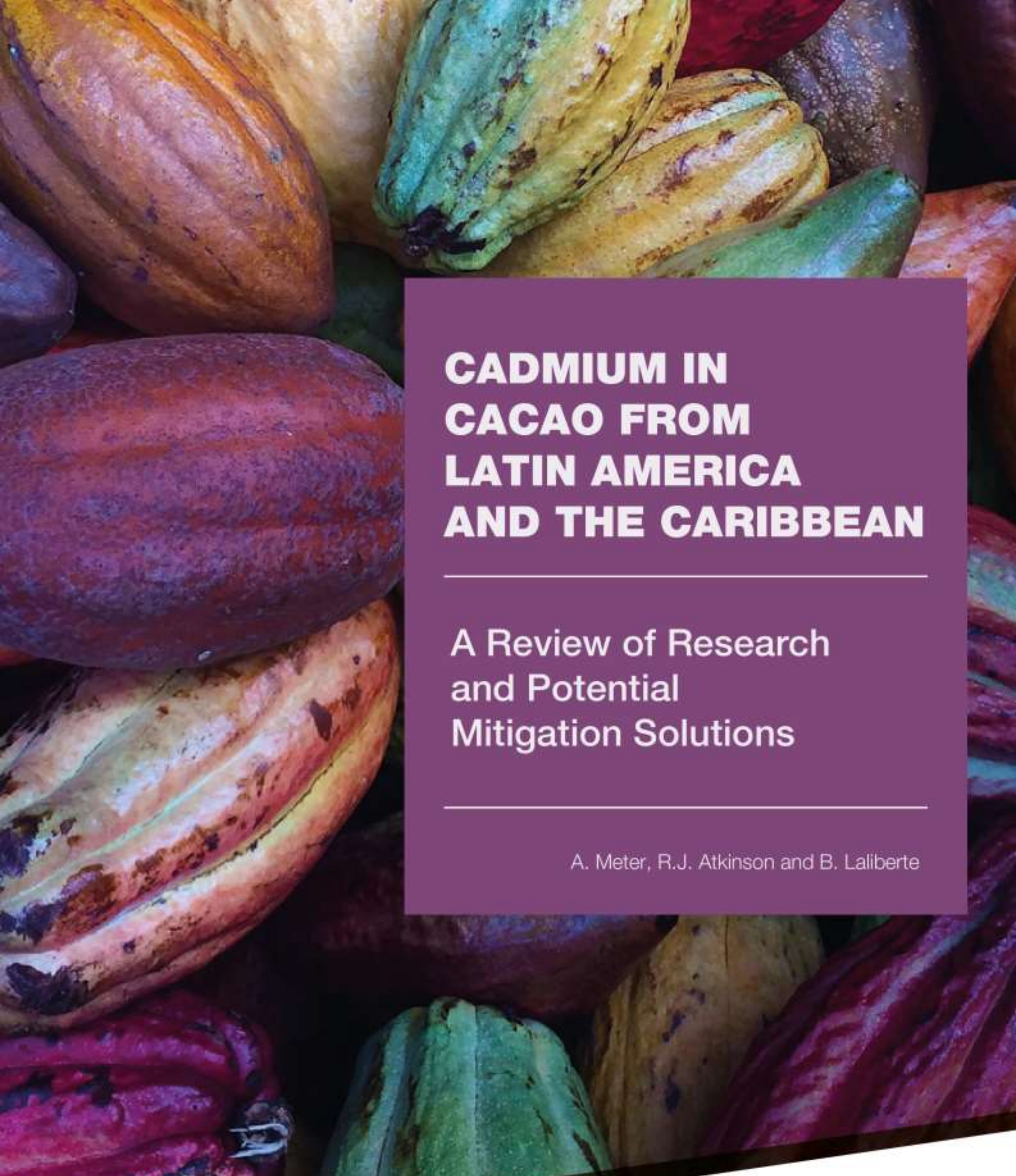

CADMIUM IN CACAO FROM LATIN AMERICA AND THE CARIBBEAN

A Review of Research and Potential Mitigation Solutions

A. Meter, R.J. Atkinson and B. Laliberte

CAF DEVELOPMENT BANK OF LATIN AMERICA

latinamerican COCOA INITIATIVE CAF DEVELOPMENT BANK OF LATIN AMERICA

Bioversity International

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Latin American Cacao Initiative (ILAC) is a project set by the Green Business Unit/DSICC – Vice-presidency of Sustainable Development of CAF. It aims to catalyse the development of fine and aromatic cacao as a sustainable economic activity and an integrating element of the people who have used it ancestrally in Latin America, achieving significant social and environmental impact on the conservation of biodiversity associated with cacao, favouring SME´s development and the entry of Latin American cacao into markets that favour biotrade and the supply of quality. **http://scioteca.caf.com/handle/123456789/1110**

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[1] Bioversity International is the operating name of the International Plant Genetic Resources Institute (IPGRI)

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ACRONYMS

AAS	Atomic Absorption Spectrometry
ABC	ATP-binding cassette
AGROSAVIA	Corporación Colombiana de Investigación Agropecuaria
AMF	Arbuscular Mycorrhiza Fungi
ATP	Adenosine tri-phosphate
BF	Bioaccumulation factor
Ca	Calcium
$Ca(NO_3)_2$	Calcium nitrate
CABI	CAB International
$CaCl_2$	Calcium chloride
$CaCO_3$	Cadmium carbonate
CADMIUMF	Cation diffusion facilitator
CAOBISCO	Association of Chocolate, Biscuit and Confectionery Industries of Europe
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica
Cd	Cadmium
CEC	Cation exchange capacity
CIAT	Centro Internacional de Agricultura Tropical
Cl	Chloride
CM	Cocoa Mass
CRA	Cocoa Research Association UK
CRC	Cocoa Research Centre of the University of the West Indies, Trinidad and Tobago
CRIG	Cocoa Research Institute of Ghana
CRIN	Cocoa Research Institute of Nigeria
DGA	Direccion General Agricola
DGT	Diffusive gradient in thin film (extraction method)
DTPA	Diethylene-triamine-penta-acetic acid
EAFIT	Universidad Escuela de Administración, Finanzas e Instituto Tecnológico, Colombia
EC	Electrical Conductivity
ECA	European Cocoa Association
EDTA	Ethylene-diamine-tetra-acetic acid
ENGIM	Ente Nazionale Giuseppini del Murialdo, Italy
ESPE	Universidad de las Fuerzas Armadas, Ecuador
ESPOL	Escuela Superior Politécnica del Litoral, Ecuador
EU	European Union
FAAS	Furnace Atomic Absorption Spectrometry
FAO	Food and Agricultural Organisation of the United Nations
FAS	Foreign Agricultural Services, USDA
FCC	Federation of Cocoa Commerce
FCIA	Fine Chocolate Industry Association, USA
FEDECACAO	Federación Nacional de Cacaoteros de Colombia
FER	Ferritin Fe (III) binding
FIBL	Research Institute of Organic Agriculture, Switzerland
FONTAGRO	Fondo Regional de Tecnología Agropecuaria
FTA	CGIAR Research Program on Forests, Trees and Agroforestry
GEF	Global Environment Fund
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit, Germany
H	Hydrogen
ha	Hectare
HCl	Hydrochloric acid
HMA	Heavy metal ATPase
HNO_3	Nitric acid
ICCRI	Indonesian Coffee and Cocoa Research Institute
ICCRI	Indonesian Coffee and Cocoa Research Institute
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Induced Coupled Plasma Optical Electro Spectrometry

ICT	Instituto de Cultivos Tropicales, Peru
IDIAF	Instituto Dominicano de Investigaciones Agropecuarias y Forestales
IDIAP	Instituto de Investigación Agropecuaria de Panamá
IGAC	International Global Atmospheric Chemistry
ILAC	Latin American Cacao Initiative
INIA	Instituto Nacional de Innovación Agraria, Perú
INIAP	Instituto Nacional de Investigaciones Agropecuarias, Ecuador
INTA	Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria, Costa Rica
IPNI	International Plant Nutrition Institute
IRD	Institut de recherche pour le développement, France
IREG	Iron-regulated transporter
K	Potassium
kg	Kilogram
KU Leuven	Katholieke Universiteit Leuven, Belgium
LAC	Latin America and the Caribbean
MADR	Ministry of Agriculture and Rural Development of Colombia
mg	Milligram
Mg	Magnesium
MINAGRI	Ministerio de Agricultura y Riego, Peru
Mn	Manganese
N	Nitrogen
$NaNO_3$	Sodium nitrate
NAS	Nicotinamide synthase
ng	Nanogram
NGO	Non-Governmental Organisation
NH_4^+	Ammonium
NO_3^-	Nitrate
NRAMP	Natural resistance-associated macrophage protein
NRCS	Natural Resources Conservation Service, USA
NRT	Nitrate transporter
P	Phosphorus
Pb	Lead
pH	Potential of hydrogen
ppm	Parts per million
PSU	Penn State University, USA
SAMS	S-adenosyl-methionine synthetase
SENASA	Servicio Nacional de Sanidad Agraria, Peru
t	Ton
TF	Translocation factor
UDES	Universidad de Santander - Colombia
UESC	Universidade Estadual de Santa Cruz, Brazil
UNA	Universidad Nacional de Costa Rica
UNAL	Universidad Nacional de Colombia
UNAS	Universidad Nacional Agraria de la Selva, Peru
UNODC	United Nations Office on Drugs and Crime
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTE	UTE Universidad, Ecuador
VLIR	Flemish University Council, Netherlands
YSL	Yellow-stripe-like transporter
ZIP	ZRT, IRT-like protein
ZnSO4	Zinc sulphate

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EXECUTIVE SUMMARY

Cadmium is a naturally occurring heavy metal, which has no known function in humans. It accumulates in the human body, primarily affecting the kidneys, but can also cause bone demineralisation and osteoporosis.

We are increasingly exposed to cadmium in our diet. In response to this, the European Union (EU) is setting maximum permissible levels in different foods. In 2014, maximum permissible levels for cadmium in cocoa and chocolate products sold in the EU were set. This has been enforced since January 1st 2019. The levels are based on estimated consumption of chocolate by different age groups.

The EU regulation sets different levels for four categories of chocolate products: 0.10 ppm for milk chocolate with < 30% total dry cocoa solids; 0.30 ppm for chocolate with < 50% total dry cocoa solids and milk chocolate with ≥ 30% total dry cocoa solids; 0.8 ppm for chocolate with ≥ 50% total dry cocoa solids and 0.6ppm for cocoa powder sold to the final consumer or as an ingredient in sweetened cocoa powder.

Limits on cadmium levels in chocolate have been put in place in Australia, Indonesia, New Zealand, Russia, and in the State of California in the USA. There is also active discussion regarding recommended limits for cadmium to be included in the *Codex Alimentarius*.

While almost all limits are set on chocolate and cacao products and not the raw product, buyers are also placing limits on cacao beans[1] to ensure the final products fall below the maximum permissible levels. These range between 0.5 and 1.1ppm.

In comparison to other cacao growing regions of the world, the levels of cadmium in cacao regularly exceed these limits in certain areas of Latin America and the Caribbean (LAC).

Much of the cacao produced in LAC is by smallholder farmers whose livelihoods are particularly vulnerable to the new regulations. Many are involved in the production of fine flavour cacao commonly used for products with high cacao content and in single origin niche products with the principal market being Europe. There is a pressing need to find short, mid and long-term solutions to address the issue.

Solutions are currently being investigated or considered based on the current state of knowledge regarding i) sources of cadmium accumulation in soils, ii) factors affecting cadmium bioavailability to cacao plants, iii) physiological mechanisms of cadmium uptake by cacao plants and accumulation in the beans, iv) genetic variation in uptake and v) effects of post-harvest processing on cadmium content in beans.

Cadmium is found in the soil where its presence is a result of a combination of natural and anthropogenic processes. Natural processes include the weathering of rock, volcanic activity, forest fires, erosion and deposition in soils through flooding events by river sediments, while anthropogenic processes include mining and industrial activities, as well as agricultural practices such as irrigation and fertilisation. It is likely that both natural and anthropogenic sources contribute to the soil cadmium content, with the relative importance of different sources depending on the area.

Not all cadmium present in the soil is bioavailable to cacao plants – i.e. readily available for uptake by the roots. While higher levels of total cadmium content imply higher potential for cadmium uptake by cacao trees, high levels of cadmium have been reported in cacao beans growing on soil with relatively low total cadmium content and *vice versa*. Bioavailability is influenced by multiple soil properties: pH, organic matter content, soil texture and

[1] In line with the industry, cacao (cocoa) seeds are referred to throughout this review as 'beans' even though they are not true beans in the botanical sense.

mineralogy, cation exchange capacity, electrical conductivity, macro- and micro-nutrient content and the presence of microorganisms. Altering these properties is key to reducing cadmium uptake by the plant

The cadmium ion is taken up by the cacao roots through specific and non-specific processes used for ion absorption. It is transported via the xylem to the leaves, and reaches the fruit through the phloem. Generally, cadmium concentration in cacao trees decreases in the following order: leaves > pod husks > seed shell > shelled nib. Various factors can affect the process of uptake and partitioning of cadmium within cacao plants, such as the age of the tree or plant nutritional status. There is variability in cadmium content across different genotypes, indicating the possibility of identifying low-accumulating cacao varieties.

Results regarding the effect of post-harvesting practices such as fermentation, drying, roasting and winnowing do not allow for clear conclusions, although it appears that these practices can potentially affect the cadmium content in cacao beans and derived products.

Only some of the proposed solutions to reduce cadmium uptake have been or are in the process of being tested in LAC. Additionally, research to date indicates that finding a single solution to reduce cadmium accumulation in cacao beans is unlikely, due the heterogeneity in environmental and soil conditions in the region, different sources of cadmium, the use of different genotypes, and the quality requirements of buyers and their markets.

Solutions also have different cost implications, and their effective implementation requires the motivation of a range of actors.

The authors suggest that a mitigation hierarchy approach can help to develop a nuanced and integrated set of solutions for reducing cadmium levels in cacao beans and thus chocolate, by considering actions from farm to final product that are adapted to the specific conditions of the cacao value chain in question:

- Avoid high risk areas for establishing plantations
- Minimize the uptake of cadmium by the cacao tree
- Reduce levels of cadmium through post-harvest processing
- Reduce high levels of cadmium in chocolate by blending

Actions are summarized in more detail below:

- ***Avoid high-risk areas for establishing plantations***: Until there are cost effective and efficient *solutions* to reduce accumulation of cadmium in cacao beans, sites where cacao is at risk from accumulating high levels of cadmium should be avoided for new plantations. While site identification is not straightforward due to the variable response of cacao trees to soil cadmium levels, areas known to produce cacao with high levels of cadmium could be avoided. It should be acknowledged that while many farmers cannot choose or change the location of their farmland, they could decide which crop to grow. It may be advisable to plant another crop, at least in the short-term in these areas.

- ***Minimize the uptake of cadmium by the cacao tree*****:** Some of the most promising strategies for reducing cadmium in cacao beans involve minimising its uptake by the trees. This can be achieved by minimising inputs of cadmium into the system through management of contaminated fertilisers, water quality and flood-drought cycles It can also be achieved by adding soil amendments that alter soil characteristics such as pH or soil organic matter content to reduce the bioavailability of cadmium to cacao plants, by increasing the nutrient status of the plant which can reduce cadmium uptake, by adding microorganisms and other plant species that sequester cadmium from the soil, and using genotypes that are naturally low accumulators of cadmium. While the theory is advanced, field trials are only just beginning.

- ***Reduce levels of cadmium through post-harvest processing:*** It appears that it may be possible to minimise cadmium levels in cacao beans through changes to the traditional post-harvest processing methods of fermentation, drying, roasting and winnowing. Further research is needed, not only to determine reduction potential of levels of cadmium, but also to understand impacts on physical and flavour qualities.

- ***Reduce levels of cadmium in chocolate by blending:*** Blending high cadmium content cacao beans with beans from other regions or even countries with a low cadmium content can be an effective short-term solution to ensure that products do not exceed the regulatory limits. However, for some areas this will result in the loss of regional identity and flavour differences that are key to the fine flavour cacao market. For fine flavour cacao that cannot be blended, a detailed traceability mechanism of cadmium levels may allow for separation of grains prior to fermentation.

Since 2014, there has been an increase in research efforts to address the problem of cadmium accumulation in cacao, with the aim of finding solutions. Currently there are at least 28 ongoing projects in LAC (Ecuador, Colombia, Peru and Trinidad and Tobago) and Indonesia. Together, these projects cover most of the short, medium and long-term solutions currently considered. Several projects are working on the evaluation of amendments to modify soil properties, identify genotypes of low accumulation and bioremediation using microorganisms. Two areas that remain particularly poorly studied are phytoremediation (the use of plants to extract cadmium in the soil) and the socio-economic aspects (the impact of regulations on the global cocoa value chain, economic viability for farmers and the extension of the potential of possible mitigation solutions). The government, private sector, researchers and producers are advancing our knowledge to understand better the applicability of the short, mid and long-term solutions currently proposed by theory.

The authors offer the following recommendations to help focus future actions:

1. Test mitigation solutions in farmers' fields across different environments and agricultural practices, focusing primarily on soil management methods that are economically and practically viable to farmers.
2. Identify low-accumulating genotypes and test their use as rootstock and scion material in the field.
3. Develop national and regional projects that use comparable methodologies, and align current research methodologies to allow for ease of comparison.
4. Identify sources of cadmium contamination and the effect of abiotic factors (temperature, water stress, shade), on cadmium accumulation in beans.
5. Fill the knowledge gap regarding the physiological basis of cadmium uptake, transport through the plant and accumulation in cacao beans.
6. Ensure that solutions consider the net gain to small-scale farmers living in difficult economic circumstances.
7. Identify and quantify the effectiveness of post-harvest solutions that might be able to reduce cadmium while maintaining the quality of the cacao.
8. Engage with the industry to better understand the changes in cadmium levels during the manufacturing of chocolate products and identify reasonable limits to apply to the raw product.
9. Establish consistency in laboratory results, focusing on standardizing sampling, cadmium extraction methods and analysis,
10. Assess the utility of other methodological approaches for estimating cadmium levels in cacao beans.
11. Assure markets and consumers that the cadmium contamination problem is localised and does not apply to an entire country or even region within the country.
12. Inform producers of the issues and research being carried out to reach solutions.

1 BACKGROUND

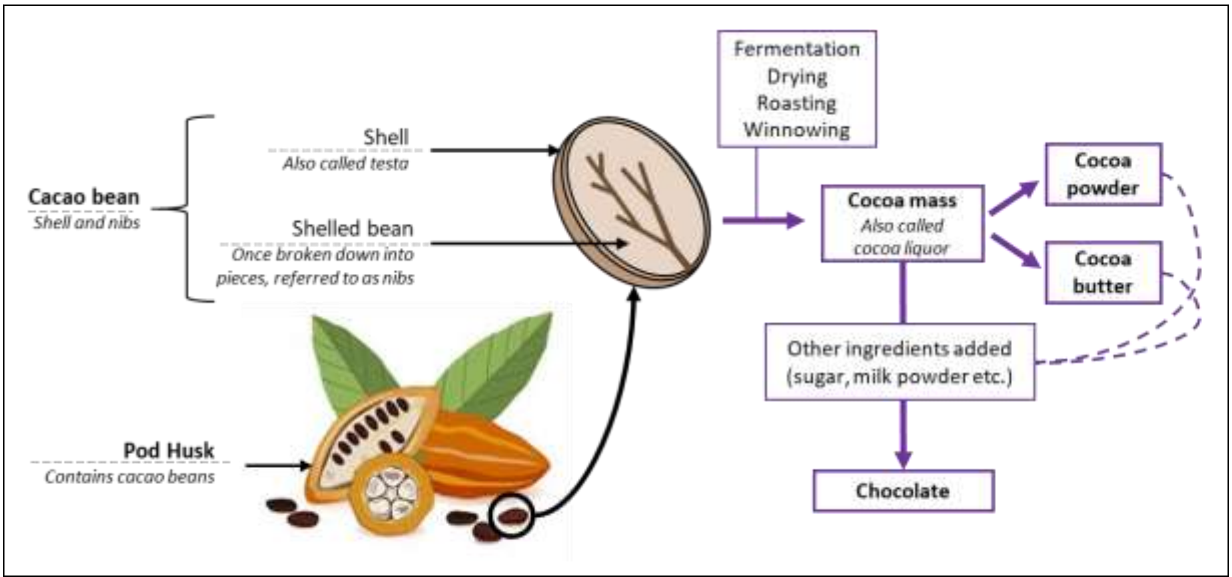
Cadmium is a naturally occurring, heavy metal, which has no known function in humans. It accumulates in the body affecting primarily the kidneys but can also cause bone demineralisation. Airborne cadmium can weaken lung function and even lead to cancer (Bernard 2008). To reduce exposure, the European Union (EU) is setting maximum permissible levels of cadmium in different foods based on dietary intake. In 2014, the EU announced maximum permissible levels for cadmium in cocoa and chocolate products sold in the EU – see Table 1. This has been enforced as of January 1st, 2019. The levels are based on estimated consumption levels of chocolate by different age groups.

Table 1 EU maximum permissible levels of cadmium in cacao and chocolate products currently enforced – adapted from European Commission regulation (EU) 488/2014 of 12 May 2014

Product	Maximum permissible level (mg/kg)
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

Source: European Commission regulation (EU) 488/2014 of 12 May 2014

Figure 1 Terms used from the cacao bean to the chocolate bar



Source: Prepared by the authors

The levels set by the EU regulation are similar to those being proposed for inclusion in the Codex Alimentarius[1] of 0.8 mg/kg for chocolate with ≥ 50% to ≤ 70% cocoa solids, and 0.9 mg/kg for chocolate with > 70% cocoa solids. The categories and limits for products with < 50% total cocoa solids and for cocoa powder (100% total cocoa solids) have yet to be defined[2].

The EU is not alone in regulating cadmium in chocolate. The Indonesian National Standard states the following maximum limits: Cocoa mass 1 ppm, Cocoa Butter 0.5 ppm, Cocoa pressed cake 0.5 ppm, Cocoa Powder 1 ppm and Chocolate products 0.5 ppm. The Australia and New Zealand Food Standards Code Standard 1.4.1 on contaminants and natural toxicants has set a maximum level of cadmium in chocolate and cocoa products at 0.5ppm not including cocoa powder, and the Russian Federation (SanPin 2.3.2-1078-01) has set the same threshold, but this covers all chocolate and chocolate products, cocoa beans and products.

The State of California USA has set maximum levels for cadmium in chocolate products under the Industrial Agreement Proposition 65 (19/02/2018) – see Table 2. Products that exceed the limits can be sold, but in this case, a warning must be put on the label.

Table 2 Maximum permissible level set under the Industrial Agreement Proposition 65 (San Pin 2.2-1078-01)

Chocolate Product Composition (% of total cocoa content)	Maximum cadmium level (ppm) set for:	
	2018-2025	2025
< 65%	0.400	< 65%
65-95%	0.450	65-95%
≥95%	0.960	≥95%

Source: San Pin 2.2-1078-01

As can be noted above, the maximum permissible levels in the EU regulation are for chocolate products and not the raw material. However, buyers need to be able to relate the level of cadmium in the cacao beans with the final product. As cocoa butter contains minimal levels of cadmium, the concentration of cadmium in cocoa mass is similar to that in the cocoa liquor, (the first product derived from cacao beans after fermenting, drying and roasting). With knowledge of the percentage of cocoa mass in the final chocolate product, the following equation can be used to estimate the maximum cadmium level in cocoa mass that will allow the chocolate product to remain below the relevant EU threshold:

$$ML_{CM} = \frac{ML_{EU.P}}{X_{\%P}}$$

Where:

ML_{CM} = Maximum level of cadmium in cocoa mass (mg/kg)
$ML_{EU.P}$ = EU Maximum permissible level in finished product P (mg/kg)

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[1] The Codex Alimentarius is a collection of internationally adopted food standards and related texts developed to assist in harmonising safety, quality and fairness of food quality at the international level. The 12th Session of the Committee on Contaminants in Foods (CCCF-12) 2018 Codex Alimentarius reviewed the proposed draft maximum levels for cadmium in chocolate and cacao derived products provided in this document

[2] Download PDF – Joint FAO/WHO Food Standards Programme, Codex Committee on Contaminants in Foods – Proposed draft maximum levels for cadmium in chocolates and cocoa-derived products

$X_{\%P}$ = percentage of cocoa mass in finished product P

For example, in the case of dark chocolate containing 70% of cocoa mass (dry cocoa solids), for which the EU regulation sets a maximum permissible limit of 0.8 mg cadmium/kg in the finished product, the maximum level of cadmium in the cocoa mass will be:

$$ML_{CM} = \frac{0.8}{0.7} = 1.1\ mg/kg$$

This can be used as an approximation for cadmium levels in cacao beans, assuming that unprocessed cocoa mass would contain similar amount of cadmium as the beans or nibs it originates from. Online tools are being developed using similar calculations for different chocolate products[1]. While this approach is useful for cases when there is a direct link between a batch of cacao beans and the cocoa mass used for making a single chocolate product, this is not always the case. As a result, many buyers appear to prefer a relatively low cadmium content to ensure that the beans can be used in any recipe and buyers are requesting limits for cadmium concentration in beans between 0.5 and 1.1 mg/kg.

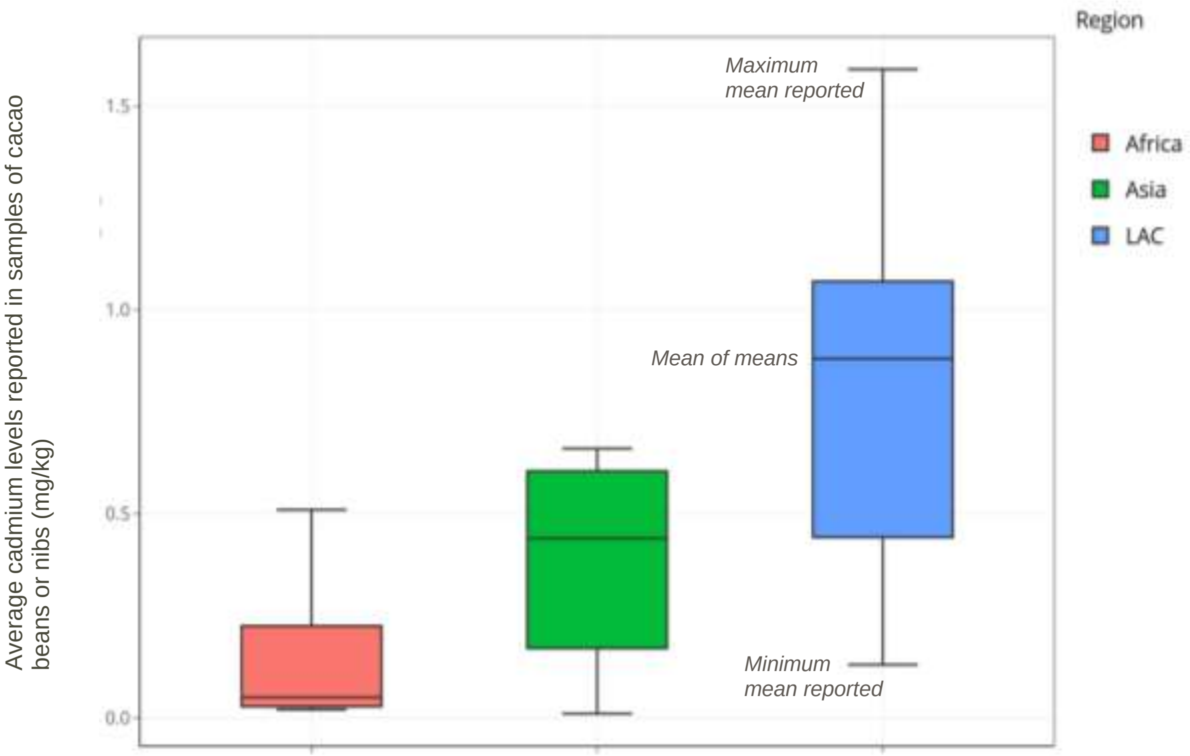
It is important to note that due to the variability of cadmium in cacao beans, and the high cost of laboratory analyses, a reliable estimate of the cadmium concentration is often not captured in the few samples analysed. This may make calculating cadmium in the final product from bean cadmium levels difficult. As the EU regulation is not written for cacao beans, there is no accepted sampling strategy. However, for other similar products sold in sacks, it is suggested that 5 samples should be taken from each 60kg sack, and for products by container, 10 samples per half a tonne (i.e. one analysis per 50kg). A sample comprises 1kg.

While these regulations have a global impact on the entire cacao supply chain, smallholder producers from Latin America and the Caribbean (LAC) countries will be the most affected. Surveys across cacao-producing regions of the world show that cadmium content in cacao beans is particularly an issue in LAC (see Figure 2 and Annex 1). Argüello et al. (2019) conducted a country-wide survey in major cacao-growing regions of Ecuador, collecting 560 samples from 159 cacao farms, and found a mean cadmium bean concentration of 0.90 mg/kg with 45% of samples exceeding 0.60 mg/kg. Similar results were also found by Barraza et al. (2017) and Chavez et al. (2015). The bean cadmium concentration from nearly 57% of sample sites in a study in Peru (n=70) exceeded 0.8 mg/kg (Arévalo-Gardini et al. 2017). Zug et al. (2019) reported a mean cadmium content of 2.46 mg/kg in a sample of 40 cacao beans from the Huánuco region of Peru, the highest value in the literature to date – although the authors measured the content in defatted powder of dried cacao beans rather than the whole seed. Cadmium content in these samples ranged from 0.02 to a maximum of 12.5 mg/kg (Zug et al. 2019).

Surveys at a farm level show considerable variation across sites, with certain areas or ´hotspots´ showing much higher cadmium levels 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 results from Ecuador found variation in cacao bean and soil cadmium content at multiple levels – among provinces, cantons and even within farmers' fields – implying a high level of heterogeneity at many scales (Argüello et al. 2019).

[1] see http://chocosafe.org

Figure 2 Distribution of reported average cadmium levels in cacao beans from Africa, Asia and LAC[1]



(see Annex 1 for more information)

Source: Prepared by the authors

Overall, values of cadmium content in cacao beans reported across LAC indicate that the new EU regulation will affect cacao producers in the region. These regulations are a potential threat to the livelihood of many smallholder farmers, particularly those producing fine or flavour cacao beans commonly used for products with a high cocoa content as well as single origin products where mixing cacaos jeopardises the niche market.

In this context, there is a pressing need to identify solutions that reduce cadmium levels in cacao beans and final chocolate products. This document reviews our current understanding and ongoing active research to help in this task. We review knowledge on sources of cadmium contamination in soils, soil properties that affect cadmium bioavailability and physiological mechanisms of cadmium uptake in cacao trees. We present results from trials to reduce cadmium uptake in cacao and other crops, and suggest directions for potential short, mid and long-term mitigation solutions. Finally, a compilation of ongoing research projects on cadmium and in cacao is presented to inform us of the results that can be expected shortly and identify possible remaining knowledge and research gaps.

[1] This graph summarises data from 21 studies that measured the cadmium content of cacao beans or nibs – Africa (7 data points), Asia (4 data points), and LAC (19 data points). The data represented in the box plots are the means reported by different studies. The graph does not take into account sample size and standard deviation for each mean or differentiate between beans and nibs. See Annex 1 for more detailed information and references.

2 SOURCES OF CADMIUM ACCUMULATION IN SOIL

The presence of cadmium in soils is a result of a combination of natural processes and anthropogenic influences. Natural processes include the weathering of rock, volcanic activity, forest fires, erosion and deposition in river sediments, while anthropogenic influences include contamination by mining and industrial activities as well as agricultural practices such as irrigation and fertilisation that can result in cadmium input into the soil.

The observed increase of cadmium levels in soils over recent years on a global scale suggests the importance of anthropogenic processes. In LAC, however, higher levels of cadmium reported in cacao beans relative to other regions, as well as localised differences, implies that the soils in some areas may be naturally rich in cadmium or have characteristics that lead to its higher bioavailability, although this does not rule out the role of anthropogenic sources, the use of different genotypes or a combination of these factors. The sources are explained in more detail in the following section.

2.1 Natural sources of cadmium

According to He et al. (2015), the contribution of natural processes to soil cadmium contamination is 3 to 10-fold lower than that of anthropogenic sources. In natural, uncontaminated soils, cadmium concentration is largely influenced by the amount of cadmium in the parent rock, and by local weathering conditions, as well as transportation by rivers and deposition in sediments and water by rivers during flooding events. Comparing different soil types, those derived from igneous rocks typically contain low amounts of cadmium, soils derived from metamorphic rocks are intermediate, and soils derived from sedimentary rocks (especially shales) contain high amounts (He et al. 2015). Gramlich et al. (2018) found that cadmium levels in cacao-growing soils varied significantly across different geological substrates in Honduras and was highest in alluvial soils originating from sedimentary material. A similar pattern was found in Ecuador in a study of 159 farms (Argüello et al. 2019). Other natural sources of soil cadmium include volcanic activity, forest fires, wind-blown soil particles and rock dust.

2.2 Anthropogenic sources of cadmium

Anthropogenic activity can increase cadmium concentration in agricultural soils through the application of phosphate fertilisers derived from sedimentary material and irrigation water from areas with high levels of cadmium. Mining and smelting of ores, burning of fossil fuels, and other industrial activities can also lead to localised cadmium contamination. Activities such as mining and land degradation on metal-rich soils upstream may be an important source of cadmium in agricultural areas downstream.

In tropical soils in humid areas, migration of naturally occurring cadmium down the soil profile is more likely to occur than its accumulation in the top layer (Kabata-Pendias 2010; Rieuwerts 2007). Thus, the results from multiple studies on cacao-growing soils of LAC that show significantly higher concentrations of cadmium in their top layer compared to other layers, and a general decrease with depth have been interpreted as the result of recent anthropogenic activity (Barraza et al. 2017; Gramlich et al. 2017; Arévalo-Gardini et al. 2016; Chavez et al. 2015; Mite et al. 2010; Rodríguez Albarrcín et al. 2019), although isotope mapping has suggested that soil-plant cycling can lead to a similar pattern (Imseng et al. 2018).

Details of key sources of anthropogenic cadmium contamination are presented in the following sections.

2.2.1 Direct input into agricultural soils

Materials directly applied to soils can contain cadmium and contaminate soils. These include sewage sludge (biosolids), compost, animal manure and phosphate fertiliser (Adriano 2001; Alloway et al. 1999; Roberts 2014).

Phosphate fertilisers are one of the most ubiquitous sources of cadmium contamination in agricultural soils throughout the world as cadmium often occurs in high concentration in the phosphate rocks from which the fertiliser is produced (Chaney 2012). Sedimentary phosphate rocks can contain cadmium in concentrations of 1 to 150 mg/kg – levels as high as 300 mg/kg have also been recorded (Fergusson 1990) – compared to volcanic sources with 1 to 4 mg/kg. It should be noted that about 85% of phosphate used in fertilisers is sourced from sedimentary deposits (Roberts 2014).

The cadmium input resulting from use of contaminated fertilisers depends not only on the concentration in the source rock, but also on the fertilisation programme being followed. Past land use may also be important as it may have resulted in an accumulation of cadmium in the soil (Alloway et al. 1999; Gramlich et al. 2018), although leaching would be expected to remove this over time (Smolders 2017).

In Peninsular Malaysia, Zarcinas et al. (2004) attributed levels of cadmium in cacao growing soils (mean of 0.11 mg/kg) and cacao beans (mean of 0.66 mg/kg) to the use of phosphate fertilisers due to a significant correlation between soil phosphorus and cadmium content (R^2=0.80, $p < 0.01$). In LAC, some studies have also indicated phosphate fertilisers as a possible source of soil cadmium (Bravo et al. 2018; Gramlich et al. 2018; Laila Marie Zug et al. 2019) but low application of fertilisers in many areas may limit the impact of this source on plantations.

Other soil amendments may also contain high levels of cadmium. These include zinc compounds and limestone, both of which are often manufactured from by-products of mining or other industries (Mortvedt 1985).

2.2.2 Irrigation water and riverine sediments

Rivers and streams running through areas with high levels of cadmium can deliver cadmium and other heavy metals to agricultural areas downstream via surface and ground water, or through irrigation systems. Cadmium may originate from weathering of the bed rock but concentrations can be exacerbated by mining and land degradation or other operations (Smolders et al. 2003; Sun et al. 2010; Zhai et al. 2008; Oporto et al. 2007; Yang et al. 2006; Takijima et al. 1973; Mortvedt 1985). Water does not have to carry high levels of cadmium to affect soil levels: both saline water conditions, and flood-drought cycles can increase the 3.1.1 of cadmium present in the soil (Singh et al. 1999).

Research in cacao plantations in LAC suggest water may be a source of cadmium contamination. Gramlich et al. (2018) suggested that the deposition of sediments from river flooding may be a key source of topsoil cadmium in their study sites in Honduras. Three studies in Ecuador have reached similar conclusions. A collaborative study between the French cooperative Ethiquable and the French Research Institute for Development (IRD) that the highest cadmium concentrations in cacao beans were found in farms that were regularly flooded by the river (with concentrations reaching 4.3 mg/kg; Maurice L., pers. com.). Chavez et al. (2015) suggested that the elevated cadmium levels in rivers used for irrigation could be the source of high levels of soil cadmium observed in their study area, and Argüello et al. (2019) mentioned that the bean samples with the highest cadmium concentration (5.28 –10.4 mg/kg) came from a farm in a region with artisanal mining. In Peru, Llatance (2018) recorded differences in cadmium concentrations in soil samples taken from non-inundated (< 0.008 mg/kg), inundated (0.043 mg/kg) and semi-inundated soils (0.11 mg/kg) in which less water is retained but for a longer period of time.

2.2.3 Atmospheric deposition

The main sources of cadmium emissions to the atmosphere are industrial processes including mining and smelting (particularly zinc), iron and steel production, oil and gas industries, waste incineration and cement production (Alloway and Steinnes 1999). The transport of cadmium emitted into the atmosphere depends upon the particle size with very high levels of cadmium contamination occurring up to 30 km from the source (Adriano 2001), and contamination via aerosols spreading over much larger distances. Cadmium from atmospheric deposition concentrates in the upper humic soil layer (Alloway and Steinnes 1999) and appears to be easily available to plants (Adriano 2001).

There is no evidence that atmospheric pollution leads to higher cadmium content in cacao beans as plantations are not normally located close to industrial zones. In Honduras, Gramlich et al. (2018) found no influence of proximity to industrial sites on the cadmium concentration of cacao-growing soils. Barraza et al (2017) found no differences in cadmium content within beans sampled at various distances from an oil refinery in Esmeraldas, or from gas flares in the Amazon region, and found low cadmium concentrations in aerosol samples collected in the farms – below the Ecuadorian legislative limit of 0.5 ng/m^3. However, Acosta and Pozo (2013) reported higher concentrations of cadmium in cacao beans from a farm close to the Santo Domingo-Esmeraldas highway (Ecuador) compared to farms further away, which they attribute to pollution, but presented no statistical evidence for their findings.

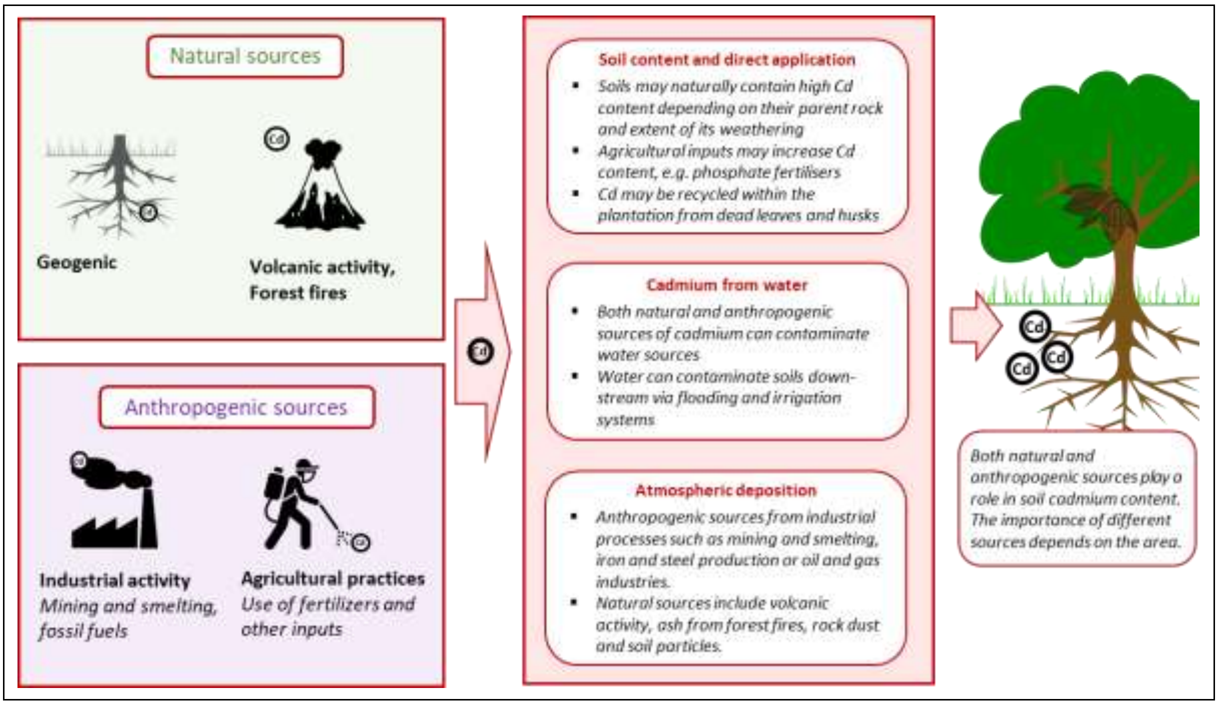
2.2.4 Recycling of cadmium within cacao production systems

Where high concentrations of cadmium are reported in cacao beans, concentrations are also high or even higher in leaves and pod husks[1] (see section **4.2**). Dead leaves and pod husks are usually left to degrade in plantations to reduce nutrient loss and improve soil organic matter. Any cadmium in these tissues will either leach into the soil or be recycled within the system.

For Mite et al. (2010), Barraza et al. (2017) and Gramlich et al. (2018), the higher cadmium concentration within top-soils relative to subsoils may be at least in part due to the accumulation over the years of cadmium from leaves and husks, although the authors do not rule out the possibility of contamination from other anthropogenic sources. In Colombia, Rodríguez Albarracín et al. (2019) found cacao leaf litter to have higher cadmium content then both cacao beans and leaves with an average of 85.5 mg/kg, which according to the authors implies a high level of cadmium cycling. However, the relative importance of this recycling process as a contributor to cadmium accumulation in cacao beans has yet to be understood. The use of stable cadmium isotopes to trace cadmium recycling between decaying and living tissue will be useful in addressing this knowledge gap.

[1] Here, pod husk refers to the cacao pod fruit shell which encloses all of the beans and pulp. The thin shell of a single cacao bean is sometimes referred to as the husk or testa.

Figure 3 Possible sources of cadmium input to cacao growing soils



Source: Prepared by the authors

3 SOIL PROPERTIES AND CADMIUM BIOAVAILABILITY

Accumulation of cadmium by cacao plants is influenced by the amount and availability of cadmium present in the soil. Higher levels of total soil cadmium content imply a higher potential for cadmium uptake. Several studies have reported positive and statistically significant low to moderate correlations between total soil and cacao bean cadmium concentrations (Ramtahal et al. 2016; Fauziah et al. 2001; Gramlich et al. 2018; Laila Marie Zug et al. 2019). This includes a nation-wide survey in Ecuador that mapped total soil and cacao bean cadmium levels and identified problem areas or ‘hotspots’ (Argüello et al. 2019).

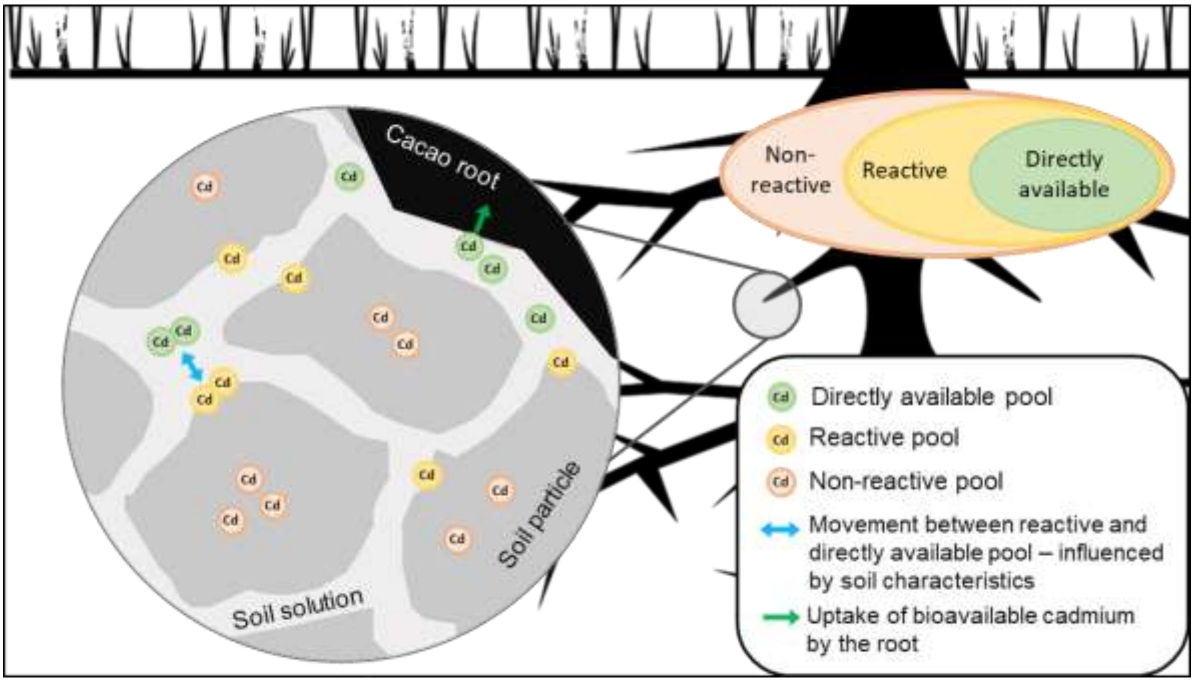
However, total soil cadmium is not always a good indicator of cadmium in cacao beans as only a part of it is available to the plants (He et al. 2015; Shahid et al. 2016; Argüello et al. 2019; Gramlich et al. 2018, 2017; Remigio 2014). The bioavailable cadmium levels in the soil shows a much stronger correlation to the bean cadmium levels than total cadmium. Understanding how soil properties affect the bioavailability of metals and specifically cadmium in cacao-growing soils is therefore key to developing effective soil mitigation strategies.

3.1 Bioavailable cadmium

3.1.1 Bioavailability and chemical speciation of cadmium

In the soil, trace metals exist in many chemical and physical forms, not all of which are available, or bioavailable, for uptake by living organisms (Adriano 2001; Singh et al. 1999). The soil cadmium content can be divided into three nested pools based on bioavailability: total, reactive, and directly available – see figure 4. The total pool contains reactive, directly available and non-reactive cadmium (cadmium that is not available and is unlikely to enter the reactive pool for decades, centuries or even longer). The reactive pool consists of cadmium ions adsorbed onto reactive surfaces of soil organic matter, short-range ordered metal-hydrous oxides, and clay particles and are potentially available for absorption by the plant. The directly available pool is composed of the free or totally dissolved cadmium ions in the soil solution that are ready to be absorbed (Shahid et al. 2016; Pan et al. 2016). Movement from the reactive to the directly available pool is affected by soil pH, organic matter content, clay content and reactivity, cation exchange capacity, the presence of metal hydroxides, electrical conductivity, macro- and micro-nutrient content and the presence of microorganisms. These conditions change in space and time and with soil depth (Welch et al. 1999; Adriano 2001; He et al. 2015; Shahid et al. 2016).

Figure 4 Cadmium pools and bioavailability in soils



Source: Prepared by the authors

3.1.2 Measuring bioavailable cadmium

3.1.2.1 Evaluating bioavailable cadmium in the soil

Quantifying the amount of cadmium in the soil in each of the pools mentioned above requires the use of different reagents. The size of the reactive pool can be determined by extraction using weak acids (e.g. 0.05 M EDTA, 0.1 M HCl, or 0.43 M HNO_3), while the directly available pool is either measured by sampling of the soil solution with soil moisture samplers or lysimeters or by a soil extraction using weak salts such as $CaCl_2$, $Ca(NO_3)_2$, $NaNO_3$ or DTPA extraction methods (Pan et al 2016). The total soil cadmium content is usually determined by digestion with a strong acid (EPA3050B for acid digestion of soils – EPA 2015).

Cadmium within the reactive pool of the soil occurs in many different fractions (e.g. exchangeable, oxide-bound, carbonate-bound, organic matter-bound). Several studies have calculated the relative importance of these fractions in the soil by comparing their concentration with the level of cadmium in plant tissues. This is carried out with multiple-step extraction methods, using neutral salts or acids of increasing strength to extract the cadmium from each fraction (Rao et al. 2008).

In the Nigerian states of Ondo and Ogun, Aikpokpodion et al. (2012b, 2012a) found that residual and oxidizable fractions account for a large part of total cadmium content in cacao-growing soils. These fractions are generally considered to be mostly unavailable to plants and are associated with weathering of parent rocks. In Ecuador , Chavez et al. (2016b) found that acid-soluble and reducible fractions accounted for most of the total soil cadmium content. Of the five fractions they considered (water-soluble, acid-soluble, reducible, oxidizable and residual), the acid-soluble fraction was most highly correlated to cadmium content in cacao beans leading the authors to suggest that this was the major contributor to the bioavailable cadmium pool in their study sites.

Although the efficiency and predictability of a single extractant to measure the reactive pool of cadmium depends on soil factors and the species being studied (Adriano 2001), Ramtahal, Chang Yen, Ahmad, et al. (2015) and Chavez et al. (2015) found that powerful extractants – metal-chelating reagents, Mehlich 3 and hydrochloric acid (HCl) – provided a better estimate of bioavailable cadmium than more neutral ones due to the relative importance of the acid-soluble cadmium fraction for cacao (Chavez et al. 2016b). Gramlich et al. (2018) found that the cadmium content in cacao beans was best predicted by measures of bioavailable soil cadmium from the diffusive gradient in thin film (DGT) and Mehlich 3 extraction methods. Novel approaches that use cadmium stable isotope compositions can also be applied to evaluate the relative importance of anthropogenic cadmium inputs and cadmium recycling, as well as the transfer of the bioavailable fraction to plants (Imseng et al. 2019, 2018). One caveat is that, as none of the extraction methods estimates bioavailable cadmium perfectly, and as different studies have used different methods, comparing levels of bioavailable cadmium across studies may not be possible.

3.1.2.2 Measuring cadmium concentration in soil and plant tissues

Reliable analysis of cadmium concentration is key to understanding the problem. The extraction protocol, choice of analytical instrument, its calibration and the quality control protocols adopted all play an important role in this. Commonly used techniques include Atomic Absorption Spectrometry (AAS), Furnace Atomic Absorption Spectrometry (FAAS), Induced coupled plasma mass spectrometry (ICP-MS) and Induced Coupled Plasma Optical Electro Spectrometry (ICP-OES). 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). Additionally, while estimation of total cadmium prepared with strong acid extraction is relatively robust, digestion with weak acids to estimate bioavailable cadmium is much more difficult to analyse reliably and may require the use of ICP-MS (McBride 2011).

3.2 Soil properties affecting cadmium bioavailability to cacao plants

3.2.1 Relationship between soil properties

The availability of cadmium to plants is influenced by multiple soil properties, which affect the chemical and physical characteristics of cadmium in the soil and can fluctuate in space and time. These include pH, organic matter content, soil texture and mineralogy, cation exchange capacity, electrical conductivity, macro- and micro-nutrient content and the presence of microorganisms (Adriano 2001; Singh et al. 1999; Shahid et al. 2016; He et al. 2015). Manipulating these properties is key to developing mitigation strategies that reduce cadmium uptake (Hamid et al. 2019).

Most of the baseline studies investigating which of these factors may influence cadmium uptake by cacao have been carried out in LAC – Bolivia, Ecuador, Honduras, Peru, and Trinidad and Tobago – see Annex 2. These studies measured various soil properties, as well as agronomic and other factors, and determined their correlation with total and bioavailable soil cadmium as well as cadmium content in cacao plant tissue (leaves, beans, pod-husks). Although many studies have investigated this, differences in soil properties measured, soil types, sampling design, sample size, study length and bioavailable cadmium extraction procedure make their comparison difficult. We consider that 9 of these studies are robust enough for the comparison presented in Table 3 (see references below table). Of these, only three (Gramlich et al. 2017, 2018; Argüello et al. 2019) allow clear conclusions to be drawn. These studies form the basis of the following sections, along with results from experimental trials to understand how changes in soil properties affect cadmium uptake by cacao.

Table 3 Summary of results from baseline studies (see Annex 2 for detailed results)

Soil properties		Reported effect on cadmium bioavailability and/or cadmium content in cacao tissues		Studies*
Cadmium content	Total soil cadmium	↑	**Consistent results across studies.**	2, 3, 4, 7, 5 (↑)
	Bioavailable cadmium *(effect on cadmium content in cacao plant parts)*	↑	**Consistent results across studies**	2, 1 (↑)
pH	Soil pH	↓	**Consistent results across studies**	1, 2, 3 (↓)
OM	Organic matter	↓	**Consistent results across studies**	1, 2, 3 (↓)
Parent material	Geological substrate	**Yes**	**Consistent results across studies**	2,3
Soil Texture	Clay content	↑ /↓	Inconsistent results	1, 2 (↑); 2 (↓)
	Sand content	↓	Too few results	8 (↓)
CEC	CEC	x	Inconclusive results	
Salinity	EC	x	Inconclusive results	
Micro-macro-nutrients or other trace metals	Zinc	x	Inconclusive results	
	Fe	↑	**Consistent results across studies**	1,2 (↑)
	P	↑ /↓	Inconsistent results	1 (↓); 5, 9(↑)
	Pb	x	Inconclusive results	
	Ca 2+	x	Inconclusive results	
	Mg2+	↓	Too few results	2 (↓)
	K	↓	Too few results	2 (↓)
	Mn	↓	Too few results	9 (↓)
Soil Microbial activity	Mycorrhizal colonisation	x	Inconclusive results	
Agronomic factors	fertiliser application	x	Inconclusive results	
	Monoculture vs agroforestry	Yes	Too few results	1
	Organic vs conventional	Yes	Too few results	3
	Age of orchard	↓	Too few results	3 (↓)
	Trunk diameter	Yes	Too few results	1
	Cultivar effect	Yes	Too few results	1
Other factors	Altitude	x	Inconclusive results	
	Proximity to industrial site	x	Inconclusive results	
	Impact of oil activities	x	Inconclusive results	4 (x)

Source: Prepared by the authors

**Studies: 1 (Gramlich et al. 2017); 2 (Gramlich et al. 2018); 3 (Argüello et al. 2019); 4 (Barraza et al. 2017); 5 (Fauziah et al. 2001); 6 (Arévalo-Gardini et al. 2017); 7 (Huamani and Rojas 2011) ; 8 (Huamaní-Yupanqui et al. 2012); 9 (Jomas 2016)*

↑ *Increase leads to higher cadmium bioavailability and/or cadmium content in cacao tissues*
↓ *Increase leads to lower cadmium bioavailability and/or cadmium content in cacao tissues*
Yes *Has an effect on cadmium bioavailability and/or cadmium content in cacao tissues*
x *No statistically significant effect on cadmium bioavailability and/or cadmium content in cacao tissues*

3.2.2 Cation Exchange Capacity

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. A higher soil CEC implies a higher capacity for soil particle surfaces to retain cations and can lead to a decrease in cadmium bioavailability. As CEC decreases there is an increased competition between H^+ and Cd^{2+} ions for binding sites which results in cadmium desorption from soil particles into the soil solution.

CEC is influenced by soil properties including texture, clay content and mineralogy, pH and organic matter content. While clay content is important in cation exchange, not all clay mineralogy has the same capacity: 1:1 clays are common in highly weathered tropical soils and have a low CEC, while 2:1 clays 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).

3.2.3 pH

Soil pH is one of the most important parameters influencing cadmium speciation, mobility, solubility and thus its bioavailability (Adriano 2001). As pH increases, so does the soil CEC. In alkaline soils, cadmium tends to be less bioavailable as it is strongly bound to soil particles. Increasing the pH of acidic soils almost always leads to lower cadmium uptake by plants (Shahid et al. 2016; Sauvé et al. 2000) – although this may be countered by salinity (see section **3.2.6**). Most studies on cacao find significant and negative correlations between soil pH and bioavailable cadmium (Low et al. 1994; Bravo et al. 2018; Barraza et al. 2017; Gramlich et al. 2018, 2017; Argüello et al. 2019) – the exception is Fauziah et al. (2001) in Peninsular Malaysia. However, the pH environment in the soil is not uniform as plants exude acids from their roots to improve the solubility of nutrients and ions (Dong et al. 2007). This means that even in neutral or alkaline soils cadmium accumulation in plant tissues may still occur. Cadmium has been found to be a significant problem in cacao grown in near-neutral pH soils in the north of Peru (Remigio 2014).

Cacao trees grow best in soils with pH levels ranging from 5.0 to 7.5. Applying certain amendments to acidic soils that increase the pH can reduce the proportion of cadmium that is bioavailable and thus reduce cadmium uptake by plants (Ramtahal et al. 2018). Commonly used amendments for this purpose include slaked lime, dolomite and zeolite (Mahar et al. 2015; Shi et al. 2009). A methodology using soil parameters to calculate liming needed to reduce cadmium uptake is presented in Ramtahal et al. (2018).

Four studies have been conducted on cadmium bioavailability in cacao-growing soils in LAC. In a field experiment in Peru, Zamora (2018) reported a significant decrease in cadmium content of beans following the application of dolomite (at 1.8, 2.7 and 3.6 kg/plant) for 12 months, although neither results from the control nor sample size are provided. Also in Peru, Schneider (2016) investigated the effect of applying slaked lime at low and high doses (2.5 and 1.85 t/ha, and 5.8 and 3.6 t/ha respectively), on the bioavailability of cadmium in cacao plantations. After 5 months, they found a significant difference in bioavailable soil cadmium content between the control and limed treatments (Schneider 2016). The effect on bean cadmium concentration was not measured due to unforeseen circumstances. Ramtahal et al. (2018) applied slaked lime to cacao trees (3 kg/tree) in a field experiment in Trinidad and Tobago and measured soil pH, soil bioavailable cadmium and cadmium content of cacao leaves every month for 18 months. Despite natural variations of pH observed in untreated soil, a significant pH increase was found for lime-treated soils. Total leaf cadmium decreased both in limed and untreated trees ($p < 0.05$), but the reduction for limed trees was 3-fold that of un-treated trees. The results will be corroborated with measurement of cadmium concentration in beans. Finally, Chavez et al. (2016a) investigated the effect of zeolite on cadmium bioavailability in a laboratory experiment, applied at 0.5 and 2% of total weight with different doses of cadmium and three soil types. An increase soil pH was not observed, and neither 0.01 M $CaCl_2$ nor Mehlich extractable cadmium levels were reduced after 28 days in any of the treatment. Chavez et al. (2016a) used natural zeolite in their experiment; synthetic forms have been suggested to be more effective in raising pH and reducing cadmium bioavailability (Shi et al. 2009; Wingenfelder et al. 2005).

One key factor to the effectiveness of pH-modifying soil amendments is to ensure that they are incorporated into the soil. In established cacao plantations this can be a challenge because of the risk of damaging the surface roots. However, it has been shown that if mixed with organic matter (composts, manures, biosolids, or green manures), and surface applied, 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).

3.2.4 Organic matter content

The organic matter content of soils plays an important role in cadmium bioavailability due to its ability to adsorb cadmium. The capacity of organic matter to bind with cadmium is due to its high CEC as well as its chelating ability (Adriano 2001; He et al. 2015). Organic matter content can also reduce cadmium 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 cadmium and increase its mobility (He and Singh, 1993; Khan et al. 2017).

Soil organic matter content in cacao plantations in Honduras and Bolivia was found to be significantly and negatively correlated to both soil bioavailable cadmium and plant cadmium content (Gramlich et al. 2017, 2018). A similar correlation was found in Ecuador between cadmium content in cacao beans and soil organic matter content (Argüello et al. 2019). Chavez et al. (2016a) interpret the higher correlation coefficient between acid-soluble cadmium and cadmium content in beans in the subsurface layer (5 - 15 cm) compared to the surface layer (0 – 5 cm) as a result of higher soil organic matter in the surface layer. A very low percentage of soil organic matter in the north of Peru (< 2%) may partly explain the elevated concentrations of cadmium in cacao beans in the region (Remigio 2014).

Studies have reported a decrease in bioavailable soil cadmium and of cadmium uptake by plants using various organic matter amendments such as biochar (Anawar et al. 2015; M. Ahmad et al. 2014), poultry, pig or cattle manure and compost (Khan et al. 2017), vermicompost (Pinto et al. 2016), activated carbon (Xu et al. 2014) or coal (Kwiatkowska-Malina 2018). Two studies have been conducted on the effect of organic matter amendments on cadmium bioavailability in cacao plantations, using compost and chicken manure (Zamora 2018), and vermicompost (Chavez et al. 2016a). Zamora (2018) applied high doses of compost and chicken manure (30, 60 and 90 t/ha) in the field and measured the resulting effect on cadmium content in beans. Although the response was variable, in general compost reduced cadmium in cacao beans and chicken manure did not. Chavez et al. (2016a) found that vermicompost at a 2% rate of application could effectively reduce 0.01 M $CaCl_2$ extractable and Mehlich 3 extractable cadmium in soils spiked with 5mg/kg of cadmium. Interestingly, Chavez et al. (2016a) reported that this effect may have been due to a substantial increase in soil pH, perhaps from mineralization of organic N.

The use of biochar and other activated carbons for heavy metal immobilisation has been shown to be effective in many other crops and is gaining attention for use in cacao – see Rizwan et al. (2016) for a review. Given that these compounds are highly variable, so is their effectiveness. The most important parameters influencing this are the choice of source material (Xu et al. 2014; Fellet et al. 2014; Yasmin Khan et al. 2017), the temperature of pyrolysis (Cui et al. 2016), and the soil characteristics of the site (M. Ahmad et al. 2014; Anawar et al. 2015).

3.2.5 Soil texture

Soil texture influences both cadmium content and its bioavailability in soils due to different cation exchange capacities of sand, silt and clay (Kabata-Pendias 2010). Fine textured soils (clays) generally have a higher adsorption capacity than coarser textured soils (sands) while total cadmium content and bioavailability appear to be higher in loamy soils (a mixture of clay, sand and silt) than in sandy soils (Adriano 2001; Kabata-Pendias 2010). However, this pattern is not always clear as the adsorption capacity of clay depends on the structure of the dominant clay mineral: 2:1-clay types have higher sorption abilities than 1:1 clay types. In the tropics, clays tend to be highly weathered and are dominated by 1:1 clay types with correspondingly lower CEC (Rieuwerts 2007). This may be the reason for the inconsistent results in correlative studies in cacao plantations where positive, negative and no correlation have been reported between clay content and cadmium bioavailability across studies in Ecuador, Honduras, Bolivia and Peru (Huamaní-Yupanqui et al. 2012; Gramlich et al. 2018, 2017; Chavez et al. 2015). Given these conflicting results, clay content does not appear to be a reliable indicator of cadmium bioavailability even though it may play an important role in cadmium bioavailability to cacao plants.

3.2.6 Electrical conductivity

Electrical conductivity (EC) is a measure of the soil's ability to conduct an electrical current. A high EC is due to a large number of cations (nutrients) held on the soil cation exchange sites and indicates a fertile soil. However, soils with a high EC due to excessive sodium and magnesium (or chloride) ions can be detrimental to plant health, and also increase the bioavailability of cadmium (Khoshgoftar et al. 2004; A. Ahmad 2017).

For cacao produced in a rain forest biome, a high soil EC is unlikely. However, in areas where cycles of flood and drought are common (naturally or due to irrigation), or where cacao is grown under irrigation with saline water (see section **4.7**), it could be a problem as Cl^- can complex with Cd^{2+} bound to soil particles and bring it into solution (Singh et al. 1999; Grant et al. 1999). In alkaline soils the concentration of chloride ions is likely to affect the uptake of cadmium more than any other soil factor (McLaughlin et al. 1998. Chaney et al 2012).

Only a few studies on cadmium uptake by cacao plants have investigated the relationship between electrical conductivity and cadmium bioavailability. Fauziah et al. (2001) found a significant and positive correlation in cacao-growing soils of Malaysia. In Ecuador, Chavez et al. (2015) found that cacao bean cadmium content was best predicted by bioavailable cadmium content and the EC of the top-soil (R^2 =0.73, $p < 0.05$). Argüello et al. (2019) found that at soil chloride ion concentrations greater than 500 ppm, cadmium bioavailability increased dramatically. This corresponds to an EC (1:5) of about 350 μScm^{-1}.

3.2.7 Macro- and micronutrients

Sarwar et al. (2010) provide a detailed discussion of the complex nature of the role of soil mineral nutrients in reducing cadmium uptake. Some ions can influence cadmium uptake directly through competition for soil exchange sites, and chelation or complexation with cadmium compounds. However, predicting the effect is rarely straightforward as it also depends on the compound applied and the mode of application which can result in a change in pH or CEC, and thus affect cadmium bioavailability.

3.2.7.1 Phosphorus

Applying phosphate fertilisers uncontaminated by cadmium has been reported to either reduce cadmium bioavailability by immobilizing cadmium in the soil, or increase it by reducing soil pH (He et al. 2015; Mahar et al. 2015). In Venezuela, Nereida (2011) found that application of phosphorus at 50 or 150 mg/kg per tree in the form of monopotassium phosphate reduced levels of bioavailable cadmium in the soil. In Bolivia, Gramlich et al. (2017) found a negative effect of P soil content on bioavailable cadmium concentration and weak negative effect on cadmium concentration in pod husks. In contrast, Huamaní-Yupanqui et al. (2012) found soil cadmium levels to be significantly and positively correlated to P content in cacao leaves, and Laila Marie Zug et al. (2019) found a modest but positive correlation between cadmium content in beans and the use of P fertilisers – which they attribute to possible additional cadmium contamination. Fauziah et al. (2001) and Zug et al (2019) also found that bioavailable P soil content was positively correlated with bioavailable cadmium soil content, but they believe this is due to the use of phosphate fertilisers contaminated with heavy metals.

3.2.7.2 Zinc

Cadmium and zinc share very similar chemical properties, and this has led to the conclusion that a relative deficiency in zinc in the soil may lead to increased cadmium uptake as they compete for the same transport membranes (Sarwar et al. 2010; Adriano 2001). This is discussed further in section 4.6. It appears that the ratio of cadmium to zinc in soils is normally 1:200-500. Soils with lower ratios than this (such as marine shales) may exhibit high cadmium accumulation by crops (Chaney 2012).

3.2.7.3 Silica

Silica (silicon dioxide-SiO_2) is known to reduce soil cadmium bioavailability as well as its uptake and movement within plants (Treder et al. 2005; Sarwar et al. 2010). A source commonly used as a cost effective and efficient filter for contaminated water is diatomaceous earth or diatomite. (Shawabkeh 2000). Diatomite can also be incorporated into soils to reduce soil cadmium bioavailability (Liva et al. 2007). Pot trials in Peru with cacao plants have shown a reduction of cadmium bioavailability especially with applications of over 5% and a field trial is currently underway (Arbulu Zuazo 2017). Ground water pollution of soils can also be prevented by placing a diatomite layer under the soil rooting layer (Liva et al. 2007).

3.2.7.4 Other elements

Many other elements in the soil are likely to influence cadmium uptake (Shahid et al. 2016; Kabata-Pendias 2010). The effect of soil nitrogen content on cadmium bioavailability appears to depend on whether a nitrate (NO_3^-) or ammonium (NH_4^+) based fertiliser is used, its application rate and timing, and the plant species studied (Sarwar et al. 2010; He et al. 2015). For example, when applied as NH_4^+, soil acidification can occur, leading to an increased bioavailability of cadmium. In Peru Zug et al. (2019) found that the use of N-fertilisers in particular dramatically increased cadmium content in cacao beans. Other elements investigated regarding their effect on cadmium bioavailability include iron, lead, calcium, magnesium, manganese and potassium. Argüello et al. (2019) found that bioavailable manganese (Mn) explained 8% of the variation in bean cadmium concentrations in a regression model with total soil cadmium, pH and total organic carbon. Manganese oxyhydroxides are known to be strong adsorbents of metals and Geeroms (2016) suggested that the ratio of Mn to Cd is important. When this ratio is more than 20 to 1, cadmium uptake appears to be reduced. Gramlich et al. (2018) found that the magnesium and potassium content in the soil had a minor negative influence on plant cadmium concentrations, which authors believe is due to ion competition. Gramlich et al. (2018, 2017), found that iron was positively correlated to DGT-available cadmium in cacao growing soils of Bolivia and Honduras. However, in Honduras, iron availability was also strongly correlated to pH which accounted for the correlation with cadmium (Gramlich et al. 2018).

3.2.8 Influence of soil microorganisms on cadmium behaviour in soils

There are positive and negative effects of soil microorganisms (bacteria, yeasts and other fungi including arbuscular mycorrhizal fungi) on cadmium uptake by plants. This is likely to be due to the range of organisms under consideration and their interactions within the soil community. Bacteria that are tolerant to cadmium come from a wide phylogenetic group that appear to show diverse mechanisms for cadmium immobilisation from experiments *in vitro* and in field (Bravo et al 2018) increasing its bio-precipitation into Otavite or secondary forms of cadmium carbonate that could form only due to microbial metabolism (carbonatogenesis) (Bravo et al. 2011). However, soil microbial activity is also known to increase cadmium availability via excretion of organic acids and subsequent solubilisation of cadmium-bearing minerals (Shahid et al. 2016). It has been observed in various studies that plants inoculated with mycorrhizae took up less cadmium and/or were more tolerant to high soil cadmium concentrations than non-mycorrhizal plants (Janoušková et al. 2006, 2005; Jiang et al. 2016). However, as mycorrhizal fungi extend the capacity of root systems to sequester soil nutrients they have also been shown to increase cadmium uptake by plants (Leyval et al. 1997; Gaur et al. 2004; Ramtahal et al. 2014). As far as we know, mycorrhizae are not a good player in the immobilization of cadmium (Ramtahal et al. 2014). However, further research should focus on the specificity between native consortia of mycorrhizae from cacao root systems to see if cadmium-blocking effect could occur in specialized interactions.

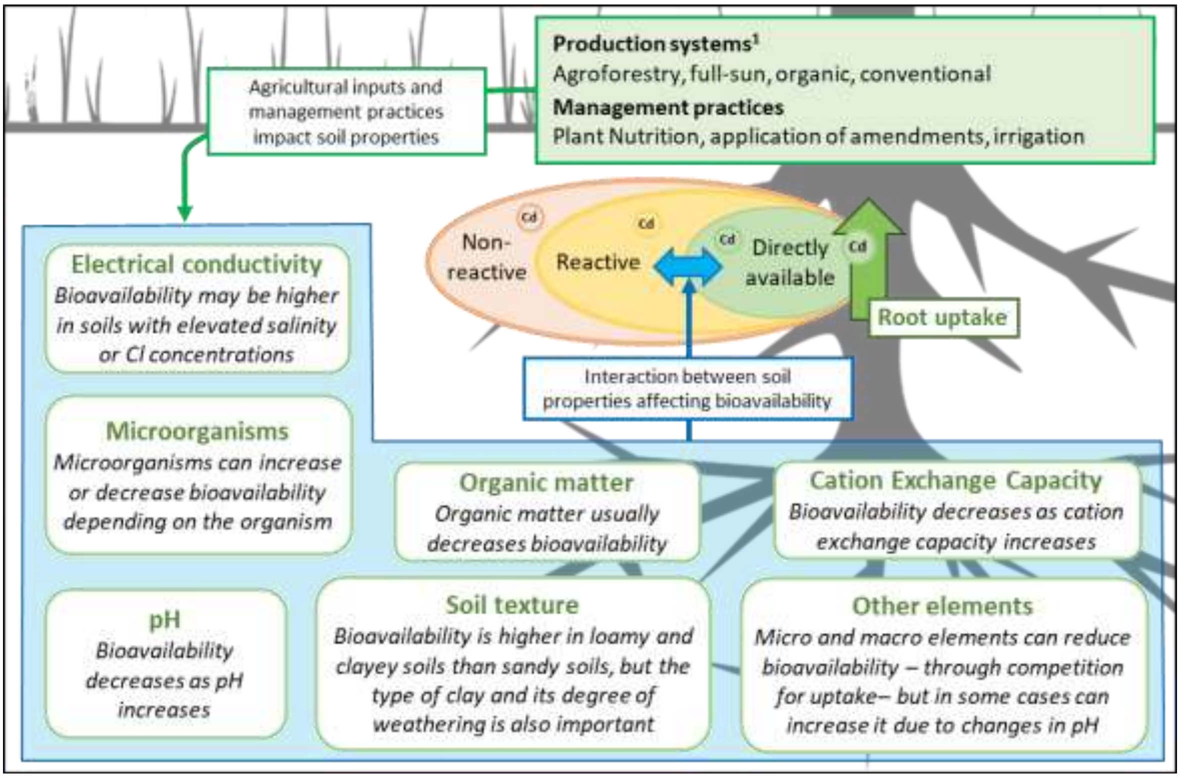
3.2.8.1 Bioremediation

Bioremediation uses living organisms to eliminate or neutralise high concentrations of contaminants from a specific site or source. Both plants (phytoremediation) and microorganisms are used for soil remediation (Alvarez and Polti 2014). Although cacao plantations do not grow on soils with exceptionally high cadmium concentrations the approach appears has received much interest.

Soil microorganisms can be used to reduce the bioavailability of a heavy metal in the soil, or increase it to facilitate its removal through other techniques (e.g. phytoremediation). Several microorganisms have been identified as highly tolerant to cadmium and capable of reducing the cadmium available to plants (Beltrán-Pineda and Gómez-Rodriguez 2016). These include microbial communities identified from cacao plantations in Colombia (Bravo et al. 2018; Caceres and Torres 2017) indicating populations of fast growing bacteria.

Three pot experiments have investigated the effectiveness of microorganisms on cadmium accumulation in cacao. Ramtahal et al. (2012) found that the addition of a commercial product containing mycorrhizae led to an increased cadmium concentration in seedlings. Using a similar product, Jacome et al. (2016) reported a decrease in cadmium concentration in seedlings in soils spiked with 12 and 24 mg/kg cadmium, but not at lower levels. Revoredo et al. (2017) investigated the effect of two different *Streptomyces* yeast strains on cadmium accumulation in cacao plants in spiked soils (100 mg/kg and 200 mg/kg). In addition to very high spiking levels, the sample size was too small to provide meaningful results. In a field experiment, Pérez Moncada et al. (2019) found a reduction in the cadmium content of cacao beans when grown in the presence of native arbuscular mycorrhizal fungi in soils spiked with 24 mg/kg of cadmium. Unpublished results using yeasts isolated from a mining site in Peru have been shown to reduce cadmium levels in cacao in pot experiments, but further validation is required (Duran pers comm).

Figure 5 Soil properties affecting cadmium availability to cacao plants



[1] Further discussed in section **4.7**

Source: Prepared by the authors

4 THE PLANT: CADMIUM UPTAKE MECHANISMS, PARTITIONING AND VARIETAL DIFFERENCES

4.1 Mechanism of cadmium uptake

Cadmium is a non-essential element for plants and its uptake is due to transport by specific and non-specific processes used for ions such as Fe^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+} and Mg^{2+} (Shahid et al. 2016). After uptake by the root system, Cd^{2+} is transported to the xylem, and moves to the leaves. In the leaves, Cd^{2+} is actively transported into the phloem from where it reaches the fruits (Shahid et al. 2016; Clemens et al. 2013). Some cadmium may also reach the fruits directly from the xylem.

Foliar uptake has received less attention then uptake by roots. While Shahid et al. (2017) suggest that for some species foliar cadmium uptake could be important, in cacao this appears to be negligible (Barraza et al. 2017).

Several important membrane transporter gene families (including NRAMP, ZIP, HMA) have been identified as playing a possible role in cadmium uptake by roots, xylem loading and transport within the plant[1] (Guo et al. 2016; Clemens et al. 2013). In many species studied, a ZIP Zn^{2+} transporter in the root epidermal cell membrane is responsible for cadmium uptake. Additionally, root cells have HMA3 transporters which pump Cd^{2+} into the root cell vacuoles

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[1] These include the Adenosine tri-phosphate (ATP) binding cassette (ABC) superfamily, HMA (heavy metal ATPase), ZIP (ZRT, IRT-like protein), NRAMP (natural resistance-associated macrophage protein), YSL (yellow-stripe-like transporter), NAS (nicotinamine synthase), SAMS (S-adenosyl-methionine synthetase), FER (ferritin Fe (III) binding), CADMIUMF (cation diffusion facilitator), NRT (nitrate transporter) and IREG (iron regulated transporter) (Shahid et al. 2016; Clemens et al. 2013)

thereby limiting its transport into the xylem. Plants with a mutant HMA3 in rice, soybean, durum wheat are much less efficient in pumping Cd^{2+} into vacuoles and thus accumulate more cadmium than expected (Wang et al. 2012; Ueno et al. 2010). Over-expression of the same HMA3 transporter lowered Cd^{2+} transport to rice shoots and grain (Ueno et al. 2010).

NRAMP, ZIP and HMA gene families are highly conserved across plant families and appear to have identifiable homologues in the cacao genome (Cryer et al. 2012). Ullah et al. (2018) identified and sequenced five genes from the NRAMP family in cacao and found that NRAMP5 encodes for a protein capable of transporting Cd^{2+} ions in yeast. However, while NRAMP proteins have been shown to be important in cadmium uptake in rice (Ishikawa et al. 2012; Sasaki et al. 2012), to date this protein has not been implicated in cadmium transport for any other crop. An increased understanding of the role of these transport genes in cadmium uptake and partitioning will help the identification of low accumulating cacao genotypes for trials in the field.

4.2 Partitioning of cadmium within the plant

Generally, cadmium concentration in plant tissue decreases from roots > stems > leaves > pod husks > seeds (Benavides et al. 2005). This appears to hold for cacao where a decrease in cadmium concentration from leaf to pod husk to shelled bean has been reported – see Table 4.

Table 4 Summary of results from studies regarding cadmium concentrations in different cacao plant parts

Study	Region	Country	Cadmium concentration in cacao plant tissues
Gramlich et al. 2018	LAC	Honduras	leaf > pod husk = shelled bean
Gramlich et al. 2017	LAC	Bolivia	leaf > pod husk > shelled bean
Barraza et al. 2017	LAC	Ecuador	leaf > pod husk = unshelled bean
Mite et al. 2010	LAC	Ecuador	Bean shell > leaf > pod husk > bean
Chavez et al. 2015	LAC	Ecuador	shelled bean > bean shell >> leaf
Tantalean et al. 2017	LAC	Peru	stem > leaf > root > unshelled bean > pod husk
Llatance et al 2018	LAC	Peru	root > stem > leaf > unshelled bean
Laila Marie Zug et al. 2019	LAC	Peru	Shelled bean (dried, powdered) > bean shell
Rodríguez Albarracín et al. 2019	LAC	Colombia	Leaf litter > leaves > shelled beans
Ramtahal et al. 2016	LAC	Trinidad and Tobago	leaf > pod husk > bean shell > shelled bean
Ramtahal et al. 2015	LAC	Trinidad and Tobago	bean shell > shelled bean
Fauziah et al. 2001	Asia	Malaysia	leaf > pod husk > unshelled bean

Source: Prepared by the authors

The transport of cadmium through the xylem may explain why other parts of the plant contain less cadmium than leaves, as active transport is needed into the phloem (Sêkara et al. 2005). In contrast, Chavez et al. (2015) reported cadmium levels in leaves that were below the detection limit while concentration in beans exceeded 1 mg/kg. Higher levels of cadmium in the bean shell compared to the shelled bean have been reported in several studies, but not all (see also Takrama et al. 2015; Crozier 2012). Such differences appear to have a genetic basis (Laila Marie Zug et al. 2019; see section **4.3**), but some of the results may be due to an artefact of material preparation (Lewis et al. 2018).

Post-harvest treatment (fermentation and drying) may also play a role in cadmium partitioning in the bean (Ramtahal et al. 2015; Alianza Cacao 2018 pers comms). Recently, Thyssen et al. (2018) mapped the distribution of several contaminants, including cadmium, in the cross section of a fermented cacao bean using a new method[1]. Their analysis suggests that cadmium is located on the inner side of the seed shell as well as in the meristematic part of the seed and has a similar distribution to zinc and to some extent magnesium, potassium and phosphorous. It remains unknown whether the same pattern is present in beans prior to fermentation.

4.3 Genotypic differences in cadmium uptake and partitioning

The use of naturally occurring low-accumulating genotypes alone, as rootstock, scions, or in combination, is seen as an integral part of reducing cadmium accumulation in edible plant tissues of many crops (Nawaz et al. 2016; Savvas et al. 2010; Zhou et al. 2017). Genotypic variability in cadmium accumulation between cacao 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). Arévalo-Gardini et al. (unpublished) found cadmium concentration in the leaves of seedlings of 60 cacao genotypes grown in cadmium-spiked soil to range from 1 mg/kg to 10 mg/kg. The International Cocoa Genebank in Trinidad and Tobago also noted differences in cadmium concentration in cacao beans between 100 of their accessions

[1] Laser ablation-inductively coupled plasma-triple quadrupole mass spectrometry (LA-ICP-TQMS)

growing in very similar soil conditions (Lewis et al. 2018). An isotope study (Barraza, Maurice et al. in prep.) showed a significant difference in cadmium transfer from soil to plant tissues between the Ecuadorian national fine flavour cacao and the CCN51 hybrid. In northern Honduras, a study comparing 11 grafted cacao cultivars found significant variations in bean cadmium content between cultivars and no relationship between bean and soil cadmium content (Engbersen et al. 2019). These results suggest that the differences in bean cadmium content could be due to genotype differences in cadmium loading during bean maturation (Engbersen et al. 2019).

A genome-wide association study of more than 600 cacao genotypes is currently underway at the University of the West Indies. The study compares phenotypic variability in cadmium accumulation with variability in genetic markers that are linked to the genes involved. There appear to be four mechanisms of importance: i) membrane transporter density and efficiency, which determines the active uptake of cadmium into the root; ii) sequestration of cadmium in root vacuoles ensuring it does not enter the xylem; iii) transport from the root to shoot through loading into the xylem, and iv) translocation from the shoot to beans via the phloem. Understanding the genetic basis of variability between genotypes in uptake, sequestration and translocation of cadmium can help in the selection of those genotypes that appear to be low cadmium accumulators for trials as rootstock and scions.

4.4 Tolerance to the toxic effects of cadmium

According to He et al. (2017), plants may exhibit signs of toxicity when the total cadmium concentration in soil exceeds 8 mg/kg, bioavailable soil cadmium concentration is > 0.001 mg/kg, or the cadmium concentration in plant tissue reaches 3 - 30 mg/kg (Solís-Domínguez et al. 2007; Chen et al. 2011). While at least two of these limits have been exceeded in many of the published studies on cacao, physiological damage has not been reported.

Hyperaccumulators are species or genotypes that are able to accumulate heavy metals in their above-ground organs at concentrations 100 to 1000 times higher than those found in non-hyperaccumulating species, without suffering any discernible phytotoxic effect (Muszyńska et al. 2016). Van der Ent et al. (2013) suggest that a cadmium hyperaccumulator should be able to tolerate levels of 100 mg/kg in leaf tissues with no side effects. Laboratory experiments with cacao seedling using soils spiked at 50 and 100 mg/kg of cadmium reported ultrastructural changes and damage to the photosynthetic machinery and anti-oxidative metabolism (Castro et al. 2015; Pereira et al. 2017), suggesting that cacao cannot be considered as a hyperaccumulator. However, Pereira et al. (2017) interpreted the fact that plants translocated cadmium to aerial tissues as a sign of tolerance to cadmium, since intolerant plants tend to have a higher accumulation in the roots (Verbruggen et al. 2009). These studies also reported evidence of genotypic differences in cadmium tolerance (Castro et al. 2015; Pereira et al. 2017).

4.5 The effect of tree age on cadmium accumulation

The age of a tree may influence its uptake of cadmium. Results from Argüello et al. (2019) and Alianza Cacao (unpublished) indicated that young cacao plants absorb more cadmium than older ones. Among possible explanations for this are that older trees have deeper roots (tapping into the sub-soil which contains less cadmium than top-soil), older plantations have higher Ca content in the top soil, blocking cadmium uptake (Argüello et al 2019), younger trees grow faster and that the high biomass of older trees reduces cadmium concentration through dilution (Lettens et al. 2011). While Gramlich et al. (2017) found a positive correlation between cacao trunk diameter and cadmium uptake, trunk diameter was strongly related to the agroforestry system and cultivar type, so it is possible that this correlation is not causal.

4.6 The effect of nutrition on cadmium uptake

The presence of micro and macro nutrients may affect the amount of cadmium absorption by the plant roots (Christensen et al. 1999; Sarwar et al. 2010). Cd^{2+} shares many properties with Zn^{2+} as well as other divalent cations including Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{2+}, and may compete with these for the same root transport membranes (Pereira et al. 2017; Castro et al. 2015; Gramlich et al. 2018).

Cd^{2+} and Zn^{2+} share the same transport protein in root epidermal cells in nearly every plant species studied. The exception is rice which accumulates Cd^{2+} via the Mn^{2+} transport protein (Green et al. 2003). It has been reported that zinc strongly inhibits cadmium uptake by wheat, spinach and lettuce (Green et al. 2003; Paul and Chaney. 2017). However, other studies found that higher zinc levels in soils led to an increase in cadmium bioavailability or uptake by plants (Adriano 2001; Sarwar et al. 2010; Kabata-Pendias 2010). A factor governing the relationship between zinc and cadmium is their relative concentration in the soil. A low Cd:Zn ratio (< 0.01) could ensure plants rapidly saturate in zinc and limit their cadmium intake as observed in several crops (Adriano 2001; Sarwar et al. 2010 see also Geeroms 2016).

The results of correlative studies of zinc and cadmium levels in soil and cacao beans have so far not increased our understanding of the role of zinc in reducing cadmium uptake. These studies have shown that a higher zinc content is not related to a reduction of cadmium uptake by cacao plants (Argüello et al. 2019; Crozier 2012; Arévalo-Gardini et al. 2017; Gramlich et al. 2017). In Honduras, Gramlich et al. (2018) found a weak but positive correlation between leaf zinc and cadmium concentrations. In studies in Venezuela and Peru, Crozier (2012) found a strong and positive relationship between bioavailable cadmium and zinc soil content. More recently, Argüello et al. (2019) found zinc concentrations in cacao leaves that were within the range of adequate plant nutrition, but leaf zinc concentration was not retained in their models predicting plant cadmium concentrations. According to the authors, this suggests that zinc deficiency is unlikely to have been a factor affecting cadmium accumulation in cacao beans – at least within their study area (Argüello et al. 2019). Similarly, Gramlich et al. (2017) found no negative correlations between soil zinc and cadmium content in cacao leaves, pod husks or beans, and concluded that higher zinc levels would not reduce cadmium uptake.

The application of zinc as zinc sulphate ($ZnSO_4$) to limit cadmium uptake has been shown to be effective in lettuce and spinach, especially when coupled with liming to prevent soil acidification (Paul and Chaney 2017). For fruit crops, there is also the option of applying zinc through a foliar application to avoid soil acidification while inhibiting cadmium transfer to fruits. In the only study so far published of the effect of adding zinc to soils with high cadmium levels, Zamora (2018) found a modest decreasing trend in cacao bean cadmium content when zinc sulphate, was applied – at 0.09, 0.18 and 0.27 kg per plant. Finally, preliminary results from a small field trial in Peru suggest that optimal fertilisation can reduce cadmium levels in beans (Zamora pers comm). Trials that are more robust are required to understand the role of fertilisation and zinc in cadmium uptake.

4.7 The effect of environmental factors on cadmium uptake

Cacao is grown under a range of production systems and agricultural practices. These range from monoculture in full sun to agroforestry systems where fruit and timber trees provide shade. Agricultural practices include organic and conventional production and water management ranges from rain fed to complete dependence on irrigation. These factors can affect soil characteristics, such as soil water balance, organic matter, and nutrient cycling and availability as well as temperature fluctuations (Deheuvels et al. 2014). As discussed in previous sections, these can all influence cadmium availability. While the use of different species of shade trees may also affect cadmium uptake by cacao (Gramlich et al. 2017;

Argüello et al. 2019), the influence of farming systems on cadmium accumulation requires further study.

Cadmium can be recycled within the system through practices such as leaving leaf litter and pod husks on the ground (see section **2.2.4**). The relative importance of this recycling process as a contributor to cadmium accumulation in beans has yet to be understood, but it is unlikely to be high compared to other processes. Moreover, reincorporating cacao biomass into the production system has several positive impacts such as increasing soil organic matter and levels of nutrients available to the cacao plant, or reducing soil erosion. Further work is needed to evaluate the contribution of this recycling process to cadmium accumulation in the beans and to assess the trade-offs involved in abstaining from reincorporating cacao biomass in production systems.

Contaminated water can be an important source of cadmium in irrigated cacao production systems (see section **2.2.2**). Assessing cadmium and chloride ion input into the farm through water as well as the impact of flooding can be conducted at a farm level to determine whether changes in water management could reduce the amount of bioavailable cadmium in the soil. Water remediation can be carried out with diatomite (Shawabkeh 2000; Liva et al. 2007) or a system of filters. There is also a possibility that bioremediation could help purify irrigation water prior to its use (Cazón et al. 2012).

4.8 Phytoremediation

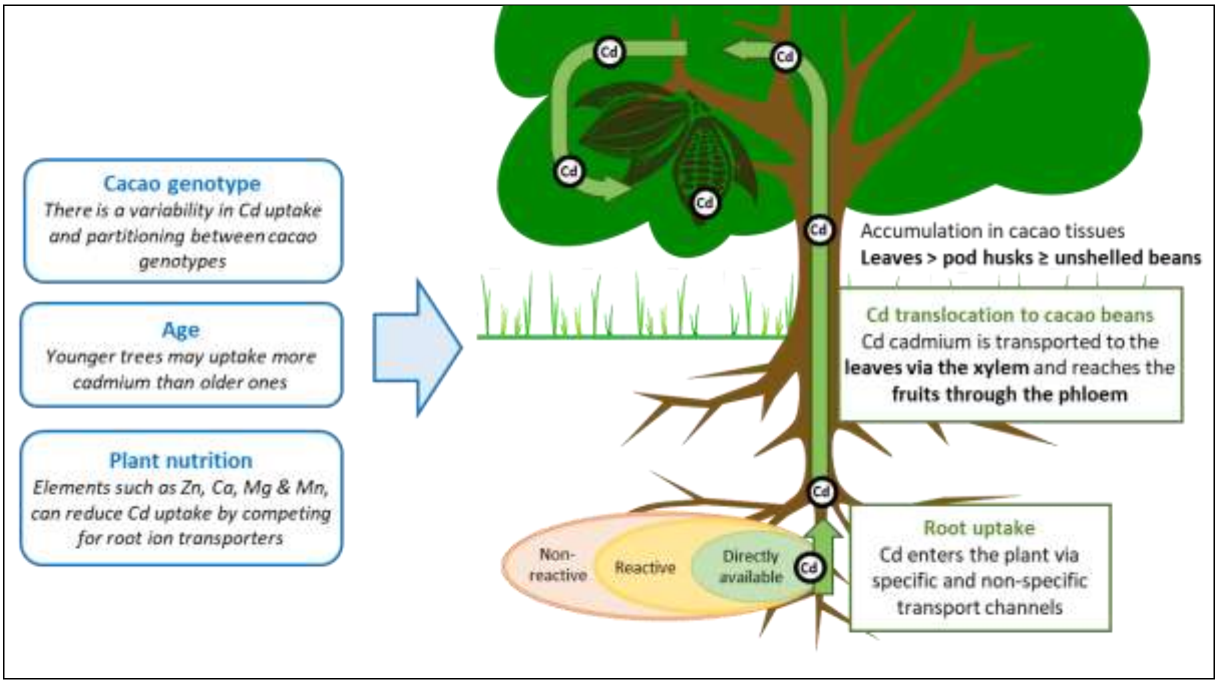
Phytoextraction is the most commonly recognized type of phytoremediation for heavy metals. It uses hyperaccumulating plants that extract and sequester heavy metals in their tissues. Harvesting these plants then removes the contaminant from the site (Ansari et al. 2016). Phytoremediators are typically used in degraded areas with very high heavy metal concentrations as a cost-effective solution to conventional remediation methods (Ansari et al. 2016; He et al. 2015).

Several studies have identified cadmium hyperaccumulators for potential use as phytoextractors (Li et al. 2012; Villafort Carvalho et al. 2013; Wang et al. 2006). Two Chinese species *Sedum plumbizincicola* and *S. alfredii*, isolated from mining sites are hyperaccumulators of cadmium, lead and zinc. They are also shade tolerant and have a high biomass yield (L. Deng et al. et al. 2016; D. Deng et al. et al. 2007) and may be candidates for use in cacao plantations.

However, there are two caveats. In order to be effective, hyperaccumulators require acidic soil conditions to ensure the cadmium is available. This contradicts the soil conditions needed for reducing cadmium accumulation in the target crop, and trials on rice in Thailand have reported difficulties in managing the conflicting conditions required (Simmons et al. 2015).

Additionally, one of the key aspects of working with hyperaccumulators is to ensure that plant material is removed from the plantation before it degrades back into the soil, and that this material is transported off site. Strategies would be necessary to make sure that plant biomass is removed in a timely manner and disposed of in facilities designed for contaminated waste, which in remote areas may be complicated. Given these numerous challenges, a thorough investigation of the utility of phytoextraction is needed to better assess its potential as a mitigation solution (Casteblanco 2018).

Figure 6 Uptake and partitioning of cadmium within the cacao plant



Source: Prepared by the authors

4.9 The effect of post-harvest processing

Once the cacao beans have been harvested from the trees, the post-harvest processes of fermentation, drying, roasting, and winnowing begin.

It has been noted that cadmium appears to migrate to the surface of the bean during fermentation and removal of the bean shell can result in a decrease in the concentration of cadmium within the bean (Alianza cacao, pers comm). However, Kruszewski et al. (2018) measured cadmium levels in raw cacao originating from Ecuador and Dominican Republic and in the processed chocolate mass materials from three different manufacturers. They found no decrease of cadmium content after winnowing and suggest that the only solution is addition of other raw materials (sugar, milk etc.), while Mounicou et al. (2003) report an increase in cadmium concentration in beans through a reduction in water content.

Given that cadmium is more soluble in acidic conditions, and that part of the fermentation process results in the production of acids, there is need for further research to understand the overall influence of traditional fermentation, drying and roasting on cadmium content of cacao beans as well as impacts on physical and flavour qualities. This information may lead to the development of new fermentation techniques. The private sector is investigating some of the aspects at the moment, using microbiology (in beans) and nanotechnology (in cocoa mass) and have managed to obtaining reduction in cadmium content between 20% and 25%, but details are so far not available. While this is a new line of research, it may prove very useful in the reduction of cadmium in cacao beans by blocking its cadmium flow. However, sensorial properties should be monitored to ensure that qualities of fine and flavour cacao are not affected.

5 MITIGATION SOLUTIONS

A mitigation hierarchy can help us develop a nuanced and integrated set of solutions to reduce the level of cadmium in cacao beans and thus chocolate by considering actions from farm to final product that are adapted to the specific conditions of the cacao value chain.

- Avoid high risk areas for establishing plantations
- Minimise the uptake of cadmium by the cacao tree
- Reduce levels of cadmium through post-harvest processing
- Reduce levels of cadmium in chocolate by blending

It is unlikely that there is a single solution to reduce cadmium accumulation in cacao beans due to the heterogeneity in environmental and soil conditions in the region, the level of cadmium in the soil and its bioavailability, different origins of cadmium, the use of different genotypes, and the demands buyers and markets are placing on the level of cadmium in the final product.

The solutions also have different cost implications, and their effective implementation requires the motivation of a range of actors. It must be kept in mind that solutions being developed for application in the plantation must be feasible to producers in the region who in general grow cacao on a small scale and with limited financial and technical resources. While it is expected that many of the solutions will improve soil health, consequently increase productivity and thus help meet any additional costs resulting from cadmium mitigation, this may not always be the case. Other solutions such as irrigation water treatment may be beyond the producers' scope and may require the intervention of the government or NGOs for implementation.

It should be kept in mind that while theories on the most important factors influencing cadmium uptake by cacao abound, only some of the solutions proposed have been or are in the process of being tested in LAC. Further research is needed to draw clear conclusions on their applicability at larger scales and across different environmental conditions. Other potential solutions remain unexplored or untested.

5.1 Avoid high-risk areas for establishing plantations

Until there are cost-effective and efficient solutions to reduce the accumulation of cadmium in cacao beans, sites at risk from cadmium contamination should be avoided for new plantations. While soil analysis can help, the identification of high risk areas is not always straightforward, as total soil cadmium content and cadmium bioavailability can vary widely within a small area (Argüello et al. 2019). Moreover, cacao grown on soils containing relatively low cadmium levels may still be able to accumulate high concentrations of cadmium in their beans. As such, regulatory thresholds of soil cadmium for agricultural land and irrigation water (e.g. Peru[1]) may not be very informative, since total soil cadmium alone is not always a reliable indicator. A better strategy may be to avoid establishment of new plantations within cacao growing areas known to have problems in selling their cacao for export. This approach requires a detailed knowledge of cadmium bean levels in a particular region, information that is being developed in many of the cacao-producing regions. A better understanding of the sources of cadmium contamination and the assessment of areas at risk based on digital soil mapping techniques using environmental covariates could help define effective guidelines for establishing new cacao plantations.

It should be acknowledged that while many farmers cannot choose or change the location of their farmland, they could decide which crop to grow on their land. Thus until solutions have

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[1] Download PDF – Decreto Supremo N° 011-2017-MINAM

been developed, farmers in areas believed to be at high risk for cadmium accumulation in cacao may be advised to plant another crop, at least in the short term.

5.2 Minimise the uptake of cadmium by the cacao tree

Some of the most promising strategies for reducing cadmium in cacao beans involve minimising its uptake by the trees. This can be achieved by i) adding soil amendments that alters soil characteristics such as pH or soil organic matter content, to reduce the bioavailability of cadmium to cacao plants; ii) increasing the nutrient status of the plant which can reduce cadmium uptake; iii) adding microorganisms and other plant species that sequester cadmium from the soil, and iv) using genotypes that are naturally low accumulators.

5.2.1 Soil management and amendments

Although development of an effective suite of solutions is still in its infancy, once high concentrations of cadmium in cacao beans have been detected in a plantation, a soil analysis can help identify which soil management approach or approaches are likely to be the most effective. In areas under irrigation, a water analysis will also be needed to measure chloride ion levels that can be key to cadmium availability.

5.2.1.1 Soil pH

Strategies aimed at raising soil pH to reduce cadmium bioavailability to cacao plants growing on acidic soils seem to have great potential as short to mid-term solutions – see section **3.2.3** – and there is a need for larger trials with a longer duration to assess the potential of these strategies across different environments. 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.

It should be noted that there are examples of cacao growing on soil with neutral to alkaline pH that have high levels of cadmium in their bean (Remigio 2014). Furthermore, in areas where some sources of liming material are forbidden due to its use in making cocaine, alternative amendments such as biochar will need to be used.

5.2.1.2 Organic matter

Increasing soil organic matter content is a promising cost-effective solution to reduce cadmium bioavailability in soils – see section **3.2.4**. This includes the use of manures, fulvic acids and biochars among others. Initial results are encouraging. Still, it should be noted that some organic matter amendments can contain high levels of heavy metals and should be analysed for cadmium content prior to their application (Khan et al. 2017).

5.2.1.3 Silica

Further research is needed through field trials to assess the potential of diatomite or other sources of silica to reduce both cadmium content in contaminated water and cadmium bioavailability in soils.

5.2.2 Plant nutrition

As nutrients and elements of the soil can influence cadmium bioavailability and uptake by cacao plants, adequate plant nutrition is likely to be important – see section **4.6**. Cacao producers in LAC commonly do not apply optimal levels of fertiliser, and more attention should be focused on including this aspect in systematic trials. For those applying phosphate and zinc-containing fertilizers it is important to ensure that they do not contain high levels of cadmium as regulations are lacking in many countries of Latin America.

5.2.3 Bioremediation

Certain plants and microorganisms have been identified as hyper accumulators of cadmium and have been proposed for soil remediation in cacao plantations although there are no results from field trials – see sections **3.2.8** and **4.8**. The concept of bioremediation was developed for soils with exceptionally high heavy metal contents, which is not the general case in cacao plantations in LAC. Their utility in soils with relatively low cadmium concentrations remains unclear. Additionally, bioremediation is more efficient in acidic soils where the cadmium is bioavailable. This condition is contrary to all the other soil mitigation solutions discussed above. An added complication is associated with timely harvesting and safe disposal of biomass from plant hyper accumulators which may be difficult to implement in rural areas. Field assessments are needed to evaluate the feasibility of using microorganisms and hyper-accumulating plants to reduce cadmium bioavailability.

5.2.4 Low accumulating genotypes

There is much variation in cadmium uptake and partitioning between cacao genotypes – see section **4.3**. Reconversion of plantations using low accumulating genotypes as rootstock or scion is likely to be a very important part of the solution in the mid- to long-term, especially in areas with high levels of available soil cadmium.

A better understanding of the genetic basis of this variation is needed to identify rootstocks with reduced cadmium uptake and translocation, and scions that transport less cadmium from rootstock to leaves and fruits. This is in addition to field trials with material already identified from phenotypic analysis.

5.2.5 Other agricultural practices

Adapting key agricultural practices may play an important role in the management of cadmium within cacao production systems although further research is needed. These include water management and irrigation, farm management, and manipulating the production system itself (organic, conventional, agroforestry, monoculture). Limiting the input of saline irrigation water may be important for some areas, as can limiting the flood-drought cycle intensity.

5.3 Reduce levels of cadmium through post-harvest processing

Investigation and advances in post-harvest processing are in their infancy and there is need for further research to understand the overall influence of traditional fermentation, drying and roasting on cadmium content of cacao beans, as well as impacts on physical and flavour qualities – see section **4.9**. It is however unlikely that these processes will reduce cadmium significantly with no effect on other properties and may only be applicable for cacao with cadmium content marginally above the regulatory thresholds.

5.4 Reduce levels of cadmium in chocolate by blending

Blending high cadmium content cacao beans with beans from other regions or even countries with a low cadmium content can be an effective short-term solution to ensure that products do not exceed the regulatory limits. However, for some areas this will result in the loss of regional identity and flavour differences that are key to the fine or flavour cacao market. Although this market represents only 6% of cacao traded, it is the most important market for producers in many areas across LAC.

For fine or flavour cacao that cannot be blended, there is a growing understanding that the fine scale variation in soil cadmium levels and bioavailability is also reflected in the bean cadmium level – thus neighbouring farms may have very different concentrations of cadmium in their harvested beans. A fine scale sampling can allow separation of beans that have acceptable levels above the EU threshold from those that do not, thus allowing for continued sale of a higher proportion of beans compared to when sampling is carried out at a larger scale (Remigio 2014). As long as the levels remain constant over time, and a cost-effective method of sample analysis can be established, this would allow continued sale of part of the harvest while mitigation measures are developed and tested in farmers' fields.

6 ONGOING RESEARCH PROJECTS ON CADMIUM AND CACAO

Several institutions have been contacted regarding information on ongoing or upcoming projects on cadmium. We received information on 26 projects across 5 countries and two regional projects:

Regional – LAC	2
Colombia	12
Ecuador	6
Indonesia	1
Peru	6
Trinidad and Tobago	1
Total	*28*

The following section presents key information about identified projects dealing with cadmium and cacao. For each project, the description of project general objectives has been broken down into different research topics or specific types of experiments.

The projects' description includes the following information (when applicable):

- **General information**
  - Project title
  - Lead/implementing institution
  - Key experts
  - Partners
  - Funding sources
  - Geographical scope
  - Start date
  - End date
- **General objective**
- **Measuring cadmium levels in soils or cacao beans and soil mapping**
  - Locations/Area covered
  - Description
- **Measuring cadmium levels in cacao plant tissue**
  - Description
- **Identifying sources of cadmium contamination in the soil**
  - Sources investigated
  - Description
- **Soil and nutrient management**
  - Type(s) of intervention
  - Description
- **Geo-chemical properties of the soil, agronomic factors and cadmium bioavailability/uptake**
  - Description
- **Phytoremediation and bioremediation**
  - Type(s) of intervention

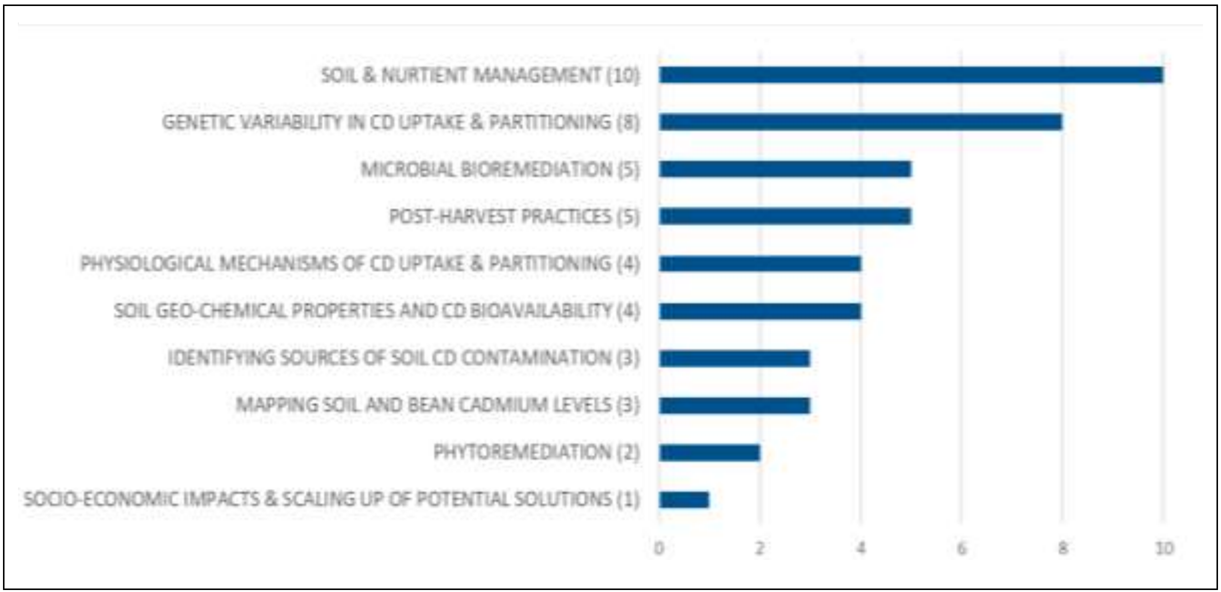
- Description
- **Genetic variability in cadmium uptake and partitioning**
    - Description
- **Physiological mechanisms of cadmium uptake and partitioning**
    - Description
- **Post-harvest practices**
    - Description
- **Socio-economics and technology transfer**
    - Description
- **Published references**
- **Expected outcomes**

This information on all projects is summarized in the following section. Detailed information on each ongoing project is provided in section **6.2**.

6.1 Synthesis of ongoing projects

Figure 7 summarizes the detailed information from each project. Each research projects may focus on one or more specific research areas.

Figure 7 Research areas of ongoing projects (*Number of Project in parenthesis)*



Source: Prepared by the authors

The section below summarises the research projects (Country and project ID) in the main research areas. The projects are detailed in section **6.2**.

Measuring cadmium levels in soils or cacao beans and soil mapping	
Ecuador P10*	Mapping of cacao growing regions of Ecuador based on bean cadmium content – 15 departments which represent 97% of the total production area (completed and published)
Colombia P18	Soil characterization (chemical, physical, cadmium) to map out suitable areas for cacao as well as to define management zones for optimal and sustainable production in Colombia.
Colombia P23	Spatial modelling of the cadmium content in soils cultivated with cocoa. Dynamics of cadmium in stony soils with high levels of the element in beans. The results show a high spatial variability of cadmium levels in soil and plant, both at the municipal level and within farms.
Colombia P24	Measuring Cd in soils and cacao beans in farms from Antioquia and Santander districts of Colombia. Performing a compilation of Cd in soils and beans of Colombia in an agreement between AGROSAVIA – FEDECACAO to develop geospatial distribution models of Cd in cacao from Colombia.
Colombia P24	Total content and availability levels of heavy metals cadmium, mercury, arsenic and lead in soil and dry cocoa beans from Colombia. This work was based on the determination of heavy metals in the soil, the presence of traces of these metals in the cocoa bean and in concentrations greater than those allowed by the international market
Measuring cadmium levels in cacao plant tissue	
Indonesia P28	Survey of cadmium contents in cacao beans produced from production areas in Indonesia and those imported from other countries
Identifying sources of cadmium contamination in the soil	
Ecuador P10	Analyse cadmium concentration in soils and cacao beans of 30 farms to identify hotspots as well as sources of contamination across different altitudes and agroforestry systems
Ecuador P13	Determine the cadmium sources and transfer processes between soil and cacao tissues in tropical environments using isotopic and biogeochemical tracers
Colombia P23	Diagnosis of cadmium concentration in soils and cocoa beans and evaluating if cadmium contents in plants is of geogenic or anthropogenic origin.
Colombia P24	Assessing both anthropogenic (mainly chemical fertilizers and manures) and geogenic sources (mainly using 2D-ERT technique) of Cd in cacao farms from Antioquia and Santander districts of Colombia.

Soil and nutrient management	
Types of intervention	• Biochar (Peru P04, Ecuador P10, Ecuador P11) • Liming material (Peru P08, Ecuador P10) • Cadmium-tolerant bacteria - CdtB (Colombia P24) • Compost (Ecuador P10) • Vermicompost (Ecuador P10) • Humic and fulvic acids (Ecuador P10) • Micronutrients (Ecuador P10) • Coffee residue (Ecuador P11) • Oil palm residue (Ecuador P11) • Quinoa residue (Ecuador P11) • Organic matter (Peru P08, Indonesia P28) • NA (Trinidad and Tobago P03, Peru P06, Ecuador P15) • Optimal fertilisation (Regional LAC P01, Peru P04) • Silica (Peru P04)
Regional LAC P01	Study of the effect of the omission of nutrients in the concentration of cadmium and the productivity of the crop.
Trinidad & Tobago P03	Field trials using amendments in collaboration with the private sector.
Peru P04	Field trials using different amendments.
Peru P06	Evaluate different organic amendments in cadmium uptake in cacao.
Peru P08	Evaluate different organic amendments and lime in cadmium uptake in cacao.
Ecuador P10	Trials on 10 model farms with different levels of pH (low, medium, alkaline), using amendments to raise pH, organic matter and foliar application of micronutrients.
Ecuador P11	Greenhouse trials using different amendments. Best performing practices will be used for field trials in Northern Ecuador, in alkaline and low pH soils.
Ecuador P14	Diagnosis of the management of the farm with agroforestry cocoa through interviews.
Ecuador P15	Sampling and analysis of amendments and cacao plantation soils from areas with high bean cadmium levels. Greenhouse trials to determine the absorption and bioavailability of cadmium.
Colombia P24, P26, P27	Evaluation of soil management strategies to reduce the presence of Cadmium in cocoa almonds.
Indonesia P28	Effect of organic matter on cadmium absorption.

Geo-chemical properties of the soil, agronomic factors and cadmium bioavailability/uptake	
Ecuador P10	Evaluation of the effect of 10 soil properties of cacao growing soils and 14 agronomic factors on cadmium concentration in soils and cacao beans through multivariate regression analysis (completed, see published references).
Ecuador P14	Physical, chemical, biological and mineralogical properties of the soils will be measured and their effect on the bioavailability of cadmium will be determined by means of correlation analysis, multiple regressions and cluster analysis.
Ecuador P15	Understand the mechanism that causes bioavailability of cadmium in cacao plantations using sequential extraction, soil physiochemical and mineralogical characterization and thermodynamics and kinetic studies.
Colombia P23	Cacao bean samples and soil samples from 100 farms analyzed to determine Cadmium levels in beans, the pseudo-total Cadmium and bio-available Cadmium and other soil properties (pH, organic carbon, P, Fe, Mn, Zn and Cu).
Colombia P24	Assessing geogenic sources (mineralogical composition of Cd using 2D-ERT technique) of Cd in cacao farms from Antioquia and Santander districts of Colombia and its analysis with soil elements and Cadmium-tolerant bacteria (CdtB).
Phytoremediation and bioremediation	
Types of intervention	• Cadmium accumulating yeasts (Peru P04) • Mycorrhizae (Peru P04, Colombia P16, P21, P24) • *Heliconia psittacorum* (Colombia P16) • Plants (Ecuador P15)
Peru P04	Field trials using cadmium accumulating yeasts.
Peru P04	Greenhouse trials using commercial mycorrhiza and bacteria.
Ecuador P15	Identification and evaluation of leguminous plants found in cacao plantations for phytoremediation.
Colombia P16	Trials in nursery using *Heliconia psittacorum* alone and associated with fungal biomass.
Colombia P17	Evaluate, in vitro, the level of tolerance to cadmium of native fungal species from cacao-growing soils of San Vicente de Chucurí.
Colombia P21	• Characterization of arbuscular mycorrhizae fungi (AMF) communities that will allow for the identification of potential stress-tolerant AMF for

	the development of mitigation strategies in cocoa plants under Cd-Zn stress. • Inoculation with AMF communities from Cd-enriched soils and commercial AMF.
Colombia P22	Cd-resistant bacteria and fungi associated to cacao rhizosphere were isolated and identified through morphological and molecular markers. Their ability to solubilize phosphorous, fix nitrogen, and degrade cellulose were also evaluated. The results of this study will provide knowledge of Cd-resistant microorganisms associated to cacao crop and highlights potential strains for biotechnology-based strategies to mitigate the cocoa Cd uptake.
Colombia P24	Assessing Cadmium tolerant bacteria (CdtB) and mycorrhizae to immobilize Cd in cacao farms from Antioquia and Santander districts of Colombia using metabolic fingerprints with isothermal microcalorimetry (IMC) and analysing its chemical conversions with XRD/XRF and MALDI-TOF, as well as, with functional genes involved in Cadmium immobilization and chelation. Monitoring its capabilities in seedlings at greenhouse experiments and amendments in experimental field pots to sequestrate Cd in soils in cacao farms from Antioquia and Santander districts

Genetic variability in cadmium uptake and partitioning	
Regional LAC P01	Cocoa genotypes with less accumulation of cadmium, proposal for gene editing.
Trinidad & Tobago P03	Field trials using promising low cadmium accumulating rootstocks
Peru P04	Identification of low accumulating genotypes (screening of > 1000 national genotypes)
Peru P04	Field trials with promising low accumulating rootstocks and scions
Peru P07	Evaluate different cadmium uptake of different cacao clones
Ecuador P11	Screening 10 different accession for low cadmium accumulation
Ecuador P13	Comparison of Cadmium transfer and recycling between soil and plant for 2 varieties
Colombia P21	Effect of grafting over Cd-Zn uptake and plant physiology of two genotypes under Cd-Zn stress. A trial experiment has been conducted using ungrafted, self-grafted and grafted plants of four different genotypes (IMC67, CAU43 as rootstocks; and FSV41, CCN51 as scions).
Colombia P25	Parental selection by attributes of interest: molecular characterization, quality criteria, disease resistance and cadmium absorption.

Indonesia P28	Screening of some rootstock on absorption of cadmium from soil
Physiological mechanisms of cadmium uptake and partitioning	
Ecuador P13	Follow the transfer and accumulation processes from soil to cacao beans using isotopic and biogeochemical tracers
Ecuador P14	Measure total cadmium content as well as micro-nutrients content in root, stem, leaves and beans
Colombia P21	Nutrition, photosynthetic efficiency, HMs partitioning and growth of two cocoa genotypes (IMC67 and PA121) are assessed in natural enriched soils with low and high Cd-Zn concentrations
Colombia P23	The Cadmium in leaf and fruit tissues (shell, bean and pod husk) was analysed. Cadmium in soil and cacao leaf litter around trees was also determined. Bioaccumulation factor (BF) was calculated as the ratio of Cadmium in leaf or bean to that in soil, and Translocation factor (TF) as the ratio of Cadmium in leaf to that in fruit tissues.

Post-harvest practices	
Ecuador P11	Monitor every two months the cadmium content of cacao beans (dried and fermented) of 15 collection centres to sort beans in batches that comply with EU regulations.
Ecuador P12	Effect of fermentation and drying processes on cadmium content in cacao beans
Ecuador P13	Monitor cadmium transfer (enrichment or loss) within each step of chocolate end-product elaboration (dry beans, fermented beans, roasted beans and cocoa liquor)
Colombia P19	Cadmium content reduction in fermented Colombian fine flavour cocoa beans grown in highest cadmium content areas by nanotechnology strategies.
Colombia P20	Cadmium content reduction in fermented Colombian fine flavour cocoa beans through the use of biotechnological strategies with microorganisms during post-harvest.
Socio-economics and scaling up of potential solutions	
Ecuador P10	Evaluation of the cacao value chain and quantification of the economic impact of EU regulations in contaminated cacao growing areas of Ecuador. The economic impact of possible mitigation strategies and scaling-up potential for small-holder cacao farmers will also be investigated.

6.2 Presentation of ongoing projects

6.2.1 Regional – Latin America and the Caribbean

projectID	P01
Project title	**Multi Agency Platform of cocoa for Latin America and the Caribbean "Cacao 2030-2050"**
Lead/implementing institution	Instituto Nacional de Investigaciones Agropecuarias (INIAP) Escuela Politécnica del Litoral (ESPOL)
Key experts	Manuel Carrillo Centeno (INIAP), Eduardo Chávez (ESPOL), Ramón Espinel (ESPOL)
Partners	Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria (INTA) Costa Rica, Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF), Instituto Nacional de Innovación Agraria (INIA) Perú, Instituto de Investigación Agropecuaria de Panamá (IDIAP), Universidad Nacional de Costa Rica (UNA), CATIE (Costa Rica), CIAT (Colombia), CEFA (Italy), GIZ (Alemania), RIKOLTO, FCIA
Funding sources	FONTAGRO
Geographical scope	Latin America and the Caribbean
Status of submission	Approved
Start date	2019
End date	2022
General objective	Develop and transfer technology for the production of fine flavour cocoa, with quality and safety in Latin America and the Caribbean, strengthening the capacities of national R & D & I systems with an impact horizon of 2030 and 2050. The specific objectives of the project are: i) to generate knowledge and alternatives for the management of cadmium in the cocoa, ii) to establish and standardize a methodology for measuring cadmium to generate maps and techniques to reduce the levels of cadmium, iii) generate socio-economic information of the impact of international regulations, and iv) disseminate and transfer the knowledge and alternatives generated by the project
Expected outcomes	• Cocoa genotypes with less accumulation of cadmium. • Study of the effect of the omission of nutrients in the concentration of cadmium and the productivity of the crop. • Proposal for gene editing. • Standardized methodology for the determination of cadmium in the region. • Report containing cadmium maps of some countries, and validation of strategies to mitigate cadmium absorption. • Report of drying and fermentation of cocoa on the content of cadmium. • Strategic framework document for the long-term Cocoa Platform 2030-2050. • Socio-economic and impact analysis of EU regulations regarding cadmium concentration. • Analysis of current regulations for the import of fertilisers in the region. • Memories of annual workshops. • Training plans for trainers, journalists, and farmers. • Virtual Repository / platform with information about cadmium in cocoa.

projectID	P02

projectID	P02
Project title	**Fostering CLIMAte-relevant and LOw CAdmium innovations to enhance the resilience and inclusiveness of the growing cocoa sectors in Colombia, Ecuador and Peru (Clima-LoCa)**
Lead/implementing institution	ALLIANCE BIOVERSITY-CIAT
Key experts	Mayesse Da Silva, Mirjam Pulleman, Andres Charry, Christian Bunn, Rachel Atkinson, Evert Thomas, Xavier Argout, Eduardo Chavez, Erik Smolders, Darwin Martinez, Rey Gastón Loor, Manuel Carrillo, Pathmanathan Umaharan, Laurence Maurice, Caren Rodriguez, Roxana Yockteng, Olivier Sounigo, Angela Castaño, Jaime Osorio, Andrea Montenegro and others in each of the implementing countries.
Partners	ALLIANCE BIOVERSITY-CIAT, CIRAD, AGROSAVIA, INIAP, ESPOL, WAGENINGEN UNIV., KULEUVEN, CRC, IRD
Funding sources	Europe Aid (DeSIRA)
Geographical scope	Colombia, Ecuador, Peru
Status of submission	Approved and contract in process of finalization
Start date	January 2020 (expected)
End date	December 2023 (expected)
General objective	Clima-LoCa will address important challenges related to the resilience, competitiveness and inclusiveness of the growing cocoa sectors of Colombia, Ecuador and Peru. Here, resilience refers to the capacity of smallholder producers, and other value chain actors, to mitigate the negative impacts of new EU food safety regulation on cadmium in cocoa; and of climate change. More specifically, the objective of the Action is to support the development, implementation and scaling of low cadmium and climate-relevant innovations that fit the diverse contexts of smallholder cocoa production.
Expected outcomes	The objectives of the project will be achieved based on 4 main outputs that are developed around 4 interdisciplinary work packages. • WP1 will develop baselines and impact assessments for cadmium and climate change, and inform public policies and interventions taking into account edaphoclimatic, cacao genetic and socio-economic variation within and between the countries • WP2 will provide scientific assessments in multilocational research trials to identify production practices and genotypes for reduced cadmium accumulation in cocoa beans, while considering effects on productivity, soil health, climate relevance and cost-benefits • WP3 will pilot low cadmium agronomic practices and genotypes in collaboration with farmer associations, and co-develop mitigation and scaling strategies in multi-stakeholder platforms • WP4 will strengthen regional research coordination and research capacity, and promote scientific exchange and training, including training of laboratories. All WPs include important activities dedicated to the dissemination of the project outputs and will develop decision support tools and training materials, targeting diverse stakeholders.

6.2.2 Trinidad and Tobago

projectID	P03
Project title	**Phase 2 "Mitigation of cadmium (cadmium) Bioaccumulation in Theobroma Cacao L."**
Lead/implementing institution	Cocoa Research Centre
Key experts	Caleb Lewis, Gideon Ramtahal
Partners	ECA, CAOBISCO, FCC
Funding sources	ECA/CAOBISCO/FCC joint research fund
Geographical scope	Trinidad and Tobago
Start date	2018
End date	2019
General objective	This project will pick up on the first phase and will intensify field trials. Activities will include: • field trials with amendments in collaboration with the private sector • field trials using promising low cadmium accumulating rootstocks
Soil and nutrient management	
Description	Field trials with amendments in collaboration with the private sector
Genetic variability in cadmium uptake and partitioning	
Description	Field trials using promising low cadmium accumulating rootstocks identified in phase 1in different environments.
Expected outcomes	• Results on the efficiency of different amendments as mitigation solutions for cadmium uptake by cacao plants • New insights on the effects of using low-accumulating rootstocks with different scions and within different environments

6.2.3 Peru

projectID	P04
Project title	**Cadmium and cacao: identifying short and long-term solutions**
Lead/implementing institution	Bioversity International
Key experts	Evert Thomas; Rachel Atkinson
Partners	MINAGRI, SENASA, INIA; U. Cientifica del Sur; U. San Marcos; U. Nacional de Piura
Funding sources	Peruvian Government
Geographical scope	Peru
Start date	2018
End date	2020
General objective	This project will first study the presence of cadmium in areas close to the project's activities and will investigate solutions at short and mid-term through: • field trials using soil amendments and cadmium accumulating yeasts • field trials testing low accumulating genotypes • greenhouse trials using mycorrhiza in soils with different cadmium and

projectID	P04
	pH levels, and combining low accumulating genotypes
Soil and nutrient management	
Type(s) of intervention	• Biochar (Inkan Negra /U. Cientifica del Sur) • Optimal fertilisation (Yara) • Diatomaceous earth (Feys Peru) • High accumulating yeast (Fertilev – Bioxlab/ U San Marcos) • Combinations
Description	Experiments using 14 treatments being carried out in established and new plantations with producers associated with the Cooperative NorAndino to determine effect on Cadmium accumulation in fruits and Cadmium availability in the soil
Phytoremediation and bioremediation	
Type(s) of intervention	• Commercial mycorrhiza
Description	Controlled experiments in greenhouses will be carried out to evaluate the capacity of commercial mycorrhiza to accumulate cadmium, combining the use of 1 to 5 promising low-accumulating genotypes within soils with different cadmium concentrations and pH levels.
Genetic variability in cadmium uptake and partitioning	
Description	The project will identify cacao genotypes native to Peru with low cadmium accumulation through a collection of > 1000 plants across the country by measuring levels of cadmium in leaves and soils (expected ~100 genotypes). This will serve as a baseline before field testing and for genetic characterization. In addition, metagenomic testing of associated mycorrhizas will be conducted. Once identified as potential low accumulating genotypes, field testing of promising rootstocks will allow to check the effect of grafting and of environment (especially alkaline soils).
Expected outcomes	• Results on the efficiency of applying different amendments and using cadmium accumulating yeasts as a solution to reduce cadmium uptake by cacao plants (first results by early 2019, more by mid-2019 and early 2020) • Results on the efficiency of applying mycorrhiza as a solution to reduce cadmium uptake by cacao plants (by early 2019) • Identified promising Peruvian genotypes which show low leaf cadmium levels growing within soils with high cadmium content. These promising genotypes could be used in further selection programs and as rootstocks in different cacao growing regions (by early 2019) • New insights on the role of genotype

projectID	P05
Project title	**Cacao Seguro USDA-FAS/MINAGRI Action Plan**
Lead/implementing institution	Instituto de Cultivos Tropicales-ICT/USDA-FAS – MINAGRI
Key experts	Harold Tarver (USDA-FAS), Benjamin Lownik (USDA-FAS), Enrique Arévalo-Gardini (ICT), Tommy Fairlie Canon (ICT/USDA-FAS)
Partners	USDA-FAS (Cacao Seguro Project), USAID, MINAGRI, SENASA, INIA
Funding sources	USAID, USDA-FAS
Geographical scope	Peru
Start date	2018

projectID	P05
End date	2021
General objective	This project seeks to stimulate the intensification of research on cadmium in cacao in Peru through actions (project funding etc.) taken by the Peruvian government. Research Topics Include: • testing of the most promising approaches to mitigate cadmium accumulation • the concerted response of Peru in the international arena, CODEX, EU, etc., • Laboratory standards and methodologies to analyse cadmium content • Outreach

projectID	P06
Project title	**Effect of organic amendments in cadmium accumulation in cacao**
Lead/implementing institution	Instituto de Cultivos Tropicales – ICT
Key experts	César O. Arévalo-Hernández, Enrique Arévalo-Gardini, Juvicksa Correa, Virupax Baligar
Partners	USDA-ARS
Funding sources	USDA-ARS, ICT
Geographical scope	Peru – Tarapoto-San Martin
Start date	2017
End date	2018
General objective	Evaluate different organic amendments in cadmium uptake in cacao

projectID	P07
Project title	**Cadmium and Lead uptake in different cacao clones used for commercial plantations**
Lead/implementing institution	Instituto de Cultivos Tropicales – ICT
Key experts	Enrique Arévalo-Gardini, Virupax Baligar, César O. Arévalo-Hernández, Jimmy Chupillon
Partners	USDA-ARS
Funding sources	USDA-ARS, ICT
Geographical scope	Peru – Tarapoto-San Martin
Start date	2017
End date	2018
General objective	Evaluate different cadmium uptake of different cacao clones

projectID	P08
Project title	**Effect of lime and organic amendments in cadmium uptake in cacao**
Lead/implementing institution	Instituto de Cultivos Tropicales – ICT
Key experts	César O. Arévalo-Hernández, Enrique Arévalo-Gardini, Virupax Baligar, Josselyn Revollar
Partners	USDA-ARS
Funding sources	USDA-ARS, ICT
Geographical scope	Peru – Tarapoto-San Martin
Start date	2017
End date	2019
General objective	Evaluate different organic amendments and lime in cadmium uptake in cacao

projectID	P09
Project title	**Inter Lab for Peruvian Laboratories (Cacao Seguro Project)**
Lead/implementing institution	Instituto de Cultivos Tropicales – ICT / USDA FAS
Key experts	César O. Arévalo-Hernández, Enrique Arévalo-Gardini
Partners	USDA-FAS (Cacao Seguro Project), USAID
Funding sources	USAID, USDA-FAS
Geographical scope	Peru – Tarapoto-San Martin
Start date	2018
End date	2019
General objective	Determination of cadmium for cacao bean and powder through Inter-Laboratory Comparison within Peruvian labs have a similar and minor variability in the results

6.2.4 Ecuador

projectID	P10
Project title	**Estudio nacional: En marcha 2017-2020. ESPOL – KU Leuven (Bélgica)**
Lead/implementing institution	ESPOL
Key experts	Eduardo Chávez, Erik Smolders, Ramón Espinel, Miet Marteens, David Argüello, José Luis Vázquez, Daniela Montalvo
Partners	KU Leuven, Colaboración con Industria y ONGs para implementar
Funding sources	VLIR-UOS
Geographical scope	Ecuador
Start date	2017
End date	2020
General objective	The project seeks to identify the agronomic and economic impacts of EU regulations on cadmium and communicate results to cacao growers. Short

projectID	P10
	and long-term mitigation strategies to reduce accumulation of cadmium in cacao beans will be evaluated through a holistic and systematic approach. The results will be transferred to all stakeholders of the cacao value chain, and in particular to small-holder cacao growers.
Measuring cadmium levels in soils or cacao beans and soil mapping	
Locations/Area covered	Cacao growing regions of Ecuador (15 departments which represent 97% of the total production area)
Description	Sampling of soils, cacao leaves and beans (n=571) in 15 departments of Ecuador for soil mapping based on bean cadmium concentration *(Finished, see published references).*
Soil and nutrient management	
Type(s) of intervention	Different liming materials, compost, vermicompost, humus, humic and fulvic acids, biochar, micronutrients.
Description	Trials on 10 model farms with different levels of pH (low, medium, alkaline). • Application of amendments to lower pH of the soil within acid soils (different liming materials). • Application of organic matter within alkaline soils (compost, humic and fulvic acids, vermicompost). • Foliar application of micronutrient (in deficient areas, both alkaline and acid soils) • Biochar in Amazonian soils
Geo-chemical properties of the soil, agronomic factors and cadmium bioavailability/uptake	
Description	Evaluation of the effect of 10 soil properties of cacao growing soils and 14 agronomic factors on cadmium concentration in soils and cacao beans through multivariate regression analysis *(Finished, see published references)*.
Socio-economics and technology transfer	
Description	Semi-structured interviews will be conducted in order to evaluate the cacao value chain and quantify the economic impact of EU regulations in contaminated cacao growing areas. The economic impact of possible mitigation strategies and scaling-up potential for small-holder cacao farmers will also be investigated.
Published references	Argüello, D. *et al.* (2019) ‘Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: A nationwide survey in Ecuador’, *Science of the Total Environment*. Elsevier B.V., 649, pp. 120–127.
Expected outcomes	• *Some results are already published (Soil map, soil properties and agronomic factors effect on cadmium concentration in beans)* • Results on the efficiency of different amendments as mitigation solutions to reduce cadmium uptake by cacao plants • Evaluation of the impact of EU regulation in contaminated cacao growing areas of Ecuador (by end of 2018) • Evaluation of the economic impact and scaling up potential of mitigation solutions for small-holders (by 2020)

projectID	P11
Project title	**Proyecto GIZ - CEFA - ESPOL Herramientas Integrales para Identificar y Mitigar Zonas Cacaoteras Contaminadas con Cadmio en la Amazonia Norte y Manabi**
Lead/implementing institution	ESPOL
Key experts	Eduardo Chávez
Partners	ESPOL, GIZ, CEFA
Funding sources	EU
Geographical scope	Ecuador (Northern Amazon and Manibi)
Start date	
End date	
General objective	• Analyse cadmium content in soils and cacao beans sampled from 30 farms to identify hotspots and sources of contamination • Trials with different amendments within model farms • Screening of accessions for low accumulating genotypes • Monitor cadmium content of cacao beans of collection centres in order to sort beans in batches that comply with EU regulations
Identifying sources of cadmium contamination in the soil	
Sources investigated	
Description	Analyse, following different methodologies, the concentration of cadmium in soils and cacao beans of 30 farms to identify hotspots as well as sources of contamination across different altitudes and agroforestry systems.
Soil and nutrient management	
Type(s) of intervention	Coffee residue, oil palm residue and quinoa residue, combination of different biocharcoals
Description	Different amendments (coffee residue, oil palm residue and quinoa residue, combination of different biochars) are being tested at the greenhouse. Three doses (0, 1, 2%), soils with different characteristics (pH of 5.2 and 7.9, cadmium content of 0.74 and 1.12 mg/kg) Best performing practices will be tested within 5 model farms in Northern Amazon provinces and Manabi province in Ecuador, in alkaline and low pH soils (by January 2019).
Genetic variability in cadmium uptake and partitioning	
Description	Screening 10 different accession for low cadmium accumulation. If results are successful, this will serve as a basis for a larger study, with identification of genes involved in cadmium uptake and subsequent study on cadmium uptake mechanisms, as well as grafting trials.
Post-harvest practices	
Description	Monitor every two months the cadmium content of cacao beans (dried and fermented) of 15 collection centres in order to sort beans in batches that comply with EU regulations.
Expected outcomes	• Identified hot-spots within 10 farms surveyed • Identified sources of contamination • Results on the efficiency of different amendments as mitigation solutions for cadmium uptake by cacao plants • Identified promising low-accumulating genotypes • Results on the efficiency of post-harvest mitigation solution based on sorting cacao beans according to their cadmium content to meet EU regulations

projectID	P12
Project title	
Lead/implementing institution	KU Leuven
Key experts	Erik Smolders, Eduardo Chávez, Ruth Vanderschueren
Partners	ESPOL, KU Leuven
Funding sources	
Geographical scope	Ecuador
Start date	
End date	
General objective	PhD project focused on post-harvest treatment to mitigate cadmium.
Post-harvest practices	
Description	First part of the project focuses on the effect of fermentation and drying processes on cadmium content in cacao beans.

projectID	P13
Project title	**CADCAO (cadmium in Cacao)**
Lead/implementing institution	Institut de Recherche pour le Développement (IRD, France)
Key experts	Dr. Laurence Maurice (IRD Ecuador/GET, Invited Professor at the Univ. Andina Simon Bolivar (Quito)), Prof. Mark Rehkämper (Imperial College, London, UK), Dr. Eva Schreck (U. Toulouse/GET), Dr. Fiorella Barraza (IRD/GET)
Partners	Laboratory Géosciences Environnement Toulouse (IRD, GET, France), Mass Spectrometry and Isotope Geochemistry Lab (MAGIC) at the Imperial College of London. Cooperative Company ETHIQUABLE (France).
Funding sources	Institut Olga Triballat (France) Cooperative Company ETHIQUABLE (France).
Geographical scope	Ecuador (Pacific Coast and Amazon Region)
Start date	December 2017
End date	July 2019
General objective	The main objective of CADCAO project is to improve the understanding of the transfer and bioaccumulation mechanisms of cadmium from soils to cacao beans, especially in the varieties cultivated in Ecuador and destined to the European market (CCN-51 and National “Fine flavour”).The aims of this study, using isotopic and biogeochemical tracers, are: • Determine the cadmium sources in tropical environments • Follow the transfer and accumulation processes from soil to cacao tissues (leaves, pod husks and beans) • Monitor cadmium transfer (enrichment or loss) within each step of chocolate end-product elaboration (dry beans, fermented beans, roasted beans and cocoa liquor)
Identifying sources of cadmium contamination in the soil	
Sources investigated	

projectID	P13
Description	Determine the cadmium sources in tropical environments using isotopic and biogeochemical tracers.
Physiological mechanisms of cadmium uptake and partitioning	
Description	Follow the transfer and accumulation processes from soil to cacao beans using isotopic and biogeochemical tracers.
Post-harvest practices	
Description	Monitor cadmium transfer (enrichment or loss) within each step of chocolate end-product elaboration (dry beans, fermented beans, roasted beans and cocoa liquor)
Expected outcomes	• Identified sources of cadmium contamination • Tracing Cadmium transfer and recycling between soil and cacao tissues • New insights on physiological mechanisms of cadmium uptake by cacao plants • Results concerning the effect of post-harvest and production practices on cadmium content in cacao beans and cacao-based products

projectID	P14
Project title	**INIAP – Regional Amazónica Ikiam University**
Lead/implementing institution	INIAP – Ikiam University
Key experts	Magdalena López PhD, Paulo Barrera MsC.
Partners	ENGIM (Italian Cooperation), GIZ (Germany Cooperation)
Funding sources	INIAP, ENGIM, GIZ, Ikiam University
Geographical scope	Ecuador – Central Ecuadorian Amazon
Start date	2/11/2018
End date	2/10/2020
General objective	Determine the dynamics of Cadmium in four zones dedicated to cocoa agroforestry system in two predominant soils in Napo – Ecuador
Measuring cadmium levels in soils or cacao beans and soil mapping	
Locations/Area covered	Tena, Archidona and Aorosemena in Napo province. Area 74340 ha
Description	16 agroforestry cocoa farms were selected, in each one 20 sub-samples of soil at two depths (0-15 and 15-30 cm) were taken to determine the total cadmium. Additionally, 5 cacao plants will be chosen at random and 4 sub-samples of litter will be taken to determine total Cadmium and soil samples at a depth of 0-15 cm to establish the cadmium contents available in each plant. These results will be correlated with the contents of Cadmium in the different parts of the cacao plants.
Soil and nutrient management	
Type(s) of intervention	Diagnosis of the management of the farm with agroforestry cocoa through interviews
Description	In 16 farms surveys and information gathering will be carried out in the field to characterize socially, economically and agronomically the different productive systems in 4 areas of Napo province. The floristic biodiversity will also be determined in the productive systems.
Geo-chemical properties of the soil, agronomic factors and cadmium bioavailability/uptake	

projectID	P14
Description	For all 5 cacao plants sampled in each farm, soil samples will be collected as well. Physical, chemical, biological and mineralogical properties of the soils will be measured and their effect on the bioavailability of cadmium will be determined by means of correlation analysis, multiple regressions and cluster analysis
Physiological mechanisms of cadmium uptake and partitioning	
Description	In 5 cacao plants, we will measure total cadmium content in root, stem, leaves and beans, for each farm and in two seasons (Dry and wet season). Macro and micro nutrients in plant tissue will also be measured

projectID	P15
Project title	**Técnicas para disminuir la disponibilidad de Cadmium en suelos de cacaoteras**
Lead/implementing institution	Instituto Nacional de Investigaciones Agropecuárias (INIAP)
Key experts	Manuel D. Carrillo Z., Luz María Martínez, Markus Gräfe, Alexis Debut, Wuellins Durango, RAúl Jaramillo y Karina Peña
Partners	INIAP, Universidad UTE, Universidad de las Fuerzas Armadas (ESPE), International Plant Nutrition Institute (IPNI)
Funding sources	INIAP, UTE, ESP, IPNI
Geographical scope	Ecuador
Start date	2017
End date	2020
General objective	Understand the dynamics of cadmium in the soil to reduce its bioavailability using methods to reduce uptake by cacao plants
Soil and nutrient management	
Type(s) of intervention	Evaluate the availability of cadmium in cacao-growing soils treated with different conventional and organic amendments
Description	Characterize soil amendments and cacao-growing soils for levels of cadmium Determine the bioavailability of cadmium under different conditions in a Greenhouse experiment.
Geo-chemical properties of the soil, agronomic factors and cadmium bioavailability/uptake	
Description	Establish mechanisms by which soil cadmium becomes available through kinetic and thermodynamic studies and using sequential extraction methods. Carry out a physicochemical and mineral soil characterization Understand the physicochemical processes of adsorption and desorption of soil cadmium Carry out fractionation of the solid phase of soil cadmium
Phytoremediation and bioremediation	
Type(s) of intervention	Identification and evaluation of leguminous plants found in cacao plantations for phytoremediation
Description	Evaluation of leguminous plants as potential phytoremediators Collection of leguminous plants and preparation of seeds for sowing Evaluation of its ability to extract cadmium

6.2.5 Colombia

projectID	P16
Project title	**Phytoremediation and Myco-remediation of cadmium in a Theobroma cacao soil at nursery level (biological strategies to improve crop quality)**
Lead/implementing institution	Universidad de Santander – FEDECACAO
Key experts	Beatriz Elena Guerra (Universidad de Santander), Jaider Muñoz (Universidad de Santander), Diannefir Duarte (FEDECACAO)
Partners	Universidad de Santander, Federación de cacaoteros de Colombia (FEDECACAO)
Funding sources	Universidad de Santander- FEDECACAO
Geographical scope	Colombia
Start date	08-2016
End date	08-2018
General objective	Evaluate the efficiency of *Heliconia psittacorum* alone and associated with fungal biomass for bioremediation in cadmium contaminated cacao-growing soils (nursery)
Phytoremediation and bioremediation	
Type(s) of intervention	*Heliconia psittacorum* alone and associated with fungal biomass
Description	The contamination of soil by various anthropogenic activities has resulted in a serious problem related to the accumulation of heavy metals with a negative impact for agriculture. Several species of plants are considered tolerant to heavy metals or adapt easily to these contaminated environments, which make them candidates to evaluate their potential as phytoremediators. *Heliconia psittacorum* is a plant that grows commonly in cocoa plantation in Santander-Colombia and grows in symbiosis with arbuscular endomycorrhizal fungi. This study will evaluate its tolerance level in soils contaminated naturally by cadmium, when it is grown alone or associated with arbuscular mycorrhizal fungi and free-living fungi.

projectID	P17
Project title	**Study of biodiversity and cadmium tolerance in native filamentous fungi native to cacao soils in Santander- Colombia**
Lead/implementing institution	University of Santander - Colombia (UDES) - Universidad EAFIT -Colombia
Key experts	Beatriz Elena Guerra (Universidad de Santander) Javier Correa (Universidad EAFIT)
Partners	Universidad de Santander, EAFIT
Funding sources	Universidad de Santander
Geographical scope	Colombia
Start date	08-2018
End date	10-2019
General objective	To evaluate the level of fungal tolerance in vitro, at high concentrations of cadmium.
Phytoremediation and bioremediation	
Type(s) of intervention	Native fungal species from cacao-growing soils of San Vicente de Chucurí

projectID	P17
Description	Fungal biomasses use mechanisms such as bioaccumulation or biosorption of heavy metals, in their tissues without affecting their metabolism. It has been reported that different fungal species have the ability to survive adapting or mutating to high concentrations of heavy metals. Hence the objective of this work is directed to the isolation of filamentous fungi from soil contaminated by cadmium from the cacao region of the municipality of San Vicente de Chucurí (Santander). For the purpose of evaluating the tolerance level for native fungal species at different concentrations of the metal heavy, assays will be evaluated through the growth and development in mycological media modified with cadmium (100, 200, 300 ppm respectively) and later will be identified molecularly more tolerant species.

projectID	P18
Project title	**Cacao for Peace - Geographical Information System Mapping for Optimized Cacao Production in Colombia**
Lead/implementing institution	CIAT (in country coordinator) and implemented together with PSU and USDA-NRCS
Key experts	Mayesse Da Silva, Gerardo Gallego, Zamir Libohova, Charles Kome, Siela Maximova, Mark Guiltinan, Patrick Drohan
Partners	CIAT, USDA-FAS, USDA-NRCS, Penn State University, FEDECACAO, IGAC, UNODC
Funding sources	USAID/USDA
Geographical scope	Colombia
Start date	2018
End date	2019
General objective	This project is part of the Cacao for Peace initiative. Its objective is to promote a deeper understanding of spatial variability of soil characteristics and cadmium, water supply for cacao cultivation, and assess the diversity of plants in the Sierra Nevada de Santa Marta in Colombia in order to define suitable areas for optimal and sustainable cacao production as well as define management zones for improved management. Definition of suitable areas takes into account soil characteristics, climate conditions, and soil cadmium content.
Soil mapping	
Locations/Area covered	Sierra Nevada de Santa Marta
Description	Soil characterization (chemical, physical, cadmium) to map out suitable areas for cacao as well as to define management zones for optimal and sustainable production in Colombia. High-resolution soil maps (30m; scale 1:25000) will be developed based on digital soil mapping approaches.

projectID	P19
Project title	**Development of bio- and nano-technology strategies for the reduction of cadmium in Colombian fine flavor cocoa beans.**
Lead/implementing institution	CasaLuker / Los Andes University.
Key experts	Johann Osma; Juan Carlos Cruz, Claudia M Rodriguez, Sergio Leonardo Florez, Ana Lucia Campana. Hector Hugo Olarte
Partners	Antioquia University.
Funding sources	CasaLuker.

projectID	P19
Geographical scope	Municipalities of the departments of Santander and Arauca.
Start date	May- 2017
End date	February 2019
Links	
General objective	Cadmium content reduction in fermented Colombian fine flavor cocoa beans grown in highest cadmium content areas by nanotechnology strategies.
Post-harvest practices	
Description	This project proposes the optimization of the bio- and nano-technological strategies previously developed by CasaLuker and Los Andes University, for the reduction of Cadmium content in fermented cocoa beans by adjusting production conditions, operation and control of these strategies on a laboratory and semi-industrial scale to achieve scaling up processes at an industrial level. Los Andes University and CasaLuker have been developing processes of cadmium reduction during post-harvest stages and subsequent processes at the laboratory and semi-industrial scale that have allowed them to approach compliance with international standards. One of the most successful approaches has been the preparation of micellar bodies on cocoa matrices between 10% and 30% of reduction, which has allowed to manufacture chemical complexes with the molecules that contain Cadmium to remove it. Another approach based on the use of heavy metal removal microorganisms has been tested and validated in laboratory conditions and at a semi-industrial scale. At the same time, a plan will be developed for the assurance of the quality of the cocoa matrix throughout the removal process, to maintain the qualities of the "Fino de Aroma" cocoa produced in Colombia. This will be done through a monitoring of the organoleptic characteristics of the grain that allows to iteratively adjusting the scaled processes.

projectID	P20
Project title	**Cadmium mitigation project with the use of microorganisms**
Lead/implementing institution	CasaLuker S.A
Key experts	Claudia Rodriguez, Johanna Hurtado, Paula Andrea Pedraza, Patricia Ahumada, Martha Cepeda, Hector Hugo Olarte, Maria José Chica – Agrosavia: Daniel Bravo, Martha Gomez, Eddy Bautista, Andrés Diaz.
Partners	CorpoGen, Agrosavia
Funding sources	CasaLuker
Geographical scope	Colombia
Status of submission	In process
Start date	2015
End date	2019
Links	
General objective	Cadmium content reduction in fermented Colombian fine flavor cocoa beans through the use of biotechnological strategies with microorganisms during post-harvest.
Post-harvest practices	
Description	The searching strategies to reduce the amount of toxic metals in food has

projectID	P20
	become an important focus for the development of studies in various parts of the world. The biotechnological strategies, in this case, consist in the application of microorganisms in cocoa cadmium decreasing processes. Bacillus and Lactobacillus from different origins with a high cadmium retention capacity have been reported in different studies. The CasaLuker´s results obtained in beans removal tests previously developed in our group, evaluated several strains selected for their potential in this application. Laboratory scale tests were carried out and those with the best percentages of cadmium removal were selected. Reference strains were also evaluated according to what has been reported in previous studies, in addition, microorganisms from the cocoa ferment in order to take advantage of the fermentation conditions. Subsequently, pilot scale trials were conducted with volumes between 10 kg and 40 kg of cocoa mass, observing removals between 13% and 28% with time of contact between 12 and 24 hours. Once the standardization of the pilot stage is concluded, it is expected to carry out in-situ and semi-industrial tests in one of the regions of Colombia where the highest cadmium contents are present in cocoa.

projectID	P21
Project title	**Effect of local arbuscular mycorrhiza fungi communities and grafting on the physiology of cacao under cadmium and zinc stress**
Lead/implementing institution	Universidad Nacional de Colombia, sede Bogotá (UNAL) Federación Nacional de cacaoteros de Colombia (FEDECACAO)
Key experts	Jhon Felipe Sandoval Pineda (UNAL), Edwin Antonio Gutiérrez Rodríguez (FEDECACAO), Alia Rodriguez (UNAL), Esperanza Torres Rojas (UNAL)
Funding sources	COLCIENCIAS, UNAL, FEDECACAO, Fondo Nacional de Cacao, Gobernación de Cundinamarca, Corredor tecnológico Agroindustrial.
Geographical scope	Colombia
Start date	2017
End date	2019
General objective	To determine the effect of local arbuscular mycorrhiza fungi (AMF) communities and grafting on the physiology of different cacao genotypes under cadmium and zinc stress
Phytoremediation and bioremediation	
Type(s) of intervention	Inoculation with AMF communities from Cd-enriched soils and commercial AMF
Description	1. Arbuscular mycorrhiza fungi (AMF) are obligate symbionts present in cacao rhizosphere and their community diversity is modified depending on several abiotic factors such as cadmium (Cd) and zinc (Zn) concentration in soils. It has been reported that Cd-Zn tolerant AMF could be used for biotechnological applications, however it is necessary to identify the role of these local communities in bioremediation processes. In this work we characterized local AMF community structure present in cacao rhizosphere with low and high natural concentrations of Cd and Zn in a cacao-producing region in Colombia. Characterization of these AMF communities will allow the identification of potential AMF stress-tolerant for the development of mitigation strategies in cacao plants under Cd-Zn stress. 2. Arbuscular Mycorrhiza Fungi (AMF) may reduce cadmium (Cd) and zinc (Zn) plant uptake under presence of those heavy metals in the soil. However, this response is dependent on different factors such as plant genotype and AMF inoculum. This research determined the effect of

projectID	P21
	local AMF communities on the physiology of two cacao-grafted rootstocks (IMC67 and CAU43, both grafted with FSV41) under Cd and Zn stress as a possible alternative for cultivating HMs-enriched soils in a Colombian region. The results of this project highlight the importance of host genotype-AMF interaction as an important factor that determines cacao physiological response under Cd and Zn stress.
Genetic variability in Cadmium uptake and partitioning	
Description	Grafting is a widespread cacao propagation technique in Colombia, this practice allows growers to clone cacao plants with better quality and yield traits. However, there is no experimental information regarding the effects of grafting over cadmium (Cd) and zinc (Zn) uptake and partitioning on cacao plants. This research assessed the effect of grafting over Cd-Zn uptake and plant physiology of two genotypes under Cd-Zn stress. We conducted an experiment using ungrafted, self-grafted and grafted plants from four different cacao genotypes, two genotypes as rootstocks (open pollinated seeds from IMC67, CAU43) and two as scions (FSV41, CCN51). This study will provide insights on plant Cd-Zn uptake and partitioning that can be used to select the lowest Cd-Zn accumulating cacao genotypes for nursery.
Physiological mechanisms of Cadmium uptake and partitioning	
Description	Cadmium (Cd) and zinc (Zn) are heavy metals (HMs) that may alter plant physiology depending on their concentration in the soil. However, in cacao these alterations only have been described under artificial conditions, which do not reflect HMs dynamics in natural soil-plant systems. In this research, nutrition, photosynthetic efficiency, HMs partitioning and growth of two cacao open pollinated genotypes (IMC67 and PA121) were assessed in natural enriched soils with low and high Cd-Zn concentrations. Results from this research provide novel information about antagonistic cationic interactions, Cd-Zn plant accumulation and physiological alterations of cacao plants grown in a natural Cd-Zn enriched soil.

projectID	P22
Project title	**Characterization of cadmium-resistant bacteria and fungi from cacao rhizosphere**
Lead/implementing institution	Universidad Nacional de Colombia, sede Bogotá (UNAL) Federación Nacional de cacaoteros de Colombia (FEDECACAO)
Key experts	Henry Novoa (AGROSAVIA, UNAL), Jeimmy Alexandra Cáceres Zambrano (UNAL), Esperanza Torres Rojas (UNAL)
Funding sources	UNAL, Gobernación de Cundinamarca, Corredor tecnológico Agroindustrial, FEDECACAO.
Geographical scope	Colombia
Start date	2015
End date	2019
General objective	To characterize Cadmium-resistant bacteria and fungi from cacao-cultivated in Cadmium natural enriched soils
Phytoremediation and bioremediation	
Type(s) of intervention	Bacteria and fungi isolated from cacao-cultivated in Cadmium natural enriched soils
Description	Heavy metal bioremediation using bacteria and fungi could be an efficient, environmentally friendly, and reasonably low-cost strategies for

projectID	P22
	management of Cadmium polluted soils. In this research Cadmium-resistant bacteria and fungi associated to cacao rhizosphere were isolated and identified through morphological and molecular markers. Their ability to solubilize phosphorous, fix nitrogen, and degrade cellulose were also evaluated. The results of this study provide knowledge of Cadmium-resistant microorganisms associated to cacao crop and highlights potential strains for biotechnology-based strategies to mitigate the cacao Cadmium uptake.

projectID	P23
Project title	**Technological improvement of cacao production in the Provinces of Rionegro and Alto Magdalena, Cundinamarca**
Lead/implementing institution	Universidad Nacional de Colombia, sede Bogotá (UNAL)
Key experts	John Fernando Soler Arias (UNAL), Heidy Soledad Rodríguez Albarracín (UNAL), Martha Cecilia Henao Toro (UNAL)
Funding sources	Gobernación de Cundinamarca (Corredor Tecnológico Agroindustrial Derivado 2)
Geographical scope	Colombia
Start date	2017
End date	2019
General objective	Assess the risk of contamination of the cacao bean with cadmium in response to the concentration of total and available cadmium in soil, in productive systems of two regions of the Colombian Andean zone
Measuring Cd levels in soils or cacao beans and soil mapping	
Locations/Area covered	
Description	1. Spatial modelling of the cadmium content in soils cultivated with cacao. An observational methodology was followed, through an exploratory sampling, to establish the relationship of the spatial variability of Cadmium and some chemical properties of the soil, with the contents of Cadmium in the plant. Zones within the region with higher concentrations of cadmium were identified. 2. Dynamics of cadmium in stony soils with high levels of the element in beans. In farms with high levels of Cadmium in cacao beans, we focused on the determination of Cadmium at two depths of the soil (0-30 and 60-100 cm), and the fractionation of the element (determination of exchangeable, carbonates, organic matter and iron and manganese oxides phases). The results show a high spatial variability of Cadmium levels in soil and plant, both at the municipal level and within farms.

Identifying sources of Cd contamination in the soil	
Sources investigated	Anthropogenic vs. natural
Description	For making a diagnosis of Cadmium concentration in soils and cacao beans and evaluating if Cadmium contents in plants is of geogenic or anthropogenic origin, a hundred farms were chosen, and one cacao tree with mature fruits was sampled in each farm for determination of Cadmium in beans. The pseudo-total Cadmium and bio-available Cadmium end other soil properties (pH, organic carbon, P, Fe, Mn, Zn and Cu), in the soils around the tree, were evaluated. Cadmium levels in fertilisers and amendments and in parent material of soils were also analysed.
Geo-chemical properties of the soil, agronomic factors and Cadmium bioavailability/uptake	
Description	For making a diagnosis of Cadmium concentration in soils and cacao beans and evaluating if Cadmium contents in plants is of geogenic or anthropogenic origin, a hundred farms were chosen, and one cacao tree with mature fruits was sampled in each farm for determination of Cadmium in beans. The pseudo-total Cadmium and bio-available Cadmium end other soil properties (pH, organic carbon, P, Fe, Mn, Zn and Cu), in the soils around the tree, were evaluated. Cadmium levels in fertilisers and amendments and in parent material of soils were also analysed.
Physiological mechanisms of Cadmium uptake and partitioning	
Description	In cacao crops selected, a plant with mature fruits was sampled. The Cadmium in leaf and fruit tissues (shell, bean and pod husk) was analysed. Cadmium in soil and cacao leaf litter around trees was also determined. Bioaccumulation factor (BF) was calculated as the ratio of Cadmium in leaf or bean to that in soil, and Translocation factor (TF) as the ratio of Cadmium in leaf to that in fruit tissues. It was evaluated that cacao plant can be considered as cadmium accumulator. The risk of Cadmium cycling in crops of the study area can be high, as litter is a direct product of foliar abscission of cacao during all year, and leaf is the organ with the highest concentration of Cadmium.

projectID	P24
Project title	**Cadmium in cocoa production and technological strategies for its management**
Lead/implementing institution	AGROSAVIA
Key experts	Daniel Bravo (Project manager and leader in bioremediation research), Nesrine Chaali, Rocio Gámez, Andrea Montenegro, Margarita Gómez, Clara León, Diana Serralde, Viviana Varón, Ruth Quiroga, Gustavo Araujo, Urley Pérez, Gersain Rengifo, Santiago López, Juan Gil.
Collaborators	FEDECACAO, ESPOL, CasaLuker
Funding sources	Ministry of Agriculture and Rural Development of Colombia (MADR)
Geographical scope	Colombia
Start date	2019
General objective	Characterize the presence of Cadmium in Cocoa cultivation to generate mitigation strategies in soils, plants and beans
Description	Although some of the aspects in the Cadmium issue have been identified, the critical aspects of contamination remain with certain complexity. The origin of cadmium should be considered – geogenic or anthropogenic, as discussed in previous studies – as well as the sources that maintain the cadmium-flow to the beans at the ecosystem level. However, given the complexity of the distribution of the metal, it must also be studied locally at the farm level, even per hectare cultivated within a farm. Indeed, there are

projectID	P24
	many questions to be answered regarding cadmium's space time behavior for different users in the value chain (i.e. producers, academy, the industry and regulators). In farms selected from Santander and Antioquia districts, we are measuring Cadmium and using CdtB and analyzing both geological and biological aspects of the Cd-flux on the system. The Cadmium in leaves and fruits, including also shells, beans and pod husk are analyzed. Cadmium in soils and cacao leaf litter around trees are also considered. As in a previous work, Bioaccumulation factor (BF) is calculated as the ratio of Cadmium in leaves or beans to that in soils, and Translocation factor (TF) as the ratio of Cadmium in leaves to that in fruit tissues. Due to the complexity of the issue, mitigating the presence of cadmium requires solutions with multi-method and multi-approach contributions that will allow i) delve into the sources of Cadmium to tackle it using a holistic perspective, which include more specific strategies at the local level; and ii) defining accurate strategies in the short, medium and long term, with impacts on national cocoa production. The portfolio of technological offers, therefore, must be varied and consistent with both regulatory impositions, as well as AGROSAVIA's corporate capabilities, expertise and international cooperation to generate potential solutions to cacao farmers of Colombia.

projectID	P25.1
Project title	**Selection of promising cocoa genotypes by agronomic attributes of interest: genetic diversity, productivity criteria, disease resistance and cadmium absorption**
Lead/implementing institution	AGROSAVIA
Key experts	Roxana Yockteng; Caren Rodríguez; Andrea Montenegro, Jaime Osorio
Funding sources	Ministry of Agriculture and Rural Development of Colombia (MADR)
Geographical scope	Colombia
Start date	2019
General objective	Perform bioprospecting of the available germplasm (germplasm banks and work collections) by means of phenotypic, molecular characterization, by cadmium absorption and compatibility to select promising genotypes ("core collection") that will feed the genetic improvement program
Genetic variability in cadmium uptake and partitioning	
Description	Colombia not only has a great genetic diversity of the Theobroma cacao species (Osorio et al. 2017), but also a great diversity of the wild sister species of cocoa. When analyzing samples from different herbaria in the country, reports were found for the species of the 22 species of Theobroma genus and 10 species of the 17 Herrania genus descriptions. Despite this intraspecific variability of T. cacao and interspecific of the sister species in Colombia, cocoa cultivation presents problems resulting from the loss of this variability. The use of monoclonal crops has led to the loss of this variability making these plantations more susceptible to diseases such as moniliasis and black cob (Evans 2007) and generating low productivity due to self-incompatibility. In addition, cocoa is a heavy metal accumulator plant, particularly Cadmium. The presence of this element in the cocoa produced in Colombia (León Moreno 2012) generates a problem of food security compared to national and international markets since cocoa cannot be commercialized if the levels of cadmium in the grain exceed the permissible levels In this project, the studies of genotype association with the phenotype will

projectID	P25.1
	continue as the phenotypic databases (incidence of diseases, data associated with productivity, etc.) are fed back

projectID	P25.2
Project title	**Identification of agronomic attributes in cocoa whose expression is affected by the pattern with a view to establishing a program of genetic improvement of patterns that contributes to enhancing the behavior of clones of interest**
Lead/implementing institution	AGROSAVIA
Key experts	Caren Rodríguez, Roxana Yockteng, Andrea Montenegro, Jaime Osorio, Xavier Argout
Funding sources	Ministry of Agriculture and Rural Development of Colombia (MADR)
Geographical scope	Colombia
Start date	2019
General objective	To study the effect of the pattern on the expression of agronomic attributes of interest in the cup oriented to establish a program of genetic improvement of patterns in cocoa
Genetic variability in cadmium uptake and partitioning	
Description	In Colombia, the propagation of Theobroma cacao is mainly carried out through the grafting technique, combining attributes of interest between cups and patterns; however, in the country the genetic basis of the materials used as standards is narrow, based mainly on the use of progenies of 3 genotypes selected for their adaptability to acidic soil conditions and resistance to Ceratocystis sp. In order to respond to the current challenges facing Colombian cocoa farming, including poor performance, limiting diseases and abiotic stress such as water deficit and cadmium absorption, it is necessary to ensure a broader genetic base that allows responding to the specific needs of producers of grain of the country, as well as international markets. In a first stage of research, the agronomic attributes for which the pattern has an effect on the expression of these in the cup will be identified. The results of the first phases will contribute to defining the strategy to be adopted for each attribute in a genetic improvement program as described below: 1. When there is no effect of the pattern on the cup, the attribute will not be considered for the selection of patterns 2. When the influence of the pattern on the cup is important, the attribute will be considered in the pattern improvement program 3. When the influence of the pattern and the cup / pattern interaction are important, the attribute will be considered and the selection of the patterns will be implemented using combinations with clones of interest such as cup 4. Once the attributes for which the pattern exerts an effect and the cup / pattern interaction are important, attributes of interest in the pattern will be combined from a program of genetic improvement

projectID	P26
Project title	**Accreditation of techniques for the determination of pesticide residues in pineapple, cashew, rice and cadmium and arsenic contaminants in rice and cadmium in cocoa.**
Lead/implementing institution	AGROSAVIA
Key experts	María Angelica Pichimata, Andrea Montenegro, Yeni Rodriguez
Funding sources	Ministry of Agriculture and Rural Development of Colombia (MADR)
Geographical scope	Colombia
Start date	2019
General objective	Prove the techniques for the determination of pesticide residues in pineapple, rice, cashew and contaminants cadmium and arsenic in rice and cadmium in cocoa, in accordance with the requirements established by the standard NTCISO / IEC 17025: 2017
Soil and nutrient management	
Description	The Corporation has been working with different projects related to food safety-quality in a transversal way in all research networks, for this reason it is important and relevant to have accredited techniques that allow obtaining reliable and traceable results at an international level with respect to to the contents of As, Cadmium and pesticide residues in food. This research project aims to accredit the techniques for the determination of pesticide residues in pineapple, rice, cashew and cadmium and arsenic contaminants in rice and cadmium in cocoa, in accordance with the requirements established by NTC ISO / IEC 17025: 2017, which will be addressed in approximately two years of execution and will generate 3 technological offers associated with the provision of laboratory services with these techniques. The results of this project may benefit the academic and scientific community so that new research projects can be generated, the monitoring and control organizations, the producers, companies and exporters that require such analysis

projectID	P27
Project title	**Determination of the origin of Cadmium and the strategies to mitigate Metal levels in cocoa beans and soils in Arauca**
Lead/implementing institution	AGROSAVIA
Key experts	Daniel Bravo; Carlos González; Mario Porcel; Gustavo Araujo; Marcela López; Rafael Novoa; Gersain Rengifo.
Funding sources	Ministry of Agriculture and Rural Development of Colombia (MADR)
Geographical scope	Colombia
Start date	2019
General objective	Contribute to the mitigation of cadmium levels in cocoa by generating new recommendations and technologies
Soil and nutrient management	
Description	Regarding safety, it is proposed to develop an innovative fertilization strategy that is based on the initial cadmium levels in the soils - cocoa beans ratio, as a starting point for soil work. With a multi-method approach with precision agriculture tools, such as geoelectric, geomicrobiology can recommend a

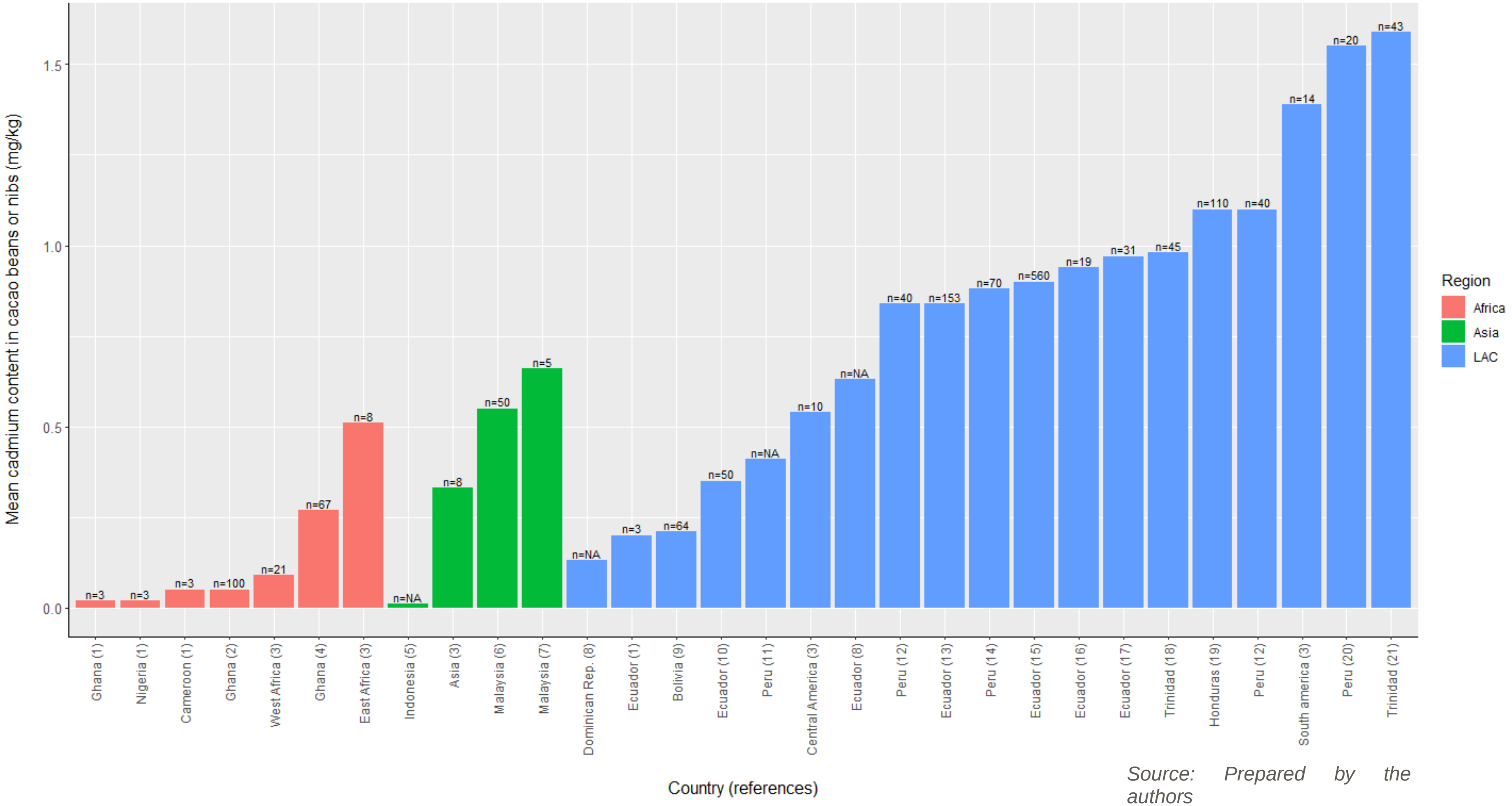
projectID	P27
	comprehensive fertilization and management recommendations will be generated to mitigate the Cadmium both in soils and cocoa beans, providing criteria of decision to reduce levels of cadmium in the medium and long term, and allowing to improve and maintain over time the state of safety of the grains in Arauca.

6.2.6 Indonesia

projectID	P28
Project title	**Cadmium mitigation through soil amelioration and genotype screening**
Lead/implementing institution	ICCRI
Key experts	Soetanto Abdoellah Soeparto, Erwin Prastowo, Niken Puspitasari, Indah Anitasari, Bayu Setyawan
Funding sources	ICCRI
Geographical scope	Indonesia
Start date	2018
End date	2020
General objective	• Screening of some rootstock on absorption of cadmium from soil • The effect of organic matter on cadmium absorption • Survey of cadmium contents in cacao beans produced from production areas in Indonesia and those imported from other countries

7 ANNEX

7.1 Annex 1 – Average Cadmium bean content reported by studies across cacao-growing regions (full references on next pages)



Source: Prepared by the authors

(1)(Vitola et al. 2016); (2)(Amankwaah et al. 2015); (3)(Bertoldi et al. 2016); (4)(Takrama et al. 2015); (5)(Assa et al. 2018); (6)(Fauziah et al. 2001); (7)(Zarcinas et al. 2004); (8)(Kruszewski et al. 2018); (9)(Gramlich et al. 2017); (10)(Acosta et al. 2013); (11)(Llatance 2018); (12)(Tantalean Pedraza et al. 2017); (13)(Mite et al. 2010); (14)(Arévalo-Gardini et al. 2017); (15)(Argüello et al. 2019); (16)(Chavez et al. 2015) ; (17)(Barraza et al. 2017); (18)(Ramtahal, Yen, Bekele, et al. 2015); (19)(Gramlich et al. 2018); (20)(Cárdenas 2012); (21)(Ramtahal et al. 2014)

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7.1.2 Annex 1.2 – Cacao bean cadmium content (mg/kg) reported in studies across Africa, Asia and LAC

Study (Full reference in Annex 1.1)	**Region**	**Country**	**n**	**Mean**	**Sd**	**min**	**max**
Vitola and Ciprovica, 2016	Africa	Ghana	3	**0.02**	0.003	NA	NA
Vitola and Ciprovica, 2016	Africa	Nigeria	3	**0.02**	0.003	NA	NA
Vitola and Ciprovica 2016	Africa	Cameroon	3	**0.05**	0.010	NA	NA
Amankwaah et al. 2015	Africa	Ghana	100	**0.05**	NA	0.005	0.095
Bertoldi et al. 2016	Africa	West Africa	21	**0.09**	0.042	NA	NA
Takrama et al. 2015	Africa	Ghana	67	**0.27**	NA	0.248	0.336
Bertoldi et al. 2016	Africa	East Africa	8	**0.51**	NA	NA	NA
Assa et al. 2018	Asia	Indonesia	NA	**0.01**	NA	NA	NA
Bertoldi et al. 2016	Asia	Asia	8	**0.33**	0.176	NA	NA
Fauziah et al. 2001	Asia	Malaysia	50	**0.55**	0.109	NA	NA
Zarcinas et al. 2004	Asia	Malaysia	5	**0.66**	NA	0.204	1.680
Kruszewski et al. 2018	LAC	Dominican Rep.	NA	**0.13**	0.031	NA	NA
Vitola and Ciprovica 2016	LAC	Ecuador	3	**0.20**	0.040	NA	NA
Gramlich et al. 2017	LAC	Bolivia	64	**0.21**	0.020	NA	NA
Acosta and Pozo,2013 (2)	LAC	Ecuador	50	**0.35**	NA	NA	NA
Llatance et al 2018	LAC	Peru		**0.41**	NA	NA	NA
Bertoldi et al. 2016	LAC	Central America	10	**0.54**	0.302	NA	NA
Kruszewski et al. 2018	LAC	Ecuador	NA	**0.63**	0.067	NA	NA
Tantalean et al. 2017	LAC	Peru	40	**0.84**	NA	NA	NA
Mite et al. 2010	LAC	Ecuador	153	**0.84**	NA	0.320	1.800
Argüello et al. 2019	LAC	Ecuador	560	**0.90**	NA	0.03	10.4
Chavez et al. 2015	LAC	Ecuador	19	**0.94**	NA	0.020	3.000
Barraza et al. 2017	LAC	Ecuador	31	**0.97**	0.84	0.09	3.51
Ramtahal 2015	LAC	Trinidad	45	**0.98**	0.248	NA	NA
Arévalo-Gardini et al. 2017	LAC	Peru	70	**0.88**	0.0.30	0.17	1.78
Gramlich et al. 2018	LAC	Honduras	110	**1.10**	0.100	NA	NA
Tantalean et al. 2017	LAC	Peru	40	**1.10**	NA	NA	NA
Bertoldi et al. 2016	LAC	South America	14	**1.39**	1.089	NA	NA
Cárdenas, A. 2012	LAC	Peru	20	**1.55**	NA	NA	NA
Ramtahal et al. 2014	LAC	Trinidad	43	**1.59**	0.152	NA	NA

Source: Prepared by the authors

n=sample size; SD=standard deviation; min=minimum; max=maximum; NA=not available

7.2 Annex 2 – Results from baseline studies

***Studies: 1 (Gramlich et al. 2017); 2 (Gramlich et al. 2018); 3 (Argüello et al. 2019); 4 (Barraza et al. 2017); 5 (Fauziah et al. 2001); 6 (Arévalo-Gardini et al. 2017); 7 (Huamani et al. 2011) ; 8 (Huamaní-Yupanqui et al. 2012); 9 (Jonas 2016)*

	Response variable	**Bioavailable Soil Cadmium**						**Bean Cadmium**												**Husk Cadmium**				**Leaf Cadmium**				
Study*		**1**	**2**	**2**	**2**	**5**	**8**	**1**	**2**	**2**	**2**	**3**	**3**	**3**	**3**	**3**	**4**	**7**	**9**	**1**	**2**	**2**	**5**	**1**	**1**	**2**	**5**	**2**
Type of analysis		MR	MR	MR	MR	PC	PC	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	SR	PC	MR	MR	MR	PC	MR	MR	MR	PC	MR
pvalue threshold		0.05	0.05	0.05	0.05		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.01	0.05	0.05	0.05	0.01	0.05
R-squared		0.38 - 0.55	0.88	0.85	0.87			NA	0.53	0.57	0.69	0.48	0.57	0.65	0.45	0.65		0.18		0.24	0.62	0.73		0.60	0.59	0.59		0.57
Cadmium content	Total soil cadmium		+	+	+					+		+	+	+		+	+	+				+	+				+	+
	Bioavailable cadmium								+												+			+	+	+		
pH	soil Ph	-		-						-		-	-	-		-						-						
OM	Organic matter		-	-									-	-		-						-		-	-			-
Soil type	Geological substrate		Yes	Yes	Yes					Yes	Yes				Yes							Yes						
Soil Texture	Clay content	+	+	+	+																-							
	Sand content						-																					
CEC	CEC																											
Salinity	EC																											
Micro-macro-nutrients	Zinc																											
	Fe	+	+		+																							+
	P	-				+													+	-								
	Pb																											
	Ca 2+																											
	Mg2+																									-		
	K																											-
	Mn																		-									

	Response variable	Bioavailable Soil Cadmium						Bean Cadmium												Husk Cadmium				Leaf Cadmium				
Soil Microbial activity	Mycorrhizal colonisation																											
Agronomic factors	fertiliser application																											
	Monoculture vs agroforestery																			Yes				Yes				
	Organic vs conventional																Yes											
	Age of orchard																-											
	Trunk diameter																								Yes			
	Cultivar effect																			Yes				Yes				
Other factors	Altitude																											
	Proximity to industrial site																											
	Impact of oil activities																											

Source: Prepared by the authors

MR/SR *Multiple/Single regression*
PC *Pearson correlation*
+ *Positive relationship (increase leads to an increase of response variable)*
- *Negative relationship (increase leads to a decrease of response variable)*
Yes *Statistically significant effect on response variable*

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