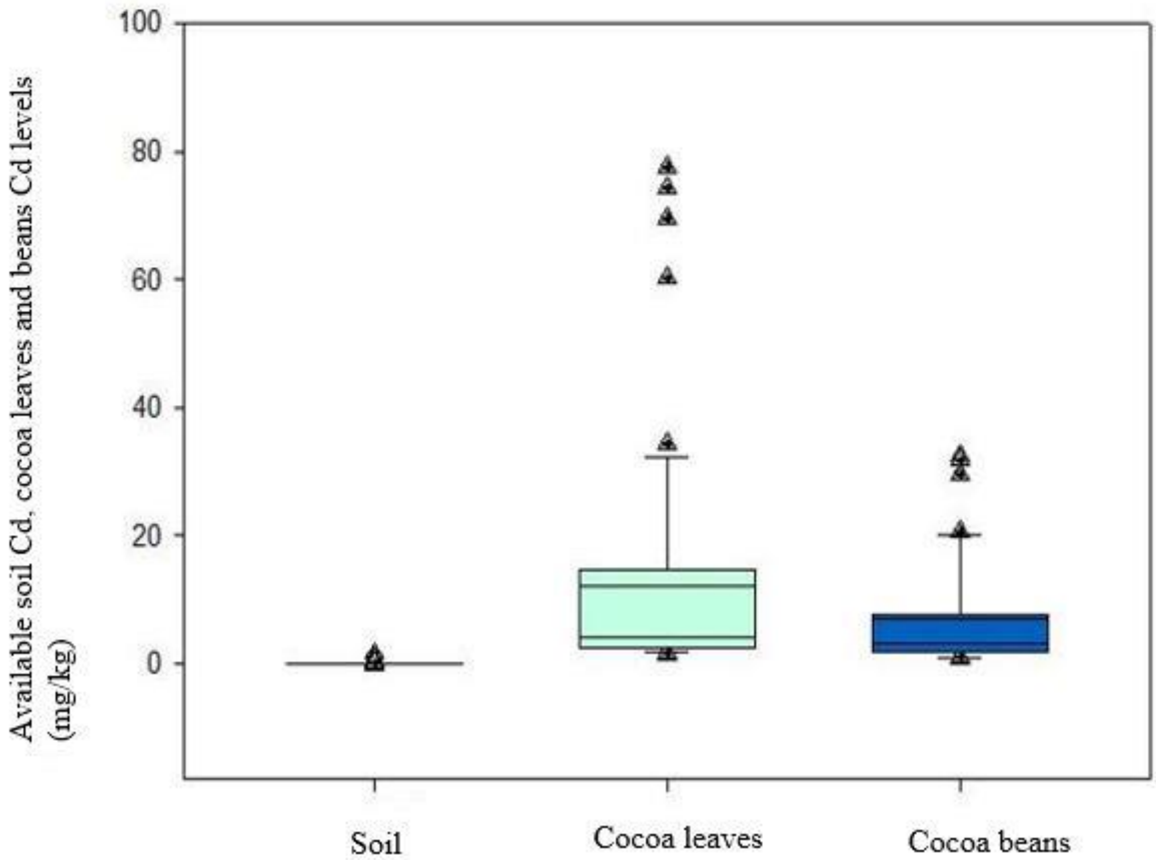

Figure 3.3: Available soil Cd concentrations, cocoa leaves and beans Cd contents (mg kg^{-1}) from farms in San Vicente Chucurí.

As stated before, the average Cd contents in cocoa beans exceeded 0.6 mg kg^{-1}, the established threshold of Cd contamination. Cadmium levels in uncontaminated soils are usually below 0.8 mg kg^{-1} (Kabata-Pendias & Szteke, 2015; Barraza *et al*., 2017); and the global average Cd level is estimated at 0.4 mg kg^{-1} (Kabata-Pendias & Szteke, 2015). Brazil, which is a country in the LAC region has a reference value of 0.5 mg kg $^{-1}$ for Cd in soil (Rueda *et al*., 2011). Bravo *et al*. (2021) reported Cd concentration in soil samples ranged from means of 0.01 mg kg^{-1} to 27.0 mg kg^{-1} in a current investigation in Colombia. Also, regarding Cd by cocoa-growing districts, soil samples in Santander had an average soil Cd content of 1.9 mg kg^{-1} which was beyond the threshold value for agricultural soils. The mean available Cd concentration in soils recorded in this study was 0.1±0.3 mg kg^{-1} which is within this specified average range.

The highest mean concentration of Cd was observed in cocoa leaves (12.2±18.3 mg kg^{-1}) however, it was not significantly different from the cocoa beans (7.9±8.5 mg kg^{-1}). Oliva *et al*. (2020) posited that cocoa leaves also accumulate high concentrations of Cd, exhibiting the minimum amounts of 0.4 mg kg^{-1} and reaching a maximum of 8.1 mg kg^{-1} in a study in Peru. It was also mentioned that previous studies had revealed cocoa's propensity to store high levels of Cd in its leaves. The results of this study confirmed that distribution of Cd in cocoa leaves and beans on plantation soils of San Vicente de Chucurí showed the following trend: Cd_{soil} < Cd_{beans} < Cd_{leaves}. The mean Cd concentrations observed in the cocoa leaves and beans were greater than the soil. The higher Cd concentrations contained in cocoa tree parts than in soil can be attributed to two factors: Cd bioavailability and the cocoa trees' propensity to accumulate Cd (Oliva *et al.*, 2020). Additionally, it has been reported that in general, the cocoa plant is vulnerable to Cd accumulation; a Cd concentration of 12.6 mg kg^{-1} was recently recorded for Peruvian cocoa, suggesting cocoa as a Cd accumulating plant species. In this investigation, the allocation of Cd concentration was observed to be greatest in the cocoa leaves as compared to the cocoa beans and soils. Many studies on Cd distribution in cocoa trees show that leaves have a higher concentration than other plant tissues (Carrera, 1994; Cárdenas, 2012; Chavez *et al.*, 2015; Gramlich *et al.*, 2018). Also, Rodríguez Albarrcín *et al.* (2019) found much higher Cd content in cocoa leaves than in cocoa beans in an investigation conducted in a cocoa-growing area of Central Colombia.

4.1.9 Correlation between available soil cadmium concentrations and the cadmium contents in cocoa leaves and beans

Results from Pearson's correlations ($p < 0.05$) revealed significant correlation between available soil Cd and the Cd contents in cocoa leaves and beans. The concentration of available soil Cd was more positively correlated with Cd concentration in the cocoa leaves ($R^2 = 0.7$) than with bean Cd concentration ($R^2 = 0.4$) as illustrated in Figure 3.4 (a, b and c). In addition, Cd concentrations of cocoa beans showed a positive relationship with cocoa leaves (R^2=0.7). Significant correlation between cocoa leaves and beans Cd levels have been reported in field studies across different countries (Ramtahal *et al.*, 2016; Barraza *et al.*, 2017; Argüello *et al.*, 2019). Rosales-Huamani *et al.* (2020) reported positive correlation between Cd concentrations in relation to the soil, cocoa beans and leaves. Figure 3.4 illustrates that the correlation between available soil Cd concentrations and the Cd contents in cocoa leaves and beans decreased in the order: leaves ($R^2 = 0.7$) ≈ beans ($R^2 = 0.4$). Also, if available soil Cd concentrations greater than 0.3 mg kg^{-1} are eliminated, there is no relation with cocoa leaves and beans. Cadmium levels in the cocoa leaves and beans analyzed were significantly ($p < 0.05$), positively correlated with each other. This indicates that Cd concentrations in cocoa leaves and beans vary proportionately with each other. It also implies that the distribution of Cd and the concentration ratios within cocoa tissues are related.

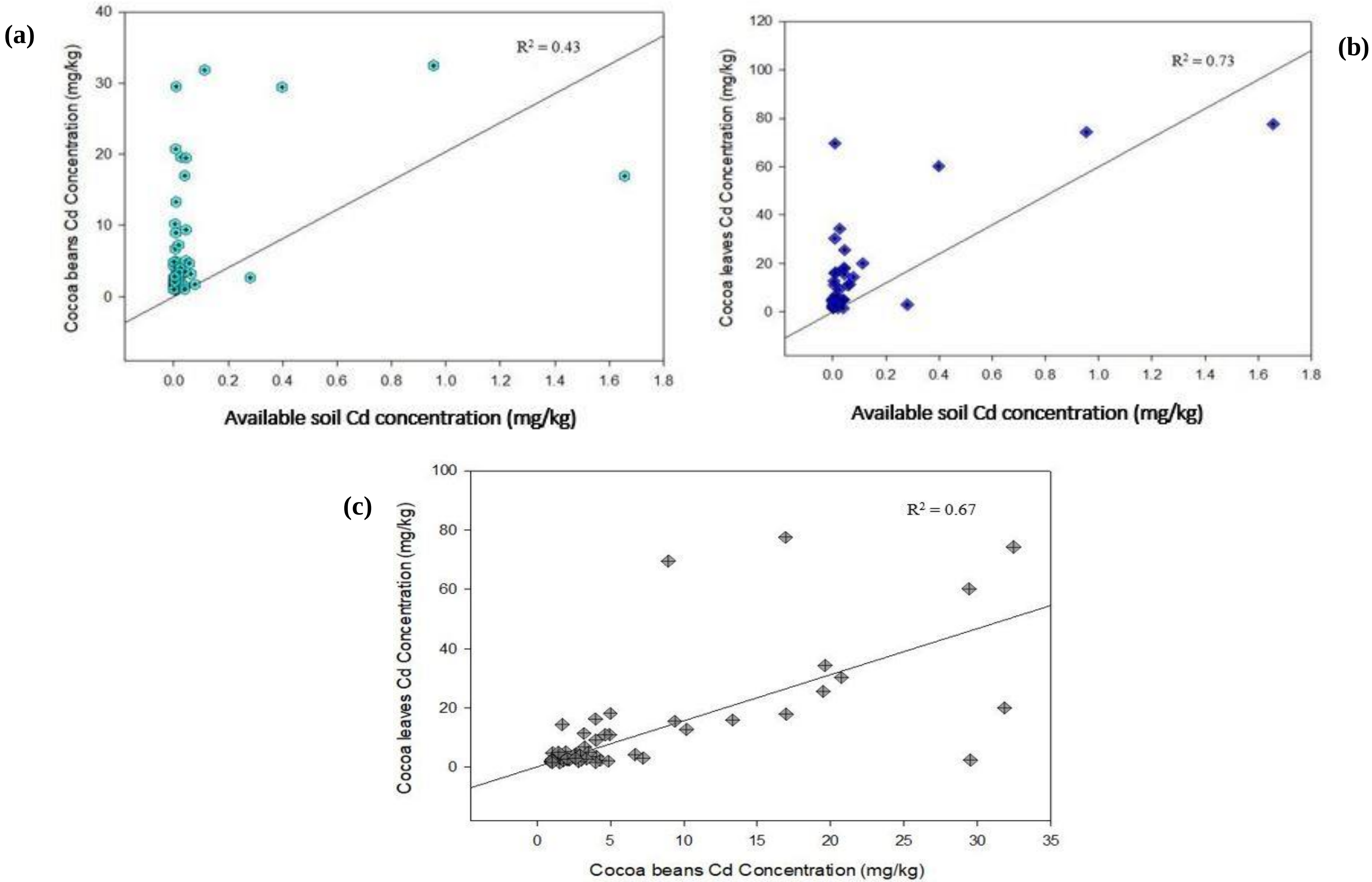

Figure 3.4: Correlation between available Cd concentration in soil and Cd contents in cocoa leaves and beans (a) cocoa beans and soil; (b) cocoa leaves and soil; (c) cocoa beans and leaves.

As a result of this correlation, it is possible to estimate Cd levels in cocoa beans using other tissues for example, when pods are not accessible. The cocoa tree exhibits variable degrees of allocation Cd from the soil to its tissues and thus considerable variation. In this study, genotypic variation of Cd levels in cocoa leaves and beans has been observed. Also, great variabilities were recorded between cocoa farms though they were located in the same geographic location. This means there are inconsistencies in Cd accumulation in different tissues of the cocoa tree. The spatial variation will affect the estimation of Cd levels in cocoa beans. Therefore, other cocoa tissues may be used in estimating the Cd levels in cocoa beans however, this is associated with some uncertainties. The available Cd levels in the soil showed a much stronger correlation with the Cd levels cocoa leaves. Understanding how soil properties affect the bioavailability of Cd in cocoa-growing soils is therefore key to developing effective soil mitigation strategies.

4.2 Soil pH-H_2O Adjustment Analysis

4.2.1 Introduction

This part of the discussion highlights the results obtained from the pH-H_2O adjustment and a week incubation of soil samples. The results of Cd concentrations were obtained for three (3) isotopes: Cd 111, Cd 112, and Cd 114. Isotope 112 was chosen for further analysis because it had the lowest LOD. The LOD (±SD) recorded for this isotope was 0.02±0.01 µg Cd L^{-1}. The concentrations of Cd were reported for all soil samples because the values were greater than the method's limit of quantification (> LOQ).

4.2.2 Available soil phosphorus and available soil cadmium after pH-H_2O adjustment

The mean available P concentration in the soil before and after pH-H_2O adjustment was significantly different (paired t-test, $p < 0.05$). Also, significant difference was observed between mean available Cd level in the soil before and after pH-H_2O increase. An average pH-H_2O increase to 7.6±0.5 resulted in a decreased available soil P concentration from 4.5±5.2 to 2.6±5.1 mg kg^{-1}, while available Cd level reduced from 106.4±108.5 to 16.1±17.9 µg kg^{-1} on the average as depicted in Table 2.1. An extremely high increase in pH-H_2O led to a reduction in available soil P concentration for all the soil samples except one sample (SV012) as visualized in Figure 3.5. Soils with inherent pH values between 6.0 and 7.5 are ideal for soil P-availability (https://www.nrcs.usda.gov; Wood & Lass, 2008). This accounted for the high available soil P concentration recorded for SV012 soil sample. Prior to incubation, the inherent pH of this soil sample was 6.3, with a corresponding available soil P concentration of 16.0 mg kg^{-1}, the highest reported among the different soils (Appendix 6). The pH-H_2O increment resulted in ca. 10% additional available soil P (17.82 mg kg^{-1}) for this particular soil.

Table 2.1: Means of soil pH-H_2O, available soil Cd and available soil P concentrations before and after pH-H_2O adjustment.

	Mean ± Standard deviation
pH before incubation	5.3±0.5
pH after incubation	7.6±0.5
P before incubation (mg kg^{-1})	4.5±5.2
P after incubation (mg kg^{-1})	2.6±5.1
Cd before incubation (µg kg^{-1})	106.4±108.5
Cd after incubation (µg kg^{-1})	16.1±17.9

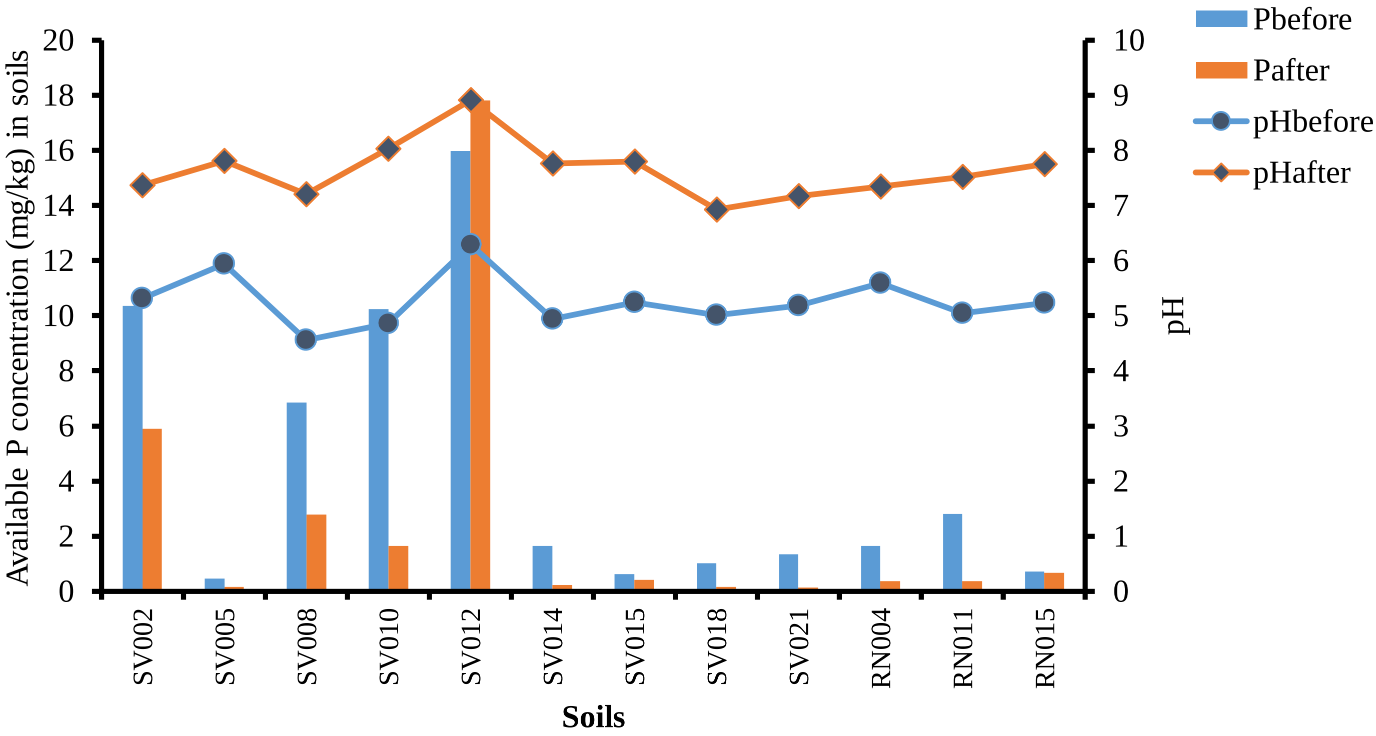

Figure 3.5: Soil pH-H_2O and available P concentration (mg kg^{-1}) in soils before and after pH-H_2O adjustment.

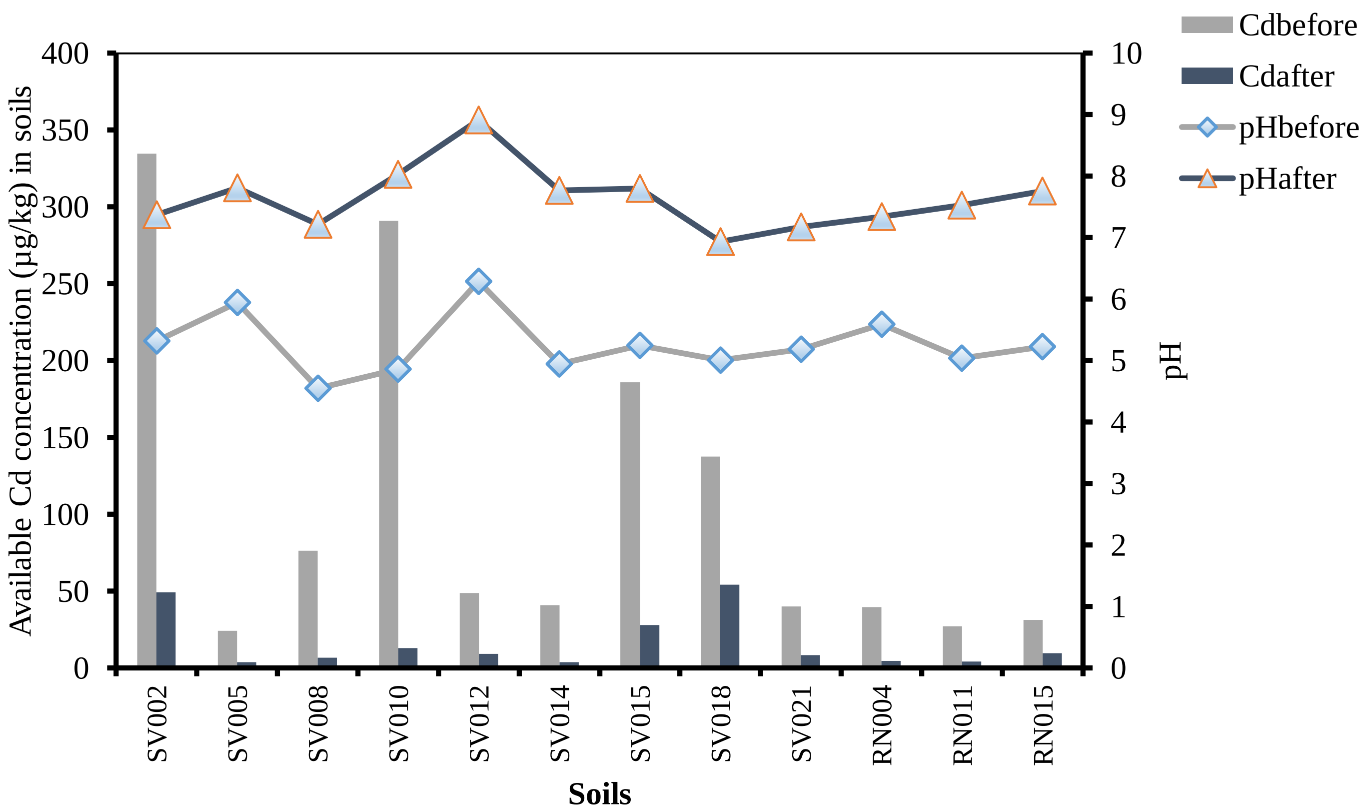

Figure 3.6: Soil pH-H_2O and available Cd concentration (µg kg^{-1}) in soils before and after pH-H_2O adjustment.

Generally, a pH-increase of 2.4 units on the average resulted in a drop in available soil P and available soil Cd by 1.9 mg kg^{-1} and 90.3 µg kg^{-1} respectively. Soil pH-increase reduced available P and available Cd concentration in soils after a week of incubation as shown in Figure 3.5 and 3.6 respectively. Plant Cd uptake is almost always reduced when the pH of acidic soils is increased (Shahid *et al*., 2016; Sauvé *et al*., 2000). Accordingly, Gramlich *et al.* (2017) emphasized that the available Cd in soil is an influencing factor of cocoa bean contamination. Negative charges with higher pH induce adsorption of Cd and, hence, a lower availability for plants (Hanafi & Maria, 1998; He & Singh, 1994). Soil pH is one of the significant factors that regulate the extractability of Cd in soils but Kirkham (2006) posited that soil pH influences Cd availability in the soil solution but increasing soil pH does not always limit plant uptake of Cd. The average pH recorded in this study was 7.6±0.5 which limited Cd bioavailability.

A pH between 6.0 and 7.5 is reported for an optimal uptake of nutrients (Wood & Lass, 2008) hence manipulating soil properties to a desirable pH range is crucial in combating Cd uptake in cocoa plants. Such manipulations should enhance the availability of plants nutrients to enhance uptake. Available concentration of Cd was decreased however; the pH was quite high (beyond the desired target) which negatively affected the availability of soil P. As a consequence, a significant reduction in extractable P concentration by ca. 42% on the average was recorded. In terms of soil nutrient requirements, cocoa is a demanding crop (Wessel, 1971). These nutrients are essential for the tree's growth and aid in flowering, pod maturity, and photosynthesis rate, among other things (Okali & Owusu, 1975). Cocoa tree requires a P uptake of 4.5 kg ha^{-1} per growing cycle to produce 1 tonne of dry cocoa beans per hectare (Landon, 2013). This means that available soil P is essential for soil ecosystem functioning and the consequence of its deficiency could have negative impacts on cocoa production in Santander. Unfortunately, this is the case in this study as low concentrations of available soil P were recorded. Sys *et al.* (1993) noted 21.8 kg ha^{-1} of P per growing cycle as the maximal limit to produce 1 tonne of cocoa beans per hectare. Since low levels of available soil P were observed due to pH-H_2O increment, agronomic solution such as fertilization must be applied to fulfil this specific need. It has been highlighted that 83% of the farmers in Santander fertilized their cocoa cultivation. Commercial mineral P fertilizers, on the other hand, contain trace elements, especially Cd. As a result, caution should be exercised when using phosphate fertilizers and/or manure, and they should be inspected to ensure that they do not contain excessive levels of Cd (CAOBISCO/ECA/FCC, 2015). Availability of P in the soil can be improved by adding organic residues to increase the organic matter content of the soil and using phosphate rock (Kongor *et al*., 2019). Phosphate rock is a low cost source of high quality phosphate mineral that can be used as direct application fertilizer in acid soils to raise pH and increase P supply (van Straaten 2002 as cited in Kongor *et al*., 2019).

4.3 Mitigating Cadmium Uptake Through Liming

The current Cd regulations in cocoa-derived products only apply to finished foods, not cocoa beans. As a result, mitigation techniques can be applied at all stages of the production process, from the tree to the chocolate bar (Vanderschueren *et al*., 2021). Mitigation practices based on soil amendments to reduce Cd uptake into the plant is crucial to achieve a cocoa final product that complies with the current EU regulation. The application of liming materials such as $CaCO_3$ has often been recommended as an agronomic practice to decrease the uptake and accumulation of Cd in edible parts of cocoa plants. A study by Ramtahal *et al.* (2019) reported the effective reduction of leaf Cd concentrations 6 months after lime application in a pot experiment. Liming soils is recommended for cocoa farmers in order to raise the pH to hinder availability of Cd (CAOBISCO/ECA/FCC, 2015). Chavez *et al.* (2016) found that soil pH and organic matter influenced the availability of Cd to cocoa plants in Ecuadorian soils, while soil pH and geology influenced bean Cd concentration (Gramlich *et al*., 2018). Liming reduces soil Cd solubility (and thus reduces available soil Cd) by raising soil pH and through interaction between Ca^{2+} and Cd^{2+} at root uptake sites (Christensen, 1984; Bolan *et al*., 2003).

The amount of agricultural liming material required to raise soil pH is determined by the amount of acidity in the soil and the quality of the liming material. The quality is ascertained by its purity and particle size distribution. A likely recommended rate of lime ($CaCO_3$) to be used as agricultural liming material proposed in this study is ca. 50 tonnes $CaCO_3$ ha^{-1} to increase the soil pH by 1 unit (Appendix 7). Locally available organic residues, agro-minerals, wood ash, biochar among others are suggested examples of materials that can be used for this purpose. These materials are cheap and internally-generated, and they could accomplish both liming and organic fertilizers purposes (Kongor *et al*., 2019). Since high levels of Cd are associated with beans from LAC, it is not advisable for the cocoa pod husks to be used although Owolabi *et al*. (2003) reported on the use of its ash together with wood ash to increase soil pH, P, K, Ca and Mg content of soil. The lack of effect of liming in the cocoa field trial may thus be related to Cd being taken up by deeper rooted soil horizons that were not affected by the lime treatment (Vanderschueren *et al*., 2021). In a pot experiment with cocoa seedlings, Argüello *et al*. (2020) showed that partially liming the rooted soil (lime on top compartment only as would be the case in field liming) increased Cd uptake from the non-limed bottom compartment. Within one (1) to three (3) years, lime moves little in the soil and neutralizes acidity only in the area where it is applied. Therefore, lime must be uniformly distributed and thoroughly incorporated to be most effective (Crozier & Hardy, 2018).

5.0 CONCLUSION

The EU needs to import all its cocoa beans to produce chocolate as there is no domestic cocoa production in Europe. Recent regulations on the maximum allowed Cd concentration in cocoa-derived products are affecting cocoa producers worldwide and are especially causing concern in Latin America. For cocoa leaf, bean, and soil samples from San Vicente de Chucuri, Colombia, a comprehensive analysis of available soil Cd, cocoa bean and cocoa leaf Cd contents was performed. Metal analysis revealed Cd contents in cocoa leaves ranged from 0.6 to 77.5 mg kg^{-1}, with a mean of 10.2±15.4 mg kg^{-1}. For cocoa beans Cd levels, 0.4 to 34.0 mg kg^{-1} with an average of 6.5±8.2 mg kg^{-1} were recorded. Available Cd concentrations varying from 0.5 to 1656.0 µg kg^{-1} with a mean of 79.2±260.5 µg kg^{-1} were observed for the soil samples.

As of January 2019, the EC established a maximum level of 0.6 mg Cd kg^{-1} in cocoa powder. Since the content of Cd in cocoa beans has not been regulated, a threshold level of 0.6 mg kg^{-1} is adopted. The present study shows alarmingly high Cd contents in cocoa beans which surpassed the permissible limit of Cd levels in cocoa and chocolate products.

The results confirmed the distribution of Cd in cocoa leaves and beans on plantation soils of Santander showed the following trend: $Cd_{soil} < Cd_{beans} < Cd_{leaves}$. This research revealed there was no statistically difference between the clones (CAU39, FEAR5 and FEC2) in their accumulation of Cd in leaves and beans. The same was observed for the available Cd concentration in soils sampled under the examined clones. However, statistically different leaves and beans Cd levels were observed among the different investigated farms thus, a spatial variability is apparently present. Also, the available Cd concentrations in the soil were statistically different for the different farms. A significant correlation between available soil Cd and the Cd contents in leaves and beans was observed. This correlation decreased in the order: leaves (R^2 = 0.7) ≈ beans (R^2 = 0.4). If the available soil Cd concentrations greater than 0.3 mg kg^{-1} are eliminated, there is no relation with cocoa leaves and beans.

The presence of Cd is undeniable, and this poses serious threat to the sustainability of cocoa production in this region of Santander. Although the quality of cocoa beans in this region seems optimal to meet the global demand, the high Cd content might constrain export. Liming which has been suggested as a mitigation strategy to limit the phyto-availability and absorption of Cd in cocoa tree was employed. Soil pH-H_2O increase generally limited the availability of Cd concentration in the soil after a week of incubation. However, the consequence of the pH-increase led to drop in the available soil P. A pH-increase of 2.4 units on the average resulted in a decrease in available soil P and available soil Cd by 1.9 mg kg^{-1} and 90.3 µg kg^{-1} respectively. The mean available P concentrations in the soil before and after pH-H_2O adjustment were significantly different. Also, mean available Cd concentrations in the soil before and after pH-H_2O increase differed significantly. Since low concentrations of available soil P were observed due to pH-H_2O increment, agronomic solution such as fertilization must be applied to fulfil this specific need. Based on the lime requirement calculations, the proposed recommended rate of lime ($CaCO_3$) to be used as agricultural liming material to increase the soil pH by 1 unit is ca. 50 tonnes $CaCO_3$ ha^{-1}.

Though a higher percentage of the farmers in San Vicente de Chucuri practice fertilization, organized efforts must be put in place to educate and assist more cocoa farmers in this region of Santander to embrace the use of fertilizers as well as liming on their farms to augment the production of cocoa. This will enable cocoa growers to meet the proposed EU standard for Cd in cocoa beans and enhance productivity.

6.0 REFERENCES

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7.0 APPENDICES

7.1 Appendix 1

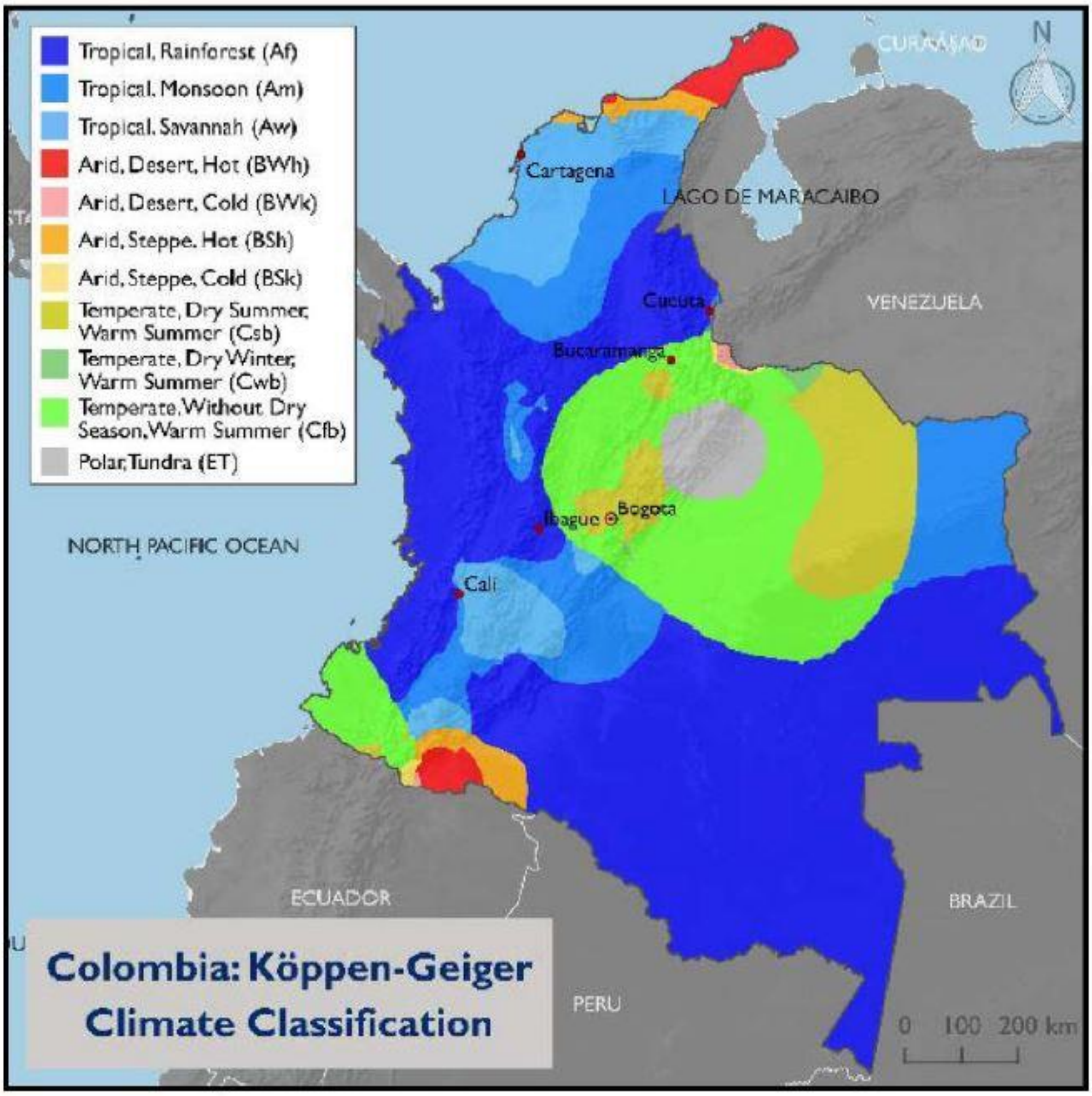

Figure 3.7: Classification of the different climates present in Colombia (USAID, 2017).

7.2 Appendix 2

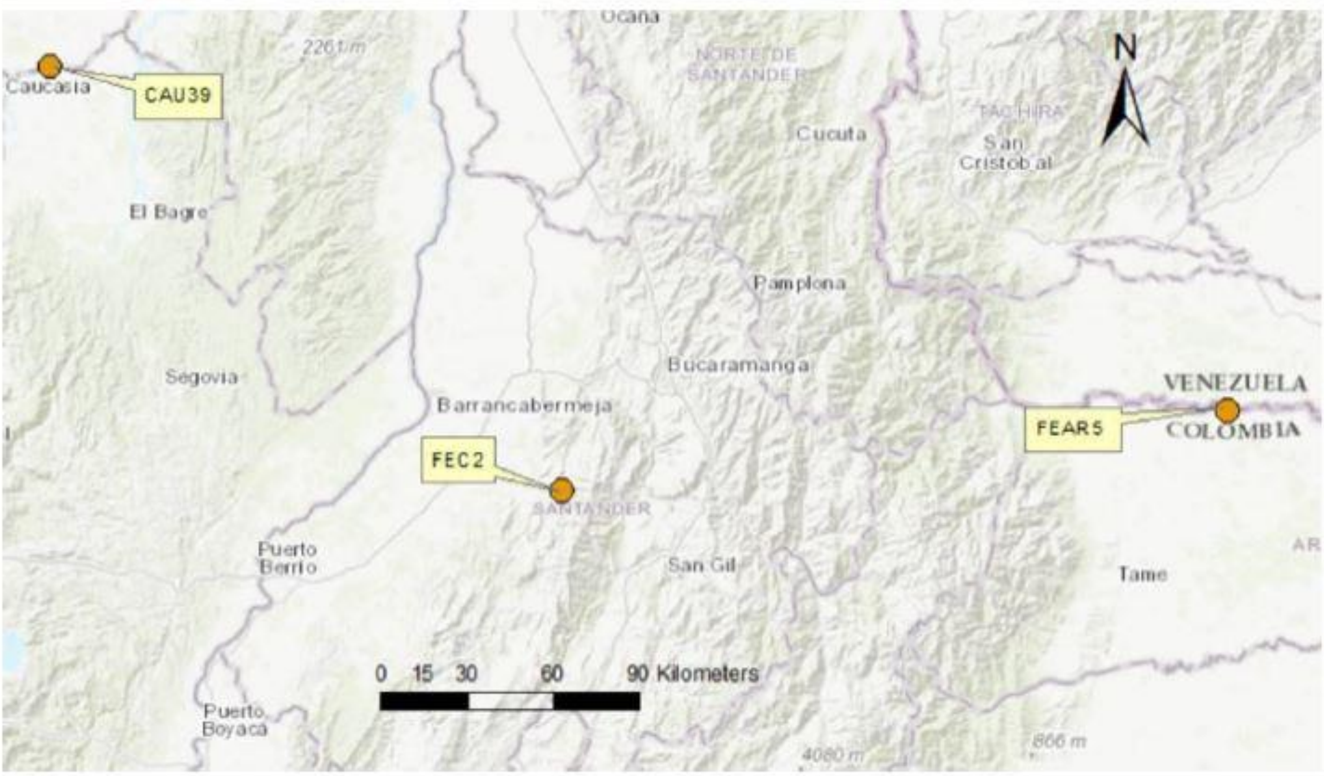

Figure 3.8: Geographical origin of the investigated cocoa clones (Hoogerwerf, 2020)

7.3 Appendix 3

Definition of terms in Table 1.2

*Spinach leaves (reference material) – This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor and trace elements in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1570a consists of 60 g of finely powdered dried spinach leaves. The certified mass fraction value reported for Cd is 2.876 ± 0.058 mg kg^{-1} on a dry-mass basis (National Institute of Standards and Technology, 2014).

**Milli-Q is obtained by purification of water using unique Jetpore ion-exchange resin, synthetic activated carbon and an ultra violet lamp to reach a resistivity of 18.2 MΩ.cm at 25°C and a total organic carbon value below 5 ppb. This water is sent through a small recirculation loop to the Application Pak, where a final purification step, critical for specific experiments, removes contaminants just before water is dispensed from the system (Merck Millipore, 2020).

***Pico-Pure HNO_3 – It is a colourless liquid with molecular weight of 63.01 g mol^{-1} and boiling point of 122^0C which makes it corrosive. It has a concentration of 67 - 69% (Pico-Pure) and is for laboratory use, ICP-MS trace analysis (www.chem-lab.be).

****Cd element standard – The quality control of ICP Cd-element standard is carried out by the accredited calibration laboratory according to ISO 17025. It is directly traceable to primary reference material from NIST. It is composed of Cd standard solution, 1000 mg Cd L^{-1} in dilute nitric acid (from $Cd(NO_3)_2$) (www.vwr.com).

7.4 Appendix 4

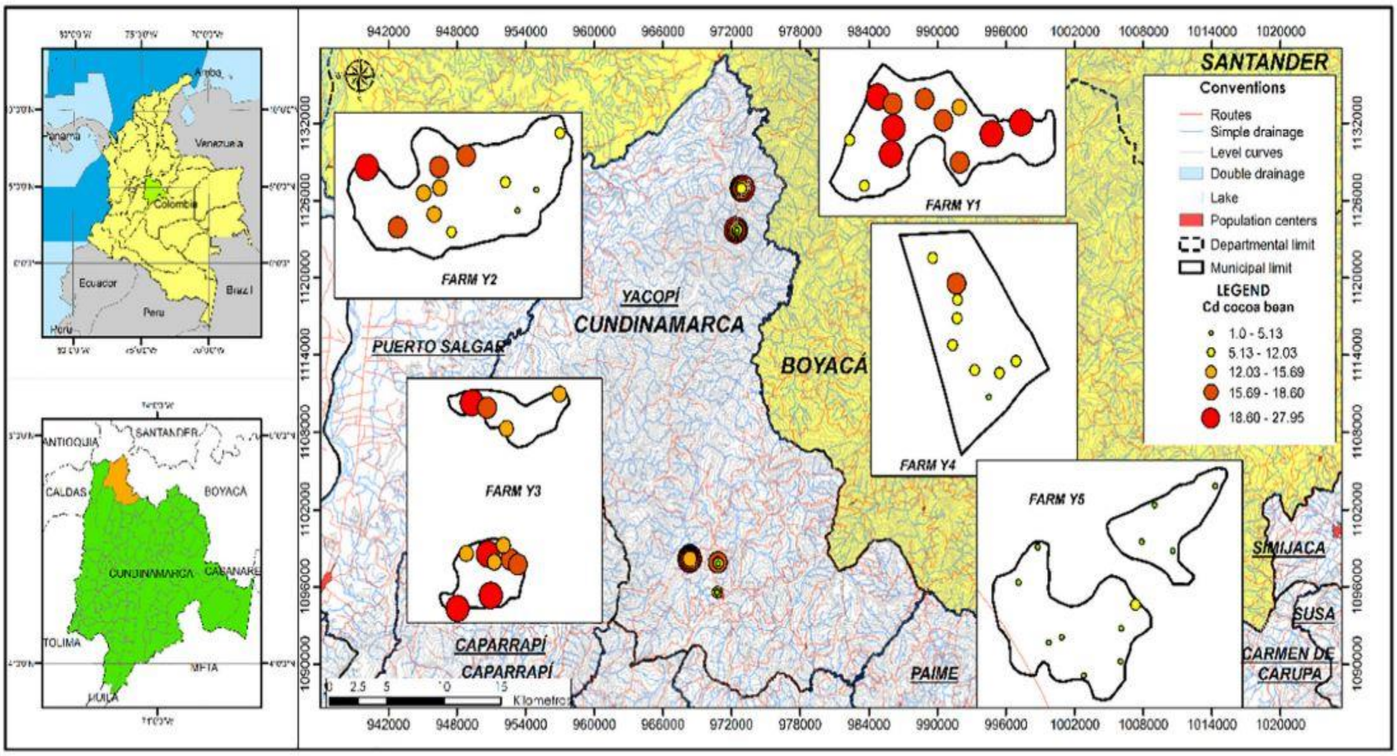

Figure 3.9: Spatial distribution of Cd in cocoa bean (mg kg^{-1}) from Yacopí (dpt. Cundinamarca, Colombia) characterized by cluster analysis (Rodríguez Albarrcín *et al.*, 2019).

7.5 Appendix 5

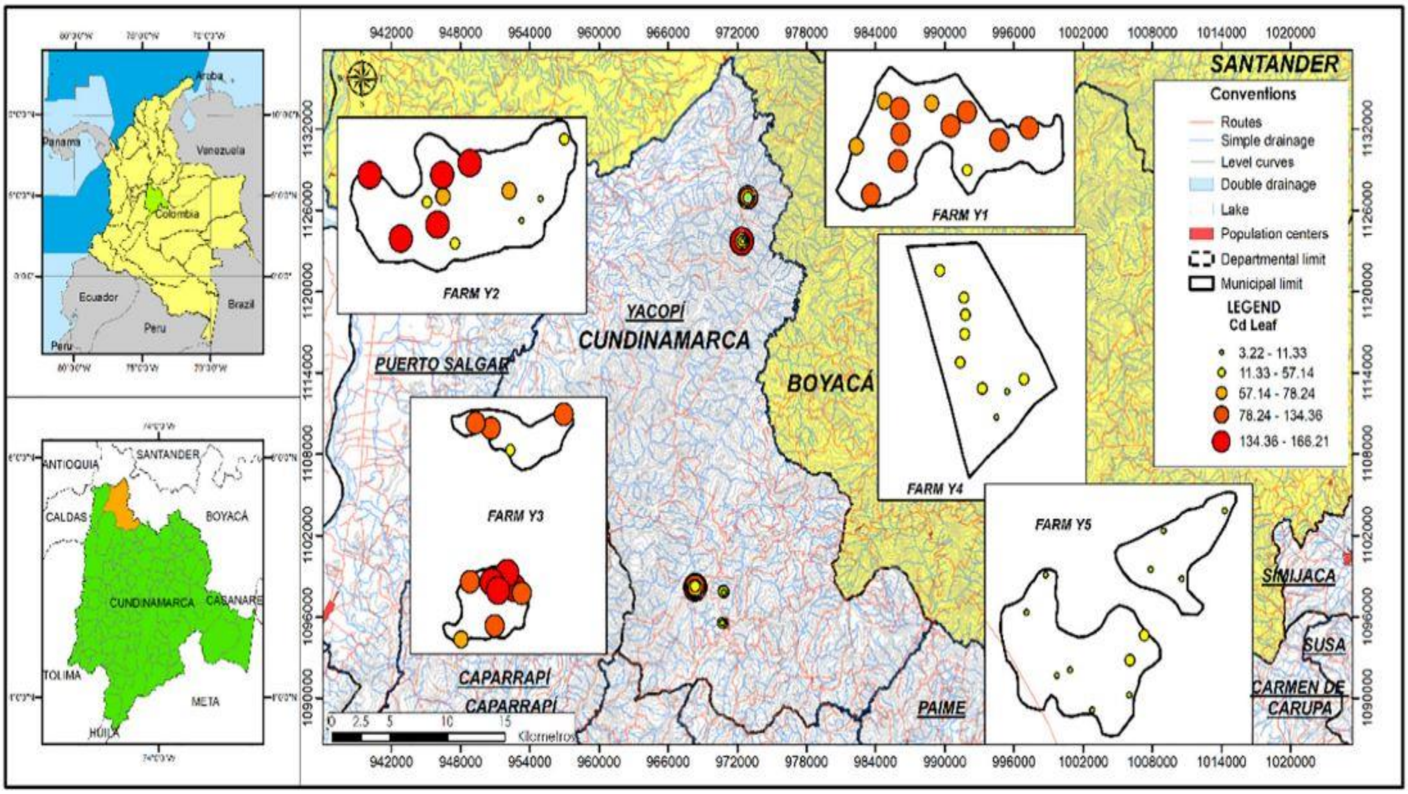

Figure 4.0: Spatial distribution of Cd in leaf litter (mg kg^{-1}) from Yacopí (dpt. Cundinamarca, Colombia) characterized by cluster analysis (Rodríguez Albarrcín *et al*., 2019).

7.6 Appendix 6

Table 2.2: Soil pH-H_2O, available soil Cd and available soil P concentrations before and after pH-H_2O adjustment.

Soil Sample	Available P concentration in soil (mg kg^{-1})		pH		Available Cd concentration in soil (µg kg^{-1})	
	Before Incubation	**After Incubation**	**Before Incubation**	**After Incubation**	**Before Incubation**	**After Incubation**
SV002	10.4	5.9	5.3	7.4	335.0	49.0
SV005	0.5	0.2	5.9	7.8	24.0	4.0
SV008	6.8	2.8	4.6	7.2	76.0	7.0
SV010	10.2	1.7	4.7	8.0	291.0	13.0
SV012	16.0	17.8	6.3	8.9	49.0	9.0
SV014	1.7	0.2	4.9	7.8	41.0	4.0
SV015	0.6	0.4	5.2	7.8	186.0	28.0
SV018	1.0	0.2	5.0	6.9	138.0	54.0
SV021	1.3	0.1	5.2	7.2	40.0	8.0
RN004	1.6	0.4	5.6	7.3	39.0	4.0
RN011	2.8	0.4	5.0	7.5	27.0	4.0
RN015	0.7	0.7	5.2	7.8	31.0	9.0
Average	**4.5**	**2.6**	**5.3**	**7.6**	**106.0**	**16.0**

7.7 Appendix 7

Lime Requirement Calculations

The average bulk density of the soils from San Vicente and Rionegro = **1.5 g cm^{-3}**

Soils were sampled at a depth of 0-30 cm (a depth of **30 cm** was considered for the calculation).

$NaHCO_3$ solution of **50 mg mL^{-1}** was used.

Average volume of $NaHCO_3$ solution added to the soil is **4.7 mL**.

a. Calculating the amount of $NaHCO_3$ that are needed

Milli-equivalent (me) of $NaHCO_3$ needed = Normality x volume (mL)

Normality ($NaHCO_3$) = 0.05 g/84 g mol^{-1} x 0.001 L

= **0.595 EqL^{-1}**

Milli-equivalent (me) of $NaHCO_3$ = 0.595 x 4.69

= **2.79 me $NaHCO_3$ 6.0 g $soil^{-1}$**

b. Calculating the weight of $NaHCO_3$ needed

Weight of $NaHCO_3$ needed = 2.79 me $NaHCO_3$ 6.0 g $soil^{-1}$

1 me $NaHCO_3$ = $\frac{23 + 1 + 12 + 3(16)}{1}$ mg

= 84 mg

Thus, 2.79 me $NaHCO_3$ = 84 x 2.79 mg

= **234.36 mg 6.0 g $soil^{-1}$**

c. Changing to unit of part per million (ppm)

6 g (6000 mg) soil needs 234.36 mg of $NaHCO_3$

Thus, 6,000,000 mg (a million mg) soil needs = $\frac{6,000,000}{6000}$ x 234.36 mg $NaHCO_3$

= **234,360 ppm**

d. Calculating the amount of $NaHCO_3$ in the top 30 cm soil in a hectare of land

Soil bulk density is 1.51 g cm^{-3} (1510 kg m^{-3})

Weight of 1 ha soil is = 1510 x 10,000 x 0.30

= **4,530,000 kg**

But 6,000,000 kg of soil needs 234,360 kg of $NaHCO_3$

Therefore, 4,530,000 kg soil needs $= \frac{4,530,000}{6,000,000} \times 234,360$ kg of $NaHCO_3$

= 176941.8 kg $NaHCO_3$ ha^{-1}

= 176.94 metric tonnes $NaHCO_3$ ha^{-1}

e. Calculating the likely recommended rate of lime ($CaCO_3$) to be applied in the field

1 milli-equivalent (me) $CaCO_3$ = 50 mg of $CaCO_3$

Thus, 2.79 me CaCO3 = 2.79 me x 50 mg $CaCO_3$

= 139.5 mg $CaCO_3$ 6.0 g $soil^{-1}$

That is, 6000 mg soil needs 139.5 mg $CaCO_3$

Thus, 6,000,000 mg soil needs 139,500 mg $CaCO_3$

Thus, 1 ha soil needs $= \frac{4,530,000}{6000000}$ kg x 139,500 kg $CaCO^3$

= 105,322.50 kg $CaCO_3$

Using calcite ($CaCO_3$) with the purity of 90%

The actual quantity of $CaCO_3$ needed is $= \frac{100}{90} \times 105,322.50$ kg $CaCO_3$

= 117 metric tonnes $CaCO_3$ ha^{-1}