

# CODEX ALIMENTARIUS COMMISSION



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Agenda Item 7

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## JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON CONTAMINANTS IN FOODS

14<sup>th</sup> Session

Utrecht, The Netherlands, 20 – 24 April 2020

### PROPOSED DRAFT CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF CADMIUM CONTAMINATION IN COCOA BEANS

(At Step 4)

(Prepared by the Electronic Working Group  
chaired by Peru and co-chaired by Ecuador and Ghana)

Codex members and observers wishing to submit comments at Step 3 on this document should do so as instructed in CL 2020/20/OCS-CF available on the Codex webpage/Circular Letters:

<http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/>

#### BACKGROUND

- At the 11<sup>th</sup> meeting of the Codex Committee on Contaminants in Foods (CCCF11, 2017), Peru presented a proposal for the development of a Code of Practice (COP) to guide Member States and the cocoa production industry in preventing and reducing cadmium (Cd) contamination in cocoa beans during the production and processing phases. The Committee agreed to establish an Electronic Working Group (EWG), chaired by Peru, to prepare a discussion paper for consideration on the opportunity to develop such COP and the risk mitigation measures available to support the development of a COP.<sup>1</sup>
- At CCCF12 (2018), Peru presented the discussion paper and stressed the usefulness of administering a survey to gather information on validated practices throughout the food chain for the prevention and reduction of cadmium contamination in cocoa prior to starting new work on the development of a COP. To gather this information, the Committee agreed that a circular letter would be prepared for the survey and distributed by the Codex Secretariat. The view was expressed that, in the conclusions, the only points that should be listed are those which are relevant for the development of the COP. The JECFA Secretariat requested the Committee to pay a particular attention to mitigation measures that would be feasible even for small-scale farmers to apply.
- CCCF12 agreed to re-establish the EWG chaired by Peru and co-chaired by Ecuador and Ghana to further elaborate the discussion paper to: (i) determine whether mitigation measures available at present would support the development of the COP and (ii) identify the scope of the COP (e.g. whether the COP will cover the whole production chain or only primary production) based on the replies provided to the survey. The EWG should focus its work on mitigation measures that are proven to be cost-effective and applicable worldwide by large and small-scale producers.<sup>2</sup>
- At CCCF13 (2019), Peru introduced the discussion paper and indicated that risk management measures available to-date support the development of a COP during primary production and post-harvest (i.e. fermentation, drying and storage processes). Such measures were validated to be feasible, cost-effective and applicable worldwide by large, medium and small farmers. Manufacturing / processing practices that could effectively reduce cadmium levels in processed products (e.g. chocolates) would not be included in the scope of the COP as they were not yet readily available. However, there was ongoing studies to reduce cadmium contamination at different stages of the processing chain that were being carried out in different countries that could be incorporated in the COP in future. The COP would help to reduce cadmium contamination in cocoa beans and their products and would facilitate the application and compliance with the MLs for cadmium in chocolates and chocolate products.

<sup>1</sup> REP17/CF, paras. 154-155

<sup>2</sup> REP18/CF, paras. 141-146

5. CCCF13 thus agreed to: (i) submit the project document<sup>3</sup> to CAC42 for approval as new work and (ii) establish an EWG, chaired by Peru and co-chaired by Ghana and Ecuador to prepare, subject to the approval of CAC42, a draft COP based on the document provided in Appendix II to CX/CF 19/13/12, for comments and consideration at the next session of the Committee.<sup>4</sup>
6. The 42<sup>nd</sup> Session of the Codex Alimentarius Commission (CAC42, 2019) approved the new work.<sup>5</sup>
7. The proposed draft Code of practice for the prevention and reduction of cadmium contamination in cocoa beans is presented in Appendix I. A background document providing the basis for the provisions in the COP is presented in Appendix II for information. The List of Participants is presented in Appendix III.

#### **RECOMMENDATIONS**

8. CCCF is invited to consider the COP as set out in Appendix I together with the comments submitted in reply to CL 2020/20-CF.

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<sup>3</sup> REP19/CF, Appendix VIII

<sup>4</sup> REP19/CF, paras. 108-112

<sup>5</sup> REP19/CAC Appendix V

## APPENDIX I

### **PROPOSED DRAFT CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF CADMIUM CONTAMINATION IN COCOA BEANS (For comments at Step 3)**

#### **1. INTRODUCTION**

1. The objective of this proposed draft Code of Practice (COP) is to provide guidance to member states and the cocoa production industry on the prevention and reduction of cadmium (Cd) contamination in cocoa beans during production and postharvest processing: fermentation, drying and storage.
2. Cd is a heavy metal that predominantly enters the environment through anthropogenic activities such as processing ores, burning fuels, and waste, and the application of phosphate and sewage-containing fertilizers. Cd can also enter the soil naturally by volcanic activity, from marine shale soils, erosion or by sea-salt aerosols.
3. Cd is toxic and persistent in soil (estimated half-life for Cd in soils varying between 15 to 1100 years). Cd is absorbed and bioaccumulated by cocoa trees (*Theobroma cacao* L), which in some cases results in unacceptably high levels in cocoa beans, so measures are required to prevent Cd presence in the soil and reduce Cd absorption.
4. Cd is not found in nature in its pure state. Its most common oxidation state is +2 and it is usually found associated with iron (Fe), zinc (Zn), lead (Pb), phosphorus (P), magnesium (Mg), calcium (Ca), or copper (Cu) through its "cation exchange capacity". The concentrations of Cd in soil solution depend mainly on soil pH, which affects Cd solubility and mobility. Most metals in the soil tend to be more available at acidic pH, which increases the availability for plants.
5. Greater adsorption of Cd on the surface of soil particles is desirable, considering that this reduces the mobility of this contaminant in the soil profile and, consequently, its environmental impact. The concentration of heavy metals (Cd) in soil solution and, consequently, its bioavailability and mobility are mainly controlled by adsorption and desorption reactions on the surface of the soil colloids. Soil factors that affect the accumulation and availability of heavy metals include pH, texture, organic material, Fe and manganese (Mn) oxides and hydroxides, carbonates, salinity and cation exchange capacity.
6. Elevated chloride contents in soils tend to enhance chloride complex formation, which decreases the adsorption of Cd on sediment, thereby increasing Cd mobility and, decreasing the concentration of dissolved Cd<sup>+2</sup> and bioavailability.
7. The understanding on how various cropping systems contribute or alleviate cadmium contamination in cocoa beans could be used to develop integrated systems for the management of cadmium levels in cocoa beans.
8. The grafting tool as a genetic strategy with low cadmium accumulation varieties is a viable option in various soil types and different Cd levels.
9. It is crucial identifying Cd areas in a country, understanding the source of Cd in each area and developing specific and general strategies to address this problem.

#### **2. SCOPE**

10. The scope of this Code of Practice is to provide guidance on recommended practices to prevent and reduce Cd contamination in cocoa beans before planting or for new plantations and during the production stage through the harvest and post-harvest phase.

#### **3. DEFINITIONS**

**Cocoa bean:** The seed of the cocoa fruit composed of episperm (integument), embryo and cotyledon.

**Cocoa pod:** The cocoa fruit pericarp that arises from the ripened ovary wall of a fruit.

**Episperm or Integument:** The protective layer of the seed also called shell when it is dried. The outermost of the two layers that constitute the tegument is called Testa.

**Pulp or mucilage:** Aqueous, mucilaginous and acidic substance in which the seeds are embedded.

**Harvesting and opening the fruits:** Fruits are manually harvested and opened using a sickle, machete or wooden baton.

**Sea-salt aerosols:** They are the most omnipresent natural aerosols over the oceanic region. Aerosols are one of the main decisive components for the radiative forcing of the Earth system. Extensive measurements reveal that processes associated with the bursting of the white-cap and wave breaking primarily generate sea-salt aerosols. In terms of primary marine aerosol, studies have confirmed a significant flux of sub-micrometer sea-spray particles, even down to 10nm sizes and it is clear that like wind speed, sea surface temperature also affects the physical sea-spray source function. In terms of secondary marine aerosol formation, significant advances identify particle production, at least in coastal zones where iodine oxides are considered the dominant species leading to particle production and contributing to growth can also be isoprene oxidation products and sulfuric acids.

**Bioremediation:** It is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms.

**Phytoremediation:** It is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil and groundwater.

**Air emissions:** They are defined as unwanted gaseous or particulate materials released to the atmosphere as a direct result of production, accumulation or consumption activities in the economy.

**Traceability:** is the ability to follow the movement of a food through specified stages of production, processing and distribution using records.

**Bioavailability:** Bioavailability of a mineral in nutrition can be defined as its accessibility to normal metabolic and physiological processes. In surface and ground water, sediment and air, bioavailability of metals (Cd) is a complex function of many factors including total concentration and speciation (physical-chemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content (both particulate and dissolved fractions), and suspended particulate content, as well as volume of water, water velocity, and duration of water availability, particularly in arid and semi-arid environments. In addition, wind transport and removal from the atmosphere by rainfall (frequency is more important than amount) must be considered. Many of these factors vary seasonally and temporally, and are interrelated.

**Geoavailability:** Geoavailability of an element or chemical compound of a terrestrial material is that portion of its total content that can be released to the surface or near the surface (or biosphere) by mechanical, chemical, or natural biological processes.

**Adsorption, Absorption and Desorption:** Physical, chemical or exchange adsorption is a concept that refers to the attraction and retention that a body makes on its surface of ions, atoms or molecules that belong to a different body. Absorption is a term that refers to the damping exerted by a body before a radiation that passes through it; to the attraction developed by a solid on a liquid with the intention that its molecules penetrate into its substance; to the ability of a tissue or a cell to receive a material that comes from its outside. Desorption is the process of removing an absorbed or adsorbed substance.

**Cation Exchange Capacity (CEC):** It is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton and Murphy 2007). The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force. This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. Mg, K and Ca).

**Redox reaction:** Reactions of oxidation and reduction that occur simultaneously and they are known to be inseparable — as one atom loses an electron, the other gains an electron, hence completing the redox cycle.

**Complexation reaction:** It is a reaction that forms a "complex". In addition, the reaction between a cation and one or more anions is very important in soil systems. Metal complexes are stable species that are less likely to participate in sorption, precipitation, and even redox reactions.

**Electrical conductivity:** Electrical conductivity in metals is a result of the movement of electrically charged particles. The atoms of metal elements are characterized by the presence of valence electrons, which are electrons in the outer shell of an atom that are free to move about. In addition, it is denoted by the symbol  $\sigma$  and has SI units of siemens per meter (S/m). Electrical conductivity of water samples is used as an indicator of how salt-free, ion-free, or impurity-free the sample is; the purer the water, the lower the conductivity (the higher the resistivity). Conductivity measurements in water are often reported as specific conductance, relative to the conductivity of pure water at 25 °C.

**Drying process:** Drying of cocoa beans either under sunlight or in mechanical/solar dryers (or a combination of both) in order to reduce the moisture content to make them stable for storage.

**Fermentation:** process designed to degrade the pulp and initiate biochemical changes in the cotyledon by enzymes and microorganisms inherent in the environment of the farm.

**Soil Amendments:** They refer to any material added to the soil to improve its physical and chemical properties. The applications of the amendments depend on the characteristics of the soils. The amendments reported in the studies for the elaboration of this COP were: compost (refers to humus that is obtained of artificial manner when organic waste is decomposed by organisms and beneficial microorganisms), magnesium carbonate, vinasse (a by-product of the production of alcohol from sugarcane), zeolite (minerals that stand out for their ability to hydrate and dehydrate reversibly, adsorbents); charcoal o biochar; calcium sulphate, lime, cachaza (by-product of sugar cane), zinc sulphate, dolomite (calcium carbonate and magnesium), vermicompost, sugar cane, palm kernel cake, phosphate rock, organic matter.

**Validation:** Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.

**Sampling:** Procedure used to draw or constitute a sample. Empirical or punctual sampling procedures are sampling procedures, which are not statistical-based procedures that are used to make a decision on the inspected lot.

**Organic agriculture:** It is a holistic production management system which promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, cultural, biological and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system. An organic production system is designed to:

- a) enhance biological diversity within the whole system;
- b) increase soil biological activity;
- c) maintain long-term soil fertility;
- d) recycle wastes of plant and animal origin in order to return nutrients to the land, thus minimizing the use of non-renewable resources;
- e) rely on renewable resources in locally organized agricultural systems;
- f) promote the healthy use of soil, water and air as well as minimize all forms of pollution thereto that may result from agricultural practices;
- g) handle agricultural products with emphasis on careful processing methods in order to maintain the organic integrity and vital qualities of the product at all stages;
- h) become established on any existing farm through a period of conversion, the appropriate length of which is determined by site-specific factors such as the history of the land, and type of crops and livestock to be produced.

**Pruning:** annually removal from shade trees and cocoa plants of branches that are dry, diseased or unbalanced.

**Shading:** Growing cocoa plants with shade trees to reduce the amount of radiation that reaches the crop and protect the crop from winds. Shading is usually more or less 50% during the first 4 years of plant life after which percentage of shade can be reduced to 25 or 30%.

#### 4. **RECOMMENDED PRACTICES TO PREVENT AND REDUCE Cd CONTAMINATION IN COCOA BEANS**

##### 4.1 **Contamination before sowing – new plantations**

11. The prevention and reduction of Cd in cocoa should begin with the physical-chemical analysis of the soil and be an integral part of the practices before sowing or establishment of a new plantation.
12. As prevention, when establishing new plantations, cocoa plantations should be located in areas where Cd content is low, so that agricultural soils should not exceed 1.4 mg/kg of Cd. and the content of Cd in irrigation water should not exceed the value of 0.005 mg /L.

13. Use a design of mixed plantations (agroforestry) with several varieties of cocoa and with different types of shade adapted to each ecological environment, instead of a monoculture of cocoa without shade.
14. The most commonly used species are musaceae (bananas, moles and cambures) for temporary shadows and legumes such as the pore or bucare (*Erythrina* sp.) and guabas (Ingas) for permanent shadows. In other cocoa plantations, other shading species are being used that provide greater economic benefits such as timber species (laurel, cedar, cenizaro or rain tree and terminalia) and / or fruit trees (citrus, avocado, sapote, breadfruit, date palm etc.).
15. Install plantations in areas far from roads or take measures to reduce the exposure of the cacao plantations to gases emitted by the combustion of vehicles because they may contain Cd (should be spaced 200 meters apart from the cacao plantation). Likewise, they should be located in areas separated from dumps in cities or mining areas.
16. Avoid flooded soils because they could be a source of Cd.
17. In new plantations, the use of cover crops of perennial legumes should be considered. Cover crops improve soil organic matter and they can protect soil from erosion and reduce the loss of nutrients, improving soil productivity through greater availability of essential nutrients and reducing the bioavailability of heavy metals.

#### 4.2 **From the production to the harvesting phase**

18. Knowledge of the sources and the distribution of Cd in the soil is important.
19. Data suggest that there is a positive correlation between higher levels of Cd in soil (as measured by soil tests) and elevated levels of Cd in plant tissues and cocoa beans.
20. Soil characterization analysis laboratories for cocoa plantations should be conducted by laboratories that are accredited with the worldwide recognized ISO/IEC 17025:2018 standard; using validated methods which include the use of certified reference materials, standards and associated uncertainties. In addition, it is very important to carry out soil analyses with internationally recognized methods (e.g. endorsed by Codex Alimentarius).
21. The soil sampling protocol should consider obtaining a representative sample because Cd content could be variable in the same production area (finca o chacra, Spanish names for cocoa farms) of cocoa.
22. The determination of soil and irrigation water salinity (Cd chloride salts) is vital since the absorption of Cd by plants increases with chloride. Therefore, it is important to determine the electrical conductivity of soil and water which should be less than 2mS/cm.

##### 4.2.1 **Strategies to immobilize cadmium in the soil**

23. Zinc has a positive effect in reducing Cd content of the cocoa beans. The application of zinc sulphate is carried out with the balanced fertilization that is carried out annually to the cocoa plantation, according to the requirements of the crop and the soil - (characterization analysis). However, with the addition of zinc sulfate, soil acidification occurs, requiring addition of limestone.
24. When there is a deficiency of Zn in the soil, soil Zn levels should be increased. Cd competes with Zn, and Cd is more likely to enter the plant and accumulate in cocoa beans when Zn soil concentration is low.
25. Liming is an agronomic management practice that reduces Cd uptake by cocoa trees cultivated on highly acidic soils, and its addition also might improve nutrition and production of cocoa trees.
26. The most effective methods developed to date to decrease Cd bioavailability is through liming the soil when soil pH is below 5.5. It has been shown that increasing the pH by 1 unit reduces Cd in the cocoa bean by 1/10.
27. Apply lime in low doses (e.g. 2 to 3 MT dolomite/ha) to gradually increase the pH and incorporate Ca and Mg which is essential for the growth of cocoa and to precipitate Cd. Over liming should be avoided.
28. A greater amount of soil organic matter causes a lower absorption of Cd. The use of organic fertilizers such as treated manure from stabled livestock, compost, etc. increases the organic matter content of the soil and improves its microbiological activity. The application of 3 to 4 % of organic matter in cocoa plantations decreases cadmium in cocoa beans.

29. The use of phosphate fertilizers and sedimentary phosphoric rock should be carried out after chemical analysis because they usually have Cd as an impurity. Nonetheless, for successful cocoa production it is vital to add phosphate fertilizers because tropical soils have very limited native phosphorus content. However, the absolute concentration of Cd in phosphate fertilizer be considered. Producers and governments should control the use of phosphate fertilizers.
30. In general, the formula for the doses of nitrogen, phosphorus and potassium (NPK) in fertilizers to be applied to cocoa crop vary according to the age of the plant and the characteristics of the soil. Verify the heavy metal analysis prior of application to ensure that Cd content is low. Soils well supplied with nutrients are less likely to bioaccumulate Cd.
31. The application of amendments (magnesium carbonate ( $MgCO_3$ ), vinasse, zeolite, humus, charcoal, calcium sulfate ( $CaSO_4$ ), cachaza and zinc sulfate ( $ZnSO_4$ ), depending on the characteristics of the soils, can help decrease Cd concentrations in cocoa beans.
32. The application of vinasse, as a liquid fertilizer is a source of K that promotes the installation of fungi that form mycorrhizas in the roots of the cacao tree, increasing the efficiency of P nutrition and immobilizing Cd.
33. Lime and sugarcane cake can reduce the flow of Cd in the soil profile. Zeolite is another option in soils with high sand content and apatite in clay-textured soils.
34. Biochar has shown to reduce the bioavailability of Cd in cocoa beans. The reduction rates are comparable to liming and have an additive influence on liming. However, biochar or activated carbon is an expensive soil amendment and may not be cost effective for farmers who grow cocoa.
35. Biochar, compost and their combination have significant effects on soil physicochemical features, metals (Cd) availability and enzyme activities in heavy metal-polluted soil.
36. The genotypes identified with low bioaccumulation of Cd have the potential to be used as rootstocks in the production of propagation material to reduce the absorption of Cd from soil; however, additional field studies are needed.
37. The *Streptomyces* sp. strain has bioremediation activity as it reduces Cd uptake in cocoa plants
38. The legumes coinoculated with plant growth promoting bacteria Cd resistant could be useful in phytoremediation of Cd-contaminated soil and biofertilization.

#### 4.3 Post-harvest phase

39. An experimental study demonstrated that the draining of the mucilage for 12 hours significantly reduced the content of Cd in cocoa beans of the clonal hybrid (cultivar) CCN-51. The quantity of mucilage removed from the cocoa beans did not affect the physical or organoleptic quality of the cocoa at the time of the evaluation.
40. After fermentation, cocoa beans should be dried on clean solid surfaces to avoid its contamination by soil.
41. Make sure that cocoa beans are not contaminated with smoke, or with gases coming from dryers or vehicles.
42. The process of fermentation of cocoa beans should be the most important practice that any export organization should carry out to reduce the levels of Cd of their cocoa beans.
43. During storage, contamination of cocoa beans due to spills of fuels, exhaust gases or fumes should be prevented.
44. The longer the fermentation process (80 %), the less Cd in cocoa beans. Cd beans can be reduced if pH is sufficiently acidified during fermentation.
45. The strain of *Saccharomyces cerevisiae* is one of the strains that intervenes in cocoa fermentation, so increasing its population in such process could improve the absorption of Cd and the safety of cocoa.

**APPENDIX II**  
**BACKGROUND DOCUMENT**  
**(For information)**

**INTRODUCTION**

1. Cadmium (Cd) is a heavy metal that predominantly enters the environment through anthropogenic activities such as processing ores, burning fuels and waste, and the application of phosphate and sewage-containing fertilizers. Cadmium can also enter the soil naturally by volcanic activity and erosion or by sea-salt aerosols. It is not found in nature on its pure state. Volcanic activity is the main natural source of Cd release into the atmosphere (U.S.E.P.A cited by Sarabia 2002), with sedimentary rocks and marine phosphates being other natural sources of this metal. Its most common oxidation state is +2, for whose chemical affinity it is associated with iron (Fe), zinc (Zn), lead (Pb), phosphorus (P), magnesium (Mg), calcium, (Ca) and copper (Cu) through its cation exchange capacity. The concentrations of available Cd in soil depend mainly on its pH, which controls its solubility and mobility. Cd is a toxic heavy metal for biotic beings, persistent in the soil and its bioavailability changes depending of the properties of the soil. Cd is absorbed and bioaccumulated by cocoa (*Theobroma cacao* L) trees, resulting in some cases in unacceptably high levels of Cd in cocoa beans, which requires measures to reduce its uptake from soils.
2. The greater adsorption of Cd on the surface of the soil particles is desirable, bearing in mind that this reduces the mobility of this contaminant in the soil profile and, consequently, its environmental impact. The concentration of heavy metals (Cd) in the soil solution and, consequently, its Cd's bioavailability and mobility are mainly controlled by adsorption and desorption reactions on the surface of the soil colloids (Kabata-Pendias & Pendias, 2001). In reference to soil factors that affect the accumulation and availability of heavy metals, Miliarium (2009) cited by Cargua (2010), mentions: pH, texture, organic matter, Fe and Mn oxides and hydroxides, Carbonates, Salinity and capacity of cation exchange. Singh and Oeste 2001, cited by Carrillo, M (2010) mention that the techniques of immobilization of heavy metals in soils, based essentially on the adsorption phenomena, depend on the nature, concentration and physical-chemical state of the contaminant and soil characteristics. Moreover, biogeochemical processes that control cadmium mobility and availability in soil depend on precipitation dissolution, chelation-dissociation-complexation, mineralization-assimilation, protonation deprotonation, metal-organic ligand formation and redox reaction. Likewise, the relative importance of each process depends on the soil type and its pH, temperature, moisture and organic matter state and is subject to rhizosphere effects.
3. Gutiérrez E. and León C. 2017 report that the German Confederation of Confectioners (BDSI) contacted the Colombian Embassy in Germany to inform about the problem of heavy metal residues in cocoa, especially the existence of Cd in imports from Latin America and recommended determining the level of cadmium in crop soils and in cocoa beans; concluding, that if the Cd levels in the cocoa beans exceed the value of 0.5 mg/kg, it is recommended to investigate the origins of Cd in the crops in order to take the necessary measures.
4. The *Code of Practice Concerning Source Directed Measures to Reduce Contamination of Foods with Chemicals* (CXC 49-2001) reports that the advantage of eliminating or correcting contamination of environmental chemical contaminants (which includes Cd in cocoa) at its source is that the preventive approach is more effective in reducing or eliminating the risk of adverse effects on health and it requires fewer resources to control food and avoids the rejection of food products. Furthermore, it emphasizes that it is important to exercise concern throughout the entire chain of production - processing and distribution since food safety and quality in other aspects cannot be "inspected" at the end of the chain.
5. The Ministry of Environment 2008 Peru, through Supreme Decree No. 002-2008-MINAM approves "National Standards for water quality" and in its inorganic parameters for irrigation of vegetables and animal drinks decrees a value of 0.005 mg /L for Cd.
6. Ministerial Resolution of Peru. Ministry of Agriculture and Irrigation. 2018. Through R.M. N° 0451-2018-MI NAGRI <http://minagri.gob.pe/portal/resoluciones-ministeriales/rm-2018?start=65> approved the document called "Sampling guidelines for the determination of Cd levels in soils, leaves, cocoa beans and cocoa products", which aims to establish the reference methods for cadmium sampling in soils, leaves, cocoa beans and products derived from cocoa, with a uniform language, according to national and international protocols, which allows the national level to obtain an official baseline, in the different state and private institutions, with the purpose of being able to adequately plan the measures of cadmium mitigation and control in the cocoa areas where it has been verified that there are concentrations in beans above those established in the national and international standards.



This document was prepared in the framework of the implementation of the Country Cooperation Strategy of the Inter-American Institute for Cooperation on Agriculture (EIP-IICA), with the approved opinions of the institutions that form part of the "National Cadmium in Cocoa Technical Group" integrated by the General Agricultural Directorate-DGA, General Directorate of Agrarian Environmental Affairs DGAAA, National Water Authority - ANA, the National Service of Agrarian Health - SENASA and the National Institute of Agrarian Innovation - INIA of the Ministry of Agriculture and Irrigation of Peru, with the participation of the General Directorate of Environmental Health (DIGESA - Ministry of Health of Peru), which specifies the sampling keys of: Cd in soils, leaves of cocoa plantations, cocoa beans, in water of cocoa agricultural areas, products derived from the cocoa (cocoa paste, powder and chocolate), as well as the accreditation of laboratories and methods of analysis by entities nationwide or international.

7. The Ministerial Resolution above cited presents the factors that determine the absorption of Cd by plants, as it's seen below:

**Table 1. Edaphic and crop factors that determine Cd absorption by plants:**

Factors	Effect on the absorption of cadmium by plants
Edaphic factors include soil texture and microbiological activity in the list.	
1. pH	The absorption increases when pH decreases (acid soils)
2. Soil salinity	Absorption increases with salinity.
3. Amount of cadmium	Absorption increases with Cd concentration
4. Micronutrients	Zinc and Mn deficiency increase absorption
5. Macronutrients	They can increase or decrease the absorption
6. Temperature	High temperature increases absorption
Crop factors	
7. Species and cultivars	Vegetables>Roots>Cereals>Fruits It reads: Vegetables absorb more than roots, roots absorb more than cereals, and cereals absorb more than fruits.
8. Plant tissue	Leaves>grain>fruits and edible roots
9. Leave age	Old leaves>Young leaves

Source: (McLaughlin, Mike. 2016)

Furthermore, soil organic matter also has an influence, higher soil organic matter results in less absorption; cocoa in agroforestry system may have lower Cd values (Gramlich 2017), more work needs to be done to confirm this.

8. John, D. and Leventhal, S. (1995) citing McKinney and Rogers (1992) mentioned that one of the metals of major interest in bioavailability studies by the U.S. Environmental Protection Agency (EPA) was Cadmium. John and Leventhal also stressed that bioaccumulation of metals by biota in surface water and by plants and animals in terrestrial environments can adversely affect humans. Moreover, their section entitled Specific Metals of Interest – Cadmium, emphasize:
- The redox potential of sediment-water systems exerts controlling regulation on the chemical association of particulate Cd, whereas PH and salinity affect the stability of its various forms (Kersten 1988).
  - In anoxic environments, nearly all particulate Cd is complexed by insoluble organic matter or bound to sulfide minerals.
  - Greenockite (CdS), chemically cadmium sulfide, has extremely low solubility under reducing conditions thereby decreasing Cd bioavailability.

- Oxidation of reduced sediment or exposure to an acidic environment results in transformation of insoluble sulfide-bound Cd into more mobile and potentially bioavailable hydroxide, carbonate, and exchangeable forms (Kersten, 1988).
  - Studies of lake and fluvial sediment indicate that most cadmium is bound to exchangeable site, carbonate fraction, and iron-manganese oxide minerals, which can be exposed to chemical changes at the sediment-water interface, and are susceptible to remobilization in water (Schintu and others, 1991).
  - In oxidized, near neutral water, CdCO<sub>3</sub> limits the solubility of Cd<sup>2+</sup> (Kersten, 1988).
  - In a river polluted by base-metal mining, cadmium was the most mobile and potentially bioavailable metal and was primarily scavenged by non-detrital carbonate minerals, organic matter, and iron-manganese oxide minerals (Prusty and others, 1994).
  - Elevated chloride contents tend to enhance chloride complex formation, which decreases the adsorption of cadmium on sediment, thereby increasing cadmium mobility (Bourg, 1988) and, decreasing the concentration of dissolved Cd<sup>+2</sup> and bioavailability (Luoma, 1983).
9. Smolders (2001) reviewing factors affecting transfer of cadmium from soil and air to plants concluded that Crop Cd concentrations are strongly influenced by soil properties that control Cd availability in soil. Even at background Cd concentrations in soil, it is likely that crop Cd concentration exceeds marketable limits. This situation has been found in soils with chloride salinity and Zn deficiency and in crops such as sunflower kernels and durum wheat grain that usually contain high Cd concentrations. Direct deposition of Cd from air is a potential direct route by which Cd may enter the human food chain. The experimental evidence reviewed here indicates that this pathway can be neglected in areas with low Cd deposition rates (<2 g Cd ha<sup>-1</sup>y<sup>-1</sup>, typical of most rural areas in Europe). However, airborne Cd can be a dominant source of Cd in crops if Cd deposition is clearly elevated (>10 g Cd ha<sup>-1</sup> y<sup>-1</sup>), a situation that may occur around pyrometallurgic smelters.
10. Schneider, L (2016) on her research applying lime carried out a field experiment using 5 to 6 year old cocoa trees (CCN-51) in the province of Tocache - Peru, showed that surface application of lime in cocoa plantations is feasible and can bring positive changes in soil properties, even within a rather short period of time. Liming enabled an increase in topsoil pH and a decrease in the availability of Cd compared to the controls.
11. Barraza et al (2019) in the first pilot investigation employing coupled Cd isotope and concentration measurements to study soil – cacao systems, carried out analyses for 29 samples of soils, soil amendments and cacao tree organs from organic farms in Ecuador that harvest three distinct cacao cultivars, concluded: first, most of the soil samples from 0 to 80 cm depth have relatively uniform  $\delta^{114/110}$  Cd of about - 0.1 ‰ to 0 ‰. However, one sample from 60–80 cm is depleted in Cd and isotopically lighter, in accord with a weathering signature. Furthermore, two 0–5 cm top soils have high Cd concentrations coupled with heavy Cd isotope compositions of  $\delta^{114/110}$  Cd  $\approx$  0.2%. This distinct isotopic signature suggests that the enrichment is unlikely to be derived from organic or mineral P fertilizers, natural or anthropogenic atmospheric contributions or sediments from a nearby stream. Rather, the Cd additions are probably sourced from decomposing tree litter. Further study is required, however, to ascertain whether the Cd enrichments reflects recent use of tree litter as fertilizer in the cacao plantations or natural accumulation of Cd from decomposing tree material over a much longer timescale. Second, the beans of the three investigated cacao cultivars appear to show differences in Cd isotope signatures. In detail, beans from CCN-51 hybrid plants show no resolvable Cd isotope fractionation relative to the leaves whilst the relatively pure Nacional cultivar has beans that display lower  $\delta^{114/110}$  Cd than the leaves, with  $\Delta^{114/110}$  Cd<sub>bean-leaf</sub> values of -0.34 ‰ and - 0.40 ‰. As such, the data may suggest that the Nacional and CCN-51 cultivars differ in the molecular mechanisms that are employed for the translocation and sequestration of Cd between cacao leaves, pods and beans. In summary, these results suggest that further, more comprehensive, coupled Cd isotope and concentration studies should be carried out to confirm whether such analyses indeed provide useful constraints on the origin and cycling of Cd in soil – cacao systems and the partitioning of Cd within cacao plant. Peruvian SENASA's consultant suggests that in general there could be differences between native cacao beans and CCN-51 hybrid beans.
12. Table 2 below reports the factors that affect the bioavailability of Cd in cocoa beans.

**Table 2. Bioavailability of cadmium by cocoa beans**

Factor	Effect on the bioavailability of cadmium in cocoa beans
<p>Soil nutrition and deficiency of specific nutrients.</p> <p>The soil analysis should recommend the appropriate levels for fertilization and make the corresponding corrections; there is also a greater dilution effect.</p>	<p>Soils that are well supplied with nutrients are less likely to bioaccumulate Cd.</p>
<p>Oxide and hydroxides minerals Fe and Mn</p>	<p>They eliminate the potential bioavailability of Cd. They are controlled mainly by adsorption and desorption reactions on the surface of the soil colloids.</p>
<p>Soil pH</p>	<ul style="list-style-type: none"> <li>• The higher the acidity, the higher the level of Cd accumulation.</li> <li>• reduces the bioavailability of cadmium and its assimilation by tissues</li> </ul>
<p>Organic matter (O.M.) content</p>	<ul style="list-style-type: none"> <li>• Reacts with Cd resulting in chelates and Cd remains in position unavailable to plants.</li> <li>• The organic matter allows a higher cationic exchange capacity.</li> <li>• Under acidic conditions the O.M. of the soil governs the availability of cadmium</li> <li>• It provides carbon to soil microorganisms by increasing their enzymatic activity, volatilizing the Cd.</li> <li>• Have negatively charged sites on their surfaces that absorb and hold positively charged ions (cations) by electrostatic force</li> </ul>
<p>Liming</p> <p>Apply before sowing depending on the characteristics of the soil and should be repeated every 2 or 3 years.</p>	<ul style="list-style-type: none"> <li>• Increases the pH in the upper part of the soil</li> <li>• Decreases the availability of Cd</li> <li>• The most effective method developed so far to decrease the bioavailability of Cd is by liming the soil when its pH is below 5.5.</li> <li>• In low doses (2 to 3 MT dolomite /ha), to gradually increase the pH and incorporate Ca and magnesium essential for cocoa growth and precipitate Cd.</li> </ul>
<p>Biochar, compost and their combination.</p> <p>These amendments are organic sources (provide carbon). Biochar has a larger specific surface area and a greater capacity to retain heavy metals.</p>	<p>They have significant effects on the availability of heavy metals (Cd) in highly contaminated soils, on the physicochemical characteristics of the soil and on enzymatic activities in soils contaminated with heavy metals.</p>
<p>Soil texture</p>	<p>Greater accumulation in medium textures (lime), these greater than in fine textures (clays) and these greater than in thick textures (sand).</p>
<p>High chloride content</p>	<p>Tend to improve chloride complex formation, which decreases Cd absorption in the sediment by increasing Cd mobility and decreasing Cd<sup>+2</sup> concentration and bioavailability</p>
<p>Oxidized water, almost neutral</p>	<p>Limits the solubility of Cd<sup>+2</sup></p>

Factor	Effect on the bioavailability of cadmium in cocoa beans
The Cd carbonate	It limits the solubility of Cd <sup>+2</sup>
Greenockite (CdS) chemically cadmium sulfide	As it has an extremely low solubility in reducing conditions, bioavailability decreases.
Soil texture	Greater accumulation in medium textures (silt), these greater than fine textures (clay) and these greater than coarse textures (sand).
Leaf crops	They are Cd accumulators
Biochar, compost y su combinación	They have significant effects on the availability of heavy metals in highly contaminated soils
Fermentation and pH	The higher the fermentation (80%) the less cadmium in the cocoa beans. The Cd in the beans can be reduced if the pH is sufficiently acidified during fermentation.
Fe and Mn oxide minerals	Eliminate the potential bioavailability of Cd
Cocoa varieties. Genetic strategy for grafting	Several cocoa genotypes have low bioaccumulation of Cd. With low accumulation varieties of Cd, it is a viable option for propagation.
Soil nutrition	Soils that are well supplied with nutrients are less likely to bioaccumulate Cd.
Coverage with perennial legume crops	These crops improve soil organic matter, protect against erosion and reduce nutrient loss, thus improving soil productivity by reducing the bioavailability of the Cd.
The concentration of physical and chemical state of the contaminant in the soil	The biogeochemical process that controls the mobility and availability of Cd in the soil depends on precipitation-dissolution, chelation-dissociation complexity, mineralization-assimilation, protonation-deprotonation, formation of metal-organic ligands and redox reaction.
Soil amendments, (CO <sub>3</sub> Mg, vinasse, zeolite, humus, charcoal, SO <sub>4</sub> Ca, cachaça and SO <sub>4</sub> Zn)	<ul style="list-style-type: none"> <li>• Depending on the characteristics of the soil, they can serve to lower the Cd concentrations in the cocoa beans.</li> <li>• Vinasse promotes the installation of fungi that form mycorrhizae on the roots of the cocoa tree, increasing the efficiency of P nutrition and immobilizing Cd.</li> </ul>
Natural geological contamination	The presence of cocoa trees in areas close to mining or volcanic centers influence the bioaccumulation of Cd.
Fertilizers	P fertilizers applied according to soil test can be useful to increase growth and thus dilute the increase in Cd in plants.
Total Cd	Total Cd in the soil is irrelevant, however, the available cadmium is more important.
Cation exchange capacity	It reduces the phyto availability of Cd, it is the surface area of the oxides of Fe and Mn and the chelation capacity of the organic matter that adsorbs Cd and reduces the phyto availability. Clays in higher CEC soils are generally coated with hydrated oxides of Fe and Mn, so the clays correlate with Cd adsorption.

Factor	Effect on the bioavailability of cadmium in cocoa beans
Soil factors including: pH, texture, organic material, Fe and Mn oxides and hydroxides, carbonates, salinity and cation exchange capacity.	Affect the accumulation and availability of Cd
Absorption and desorption reactions on the surface of the soil colloids	The concentration of Cd in the soil solution and its bioavailability and mobility are controlled
The total concentration, speciation (physical or chemical forms) of metals, mineralogy, pH, redox potential, temperature, total organic content, water volume, wind transport, water speed, duration and availability of water, particularly in arid and semi-arid environments, wind transport and the removal from the atmosphere by rainfall, many of these factors vary seasonally and temporally, and are interrelated.	These factors predispose the accessibility of a mineral nutrient to normal metabolic and physiological processes in surface and groundwater, sediments and air affect the bioavailability of the Cd.

Source: Prepared by the EWG based on the revised references

## 2. DEFINITIONS

**Shoots:** bud, scion, or stem that sprouts again in cocoa plants.

**Cocoa bean:** The seed of the cocoa fruit composed of epispem (integument), embryo and cotyledon.

**Cocoa pod:** The cocoa fruit pericarp that arises from the ripened ovary wall of a fruit.

**Epispem or integument:** The protective layer of the seed also called shell when it is dried. The outermost of the two layers that make up the integument is called "testa".

**Pulp or mucilage:** Aqueous, mucilaginous and acidic substance in which the seeds are embedded.

**Harvesting and opening the fruits:** Fruits are manually harvested and opened using a sickle, machete or wooden baton.

**Sea-salt aerosols:** They are the most omnipresent natural aerosols over the oceanic region. Aerosols are one of the main decisive components for the radiative forcing of the Earth system. Extensive measurements reveal that processes associated with the bursting of the white-cap and wave breaking primarily generate sea-salt aerosols. Moreover, O'Dowd Colin D and de Leeuw Gerrit (2007) concluded that in terms of primary marine aerosol, studies have confirmed a significant flux of submicrometre sea-spray particles, even down to 10nm sizes and it is clear that like wind speed, sea surface temperature also affects the physical sea-spray source function. In terms of secondary marine aerosol formation, significant advances identify particle production, at least in coastal zones where iodine oxides are considered the dominant species leading to particle production. Contributing to growth can also be isoprene oxidation products and sulphuric acids

**Bioremediation:** It is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms.

**Phytoremediation:** It is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil and groundwater.

**Air emissions:** They are defined by the UN System of Environmental Economic Accounts (SEEA) as unwanted gaseous or particulate materials released to the atmosphere as a direct result of production, accumulation or consumption activities in the economy.

**Traceability:** It is the ability to follow the movement of a food through specified stages of production, processing and distribution using records.

**Geoavailability:** Geoavailability of an element or chemical compound of a terrestrial material is that portion of its total content that can be released to the surface or near the surface (or biosphere) by mechanical, chemical, or natural biological processes.

**Adsorption, Absorption and Desorption:** Physical, chemical or exchange adsorption is a concept that refers to the attraction and retention that a body makes on its surface of ions, atoms or molecules that belong to a different body. Absorption is a term that refers to the damping exerted by a body before a radiation that passes through it; to the attraction developed by a solid on a liquid with the intention that its molecules penetrate into its substance; to the ability of a tissue or a cell to receive a material that comes from its outside. Desorption is the process of removing an absorbed or adsorbed substance.

**Cation Exchange Capacity (CEC):** It is a measure of the soil's ability to hold positively charged ions. It is a very important soil property influencing soil structure stability, nutrient availability, soil pH and the soil's reaction to fertilizers and other ameliorants (Hazleton and Murphy 2007). The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic force. This electrical charge is critical to the supply of nutrients to plants because many nutrients exist as cations (e.g. magnesium, potassium and calcium).

**Redox reaction:** reactions of oxidation and reduction that occur simultaneously and they are known to be inseparable — as one atom loses an electron, the other gains an electron, hence completing the redox cycle.

**Complexation reaction:** it is as a reaction that forms a "complex". In addition, the reaction between a cation and one or more anions is very important in soil systems. Metal complexes are stable species that are less likely to participate in sorption, precipitation, and even redox reactions.

**Electrical conductivity:** Electrical conductivity in metals is a result of the movement of electrically charged particles. The atoms of metal elements are characterized by the presence of valence electrons, which are electrons in the outer shell of an atom that are free to move about. In addition, it is denoted by the symbol  $\sigma$  and has SI units of siemens per meter (S/m). Electrical conductivity of water samples is used as an indicator of how salt-free, ion-free, or impurity-free the sample is; the purer the water, the lower the conductivity (the higher the resistivity). Conductivity measurements in water are often reported as specific conductance, relative to the conductivity of pure water at 25 °C.

**Drying process:** Drying of cocoa beans either under sunlight or in mechanical/solar dryers (or a combination of both) in order to reduce the moisture content to make them stable for storage.

**Fermentation:** process designed to degrade the pulp and initiate biochemical changes in the cotyledon by enzymes and microorganisms inherent in the environment of the farm.

**Soil Amendments:** They refer to any material added to the soil to improve its physical and chemical properties. The applications of the amendments depend on the characteristics of the soils. The amendments reported in the studies for the elaboration of this COP were: compost (refers to humus that is obtained of artificial manner when organic waste is decomposed by organisms and beneficial microorganisms), charcoal or biochar, magnesium carbonate, vinasse (a by-product of the production of alcohol from sugarcane), zeolite (minerals that stand out for their ability to hydrate and dehydrate reversibly, adsorbents); Calcium sulphate, lime, cachaza (by-product of sugar cane), zinc sulphate, dolomite (calcium carbonate and magnesium), vermicompost, sugar cane, palm kernel cake, phosphate rock, organic matter.

**Validation:** Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome. (CXC 1 - 1969), supported by (CXG 69-2008)

**Sampling:** Procedure used to draw or constitute a sample. Empirical or punctual sampling procedures are sampling procedures, which are not statistical-based procedures that are used to make a decision on the inspected lot. (CXC 50-2004).

### **Organic agriculture**

The Codex Committee on Food Labelling in the *Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods* (CXG 32-1999) stated that Organic agriculture is a holistic production management system which promotes and enhances agroecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, cultural, biological and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system. An organic production system is designed to:

- a) enhance biological diversity within the whole system;
- b) increase soil biological activity;
- c) maintain long-term soil fertility;

- d) recycle wastes of plant and animal origin in order to return nutrients to the land, thus minimizing the use of non-renewable resources;
- e) rely on renewable resources in locally organized agricultural systems;
- f) promote the healthy use of soil, water and air as well as minimize all forms of pollution thereto that may result from agricultural practices;
- g) handle agricultural products with emphasis on careful processing methods in order to maintain the organic integrity and vital qualities of the product at all stages;
- h) become established on any existing farm through a period of conversion, the appropriate length of which is determined by site-specific factors such as the history of the land, and type of crops and livestock to be produced.

### Pruning

Rodriguez, T. (2002) cited by Torres, O. (2016) mentions that an annual pruning is sufficient but this should be as complete as possible. First the shade trees must be pruned and then the cocoa plants, to which dry, diseased branches or that deteriorate the balance of the plant are eliminated. The removal of shoots and general cleaning should be done several times a year, whenever necessary to maintain health and proper development of the tree and the harvest.

### Shading

Alvim (2000) cited by Torres, O. (2016) expresses that cocoa is typically an umbrophilic crop. The objective of shading at the beginning of the plantation is to reduce the amount of radiation that reaches the crop to reduce the activity of the plant and to protect the crop from winds that may harm it. When the crop is established, the percentage of shade can be reduced to 25 or 30%.

### 3. Strategies for the mitigation of cadmium in cocoa beans

13. To avoid the bioaccumulation of Cd in cocoa beans requires the implementation of different strategies considering the particularities of each agroecological and production system (organic or conventional or cocoa in agroforestry system), so that together they contribute mitigating the levels of Cd in those plantations that require it.

3.1. According to the available research works it is recommended the following:

#### 3.1.1. In new plantations:

14. Install plantations on agricultural soils that have less than 1.4 mg/kg of total Cd. (CCME from Canada, 1999; DS 011-2017 MINAM Peru). Moreover: Limits for soil Cd in new plantations would need to be related to soil properties: mainly pH, chloride, and soil Zn would be important in siting decision, less important are organic matter, clay, Fe, and Mn oxides.
15. Use a design of mixed plantations (agroforestry with different varieties of cocoa and with different types of shade (bananas, inga, etc.), adapted to each ecological environment, instead of a monoculture of cocoa, without shade). Switzerland comment: Gramlich (2017) showed that there might be a positive effect, however more research is needed on this field. The Association of Exporters ADEX - Peru states that according to evaluation of farms already in production, the agroforestry system works well in the first 2-3 years of planting, as young plants develop better under shade, which helps them to be better suited to resist the periods of natural drought in the area. In adult plants the shade favors the proliferation of diseases (moniliasis and witches' broom).

Sources: <http://www.senasa.gob.pe/senasa/moniliasisdel-cacao/>; <http://www.agrobanco.com.pe/wp-content/uploads/2017/07/010-f-cacao CULTIVOS. pdf>

16. Install plantations in areas far from roads or take measures to prevent the contact of the cacao plantations with the gases that emit the combustion of the vehicles because they may contain Cd. Also, in areas far from dumps in cities or mining areas. In this regard, in large cacao farms, roads must be an important consideration and should be spaced 200 meters apart (SMIARC Technoguide 2014) from the farms. The US FDA comments that automotive traffic is not a practical source of Cd., and that tires are very low in Cd but very rich in Zn and there is no risk of Cd. It also indicates that during a period between the 1970s and approximately in 2000, some automobile radiators used Cd welding, but they have stopped using Cd. Because gasoline does not contain Cd, the exhaust gases do not cause Cd contamination. The Swiss FSVO emphasizes that the sediment from the contaminated water from the mining area could become a problem away from the mining center depending on the size of the emitted particle, and the deposition distance from the emitting source.

17. In new plantations, use of perennial legume cover crops should be considered. Cover crops improve soil organic matter. Cover crops can protect soil from erosion and reduce nutrient loss, thereby improving soil productivity through greater availability of essential nutrients and reduction in heavy metal toxicity (US FDA comment).
- 3.1.2. **In plantations already installed:** Strategies to immobilize Cd in soil and decrease its availability in soil
18. Guo, G., et al (2006) mention that organic amendments for cadmium immobilization are: bark saw dust (from timber industry), chitosan (from crab meat canning industry), poultry manure ( from poultry farm), cattle manure (from cattle farm), rice hulls (from rice processing), sewage sludge, leaves and straw. Likewise, report the following inorganic amendments: lime (from lime factory), phosphate salt (from fertilizer plant), hydroxyapatite (from phosphorite), fly ash (from thermal power plant) and slag (from thermal power plant).
19. Increase the levels of Zn and Mn in the soil. It has been demonstrated that when there is a deficiency of these micronutrients, Cd is more likely to enter the plant and the cocoa bean. The scientific analysis shows that the imbalance between micronutrients and Cd has a great impact on the absorption of Cd and the high content of Cd in the cocoa bean. For “organic plantations”, there are no commercial “organic” sources of Zn except ground ores, but ores contain Cd at about 1 % of the Zn content and should not be used as “organic” Zn fertilizers. Application of ZnSO<sub>4</sub> or other soluble Zn salts or chelates to the leaves of cocoa trees may be able to inhibit Cd transfer to fruits. Spray Zn has been shown to reduce Cd translocation to several cocoa beans in field testing. The ability of spray Zn to inhibit Cd movement to cocoa fruits could be assessed using a multi-year field trial. The action of the Zn spray on leaves is separate from possible effects from the use of Zn fertilizers to inhibit Cd uptake by roots (note again the need to include limestone to counteract acidification resulting from ZnSO<sub>4</sub> or other Zn salt applications to soils).
20. Apply liming levels in low doses (3 t/ha/year) and preferably dolomite [CaMg (CO<sub>3</sub>)<sub>2</sub>] to gradually increase pH and incorporate Ca and Mg that are essential for the growth of cocoa and can precipitate Cd decreasing its bioavailability. This requires further research because there are varieties of cocoa that grow well on slightly acid soils, and could be affected by the increased pH. It is important to recognize that surface application of “lime products” in established cocoa farms cannot increase the pH of the root zone for decades because of the low water solubility of CaCO<sub>3</sub> and hence low rate of leaching of alkalinity from surface applied limestone amendments. Several studies of methods to raise the pH of subsurface soil layers have found that combining biodegradable organic matter with lime products allows the metabolism of the organic matter to provide a way to obtain leaching of alkalinity into the subsurface soil (See Brown et al., 1997; Tester et al. 1990; Liu and Hue al. 2001; and Tan et al., 1986). Ramtahal et al, 2019 studying biochar and lime amendments in vitro, greenhouse and field experiments concluded that their study implies that the two amendments were complementary in their action and can be used in the recommended rated to reduce Cd bioaccumulation. However further studies are required on the placement of amendments to improve their effectiveness and longevity particularly under field conditions. Besides, it is important to adjust lime levels based on soil analysis from a reputed soil testing laboratory. Cd in soils cannot be forced to precipitate except in highly calcareous soils. Instead, raising pH causes soil Cd to be adsorbed more strongly by Fe and Mn oxides and organic matter in the soil. Over liming should be avoided. FSVO (Food safety and veterinary Office) from Switzerland mentions that no field research in existing plantations has been published to show dosage, etc., and its effect on Cd uptake and Cd bean concentration.
21. A field experiment, lasting 18 months, was performed to assess the effectiveness of liming on pH, bioavailability of Cd in soils and its uptake in cacao tissues concluding that remediation by liming of cacao soils contaminated with Cd to reduce its uptake appears feasible, based on the results of laboratory and field trials in Trinidad and Tobago (Ramtahal, G. et al., 2018).
22. Increase the content of organic matter in the soil and improve its microbiological activity using fertilizers or organic fertilizers such as treated manures from feedlots, stabled cattle in farms, compost, bokashi, among others. For this action it is important to previously know the contents of Cd in the inputs to be used. Table N° 2 shows estimated contributions, minimum and maximum range of Cd added to agricultural soils by different sources (mg/kg) and table N ° 3 exhibits concentration of cadmium in rocks.



**Table 3: Estimated contributions of heavy metals added to agricultural soils by different sources (mg/ kg), range (minimum to maximum)**

Heavy metal	Phosphate fertilizers	Nitrogen Fertilizers	Plant-protection products (pesticides)	Manure	Sludge from sewage
Cd	0.1 - 170	0.05 - 8.5	1.38 - 1.94	0.3 - 0.8	2 - 1500

Source: Sánchez, 2003; Mico, 2005; Peris, 2006; Delgado, 2008. Cited by Rueda, Rodríguez y Madriñan, 2011

**Table 4: Cadmium concentration on rocks**

Type of rock	Range mg/kg	Average mg/kg
Igneous rocks		
Rhyolites	0.03 – 0.57	0.230
Granites	0.01 – 1.60	0.200
Basalt	0.01 – 1.60	0.130
Sedimentary rocks		
Schists and clays	0.017 – 11	-
Black schists	0.30 – 219	
Sandstones and conglomerates,	0.019 – 0.4	-
Carbonates	0.007 – 12	0.065
Phosphorites	<10 – 980	-
Coal	0.01-300	-
Sulphur mineral deposits		
Sphalerite (SZn)	0.02 – 0.4 (<5%)	-
Galena (SPb)	<0.5%	
Tetrahedrite, Tennantite (CuSZn)	0.24%	
Metacinnabar (HgS)	11.70	

Source: Alloway, 1995

23. In order to provide more information on the influence of agricultural practices, the arithmetic means which is a better measure of central tendency are reviewed for the purpose of better explanation of statistical inference of the cadmium concentrations exposed in Table 2. For that, the following paragraphs show some sources cited by Rueda, Rodríguez and Madriñan (2011);
24. De Meeûs, C.; Eduljee, G.H.; Hutton, M. (2002) cited by Mico (2005) found in 1989, that the average contribution of Cd from phosphate fertilizers was approximately 2.5 g of Cd per ha and year, for agricultural soils of the countries of the European Union, which comes to suppose 50% of the total contribution in soils not affected by other polluting activities such as, for example, industrial.
25. Mico (2005) cited some studies, e.g., Jinadasa, K.B.P.N.; Milham, P.J.; Hawkins, C.A.; Cornish, P.S.; Williams, P.A.; Kaldor, C.J.; Conroy, J.P. (1997) that corroborate the results cited in the previous paragraph, as they find higher Cd contents in cultivated soils (average 1.3 mg/kg) than in uncultivated soils (average 0.36 mg/kg), due to the intensive use of phosphate fertilizers.

26. Mico (2005) in its comparative study of total heavy metal contents (in mg/kg dry soil) in soils with different textures ( $\bar{X} \pm DE$ ) found for Cd the following values for: Fine textures (n=36)  $0.34 \pm 0.17$ ; medium textures (n=15)  $0.35 \pm 0.25$ ; coarse textures (n=3)  $0.26 \pm 0.22$ ; concluding that the sequence of accumulation of heavy metals, depending on the texture of the soil, follows the following order: Cd: Medium Textures > Fine Textures > Coarse Textures and that in the case of Cd, soils with medium textures contain an average value slightly higher than those with fine textures, although both results are similar and can be considered to be comparable.
27. Mico (2005) found that extractable contents of Cd in the study area ranged from 0.01 mg/kg (MPA - 02 and 28) to 0.14 mg/kg (MPA - 18 and MPA - 47), with an average value of 0.08 mg/kg. Furthermore, it reports that in the international field McLaughlin, M.J.; Maier, N.A.; Ryament, G.E.; Sparrow, L.A.; Berg, G.; McKay, A.; Milham, P.; Merry, R.H.; Smart, M.K. (1997) obtain an average value of 0.18 mg/kg in soils cultivated with potatoes in Australia; while McGrath (1996) obtains an average value of 0.52 mg/kg in agricultural soils of Ireland.
28. Peris (2006) studied soils with horticultural crops in the province of Castellon-Spain from 77 samples taken in randomly selected plots. The plots were characterized by the analysis of soil characteristics and edaphic properties and the total and extractable contents of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soils. In addition, the contents of metals in three characteristic crops of the study area, such as chard, lettuce and artichoke, were analyzed in 30 plots. The results obtained are:
- Soils are generally basic (pH=8.1 of medium value; with a range of variation from 7.5 to 8.5) and carbonate (32.8% of medium value; with a range of variation from 8.8 to 65.7%).
  - In 66% of the plots the electrical conductivity, in the saturated paste extract, indicates that they are soils without salinity problems (<2 dS/m), in 31% of the plots there may be salinity problems, since the soils are slightly (2-4 dS/m) or moderately saline (4-8 dS/m), and in 3% of the plots the soils are strongly saline (>8 dS/m).
  - The organic matter content is high and even very high in some plots (4.2% medium value, with a range of 1.8 to 10.2%). The cation exchange capacity of soils is from very low to high (18.3 cmolc (+)/kg medium value; with a range of variation from 3.4 to 39.6 cmolc (+)/kg) and the texture is mostly Frank, Frank-clay or Frank-silty.
  - The mean concentrations of "pseudo-total" metals (mg/kg) in the study area are: 0.328 for Cd, 55.8 for Pb and 78.5 for Zn. These results mostly reflect normal levels for agricultural soils based on comparison with other studies described in the literature. Therefore, agricultural practices in the area generally do not appear to provide an excessive level of metals.
  - The results of multivariate analyses show the existence of two groups of heavy metals that differ in their origin. Thus, the first group is formed by Fe, Ni, Co and Mn, which in the analyzed plots their origin is mainly due to the alteration of the original material. Therefore, this group has been called lithogenic factor. The second group is made up of Cd, Pb, Cu, Cr and Zn, which are grouped by generally having an anthropogenic origin in the plots, being called anthropogenic factor.
  - The content of Ethylenediaminetetraacetic Acid (EDTA) extractable fractions with respect to their "pseudo-total" content is less than 10% for Co, Cr, Fe and Ni, 10% for Mn and more than 10% for Cd (38%), Pb (19%), Zn (17%) and Cu (15%). These results indicate that the metals with the greatest transfer potential (mobility) in the studied soils are Cd, Cu, Pb and Zn, probably due to their anthropic origin in the study area.
  - In addition to the "pseudo-total" content, the concentration of the extractable fraction is also influenced by the characteristics and properties of the soil. Thus, correlations and/or regression lines indicate that the EDTA extractable fraction of Cd, Co, Cu, Fe, Mn, Ni and Pb is positively related to CIC and/or negatively to carbonates. However, the edaphic characteristic that most influences the extractable Zn is clay. In addition, for some metals the relations established between the extractable fraction with EDTA and the "pseudo-total" content of other metals are important. Generally, extractable fractions are related to the "pseudo-total" contents of metals with a similar origin. Thus, extractable fractions of metals that have a lithogenic origin are related to "pseudo-total" metals of lithogenic origin, while metals of anthropic origin are related to each other.

- In the plots with lettuce, chard or artichoke, samples of crops were taken. The concentrations of metals in the crops are grouped in leaf crops (lettuce and chard) and inflorescence crops (artichokes). Thus, the average metal contents, expressed in mg/kg dry weight, in leaf crops are 1.47 for Cd, 0.57 for Co, 3.35 for Cr, 13.2 for Cu, 431 for Fe, 63 for Mn, 3.84 for Ni, 1.99 for Pb and 41.7 for Zn, while in inflorescence crops are 0.24 for Cd, cannot be quantified for Co, 0.68 for Cr, 8.7 for Cu, 65 for Fe, 21 for Mn, 1.32 for Ni, 0.28 for Pb and 44.3 for Zn. Therefore, the elements with the highest concentration are the micronutrients (Cu, Fe, Mn and Zn). The results obtained from the concentrations of metals in the sampled cultures seem to indicate that, in general, these should not have problems to develop properly. However, some cultures present too high values of some metals, specifically, one sample for Cd, another for Pb, and 3 for Ni and others present deficient values of Mn or Zn. In addition, on plots that could not be analyzed for crops, the values of the extractable micronutrient fractions (Cu, Mn and Zn) reflect that the possible crops that develop on these plots should not be lacking in these elements, as indicated above.
  - The possible differences between species in the absorption and / or accumulation of metals in the crops were analyzed by comparing the metal contents between the two types of crops analyzed. The results show that the contents of Cd, Co, Cr, Cu, Fe, Mn, Ni and Pb are higher in leaf crops. The different concentrations observed between the two types of crops must be mainly due to physiological differences, which fundamentally cause leaf crops to be metal accumulators, since there are no significant differences in the metal content in the soils of both types of crops, or when there are higher concentrations in soil crops with lower concentration. In addition, for metals such as Cd and Pb, a greater amount of atmospheric deposition can enter through the leaves. On the other hand, the average value of Zn is slightly higher in inflorescence crops than in leaf crops, although the difference is not statistically significant, despite having much higher concentrations in soils with inflorescence crops. This reflects the physiological differences between the two types of crops, with the leaf crops being accumulators.
  - The relations established between the "pseudo-total" content of heavy metals in the soil and in crops are negative in all crops for Cd and Ni, in leaf crops for Cd, Co and Mn, and in artichokes for Cd. These negative relations seem to reflect the greater importance in the entry of metals, such as Cd, to crops by atmospheric deposition versus transfer from the soil. On the other hand, the non-existence of relations between the extractable fraction of metals and the content of crops is perhaps indicating that the EDTA extraction method is not the most suitable to value the bioavailability of metals in these soils for these crops.
  - Among the different elements there are correlations, positive or negative, that are indicating that the absorption of a metal by the plant increases or decreases in the presence of other metals in the soil. For example, in the set of crops, the content of Cd is lower the higher the concentration of Zn in soils. Therefore, to study the possible accumulation of toxic elements in crops, as important as knowing the concentration of metals in the soil and in the crop, is to know the interactions that may be occurring between the elements.
29. Tang, J. et al (2020) investigating the effects of biochar, compost and their combination on availability of heavy metals, physicochemical features and enzyme activities in soil showed that adding amendments to polluted soil significantly altered soil properties and compared to the separate addition of biochar or compost, their combined application was more effective to improve soil pH, organic matter, organic carbon and available potassium. Moreover, all amendments significantly decreased the availability of Cd and Zn, but slightly activated As and Cu and soil enzyme activities were activated by compost and inhibited by biochar, but exhibited highly variable responses to their combinations. Pearson correlation analysis indicated that electrical conductivity and available potassium were the most important environmental factors affecting metal availability and soil enzyme activities including dehydrogenase, catalase,  $\beta$ -glucosidase, urease, acid and alkaline phosphatase, arylsulfatase except for protease and invertase.
- Availability of As, Cu, Cd and Zn affected dehydrogenase, catalase and urease activities. These results indicated that biochar, compost and their combination have significant effects on physicochemical features, metals availability and enzyme activities in heavy metal-polluted soil.
30. Regarding microbiological activity, the effect of microorganisms in the decrease of cadmium intake in cocoa is well documented: Bravo et al (2018), and Revoredo, A. and Hurtado, J. (2018), who have shown that microbial inoculum are effective in cocoa crops. Revoredo and Hurtado determining the bioremediation activity of 3 *Streptomyces* strains: *Streptomyces variabilis* (AB5 and X) and *Streptomyces* sp. (C2) using two different concentrations of Cd: 100 and 200 ppm, concluded that the C2 strain has bioremediation activity since it reduces the absorption of Cd in cocoa plants, by an average of 39.67% in the treatment with Cd 100 ppm and an average of 21.13% in the treatment with Cd 200 ppm.

31. Avoid fertilization with phosphate fertilizers and sedimentary rock containing phosphorus because they usually have Cd as an impurity; cadmium being lower in phosphorus' rocks of igneous origin. Smolders (2017) expresses that for successful cacao production it is vital to add phosphate fertilizers because tropical soils have very limited native phosphorus content. It is not the type of phosphate fertilizer but the absolute Cd concentration that should be the basis for guidance. These farms are reported to have very low levels of plant available P based on published surveys of soil P fertility, so P fertilizers may be useful to increase growth and hence growth dilution of Cd in the plants. The EU, US and many other nations have limits on Cd in P fertilizers including rock phosphates that may be sold commercially. Smolders (2017) summarizes the current best advice about appropriate limits for Cd in P fertilizer products. One major problem in the Latin American and Caribbean nations producing cocoa is the failure to verify Cd in fertilizer products and the failure to tightly regulate Cd in fertilizers. Such regulations need to be developed and enforced in cocoa fertilizers.
  32. Use nitrogen and potassium fertilizers because they usually have very low cadmium content and preferably compound fertilizers such as 20-20-20 (N-P205 and K20), verifying their heavy metal analysis. It is demonstrated that in well-supplied soils the chances of bioaccumulation of cadmium are lower. It must be emphasized that nitrogen and potassium fertilizers should not be used in organic cocoa plantations.
  33. Preparation and use of activated carbon using different types of materials, preferably local (residual biomass or stubble) can be applied to decrease the availability of Cd in soil by the adsorption mechanism. However, activated carbon or biochar are expensive soil amendments and not likely economic for cocoa farms, especially the small ones. It should be kept in mind that activated carbon should not be used in organic cocoa plantations.
  34. The Inter-American Institute for Cooperation on Agriculture (2017b) emphasizes that there are elements which indicate that the adequate and permanent pruning of trees significantly reduces the presentation of Cd in the husk. Surface pruning of the cocoa tree has a significant impact on the root architecture and is a significant element in blocking the absorption of Cd from the soil; especially, in volcanic soils.
  35. Applying vinasse (by product of the cane industry), a rich liquid fertilizer as a source of potassium, it also can promote the installation of fungi that form mycorrhizas in the roots of the cacao tree and increase the efficiency in the nutrition of phosphorus in this crop, give resistance to drought, protection against diseases, and immobilization of cadmium.
  36. Use preferably native mycorrhizae of the area and other bio remediators that "capture" Cd present in soil making it unavailable for uptake by cocoa beans.
- 3.2. Phyto extraction of heavy metals (Cd).
37. It is a technique that involves planting plants (trees, shrubs, herbaceous, cover crops) in soils contaminated with heavy metals in order to extract them through the root system and transferred to the leaf mass, which is harvested and incinerated (450° C) to turn it into ashes and then decide whether to go to confinement or to an analytical or industrial chemistry laboratory so that they can reuse these metals. It is worth mentioning that the application of this technique requires having an implemented biosecurity system to prevent foliage from being used for food or feed. Chaney, R.L y Baklanov. 2017 report that unfortunately, plant species with demonstrated very high Cd accumulation are not adapted to tropical environments and growing another crop under the cocoa trees and harvesting the above ground biomass annually to remove Cd would be very difficult in cocoa plantations.
  38. Jebara, S.H., et al 2019 expressed that legumes coinoculated by cadmium-resistant, plant growth promoting bacteria (PGPB) contribute to a type of phytoremediation for alleviating Cd contamination in the soil. Furthermore, the legumes-PGPB symbioses are reported to influence Cd bioavailability in legumes by various mechanisms, such as bioaccumulation, precipitation, complexation, and chelation. Cd toxicity has induced a wide range of physiological and biochemical tolerance mechanisms in legumes, such as the gene expression of metal binding proteins involved in chelation, and the transport of Cd, such as phytochelatin synthase (PCS) and metallothionin (MTs), and the activation of the antioxidant defense system.
- 3.3. **Agronomic management of the cocoa crop and Cd bioaccumulation**
39. In the efficient agronomic management of the crop, the following factors are important: the pruning, the number of plants per hectare, the shade systems, the humidity regime in the soil, the application of fertilizers and amendments, the doses and moments of performing these tasks, which allow that the cocoa metabolism be adequate and there is less likelihood that cadmium enters the roots because this metal normally bioaccumulates in greater quantities in low fertility, sandy soils, poor in organic matter, low concentrations of zinc and manganese, strongly acids (pH <5.5) and with poor handling.

40. When the physiology of cocoa is adequate, the production and functioning of enzymes will favor their normal metabolic processes, decreasing the bioaccumulation of cadmium in cocoa beans, since there are self-defense mechanisms of plants against contaminants that are activated when plants are healthy and well nourished. USA comment: There are no known special physiological activities of normal well growing plants that reduce Cd accumulation. Stunting of growth, especially by excessive soil acidity, causes higher Cd accumulation. Table 5 below presents a list of conditions that generate bioaccumulation of Cd in cocoa beans and their proposed mitigation measures:

**Table 5: Conditions of soil and water that favor Cd bioaccumulation in cocoa beans.**

<p>Conditions of soil and water that generate cadmium bioaccumulation in cocoa beans the USA indicates that it is very unlikely that saline irrigation waters are a problem in cocoa production and Cd. Providing a warning about high levels of chloride in irrigation water, fertilizers, and other soil amendments is appropriate. And it is specifically soil chloride, not salinity that causes higher Cd accumulation in all plant species. (McLaughlin, 2016).</p> <p>Also, advice about soil pH should be more specific. Surveys of soil properties in cocoa plantations in several nations reported soil pH as low as 4.5 which strongly promoted Cd accumulation. Soils in the rooting zone should be limed to reach a pH of 6.5 if cocoa Cd levels need to be reduced. And if the soil has naturally high Cd levels, soils should be made calcareous to minimize Cd accumulation, or other crops should be grown.</p>	Proposed mitigation measures
Soils of low natural fertility	Fertilize the soil with good nutrient
Low content of organic matter in the soil	Increase organic matter (> 4%)
Low concentration of Zn	Incorporation of Zn
Sandy soils	Avoid sowing in sandy soils, preferably use loam to clayed soils
Saline waters (2 mS/cm) with high chloride content. In the unit mentioned S stands for Siemens	Treat water to lower its salinity and decrease chlorides
Strongly acid soils	Liming the soil to moderately acidic to neutral levels

41. Planting areas: As prevention, the planting of cocoa trees should be in areas where there is no high Cd content, so agricultural soils should not have more than 1.4 mg/kg of Cd (CCME of Canada, 1999; DS 011-2017 MINAM Peru).
42. Gramlich, A., et al. (2017) referring to Cd uptake by cocoa trees in agroforestry and monoculture systems under conventional and organic management determined that production systems and cultivars alone had no significant influence on leaf Cd. However, they found lower Cd leaf contents in agroforestry systems than in monoculture when analysed in combination with DGT-available soil Cd, cocoa cultivar and soil organic matter. Overall, this model explained 60% of the variance of the leaf Cd concentrations. Moreover, they explain lower leaf Cd concentrations in agroforestry systems by competition for Cd uptake with other plants, and also that the cultivar effect may be explained by cultivar specific uptake capacities or by a growth effect translating into different uptake rates, as the cultivars were of different size.

43. Gramlich, A., et al. (2018) in their paper of soil Cd uptake by cocoa in Honduras highlighted that: a) Bean Cd exceeded European standards in some areas, although soils were uncontaminated., b) DGT-available soil Cd ( $Cd_{DGT}$ ) best predicted bean and leaf Cd. Furthermore, Cd concentrations in cocoa beans were highest on alluvial substrates. On the other hand, as they found no influence of fertilizer application nor vicinity to industrial sites, concluded that differences in soil Cd between sites were due to natural variation and that of all factors included DGT-available soil Cd was the best predictor of bean Cd. Cd ( $R^2 = 0.5$ ) and when DGT was not considered, bean Cd was best predicted by “total” soil Cd, pH and geology.

#### 4. FIELD PRODUCTION MEASURES TO PREVENT AND REDUCE CADMIUM CONTAMINATION IN COCOA BEANS

44. Knowledge of the sources of cadmium and its distribution in the soil are also important. Several soil-plant surveys of Cd in Latin American countries have found substantial Cd enrichment of some of the national soils used in cocoa production. The somewhat higher Cd in topsoil than subsurface soils could arise from natural soil formation processes and application of fertilizers (particularly phosphate products), or aerosol emissions from industrial sources. Natural soil metal enrichment can arise from mineralization (both Zn and Cd) are enriched near mines of Zn, Cu and Pb (Cd:Zn ratio typically 0.005 to 0.01  $\mu\text{g Cd}/\mu\text{g Zn}$ ). Marine shale rock can yield Cd with high Cd:Zn ratio which has greater plant availability than the Zn ore type of contamination. And several areas in Latin America have been found to have significant and even extreme local Cd contamination from these marine shale sources (Garrett et al., 2008); some of the Jamaican soils have higher Cd than Zn, an extremely rare event. Where Cd is above background levels (say 0.2-0.5 mg Cd/kg dry soil), other elements (Zn) help clarify the source. Soils developed from phosphate deposits are rich in P, Cd and Zn with typical Cd:Zn ratio of 0.1. Thus at least the Zn and P levels of suspect high Cd soils should be measured to help clarify the source. (Garrett, R.G., A.R.D. Porter, P.A. Hunt and G.C. Lalor. 2008). Rodriguez, et al (2019) using data from an existing study done in a cocoa producing area of central Colombia, selected several farms with high Cd concentration, and in these farms studied the spatial variability of Cd in soil and plant tissues, along with other soil chemical properties. Some of the findings were: At increased soil depths there was a reduction in total Cd as well as in available levels of the element, spatial regression modeling explains the Cd in cocoa beans relating edaphic chemical properties, location and depth of soil.

45. Soil analysis should be a requirement for current and new cocoa farmers to identify soil and water with lower levels of cadmium. Those areas with higher levels should be designated for other types of cash crops, such as coffee or those plants with lower cadmium absorption. The Association of Exporters of Peru - ADEX states that coffee and cocoa are produced in different ecological levels, so it cannot be suggested to change cocoa plantations for coffee. Quality coffee grows above 1000 meters above sea level - <http://www.minagri.gob.pe/portal/especial-iv-cenagro/24-sector-agrario/cafe/204-cafes-especiales-en-el-peru> and cocoa between 300 and 900 meters above sea level - <https://www.sierraexportadora.gob.pe/programas/cacao/que-significa.php>. The FSVO of Switzerland states that soil sampling should also be addressed, since the contents of Cd are not homogeneous. In addition, factors such as pH or soil organic matter should also be considered. Davila, C. b (2018) for the characterization of the soils contemplated in their analysis: pH, electrical conductivity, calcium carbonate, organic matter, phosphorus, potassium, mechanical analysis (sand, silt, clay), textural class, exchange of cations, changeable cations (Ca, Mg, K, Na, Al + H), sum of cations, sum of bases, and saturation of bases).

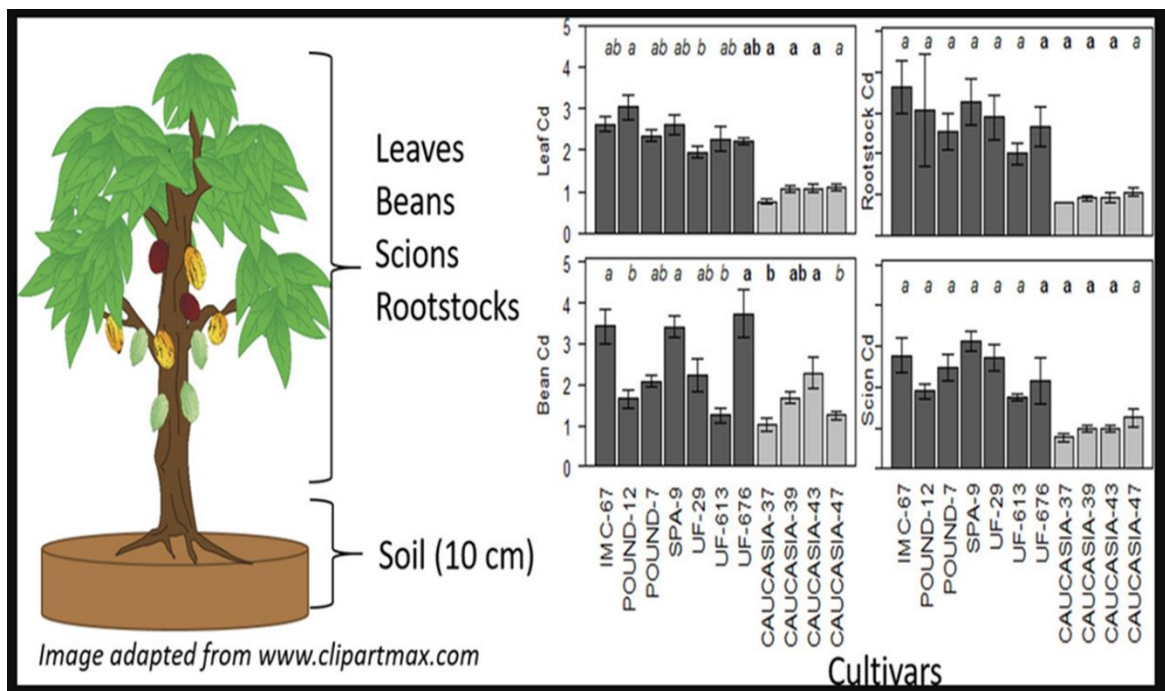
46. For the analysis of Cd, several methods not included in CODEX STAN 228/2001 may be used, but the selected method should meet the performance criteria required for the maximum levels above 0.1mg/kg established in the Commission's Procedural Manual of Codex Alimentarius that are the same as those established in the European Union regulation (EFSA, 2009) for limit of detection (LOD), limit of quantification (LOQ) and precision. Recovery must have a range of 80% to 110%.

47. Methods of analysis to determine Cd in cocoa beans:

Technique	Detection limit ( $\mu\text{g/L}$ )
Flame Atomic Absorption Spectrometry F-AAS	0.8 – 1.5
Inductively Coupled Plasma Optical Emission Spectrometry (ICP-ES) ICP-OES	0.1 – 1.0
Graphite Furnace with Atomic Absorption Spectrometry - GF-AAS	0.002 – 0.02
Inductively Coupled Plasma Mass Spectrometry (ICPMS). ICP-MS	0.00001 – 0.001

48. Use the results of the soil tests to determine if there is a need to apply fertilizers or soil amendments to ensure adequate soil pH and plant nutrition to avoid plant stress, especially during the development stage of the seeds, unless the producer can prove that his proposed action plan reduces the risk to allowable levels. It can be said that cocoa is a plant that thrives in a wide variety of soil types. van Vliet and Giller 2017, reviewing Mineral Nutrition of Cocoa established that among production constraints met by cocoa farmers is nutrient limitation. In their review they compile current knowledge on nutrient cycling in cocoa production systems, nutrient requirement of cocoa, and yield response to fertilizer application in relation to factors such as management, climatic, and soil conditions.
49. Soils with a higher cation exchange capacity (59.0-60.6 meq/100g) would have a greater capacity to fix metals (Cargua, J. et al., 2010). USA comments that "CEC 59.0 a 60.6 meq/100g" does not appear to be correct. Typical CEC of cocoa soils is <15 in the published literature. Sandy soils have low CEC, some as low as <5. Also, it is not CEC per se that reduces Cd Phyto availability, it is the surface area of Fe and Mn oxides and chelation ability of organic matter which adsorbs Cd and reduce Phyto availability. Clays in higher CEC soils are usually coated with Fe and Mn hydrous oxides, so clays are correlated with Cd adsorption.
50. Cargua et al 2010 cites Miliarium (2009) who also observed that clay tends to adsorb heavy metals that are retained in their exchange positions and that; on the contrary, sandy soils lack the capacity to fix heavy metals. which pass quickly to the subsoil and can contaminate groundwater levels.
51. Before harvesting, make sure that all the equipment, which will be used for harvesting, drying, cleaning and storing the crops, is in good working order and the waste, cocoa beans and dust are cleaned as much as possible. A breakdown of the equipment during this critical period can cause losses in the quality of the cocoa beans and increases the appearance of cadmium. Make sure that the equipment necessary for moisture content measurements is available and calibrated.
52. Harvest: avoid harvesting immature cacao fruits, since they have a solid pulp without mucilage and the cocoa beans are difficult to separate from the pod and do not ferment properly. Furthermore, the Inter-American Institute for Cooperation on Agriculture, IICA (2017b) reports that the reduction of Cd contamination in cocoa beans depends considerably on harvest management; being very clear evidence that the highest concentrations of cadmium are in the pod, which is why it is recommended to separate the beans from the pods early on.
53. Fertilization: The use of long-term phosphate fertilizers causes high levels of Cd in the arable layers of the soil (IPCS 2010), although USA FDA comments that it is important to maintain adequate P fertility so that trees are not stunted by low P and accumulate high Cd in the stunted trees, it is better to advice use of P fertilizers with limits on Cd in the products. Ensuring regulations governing the sampling, monitoring and enforcement of P-fertilizer limits is what is needed. Such limits may also be needed on Zn fertilizer products because some byproduct-derived Zn fertilizer products contain much higher Cd than any P fertilizer.
54. Cost-effectiveness of Cd mitigation measures: Treatment of soil contaminated with metals by immobilization with chemical amendments such as dolomite, limestone can provide less expensive and viable alternatives to reduce the availability of metals (Trakal et al., 2011). Furthermore, personal communication from International Researchers participating in the Forum (MINAGRI-IICA 2018) emphasized that the use of said chemical amendments increases the production of the cocoa crop. Production is increased because nutrient limitation is reduced.
55. The Guide for Phytosanitary Management and Safety in the cocoa farm (MINAGRI-SENASA-IICA, 2017), mentions that the management of the cocoa crop with an agroforestry system reduces the concentration of cadmium. On the other hand, it provides differentiated environmental services to society, constitutes an environmental alternative and contributes to the mitigation of cadmium. See "Use of timber and non-timber forest resources of the cocoa agroforestry system" (Theobroma cacao L.) [https://www.academia.edu/28727375/USODERECURSOS\\_FORESTALES\\_MADERABLES\\_Y\\_NO\\_MADERABLES\\_DEL\\_SISTEMA\\_AGROFORESTAL\\_CACAO\\_Theobroma\\_cacao\\_L.\\_USE\\_OF\\_TIMBER\\_AND\\_NON-TIMBER\\_FOREST\\_RESOURCES\\_IN\\_THE\\_CACAO\\_Theobroma\\_cacao\\_L.\\_AGROFORESTRY\\_SYSTEM](https://www.academia.edu/28727375/USODERECURSOS_FORESTALES_MADERABLES_Y_NO_MADERABLES_DEL_SISTEMA_AGROFORESTAL_CACAO_Theobroma_cacao_L._USE_OF_TIMBER_AND_NON-TIMBER_FOREST_RESOURCES_IN_THE_CACAO_Theobroma_cacao_L._AGROFORESTRY_SYSTEM)

56. Engbersen, N. et al (2019) in their research, used the long-term cacao cultivar trial CEDEC-JAS in northern Honduras to investigate differences between 11 cultivars in Cd uptake and translocation. Sampling of rootstocks, scions, leaves and beans was conducted at each site from three replicate trees per cultivar and the soil around them. Results indicate that concentrations of available soil Cd were more closely correlated with Cd concentrations of the rootstocks ( $R^2=0.56$ ), scions ( $R^2=0.59$ ) and leaves ( $R^2=0.46$ ) than with bean Cd concentrations ( $R^2=0.26$ ). In addition, Cd concentrations of rootstocks, scions and leaves showed close relationships to available soil Cd concentrations, with no significant differences between the cultivars. In contrast, bean Cd concentrations showed only weak correlations to available soil Cd and Cd concentrations in the vegetative plant parts, but significant variation among cultivars. Three cultivars, which were analyzed in more detail, showed significant differences in Cd concentrations of mature beans, but not of immature beans. These results suggest that cultivar-related differences in bean Cd concentrations primarily result from differences in Cd loading during bean maturation, possibly due to cultivar-specific differences in the xylem-to-phloem transfer of Cd. The results show that selection of cultivars with low Cd transfer from vegetative parts into the beans has high potential to keep Cd accumulation in cacao beans at levels that are safe for consumption. The figure below shows Cd accumulation and allocation in different cacao cultivars.



57. Chavez, E., Z.L.He, P.J Stofella, R.S. Mylavarapu, Y.C.Li, B. Moyano and V.C. Baligar (2015) cited by McLaughlin Mike. 2016, show in Table 5 the relationship between soil parameters and Cd concentration in cacao beans for the 0 – 5 and 5 – 15 cm depth in southern Ecuador.

**Table 6**

Table 5  
Relationship between soil parameters and Cd concentration in cacao beans for the 0-5 and 5-15 cm depth.

<u>Soil properties</u> (0-5 cm)	$R^2$	<u>Soil properties</u> (5-15 cm)	$R^2$
M3, EC, pH, % clay, Total C, TR, CEC	0.77	M3, total C, % clay, pH, TR, EC, CEC	0.84
M3, EC, pH, % clay, total C, TR	0.77	M3, total C, % clay, pH, TR, EC	0.84
M3, EC, pH, % clay, total C	0.76	M3, total C, % clay, pH, TR	0.82
M3, EC, pH, % clay	0.76	M3, total C, % clay, pH	0.79 <sup>*,+</sup>
M3, EC, pH	0.73 <sup>*,+</sup>	M3, total C, % clay	0.72
M3, EC		M3, total C	

Nomenclature: TR = total recoverable Cd, Total C = total carbon, CEE = effective cation exchange capacity, EC = electrical conductivity.

\*  $P < 0.05$ .

+ Best model.



58. The Inter-American Institute for Cooperation on Agriculture, IICA (2017b) refers that experiments conducted by the Instituto Nacional de Investigaciones Agropecuarias – INIAP in Ecuador show that contamination by Cd is most evident in the first 5 cm of the soil.
59. Chupillon-Cubas J, Arévalo-Hernández, C; Arévalo-Gardini, E; Farfán-Pinedo, A; Baligar V. (2017) studying the accumulation of Cd in six cocoa genotypes used as a rootstock concluded that the IMC67 clone, presented the lowest Cd content both in the aerial part and in the root, with significant differences to the control, being the clone most suitable for use by farmers.

## 5. POST-HARVEST MEASURES TO PREVENT AND REDUCE CADMIUM CONTAMINATION

The Inter-American Institute for Cooperation on Agriculture (2017b) mentions that recent studies have found that the greatest amount of Cd is in the mucilage, so measures must be taken in the post-harvest period. It is estimated that mucilage has 4.5 times more Cd than the testa and 5.7 times more than the bean.

60. Mucilage drainage for 12 hours during fermentation significantly reduced cadmium content in CCN-51 cocoa beans. Davila, C. 2018.
61. Drying: Ensure beans cannot be contaminated by smoke, fumes from dryers or vehicles. Cocoa beans should be dried off the ground so that they are not in direct contact with soil, tarmac or concrete and are inaccessible to animals. (CAOBISCO/ECA/FCC.2015).
62. Storage: Ensure stores are not contaminated by fuel spills, exhaust fumes or smoke. (CAOBISCO/ECA/FCC.2015).
63. The risk of contamination of cadmium after harvesting during cocoa beans storage periods can be managed in a more predictable manner through Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) that ensure that moisture levels in the cocoa beans stored below the levels that are propitious to mitigate Cadmium according to environmental conditions present in the region. The moisture content of the stored cocoa beans should be checked periodically and kept below 8 % on drying. (CAC/RCP 72-2013)
64. Although it is practiced with some large purchases of cocoa beans, analyzing the shipment of batches of cocoa beans before the process should become a standard practice for mixing cocoa beans with higher levels of cadmium with cocoa beans of lower levels of cadmium. This practice is being done in some South American countries. ADEX, adds that this practice is common during the processing of grain to cocoa derivatives, not only to decrease levels of cadmium, but, mainly, to achieve the organoleptic characteristics requested by the target market. The representative of the Ministry of Agriculture and Irrigation of Peru specifies that, as far as possible, the mixing of grains should be avoided since it is a short-term commercial solution, but the problem is not solved, identity and origin that characterizes is lost to countries that produce fine of aroma and flavor cocoa. It also indicates that it is necessary to implement a traceability of the cadmium content.

## 6. VALIDATED MEASURES APPLIED IN PRODUCING COUNTRIES AND OTHERS OBTAINED WITH SUPPORT FROM INDUSTRIALIZED COUNTRIES

### 65. COLOMBIA

The National Codex Alimentarius Committee of Colombia, in response to circular letter CL 2018/73-CF, forwarded the requested information with the following details:

Summary of the demonstrated measure: Study of the microbial diversity associated with cocoa soil with presence of cadmium (Cd) and evaluation of its bioremediatory potential.

Description of the measure: Characterize the populations associated with cocoa soils with the presence of Cd and evaluate the bioremediation potential of some isolated microorganisms, both at laboratory level and in greenhouse bioassays. Cultivation-dependent techniques (isolation, phenotypic and genotypic characterization and analysis of the potential of biological activity) and culture - independent techniques (last generation sequencing techniques [NGS] and analysis of marker genes (RNAr 16S)) that are complementary to each other and allow, on the one hand, to study the structural diversity of these microbial communities and on the other, allow to perform a bioprospecting of the isolated organisms. For the characterization of the microorganisms involved, microbiology methods dependent culture and molecular techniques will be used to elucidate the identity and characteristics of the spices involved in the process.

Location of the study:

Finca 1: Latitude 06-55-24.3 Longitude 073-28-40.5; Finca 2: Latitude: 06-53-10.2 Longitude 073-23-13.8; Finca 3: Latitude: 06-54-14.3 Longitude 073-22-15.6; Farm 4: Latitude: 06-54-494 Longitude 073-44,1-178.

Finca 1 (Finca is the Spanish name for cocoa farm) pH acid soil / high total Cd in soil. Finca 2 pH acid soil and low total Cd in soil. Finca 3 pH neutral / basic soil and soil high total Cd. Finca pH soil neutral/basic and soil low total Cd.

The study began in 2015 and will end in 2019

Study area and plot size: Total trees 3,200, approximately 3.5 Has.

The varieties of cocoa under study are: ISC95, ISC60-39, CCN51, new varieties (SYS).

Planting time: Farm 1: 15-20 years, Farm 2: 40-50 years, Farm 3: 5-10 years, Farm 4: 6-80 years.

Sampling date with respect to the application of the measure: Semester I, 2017. 3 samplings per farm, spaced approximately every 2 months.

Number of samples taken: A total of 12 samples (3 samples per farm). Each composite sample was formed from subsamples ( $n = 18$ ), selecting at random during the Zigzag run, cocoa trees with good phytosanitary status, cleaning the surface of the land (under the tree's leak) and introducing a hole to the indicated depth. For the collection and final handling of the samples, the NTC 4113-6 (2) was followed. Each composite sample (2 Kg).

Cd concentrations in the samples: The concentration of cadmium in soil is variable, samples were found with amounts of 44 mg / 100 g of sample, up to 0.01 mg / 100 g. This is the before. The after has not yet developed. USA mentions that the listed concentrations are confusing. 44 mg/100 g is 440 ppm which would be very highly contaminated; but "up to 0.01 mg/100 g is equal to 0.1 mg/kg which would be a relatively low Cd soil concentration.

#### 66. COLOMBIA

Gutiérrez E. y León C. 2017:

Study "Evaluation of amendments in order to solve the problems of Cd concentrations in soil and dry grain". Year 2010 to 2012- Santander: The following details are precised:

Kelley Directives were followed for the classification of soil contaminated with Cd; which values in mg/kg dry soil vary from:

Typical values for uncontaminated soils (mg/kg soil)	Light contamination (mg/kg soil)	Contamination (mg/kg soil)	High contamination (mg/kg soil)	Contamination unusually high (mg/kg soil)
0 – 1	3 – 5	5 - 10	10 - 20	> 20 These suggested ranges of Cd in different soil contamination classes are confusing. Soils with over 1.0 mg Cd/kg are already unusual. Background soils are usually defined as 0-0.7 by some authors. And as noted above, when soils are rich in Cd but not in Zn, that soil Cd comprises much higher risk than when usual soil Cd/Zn ratios occur (Garrett et al, 2008; Chaney et al, 2009).

595 samples were taken in 59 municipalities within eleven (11) provinces.

In the soil samples the following analyses were carried out: Chemical: Complete + minor elements less from 0 to 30 cm., Physical: Apparent and real density, and texture by Bouyucos at 0 -30 cm, Total Cadmium and Cadmium available at 0-30 cm and total and available Cadmium at 30-60 cm.

In the sampling of pods, the following were analyzed: Dry cocoa bean with shell and dry cocoa bean without shell.

Service laboratory - Area: Analytical chemistry were used: AA spectrophotometers (240FS / 280FS) and Spectrophotometer (ICP-OES) ICAP 6500 -Thermo Scientific.

The results obtained were

Baseline defined by the total concentration of Cd, present in soils of the cocoa areas: Of the 207 samples analyzed, highly variable Cd levels were found in the different sampled areas (between 0-1 mg/kg to > 10mg/kg).

The cocoa bean samples for which there is a maximum allowed limit of Cd of 0.5 mg / kg: 175 (84.5%) were below this legal limit and 32 (15.4%) exceeded it. In the latter areas, a highly significant correlation ( $r = 0.652$  and  $p > 0.001$ ) was found between the cadmium content of the bean with respect to cadmium available in the soil.

In the evaluation of the nutritional status of the cocoa soils, it was found:

The average value of soil pH in the cocoa municipalities, where 40 % of the soil have interchangeable Al contents is 5.6 which is considered moderately acidic.

76% of the soils have low organic matter content.

49% of cocoa soils have P deficiencies, a response commonly found in more than 70% of the country's soils due to the acidity of the soil.

With reference to the bases of soil K, Ca, Mg and their relationship, we find that 85% of cocoa soils have K deficiency, 16% of soils have low Mg content and 65% of soils have an adequate ratio Ca/Mg.

The effects of three treatments (compost and/or biofertilizers of ECOCACAO), organic biofertilizer (vermicompost and/or poultry manure) + mycorrhizae + dolomitic lime, and a combination of dolomitic lime, phosphoric source, potassium source, mycorrhizal soil and organic fertilizer were evaluated. It was carried out in six farms in an experimental plot of approximately 1 200 m<sup>2</sup> (144 cocoa trees).

The levels of each application of fertilization are dolomitic lime (1.5 - 2.5 kg/plant), K (300-350 g/plant), P (35-50 g/plant), and organic matter (350 g/plant) and mycorrhized soil (20: 1).

The analysis of variance does not show statistical differences in the properties of the soil due to the effect of the remediation treatments applied to the soil and show no changes in soil properties.

For the presence of total and available cadmium in soils, in cocoa beans and foliar tissue (leaves), the analysis of variance does not identify differences among the treatments in the evaluated municipalities.

## 67. ECUADOR

SENESCYT (2011). Project: Recovery of contaminated soils due to the presence of cadmium in the most polluted areas of the provinces of Manabí, Santa Elena and El Oro.

The details of the study are:

The study was conducted from July 2012 to December 2014.

The samples were taken in El Oro Province, Pasaje locality and in the Rio Grande farm; Peninsula Province of Santa Elena, Cerecita locality, La Mejor farm and in the Manabí province, locality Canuto and "Experimental" farm. The experimental plots contained 20 plants each, of which six central plants were identified to monitor the contents of Cd over time.

Eight amendments were evaluated: Co3Mg, vinasse, zeolite, humus, charcoal, CaSO<sub>4</sub>, cachaza and ZnSO<sub>4</sub>; applied in two doses in the varieties of Cacao CCN51 in the provinces of Santa Elena and El Oro and the National variety of cocoa in the province of Manabí.

The application of the amendments was made depending on the characteristics of the soils,

The results are:

In Santa Elena Peninsula, El Oro and Manabí, the soil responded to the application of 1 MT/ha and 2 TM/ha of the eight amendments, since it was possible to reduce the Cd contents significantly in the four depths evaluated (0 - 5cm., 6 - 10 cm., 11 - 15 cm., and 16 - 20 cm.) compared to the contents of the control (treatment without amendment).

In the soil of Santa Elena Peninsula, the application of the dose of 1 MT/ha of humus and calcium sulphate decreased the Cd contents of 1.76 mg/kg present in the control to 1.10 mg/kg of Cd in the first 5 cm of the ground. With the application of 2 TM/ha, it was reduced from 1.76 mg/kg in the control to 1.02 mg / kg when using charcoal.

In general, it was observed that all the amendments applied to the soil influenced the decrease in Cd concentrations in cocoa beans. At the end of the study it was determined that calcium sulfate and cachaza managed to reduce the concentrations of Cd in cocoa beans in the Santa Elena Peninsula by 46 and 44%, respectively,

In El Oro, when 1 MT/ha of vinasse was applied, the contents of Cd in the soil are reduced from 4.87 mg / kg to 2.38 mg/kg and when 2 MT/ha was applied, the zeolite lowers these contents to 2.29 mg/kg.

In the case of El Oro, the dolomite and vinasse amendments lowered the contents of Cd in beans of cocoa by 48 and 45%, respectively.

In Manabí the control presented 1.35 mg/kg of Cd in the first 5 cm of the soil, gradually decreasing as the depth increases. With 1 MT/ha. Ca carbonate was able to lower the contents to 0.57 mg/kg and with the high dose 200 kg/ha of zinc sulfate the contents were reduced to 0.59 mg/kg.

Finally, in Manabí all the amendments with the exception of Mg carbonate had a similar behavior reducing by 30% the contents of Cd in cocoa beans.

#### 68. BRAZIL

Carrillo, M., et al. 2010.

Estudio: Efeito of different conditioners na mobilidade of cádmio em dois latossolos brasileiros.

The details of the study are:

The objective of the study was to evaluate the movement of Cd in the profile of the soil affected by the addition of three organic conditioners (vermicompost, cake of sugarcane and palm kernel cake and three minerals (limestone, apatite and zeolite), in two Brazilian Oxisols ("Tiro de Guerra" and Tres Mariás) with clay and medium texture, respectively, to know the retention of Cd in soils, using the leaching column method.

Results:

Lime and sugarcane cake have greater potential to reduce the flow of Cd in the soil profile.

Alternatively, another option is zeolite, in soils with high sand content and apatite, in clay-textured soils.

#### 69. INTERNATIONAL CONFECTIONERY ASSOCIATION

The International Confectionery Association based in Brussels in its comments in response to Circular Letter CL 2018/73-CF on Research in mitigation refers to a study conducted from 2014 to 2017 at the Cocoa Research Center, University of the West Indies and supported by the Joint Research Fund of the ECA / CAOBISCO / FCC, which leads to a better understanding of the different factors that affect the availability of the Cd and the absorption of the metal.

Three possible solutions for mitigation have been proposed:

Grafting plants with rootstocks with low cadmium content.

Obtain new varieties that are not as prone to the absorption of Cd and modify soils to reduce the absorption of Cd by plants.

On the other hand, in the summary of the measures demonstrated, it specifies:

Soil analysis have shown a positive correlation between the highest levels of Cd in the soil and in the tissues of the plants and cocoa beans.

There also seems to be a high correlation between the absorption of Cd by cocoa plants and the acidity of the soil. A high acidity in the soil generally corresponds to a higher level of cadmium accumulation.

There seems to be some correlation between the use of phosphate fertilizers and the absorption of cadmium by the cocoa plant.

Cocoa beans from different growing areas in the same country and in different countries have a wide range of cadmium levels.

Citing as remediation for the measures mentioned above, the following:

Soil analysis should be a requirement for all new cocoa farmers to identify the soils with the lowest levels of cadmium. Areas with high levels of cadmium should be assigned to other types of commercial crops such as coffee or those plants with lower cadmium absorption or used for cocoa farming after the soil Cd levels are lowered to an acceptable level. The use of phosphate fertilizers should be minimized.

#### 70. FOODDRINK EUROPE

FOODDRINK EUROPE based in Brussels.

In its summary of the measures demonstrated, it specifies the following:

Cd contamination of cocoa beans depends to a large extent on the content of Cd in the soil and its bioavailability.

The bioavailability of cadmium depends on:

- Soil pH
- Content of organic matter.
- Deficiency of specific nutrients.
- Cl<sup>-</sup> ions in soil.

The work of the Cocoa Research Center supported by the Joint Research Fund of the FCC / ECA / CAOBISSCO is focusing on the management of all the mentioned parameters, except the Cl<sup>-</sup> ions in the soil.

The most effective methods developed so far are the liming of soils below pH 5.5.

It has been shown that increasing the pH by 1 unit reduces Cd of the bean by 1/10.

The application of biochar has also been shown to reduce the bioavailability of Cd in cocoa beans. The reduction rates are comparable to liming and have an additive influence on liming.

Cd contamination of cocoa beans is also a function of the cocoa variety. In the CRC study mentioned above, 10 genotypes with low bioaccumulation of Cd have been identified that can reduce the Cd levels in grains by 7 times.

In addition to the previous measures that reduce Cd contamination of cocoa beans due to natural geological of cocoa contamination, the contamination of the soils can come from:

- Sources of contaminated fertilizers
- Contaminated irrigation water
- Contaminated floodwater

Any additional contamination should be avoided by careful selection of fertilizers, irrigation water and flood prevention and cites as a description of the measure:

Handling soil pH by liming.

As a first step, the following soil parameters should be recorded: total Cd, bioavailability of Cd, soil pH, physical composition of the soil, organic matter content and cation exchange capacity with the support of certified laboratories. Based on this requirement, liming can be calculated and, depending on its effectiveness, liming rates can be adjusted.

In the second phase of the project, field trials are being carried out to further determine the effectiveness of the use of lime and biochar, including the frequency of application and application methodologies. Additional parameters are being investigated, such as nutrient availability and productivity.

The genotypes with low bioaccumulation of Cd identified in our study (Lewis et al., 2018) can be used as rootstocks in the production of propagation material to reduce Cd absorption.

Additional studies are being conducted in hydroponic experiments to understand whether the differences observed by Lewis et al 2008 are due to differences in root morphology or genetics. Bio accumulators with low Cd content are being tested as rootstocks because of their effectiveness.

71. PERU

García, J. and García L. 2018. Genetic selection with reduced accumulation of cadmium

In order to study the kinetics of heavy metal accumulation of Cd and Pb in different cocoa clones and to identify those with reduced accumulation in reproductive organs, this June-November, 2017 trial was run at the Station Tulumayo, Tingo Maria. The promising genetic material (clones) of cocoa used was the result of a process of individual selection-hybridization-genealogical selection, carried out by the Cocoa Genetic Improvement Program initiated in 1995 and directed by M.Sc. Luis García, Associate Professor of the National Agrarian University of La Selva - Tingo María, Huánuco (Peru). Except for clone S-60 (selection in the farmer's field) of San Martin; the other clones have their respective genealogies that were characterized morpho agronomically and published in the Catalog of Cocoa Cultivars of Peru (2012). Clones S-8 and S-12 are hybrids from the crossing between 2 Trinitarian clones. Clone S-23 is a hybrid of the cross between 2 clones Forastero Alto Amazonico, with mother from Iquitos and father from Cusco. Clone S-28 is a hybrid of the cross between 2 clones, the first Trinitarian and the second Stranger of the Upper Amazon (Ucayali):

N °	CODE	GENEALOGY	PLACE OF SELECTION
1	S-8	(ICS-95 x UF-296), 8	Tulumayo Station
2	S-12	(ICS-95 x UF-296), 12	Tulumayo Station
3	S-23	(IMC-67 x U-68), 16	Tulumayo Station
4	S-28	(ICS-1 x SCA-6), 20	Tulumayo Station
5	S-60	Unknown	San Martin Farmer's Field

As a result, there was a reduction tendency of the Cd from June to September (months of lower precipitation) and an increase in October and November (months of higher precipitation). In general, the accumulation of Cd was greater in the leaves (1,288 ppm) and lower in seeds (0.894 ppm) on average. Likewise, there were differential responses between clones, with clones S-08 and C-60 having the lowest accumulations of Cd with 0.650 ppm and 0.815 ppm respectively in the bean. In addition, relevant synergistic interactions were found between Cu/Zn, Mn/Fe, Fe/Zn and Cu/Pb, and antagonistic interactions between Cd / Zn and Cd / Cu.

Future studies of kinetics of accumulation of Cd and other heavy metals in cocoa beans should be carried out all year and for each phenological phase, including other cocoa clones of different genetic origin and hypo accumulative potential.

72. PERU

Davila, C. 2018 a. Technological package to decrease Cd content in cocoa beans.

The Cooperativa Agroindustrial Cacao Alto Huallaga (CAICAH) located in Tingo Maria, Huánuco made the diagnosis of Cd of the cocoa beans of its associates, determining an average content of 0.84 ppm.

To date, CAICAH has been developing a series of technologies at the field level to reduce the cadmium content in cocoa beans.

After 2 years of research we can mention the most efficient technologies for this purpose:

Soil liming (pH less than 5.5). In acidic soils, Cd is more available and can be easily absorbed by plants. Liming provides calcium to the soil solution, which is antagonistic to Cd; it also allows increasing the pH, increasing the negative charges of the soil and therefore facilitates the adsorption and complexation of cadmium in the soil, making it not available for plants. Floating materials that can be used to increase soil pH can be: agricultural lime [Ca (OH)<sub>2</sub>, SiO<sub>2</sub>, CaSO<sub>4</sub>], slaked lime [Ca (OH)<sub>2</sub>], dolomite [Ca Mg (CO<sub>3</sub>)<sub>2</sub>]. The project area of CAICAH evaluated the effect of dolomite on the decrease of cadmium in cocoa beans, three treatments were used (1.80 kg of dolomite/plant, 2.70 kg of dolomite/plant and 3.60 kg of dolomite/plant, obtaining the following results after one year of 0.52 ppm, 0.47 ppm and 0.45 ppm, respectively, of Cd in cocoa bean, without peel, using the Official method 999.1 1-AOAC

Conclusion: The application of dolomite in cocoa plantations decreases the content of cadmium in the beans, positive effect.

### **Application of organic matter (compost, poultry manure, humus, manure, etc.)**

The organic matter thanks to its functional groups (OH, COOH, NH<sub>2</sub>, CONH<sub>2</sub>, CO, quinones, etc.) of the humic substances react with Cd, giving rise to complexes of Cd or chelates; resulting in the availability of Cd uptake in plants. The organic matter provides carbon to the microorganisms of the soil, which allows an increase in the microbial population and enzymatic activity; soil microorganisms allow: precipitate, sequester, volatilize and complex the cadmium, favoring the adsorption of Cd in the soil. You can use compost, poultry manure, humus, manure, etc.; the quantities of these materials to be applied per ha. will depend on the organic matter content of the soil, indicated in the characterization analysis; in general, the soil should be handled with an average level from 3 to 4%, to preserve the physical, chemical and biological characteristics. CAICAH evaluated the effect of compost and manure on the reduction of Cd in cocoa beans.

The following treatments were used: compost - dose of 27.00 kg/plant or 30.00 MT/ha, 54.00 kg/plant or 60.00 MT/ha and 81.00 kg/plant or 90.00 MT/ha; poultry manure - dose of 27.00 kg/plant or 30.00 MT/ha, 54.00 kg/plant or 60.00 MT/ha and 81.00 kg/plant or 90.00 MT/ha and Control - dose of 00.00 kg/plant or 0.00 MT/ha; after one year of evaluation, the Cd content observed from each compost treatment was 0.08 ppm, 0.17 ppm, 0.11 ppm; of chicken manure 0.09 ppm, 0.22 ppm, 0.28 ppm and in the control 0.19 ppm. Conclusion: The application of organic matter in cocoa plantations, results in the decrease of cadmium in cocoa beans, reaching very small values of up to 0.08 ppm. USA comments that Cd cannot be volatilized under about 800°C. Soil Cd can be more strongly adsorbed and leached, but not volatilized. In addition, the organic matter allows you to increase the capacity of cation exchange.

### **Use of zinc sulphate in fertilizer formulas**

Zn has an antagonistic effect with Cd; Venezuelan studies determined that the Zn/Cd ratio higher than 1,000 meant a decrease in Cd adsorption in cocoa beans, being determined up to 0.05 ppm.

CAICH conducted studies using three (03) doses of zinc sulfate to decrease Cd content in cocoa beans, using the following treatments: 0.00 kg of Zn sulphate/plant, 0.09 Zn sulphate/plant, 0.18 Zn sulphate/plant and 0.27 Zn sulphate/plant. After one year of evaluation, the following results were obtained: 1.92 ppm, 1.83 ppm, 1.86 and 1.49 ppm respectively.

In conclusion: Zn sulphate has a positive effect on the decrease of cadmium content of cocoa beans. The application of zinc sulphate was carried out with the balanced fertilization that is carried out annually to the cocoa plantation, according to the requirements of the crop and soil (characterization analysis).

### **Post-harvest treatment of cocoa beans**

Cocoa CCN-51 is a clone with a high content of mucilage, superior to Creole cocoas. Studies carried out in the CAICH showed quantities of Cd in the mucilage higher than the shell and the grain. It is suggested that during the process of fermentation of the grain there could be entry of Cd from the mucilage to the cotyledons. To clarify this hypothesis, the mucilage of the cocoa beans was drained for 24 hours in meshes, before entering the fermenting boxes; obtaining a reduction of 29.12% of the content of Cd in the beans with respect to the control or control (cocoa beans without draining the mucilage). The results obtained on the content of Cd in cocoa beans were: T1 (Yeast LB', without draining = 0.48 ppm), Control (Without yeast, without draining = 0.46 ppm), T2 (Drained 24 hours = 0.33 ppm), T3 (Yeast LB', no draining, bean washing = 0.63 ppm).

Conclusion: The draining of the mucilage had a positive effect on the decrease of Cd in cocoa beans.

Note: The amount of mucilage evacuated from cocoa beans did not affect the physical or organoleptic quality of the cocoa at the time of evaluation.

## **73. PERU**

Dávila, C. 2018c. "Effect of compost and chicken manure on the physicochemical properties of the soil and cadmium content of the dry cocoa beans (*Theobroma cacao L*), CCN-51"

Organic matter was studied as a sequestering and complexing source of cadmium and that in cocoa beans its level does not exceed 0.50 ppm.

It was evaluated the effect of compost and chicken manure on the physicochemical properties of the soil and on the Cd content of the dry cocoa beans CCN-51, of the "San Pedro" farm, Trampolín farmhouse, Daniel Alomía Robles district, Leoncio Prado province, Huánuco region, during the months of February to November 2015.

The study was conducted in a CCN-51 clone cocoa plantation of 8 years old in production, in degraded soils where coca was previously grown. With initial levels of cadmium in grains of 3.55 ppm (maximum permitted levels of Cd is 0.50 ppm), currently this plantation has organic certification, with an approximate production of 500.00 kg/ha/year. The physiographic unit corresponds to Lomada, climate of Tropical Very Humid Forest (vhf-T), with average temperature of 25.53 °C, average rainfall of 219.13 mm/month, average relative humidity of 83.4%, average hours of sunshine of 158.63, which in the August was greater, at an altitude of 740 meters above sea level and geographic coordinates: mN 8981978, mE 395270.

To its physical-chemical analysis, the soil is of frank texture, slightly acidic pH, medium organic matter, N at a medium level, P with high level and K with low level, a cation exchange capacity equal to 10.03 meq/100 g of soil; with available Cd of 2.72 ppm.

Weeding was done manually (machete) and mechanically (motoguadaña); then, the treatment was to use compost and chicken manure in different proportions: 27.00, 54.00, 81.00 kg / plant each, using 30.00, 60.00, 90 MT/ ha for each combination, with 6 treatments and a control group. Standard fertilization used was the fertilizer formula (60 - 70 - 60), calculated for an estimated production of 1000 kg of dry beans per ha for year 2015. The inputs used as macronutrients were sulphomag, island guano which P<sub>2</sub>O<sub>5</sub> content covered the needs of the plantation, because the soil has a pH > 5.5. The micronutrients used were ulexite (B) at a dose of 20.00 kg / ha, Zn sulfate, Fe sulfate and Cu sulfate, each of the sulfates was used at a rate of 5.00 kg /ha.

The experimental design was that of completely randomized blocks (DBCA), with 7 treatments and 4 repetitions, for the analysis of variance and the comparison of averages the Duncan test with a significance level of  $\alpha = 0.05$  was used.

The analysis of total cadmium in the cocoa samples was carried out by the wet method, excluding the seed testa, according to the protocol, and the quantification of the available Cd in soil was carried out by the method according to WETERMAN.

It was shown that Cd increased in soil as higher doses of compost and chicken manure does, not exceeding the environmental standard for agricultural land that is 1.40 ppm of Cd (Ministry of Environment, 2017). In cocoa beans the effect of compost and chicken manure on the content of Cd has no statistical significance between treatments, but mathematically yes, it was determined that the T1 treatment (compost at 30 Mt/Ha) had lower Cd content in the beans with 0.076 ppm (decreasing 59.114% of Cd with respect to the control) and organic matter of 3.74%, which explains that the higher content of organic matter in the soil prevents the absorption of Cd by plants in various organs. The registered pH was 4.87 confirming that, in acidic conditions, the organic matter of the soil is the most important variable that governs the bioavailability of Cd in the soil.

Regarding the chicken manure: the most effective treatment to reduce the content of Cd in cocoa beans was T4 (chicken manure at 30.00 t/ha) with 0.086 ppm of Cd which is a result of having the highest content of organic matter in the soil (3.82%), corroborating that plants absorb less Cd when the soil has a higher organic matter content. Doses greater than 30 t/ha of compost and chicken manure, increased the Cd content in dry cocoa beans, with respect to the T1 treatment.

Treatments T6 and T5 have the highest content of Cd in cocoa beans by applying amounts greater than 30 t / ha of chicken manure, which contributes to the absorption of Cd by cocoa, indicating that volumes greater than 30 t / ha of compost and chicken manure are not necessary to decrease Cd levels in cocoa beans so lower amounts should be used.

The researcher recommends continuing investigations of bioremediation of Cd in the cultivation and production of cocoa, in other types of soils and different places with significant contents of Cd in the beans; indicating that the process of humification of the organic matter in the soil is slow; also, suggests that works of this nature should be evaluated at least two or three years to obtain better results.

#### 74. PERU

Falcon, G y Davila, C. 2019. "Effect of fermentation on the content of Cd and total polyphenols of cocoa beans (*Theobroma cacao* L.) Clon CCN-51.

In the present investigation the fermentation of the beans was carried out under certain conditions, such as draining of the juice and use of yeasts; given that cocoa juice and pulp contain an average of 6.80 ppm of cadmium (IPNI, 2015) and microorganisms such as yeasts, have the property of sequestering Cd in their cell walls and therefore decreasing the concentration of Cd in cocoa beans.

General objective: To evaluate the effect of the fermentation process on the content of Cd and total polyphenols of the cocoa beans clone CCN-51.



Specific objectives: To determine the effect of fermentation on the macro and microelements (Cd) content of the cocoa beans clone CCN-51.

The present investigation was carried out in the months of October, November and December of year 2018, in the benefit center of the Cooperativa Agroindustrial Cacao Alto Huallaga (CAICAH), located in the district of Castillo Grande, province of Leoncio Prado, Huánuco region. According to the classification of the American scientist Holdridge, this area corresponds to a climate of Tropical Very Humid Forest (vhf - T), average temperature 26 ° C, relative humidity 84%, geographical coordinates: mE 0389535 and mN 8974399 with an altitude of 654 meters above sea level.

The plot from which the cocoa beans were extracted is called "El Mirador", belonging to Mrs. Cecilia Huancho Hualinga, located in the hamlet of Venenillo, district of José Crespo y Castillo, province of Leoncio Prado, region Huánuco; It is a plot of 8 ha, it has a shade-free cultivation CCN-51 of 12 years, currently this plot has organic certification, with an estimated production of 1000 kg/ha/ year of dry bean. The physiographic unit where the cocoa plantation is installed corresponds to a medium terrace, whose initial content of available Cd in the soil and total Cd of cocoa bean was 0.18 and 1.80 ppm respectively. The meteorological data, obtained from the Experimental Meteorological Station "José Abelardo Quiñones" of Tingo María, corresponding to the year 2018 shows the following average values: temperature 25.41 °C, precipitation 297.94 mm/month, relative humidity 84.08% and sun hours were 145.59, being higher in the month of August.

Table 7 shows the physical - chemical soil analysis of the plot El Mirador.

**Table 7. Physical - chemical analysis of the soil (plot).**

Parameter	Value	Method used
Physical analysis:		
Sand (%)	16.00	Hydrometer
Clay (%)	19.00	Hydrometer
Silt (%)	65.00	Hydrometer
Textural class	Silty loam	Textural triangle
Chemical analysis:		
pH (1: 1) in wáter	7.15	Potentiometer
M. O. (%)	1.48	Walkey y Black
N - Total (%)	0.07	% M.O. x 0.05
Available phosphorus (ppm)	10.00	Olsen Modified
Available potassium (ppm)	71.00	Ammonium acetate
Pb available (ppm)	-	-
Cd available (ppm)	0.18	EDTA - EAA
Changeable Ca (cmol+)/kg)	9.66	EAA
Changeable Mg (cmol+)/kg)	1.41	EAA
Changeable K (cmol+)/kg)	0.06	EAA
Na Changeable (cmol+)/kg)	0.94	EAA
Al changeable (cmol+)/kg)	....	Yuan
H changeable (cmol+)/kg)	....	Yuan
CIC	12.07	EAA
Bas. Camb. (%)	100.00	Ca + Mg +K+ Na/CIC x 100
Ac. Cam. (%)	0.00	CIC - Bas. Camb.
Sat. Al (%)	0.00	

Source: Soil laboratory of the National Agrarian University of La Selva - UNAS.

Methods - Components in study

It was used fresh CCN-51 cocoa beans, drained and undrained, that have reached the optimum physiological maturity of a 12-year-old adult plantation.

The yeast used in this research is the *Saccharomyces cerevisiae* strain, which has a high affinity for heavy metals, obtained in the Tingo María market.

The description of the treatments under study is presented in Table 8.

**Table 8. Description of the treatments under study**

Treatments	Description
T <sub>0</sub>	Undrained fermented bean
T <sub>1</sub>	Fermented bean drained for 36 hours
T <sub>2</sub>	Undrained fermented bean + <i>Saccharomyces cerevisiae</i> at 1% p/p
T <sub>3</sub>	Unfermented bean (control)

#### Research execution

##### Selection of the cocoa plot clone CCN-51

Before starting the study, sampling and quantification of total Cd from several cocoa plots was carried out in order to identify one that has a Cd content greater than 1.50 ppm. The selected cocoa plot had a total Cd content of 1.80 ppm, excluding the shell.

##### Collection of cocoa beans

The physiologically mature pods of the selected cocoa plot were harvested. The breakage of the pods was made using a machete without edge; depositing cocoa beans in plastic buckets, avoiding the draining of the mucilage at all times; in the afternoon at averaging 4.00 pm the beans were transferred in timbos (timbo wood containers) of 200 L to the CAICAH collection center. Beans were transported on the same day of harvest, using motorized units.

##### Treatment implementation – fermentation

The implementation of the treatments was carried out according the following methodology:

##### Treatment T<sub>0</sub>:

The fresh cocoa bean, arrived from the field, was fermented in the wooden boxes; The cocoa mass was covered with jute sacks, as were all treatments. The first removal of the beans was done at 48 hours and turned every 24 hours. It fermented 6 days in total. 60.00 kg of fresh bean was used for each repetition. This CAICAH fermentation method was used for cocoa volumes.

##### Treatment T<sub>1</sub>:

For this work a simple mesh of 0.50 cm in diameter was used; suspending the mesh with the fresh beans of a wooden stringer for 36 hours, to guarantee the draining of the beans. After that, the cocoa mass to be fermented was just placed in the wooden boxes, the first removal was carried out at 48 hours and then every 24 hours. It was fermented for a period of 6 days. 60 kg of fresh bean was used for each repetition.

##### Treatment T<sub>2</sub>:

The strains of *Saccharomyces cerevisiae* were applied to the fresh bean arrived from the field. For this, 60 g of yeast was weighed, activating them for a period of 20 minutes in 120 g of sugar diluted in 420 ml of water at a temperature of 37 °C; once the yeast strains have been applied; the fresh mass was covered with jute sacks, beginning its fermentation process. The first turn of the dough was done at 48 hours and then every 24 hours. The fermentation time of the cocoa beans was for a time of 5 days. 60 kg of fresh bean was used for each repetition.

##### Treatment T<sub>3</sub>:

Fresh cocoa beans from the field were dried by direct solar radiation. Three (3) kg of fresh grain was used for each repetition.

During the experiment, the daily temperature was recorded for each treatment.

##### Drying of cocoa beans

Using first-use polypropylene sacks, the cocoa samples were dried through direct solar radiation, to an approximate humidity of 7%. Bean samples at the time of drying were correctly identified by treatment, using indelible marker and masking tape. Once the cocoa samples of each treatment were dried, they were coded and sent to the laboratory for their respective analysis of Cd and polyphenols.

#### Cd content in cocoa bean

The total Cd content of cocoa beans was quantified by flame atomic absorption spectrophotometry (FAAS), using the official method of AOAC 999.11. The analyzes were carried out in the "Valle Grande" Agricultural Chemistry Laboratory, Cañete, Lima. The method used for the quantification of total Cd bean excludes the testa or shell.

The value of the coefficient of variation (C.V.) for the content of Cd was 17.97%.

The T<sub>3</sub> treatment (unfermented bean) has the highest average contents of Cd with a value of 1.21 ppm; for Cd the lowest value is shown in the T<sub>2</sub> treatment (undrained fermented bean + *Saccharomyces cerevisiae* at 1%) with 0.54 ppm.

The content of Cd obtained in this research is similar to that reported by Tantalean (2017) who performed the nutritional characterization of cocoa beans from an alluvial soil in the Alto Huallaga Valley, whose average content was 0.84 ppm.

In general, the T<sub>3</sub> treatment (unfermented bean - control), contains the highest contents of macro and microelements with respect to the other treatments, this treatment reached the lowest percentage of fermentation (68%); which would be influencing the nutrient content; that is, the lower the fermentation content, the higher the nutrient content in the cocoa bean cotyledon; it seems that when there are low fermentation percentages, the organic compounds present in the cocoa bean cotyledon would not be degrading, therefore there would be no release of substances to the external environment of the bean.

Remember that during the fermentation stage the bean moisture remains above 35%, allowing enzymatic activity (Thompson et al., 2001, cited by Pancardo, 2016). The T<sub>2</sub> treatment (undrained fermented grain + *Saccharomyces cerevisiae* at 1%) obtained the lowest cadmium content (0.54 ppm), this is perhaps linked to the biological activity of yeasts which have the property of absorbing heavy metals (Cd, Pb and Zn) in their cell walls thanks to organic compounds called peptidoglycans specific for *Saccharomyces cerevisiae*. The strain of *Saccharomyces cerevisiae* is one of the native strains that intervened in the fermentation of cocoa, increasing its population in this process could improve the absorption of cadmium and improve the quality of cocoa (safety). The biosorption capacity of these microorganisms is due to the quantity of organic compounds capable of sequestering and/or exchanging metal ions, among which polysaccharides, glycoproteins, flavonoids, etc., in which the cation attracting centers are the groups functional amino, hydroxyl, carboxylate, phosphate and sulfhydryl (Chávez et al., 1993). Lanza et al. (2016) when analyzing the Cd content of the cotyledons of a hybrid cocoa from Venezuela; fermented and without ferment found differences, whose values were: fermented cocoa (1.74 ppm) and cocoa without fermentation (2.09 ppm). During the fermentation process metals could be moving from the cotyledon to the bean shell by mass flow. The chemical composition (macro and micronutrient content) of cocoa beans can vary depending on the type of bean, fermentation, drying and subsequent processing (Arvelo et al., 2017).

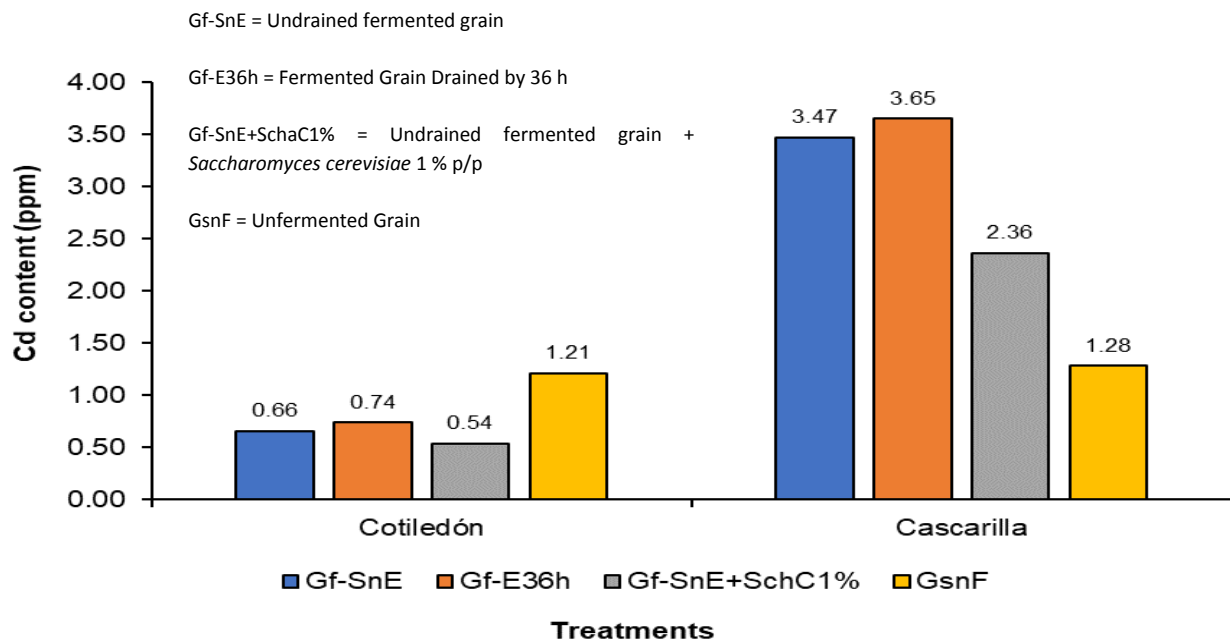
Regarding the content of Cd in the shell of cocoa beans in relation to the treatments under study.

The treatments T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub>, have the highest values of Cd in the shell, possibly increasing its concentration due to its migration of cocoa bean cotyledons, as a result of the biochemical process of fermentation. The T<sub>3</sub> (Unfermented bean treatment) has the highest Fe contents, probably because these beans underwent major chemical changes due to the fermentation without loss of organic substances in the shell. Finally, it is evident that the shell contains more Fe, followed by Zn, Cu and Cd.

In recent years one of the microelements of cocoa that has taken great relevance worldwide is Cd, which is analyzed independently both in the cotyledon and in the cocoa bean shell. In Figure 1, it is shown that the content of cadmium is higher in the shell than in the cotyledon of the cocoa bean. On average, 0.79 ppm was obtained in the cotyledon and 2.69 ppm of Cd in the shell, yielding a shell/cotyledon ratio equal to 3.40. When the cocoa beans reach a fermentation percentage greater than 80% as in the case of the present study, the cadmium content of the cotyledons is reduced as a result of the migration to the testa and the external environment. Unfermented beans have very similar Cd contents both in the cotyledon (1.21 ppm) and in the shell (1.28 ppm).

Figure 1 clearly shows that fermentation of the bean under any modality or treatment decreases Cd content of the cocoa bean cotyledons.

**Figure 1. Cadmium content in cotyledon and cocoa bean shells with respect to the treatments under study.**



75. PERU

Arviles, M. 2012. "Prospecting cadmium and lead in three cocoa cultivars in soils of the Kumpirushiato Valley, Echarati," La Convention. Cusco

Study environment: Location Echarati district sectors of Kuviriari, Alto Kepashiato, Cigakiato, Puguientimari in the Kumpirushiato valley, Echarati-La Convencion-Cusco. Semi-warm rainy climate with dry winter, with annual rainfall of 1 211.5 mm and average annual temperature of 24.9 °C. The rains are distributed in a dry period between the months of May to July and a period with abundant rainfall between the months of December to March. They are areas with anthropic intervention in which an intense agricultural activity takes place, subtropical humid forest life zone, of rugged topography with predominant slopes in the sectors of Kuviriari (45%), Alto Kepashiato (33%), Cigakiato (42%), Puguientimari (48%) that are distributed in a considerable and dispersed way throughout the valley.

Methodology and results: She worked on twelve (12) samples of cocoa beans (*Theobroma cacao*): three CCN-51 (Castro Naranjal Collection -51), three ICS-1 (Institute College Selection -1 Collection), three ICS -95 (Institute College Selection Collection - 95) of each of the sectors and in 04 soil samples: one from each sector extracted at 30 cm. depth according to what is established in the Mexican standard NMX-AA-132-SCFI-20 (soil sampling for the identification and quantification of metals, metalloids and sample handling), concluding that in the four sectors in study, cadmium has not been detected in the soil or, failing that, it is found in levels that are not noticeable. These results are below the environmental quality standards for the soil decreed in the D.S. 002-2013-MINAG (2013). The method used for the determination of cadmium is the EPA 6010B method "Determination of metals and Trace Elements, Wastes, Soils, Sludge, Sediments, and other Solid Wastes by Inductively Couple Plasma - Atomic Emission Spectrometry". Rev. 2. January 1995. The samples were analyzed in Envirolab-Peru laboratory. Cadmium was detected in cocoa beans only in ICS-95 (0.3 mg / kg) and CCN-51 (0.2 mg / kg) cultivars in the Cigakiato sector plot.

76. PERU

Marie Zug, K, Huamaní Yupanqui, H, Julia Susanne Frank Meyberg and Cierjacks Arne Cierjacks. 2019. Cadmium Accumulation in Peruvian Cacao (*Theobroma cacao* L.) and Opportunities for Mitigation. Water, Air, & Soil Pollution

Crops are the main source of toxic Cd for humans due to uptake from naturally or anthropogenically polluted soils. Chronic Cd ingestion causes kidney, liver, and skeletal damage along with an increased risk of cancer. Cacao is known to accumulate Cd and may therefore be potentially harmful to human health. Consequently, cocoa production on intensely polluted soils should be avoided. Cocoa products from South America often exceed the limits for Cd, but the factors that drive Cd uptake are as yet poorly studied. In this study, we measured Cd concentrations in defatted cocoa powder from unfermented seeds of 40 different trees on 20 farms in the Huánuco Region, Peru, and associated the Cd levels with the farms' soil, field management, and nearby vegetation diversity.

The mean Cd concentration found in cocoa of the study region was 2.46 mg kg<sup>-1</sup> with a range of 0.2–12.56 mg kg<sup>-1</sup>. The maximum content measured was an order of magnitude higher than the allowed limit of 1.5 mg kg<sup>-1</sup> and was the highest reported so far in the literature. Soil Cd content was the most relevant driver of Cd concentration in cocoa. In addition, fertilizer use caused significantly higher Cd concentration in cocoa. Higher biodiversity of herbs was positively correlated with Cd contents in cocoa. The study shows that, apart from the known correlation of soil conditions with Cd accumulation in cocoa seeds, changes in fertilization and plant composition may be promising measures to counteract Cd contamination in regions with high soil Cd content.

The online version of this article (<https://doi.org/10.1007/s11270-019-4109-x>) contains supplementary material, which is available to authorized users.

## 77. PERU

Condori, D. 2018. Peru. Informe de Estudio: “Cadmio en el cultivo del cacao”

OBJECTIVES: a) Conduct an updated bibliographic review on the sources, causes, effects, contents and implications of Cd content in cocoa crop and b) Describe proposals for mitigation and / or remediation of cadmium in areas of crops and cocoa beans.

The release of Cd into the environment is increased by the action of man (burning of fossil fuels, metallurgy, manufacturing of batteries, pigments, incineration of garbage), by the use of phosphate-based fertilizers and agrochemicals; in addition, smoking has been identified as a possible significant contributor to Cd exposure.

The increase in Cd levels in the plant may be due to various factors, such as:

### Edaphic factors (Soil)

- pH, adsorption increases when pH decreases
- Soil salinity, adsorption increases with salinity
- Cd content, adsorption increases its concentration
- Micronutrients, Zn deficiency increases Cd adsorption
- Organic matter content, adsorption decreases with its increase

### Crop factors

- Crops, certain types of cocoa adsorb Cd more than others
- Plant tissue, leaf > almond (mucilage > testa > cotyledon) > roots
- Leaf age, old leaves > young leaves

### Anthropogenic factors

- Excessive use of pesticides that contain traces of Cd, eliminate efficient microorganisms in the soil.
- Excessive use of phosphate fertilizers, contains traces of Cd (0.1 – 170.00 mg / kg)
- Excessive use of island guano, may contain traces of Cd (2.44 – 5.50 mg / kg)
- Irrigation with contaminated rivers, especially in mining or former mining areas.

### Proposals for the mitigation and / or remediation of Cd content in cocoa crop

Soil remediation can be one of several viable tools to reduce the solubility of Cd, thus preventing excessive uptake and accumulation of Cd in plants. Based on findings in various studies, a series of preliminary recommendations are presented to help cocoa producers cope with the potential contamination of Cd in cocoa cultivation.

Prior to mitigation and/or remediation:

Chemical analysis of the soil (pH, macro and micronutrients)

Evaluation of the content of Cd in soil, irrigation water and cocoa beans

Strategy in new plantations:

Install plantations in agricultural soils that have less than 1.4 ppm of total Cd and irrigation water whose contents do not exceed 0.005 mg / L.57

Install the plantations in areas away from the roads or take measures to prevent the contact of cocoa with the gases that emit the combustion of vehicles. Also, in areas away from dumps of cities or mining areas.

Strategy in plantations already installed:

Increase levels of Zn and Mn in the soil. Zn sulfate has a positive effect on decreasing the Cd content of cocoa beans. The application of Zn sulfate should be done in a balanced way according to the requirements of the crop and soil.

Apply limestone levels in low doses (dolomite -  $\text{CaCO}_3 \cdot \text{MgCO}_3$ ) to gradually increase the pH and incorporate Ca and Mg, and reduce the absorption of Cd. Lime could also be applied to correct the pH and obtain the same result.

Increase the organic matter content of the soil and improve the biological activity using fertilizers or organic fertilizers (compost, humus, bocashi, among others).

Avoid the use of phosphorus fertilizers and phosphoric rock that may contain high Cd concentrations, and verify the heavy metal content. The EC recommended is 30 ppm Cd / Kg maximum.

Use nitrogen and potassium fertilizers (NPK), but previously verify the content of heavy metals.

Using activated carbon or biochar (biocarbon), this can adsorb cadmium and reduce its bioavailability towards the plant.

Use of yeast-based biofertilizers as the product FERTILEV®, EM®(efficient microorganisms) and/or mycorrhizal fungi, these organisms have the ability to adsorb cadmium and bioaccumulate or immobilize it.

In plantations with high Cd content in leaf, remove them from the plot after pruning.

Reduce or avoid the use of pesticides such as fungicides or insecticides, as is done in organic agriculture. Pesticides could increase the Cd content by having a negative effect on native soil microorganisms.

Selection of cocoa varieties that do not accumulate high levels of heavy metals.

During the cocoa post-harvest

Draining the beans before fermentation (12 to 24 hours), reduces the content of cocoa mucilage and consequently the content of Cd in the bean.

Use of yeasts during fermentation, yeasts such as *Saccharomyces cerevisiae* or other used for the production of wine or champagne, reduces the content of Cd in the bean.

Avoid drying cocoa beans on the roads and / or exposed to vehicle smoke, these practices could increase the content of Cd in the bean.

The mitigation and/or remediation of Cd should be addressed in an integrated manner, and these recommendations to reduce Cd levels should be implemented progressively to agricultural practices and post-harvest operations.

It is important to mention that Peru, as a member of FAO / WHO, is developing a "Code of practice to prevent and reduce the contamination of cocoa by Cd", which is being evaluated by JECFA. The development of a COP will help prevent and reduce Cd contamination of cocoa and cocoa products, thus helping to reduce exposures to this contaminant and possible alterations in international trade.

## Conclusions

Peruvian cocoa is in the process of development and growth, and its main markets are Europe (cocoa bean) and North America (chocolate).

In some cocoa producing areas in Peru, as well as in the region, cadmium content in cocoa beans is high (> 0.8 to 3.14 ppm) in relation to the maximum level (ML) described in the European Regulation 488/2014 (0.8 ppm) and that requested by importers.

The content of cadmium in the various types of chocolates marketed in the country seems to comply with the NMs of European Regulation 488/2014 (0.8 ppm). There is still more data needed to verify this conclusion.

The application of the European Regulation 488/2014 could limit Peruvian exports of cocoa beans, generating a negative social and economic impact on cocoa-producing families and exporting institutions.

High Cd content in cocoa and derivatives is a real and current problem, the solutions should be given from each phase of the value chain.

The mitigation and/or remediation of Cd in the cocoa chain should be approached in an integrated and progressive manner, starting from agricultural practices, followed by post-harvest operations.

The establishment of NM seems to have two connotations. The first is related to safety, to reduce the exposure of Cd to the consumer through food. The second is of an economic nature for the commercial negotiation of raw material.

78. PERU

Rojas, R., Rodríguez, C., Ruiz, C., Portales, R., Neyra, E., Patel K., Mogrovejo J., Salazar G., Hurtado, J. 2017. Cacao Chuncho from Cusco

The CACAO CHUNCHO DEL CUSCO publication summarizes the main findings obtained during the execution of the Project "From Cocoa to Chocolate: Morphological, Genomic, Metabolomic and Sensory Study of Chuncho Cocoa from Cusco for Quality Assurance and Innovation of the Peruvian Chocolate Industry" (Agreement 159-PNICP-PIAP-2015), which was co-financed by Innovate Perú.

This project had as applicant entity the Peruvian University Cayetano Heredia (UPCH) and as associated entities to the Institute for Development Research (IRD), APPCACAO, La Fabrica de Chocolates La Iberica, Chocomuseo and the Cocoa Innovation Center (CIC).

The objective of the study was to characterize morphologically, genetically, chemically and sensorially 11 "Chuncho" cocoa cultivars from Quillabamba, province of La Convencion, Cusco.

**Note:** For this work, from the aforementioned publication it has only been taken the points regarding the Proposed Development of A Code of Practice for the Prevention and Reduction of Cadmium Contamination in Cocoa Beans, but always keeping in mind the nutraceutical benefits and the genetic improvement for cacao crops exhibited by the Cacao Chuncho from the Cusco Region.

#### **Fermentation process and drying, and elaboration of cocoa paste**

The harvest and benefit of the 11 cocoa cultivars was carried out with the support of the producers, making the pod harvest by type of cocoa and plant by plant, only mature pods were used, avoiding harvesting sick, overripe and green pods. Subsequently, the cocoa beans were placed in cloth bags and then introduced into the fermentation drawers, a process that lasted between 4 and 5 days depending on the size of the almonds. The fermenting drawers were provided by the producers, located in sheltered places in Calzada and Cacaopampa in order to guarantee the due fermentation process.

The drying process was carried out in the city of Quillabamba using wooden pallets for a period of 5 days, with 2 to 4 hours of daily sun exposure. The drying was developed according to criteria of Good Practices of Harvest and Benefit of Cocoa adapted for Chuncho cocoa. At the end of the drying process, the bagging was carried out in jute bags, with the respective labels for the correct identification of the 11 samples of Chuncho cocoa.

The samples were subjected to the roasting process at a temperature of 120 ° C for a period of 20 minutes. After removing the shell and obtaining the cocoa nibs, the process of repeated grinding was continued for a period of 24 hours until obtaining cocoa liquor. For this, a manual mill and a Cocoa Town grinding equipment were used.

#### **Heavy Metals**

Other analyzes of Minerals/Heavy Metals: Cd, Al, Antimony (Sb), As, Barium (Ba), Beryllium (Be), Boron (B), Cerium (Ce), Strontium (Sr), Lithium (Li), Mercury (Hg), Nickel (Ni), Silver (Ag), Pb, Titanium (Ti) and Vanadium (V) were performed by Laboratorios CERPER S.A. using the ICP-AES technique (EPA Method 200.7. 1994. Inductively Coupled Plasma Atomic Emission Spectrometric Method for trace Element Analysis of Water and Wastes).

#### **Genetic analysis**

After the morphological characterization of the selected cocoa cultivars, it was taken between 2 and 6 leaf samples of each of them; continuedly, the leaves were transported in ziploc® bags with silica gel to the UPCH Genomics Unit with the following codes:

Cultivar	Code	Repetitions
Chuncho común	Muestra 1	M1-1, M1-2, M1-3, M1-4, M1-5
Común	Muestra 2	M2-1, M2-2
Chuncho	Muestra 3	M3-1, M3-2, M3-3, M3-4, M3-5
Común liso	Muestra 4	M4-1, M4-3, M4-5, M4-7, M4-8
Común rugoso	Muestra 5	M5-1, M5-3, M5-4, M5-5
Común manzana	Muestra 6	M6-1, M6-2, M6-3, M6-4, M6-5
Cáscara de huevo	Muestra 7	M7-1, M7-2, M7-3, M7-4, M7-5
Señorita	Muestra 8	M8-2, M8-3
Achoccha	Muestra 9	M9-1, M9-2, M9-3, M9-4, M9-5, M9-6
Chuncho de montaña	Muestra 10	M10-1, M10-4, M10-5
Pamuco rugoso	Muestra 11	M11-1, M11-2, M11-3, M11-4

For the genetic analysis using the AFLP (Amplified fragment length polymorphism) technique, Doyle & Doyle (1990) protocol was followed, with some modifications:

Results and Discussion

Cadmium content in beans

CULTIVAR	PRODUCER	ZONE	Concentration (Cd mg/kg)
Chuncho	Ricardo Quintanilla Gamarra	Santa Ana – Cacaopampa	0.11
Comun	Ricardo Quintanilla Gamarra	Santa Ana – Cacaopampa	0.11
Señorita	Francisco Torres Baca	Echarati-Pispita	0.07
Achoccha	Francisco Torres Baca	Echarati-Pispita	0.10
Cáscara de huevo	Francisco Torres Baca	Echarati-Pispita	0.05
Común manzana	Lorenzo Bedoya Farfán	Santa Ana - San Pedro	<0.05
Común liso	Orlando Tupayachi Muñoz	Santa Ana – Cacaopampa	0.07
Común rugoso	Orlando Tupayachi Muñoz	Santa Ana – Cacaopampa	0.08
Chuncho de montaña	Francisco Torres Baca	Echarati-Pispita	0.08
Chuncho común	Demetrio Villavicencio Pereira	Echarati - Chahuare	<0.05
Pamuco rugoso	Francisco Torres Baca	Echarati-Pispita	0.09

Genetic analysis

The extraction process with CTAB (Cetyltrimethylammonium Bromide) was successful, obtaining a DNA concentration in the order of 57.9 to 211.6 ng /  $\mu$ L with a ratio of A260 / A280 close to 2, thus obtaining a good quality of DNA to work in the AFLP reactions. The basic matrix of presence and absence band analysis data considering a total of 21 polymorphic bands is in the range of 80 to 350 pb.

In conclusion, the use of the AFLP technique allowed the generation of molecular patterns to genetically differentiate cocoa cultivars. The cultivars "Chuncho Común" and "Común" are the most genetically distant from the other cultivars. Clusters could be proposed for the other cultivars, such as: "Señorita" with "Achoccha", "Pamuco rugoso" with "Chuncho de Montaña" and "Chuncho" with "Común Liso".

Microbiological study of the Fermentation Process of the cultivar "Cáscara de huevo".



During the fermentation process of the Chunchu cocoa cultivar "Cáscara de huevo", a variation in the pH values of the mass was observed due to the action of the microorganisms present in the fermentation box. The initial pH of the fermentation mass was 4.0, which is consistent with that published in other scientific papers where pH values in the range of 3.5 to 4.5. are reported. The variation in pH occurs possibly due to citric acid consumption by yeasts, which in turn allows the growth of lactic acid bacteria (BAL). In addition, an increase in temperature was observed until reaching 42.8 ° C on day 4 of the fermentation, this is because the metabolic reactions are highly exothermic in yeasts and bacteria during the development of fermentation.

At the microbiological analysis a succession of microorganisms was observed throughout the fermentation process. As of the second day, a greater number of microorganisms was evident in the MRS medium (Agar De Man, Rogosa, and Sharpe), which included lactobacilli and yeast. Subsequently there was a marked increase in growth in the Sabouraud medium that indicated the presence of yeasts. From the fourth day the growth in the GYC medium (Chloramphenicol Glucose Agar) was increased, which is related to the increase in acetic acid bacteria (BAA), keeping this growth until the end of fermentation.

#### Isolation and Characterization of Yeasts

During the cocoa fermentation process, of Chunchu cultivar "cáscara de huevo" it was isolated 26 yeasts, identifying the presence of *Saccharomyces cerevisiae*, *Kloeckera apiculata* (anamorf or *Hanseniaspora uvarum*) and *Candida* sp. The yeast *S. cerevisiae* dominates the course of fermentation; therefore, it can be found throughout sampling, has a rapid growth at a slightly high pH and can tolerate high concentrations of ethanol and temperature.

## 7. CONCLUSIONS

The prevention and reduction of Cd in production systems requires a comprehensive approach to understand the soil factors that lead to high Cd uptake by cocoa crops in order to define strategies to avoid its bioaccumulation. Available or extractable elements are those that can participate in chemical or biological reactions. The roots of cocoa plants can only absorb ions when these are available in the soil solution.

McLaughlin, M. and Singh B. R. in 1999 foreseen that the prevention and reduction of Cd in production systems requires a comprehensive approach to understand the soil factors that lead to high Cd uptake by cocoa cultivation in order to define strategies to avoid its bioaccumulation. Available or extractable elements are those that can participate in chemical or biological reactions. The roots of cocoa plants can only absorb ions when these are available in the soil solution. In addition, the availability of soil-Cd to food crops is dependent on the physical, chemical, and biological processes that control the solubility and form of Cd in soil solution, especially in the rhizosphere. Important non-biotic factors include soil pH, clay content, carbonates, iron and manganese oxides, redox potential, type and content of organic matter, complexing ligands, and water content, as well as soil management practices, including crop rotations and soil amendments such as phosphate fertilizers, manures, sewage sludges and agricultural lime. Known important biotic factors include plant species, crop mechanisms of Cd uptake by plants, cultivars, root activity, rooting patterns and rhizosphere root-associated microorganisms (such as mycorrhizal fungi), all of these factors interact to influence Cd availability to plants, more research is needed on the mechanisms of uptake by roots, translocation, retranslocation and deposition of Cd in plants. Last but not least, factors controlling the bioavailability of Cd from plant foods and the interactions of various dietary constituents and individual nutritional status on Cd bioavailability from these foods need to be studied in greater detail before a full understanding of the significance of current levels of Cd in plant foods on human health and well-being can be assessed with any certainty.

**8. RECOMEDATIONS**

Several of the studies cited in the COP have been validated although some of them are obtaining their results this year. Ecuador has various studies completed and will be included in this COP as soon as possible. The EWG acknowledges that the proposed COP with the necessary changes on the mechanisms of uptake by roots, and deposition of Cd on cocoa beans could be accomplishing its objective.

**9. ACKNOWLEDGMENT**

The EWG chaired by SENASA - Peru Eng. Javier Aguilar Zapata and cochaired by Ghana and Ecuador wishes to deeply thank the United States Food and Drug Administration, the European Cocoa Association, the Ministerio de Economía, Industria y Comercio de Costa Rica, the Brazilian Health Regulatory Agency, the Agencia de Regulacion y Control Fito y Zoosanitario de Ecuador, the Ministry of Health Malaysia Food Safety and Quality Division and to the Ministerio de Agricultura y Riego de Peru for their valuable comments for the elaboration of the Draft "Development of a Code of Practice for the Prevention and Reduction of Cadmium Contamination in Cocoa Beans"

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**APPENDIX III**  
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