



HEAVY METALS REMEDIATION WITH POTENTIAL APPLICATION IN COCOA CULTIVATION

TÉCNICAS DE REMEDIACIÓN DE METALES PESADOS CON POTENCIAL APLICACIÓN EN EL CULTIVO DE CACAO

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Abstract

Cacao (*Theobroma cacao*) worldwide has increased its area sown and yield per hectare, however currently producers are facing strong legislation issued by the European Union regarding the maximum levels of lead and cadmium that must have chocolates that contain an amount greater than or equal to 50% cocoa solids. Based on a review of the work carried out around the world and published in the last three years in global databases are presented, in the first instance, the problems caused in people by the consumption of food contaminated by heavy metals and the routes through which the cocoa can be contaminated, from its planting to its processing. The remediation techniques (phytoremediation and bioremediation) that have obtained good results regarding the cleaning of contaminated soils or that avoid the transfer of the contents of lead and cadmium from the soil to several crops of commercial interest to have options of potential application in the cacao areas of Colombia or anywhere in the world. The results show the importance of implementing an integrated soil remediation system that includes the gradual incorporation of native trees, herbaceous plants, aquatic plants, biochar, bacteria and arbuscular mycorrhizae.

Keywords: Phytoremediation, bioremediation, lead, cadmium

Resumen

El cacao (*Theobroma cacao*) a nivel mundial ha aumentado su área sembrada y rendimiento por hectárea, sin embargo actualmente los productores se enfrentan a una fuerte legislación emanada por la Unión Europea respecto a los contenidos máximos de plomo y cadmio que deben tener los chocolates que contienen una cantidad mayor o igual al 50% de sólidos de cacao. En base a una revisión de los trabajos realizados alrededor del mundo y que han sido publicados en los últimos tres años en bases de datos mundiales se presentan, en primera instancia, los problemas ocasionados en las personas por el consumo de alimentos contaminados por metales pesados y las rutas a través de las cuales se puede contaminar el cacao, desde su siembra hasta su procesamiento. A continuación y dando cumplimiento al objetivo de la revisión se muestran las técnicas de remediación (fitoremediación y bioremediación) que han obtenido buenos resultados respecto a la limpieza de suelos contaminados o que evitan la traslocación de los contenidos de plomo y cadmio del suelo a varios cultivos de interés comercial para tener opciones de potencial aplicación en las zonas cacaoteras de Colombia o cualquier parte del mundo. De los resultados obtenidos se resalta la importancia que tiene la implementación de un sistema integrado de remediación de suelos que incluya la incorporación gradual de árboles nativos, plantas herbáceas, plantas acuáticas, biochar, bacterias y micorrizas arbusculares.

Palabras clave: Fitoremediación, bioremediación, plomo, cadmio.

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1 Introduction

Cocoa is a food product that has had a quite marked growth in producing countries due to its easy handling and excellent economic benefits it presents (TECHNOSERVE, 2015). However, cocoa producers around the world were disturbed when the European Union announced plans to apply regulations on chocolate containing 50% or more of cocoa solids which should contain a maximum of 0.3 mg/kg of cadmium and 1 mg/kg of lead (CODEX Alimentarius, 2015).

Lead or cadmium in soils may have a natural or anthropic origin, but independently of this the plants absorb it and can accumulate it in different structures and proportions (Londño Franco, Londño Muñoz and Muñoz García, 2016; Covarrubias and Cabriales, 2017). This situation occurs in cocoa with significant accumulation in its seeds and leaves (Guerra Sierra et al., 2014) and since most of the products derived from cocoa are consumed by children it is necessary to minimize the presence of these metals in the final product (CODEX Alimentarius, 2015).

The objective of this review is to point out part of the research that has been worldwide developed over the last three years regarding the remediation techniques (phytoremediation and bioremediation) of heavy metals present in the soil, with emphasis on lead and cadmium, and that could potentially be used to manage the levels present in the producing areas of Colombia or anywhere in the world.

2 Referential Framework

The cacao tree is grown in tropical regions. It is commercially cultivated between 15° north and 15° south of the equator. The average annual temperature range goes from 23 to 30°C. It is cultivated from sea level to up 1 200 masl. Likewise, it needs average annual relative humidity of between 70% and 80% (Vizcaíno Cabezas and Betancourt, N.d.; Ramírez Sosa and Orrego Suaza, 2014).

World production of cocoa (*T. cacao* L.) has increased linearly from 1.2 million tons in 1961 to 4.6 million tons of dry cocoa in 2013. During the same period, the annual cocoa area had an average annual increase of 2.5%, (Vanhove, Vanhoudt and Damme, 2016) and although the world demand for cocoa continues to increase, it also shows that production declines as a result of multiple factors, in-

cluding pests and diseases, decrease in soil fertility and an increasingly hot and dry climate (TRANS-MAR GROUP, 2014; Blaser et al., 2017), and this has been accompanied by an increase in international cocoa marketing prices due to the rise in oil prices, which increases the production of fertilizers, pesticides and fuels (Callejas, 2016). Even as of 2001, there was talk of a cocoa deficit that would consolidate by 2020, due to the decrease in production, generated by the poor conditions of growers in the main countries of origin, in addition to the increase in industrial consumption, which it has been stable in recent years (ProExport Colombia, 2014).

World cocoa production is absorbed mainly by three countries (60% of imports), with Switzerland being the largest importer (29.4%), followed by the United States (16.7%) and Germany with 13.9%. Most of the exports will continue to be cocoa beans, although there are attempts by the producing countries to add more value and it is to be expected that cocoa will be so scarce in 20 years that it will turn chocolate into a very expensive luxury product, the authentic chocolate will be within two decades a very expensive and scarce product that will honor its origin: *Theobroma cacao*, "the food of the gods" (Anga, 2015).

The high international prices and the demand by the processing industries has generated an active global dynamics of cocoa, thus encouraging the expectations of the Colombian producers since the above is added in the work done by the National Federation of cacaoteros, FEDECACAO, related to the positioning and recognition of the quality of cocoa at an international level, which translates into new market opportunities for high quality grains produced by cocoa farmers from all regions of the country. In this sense, it is expected to continue increasing the presence in international markets where the Colombian cocoa is already recognized by its fine flavor and aroma and it is expected to be able to obtain extra premiums on the base price, which also translates into better income and services for cocoa farmers (Cacao de Colombia, 2014).

Given this social and economic importance of cocoa, several producing and exporting countries, including Colombia, are interested in knowing the levels of heavy metals present in the cultivated areas (Jaramillo, 2013; Reyes et al., 2016; CAOBISSCO/ECA/FCC, 2015) since in recent years there have been progressive regulations of the European Community, where maximum limits are established

regarding the allowable content of lead and cadmium in chocolate and other cocoa derivatives marketed in Europe, Table 1, according to the regulations EC 1881/2006 and EC 488/2014 (FAO/WHO, 2014; Lanza et al., 2016; Chavez et al., 2015).

Table 1. Maximum permissible contents of lead and cadmium in chocolate (CODEX Alimentarius, 2015). The high assigned value for chocolates with % dry matter total $\geq 50\%$ stands out.

Product	% of dry matter	Maximum limit of cadmium allowed**
Milk chocolate	<30%	0.10 mg/kg
Milk chocolate	$\geq 60\%$	0.30 mg/kg
chocolate	<50%	0.30 mg/kg
chocolate	$\geq 50\%$	0.80 mg/kg
Cocoa powder*	-	0.60 mg/kg

* This limit is applied to both the powder chocolate and cocoa sold to the final consumer as well as the sweetened cocoa powder used as ingredient, also sold to the final consumer (chocolate for drinking)

** These limits will take effect from January 1, 2019.

Consequently, it is essential for any country to have reference data on the content of lead and cadmium in food, especially in those that are preferably consumed by children, such as cocoa, which will allow them to establish a clear position before the international regulations that could represent a risk for the exports of the product and direct the necessary investigations to manage the incidence and severity of this problem on the health of people (Uytendaele, Boeck and Jacxsens, 2016), emphasizing the natural and easy techniques access for farmers known as remediation, including concepts such as phytoremediation (use of plant material that decreases the presence of metal in plants of interest) (Ahkami et al., 2017; Tahir, Yasmin and Khan, 2016; Luo et al., 2016); bioremediation (use of microorganisms alone or in combination of plant material that decreases the presence of metal in plants of interest (Brown et al., 2017; Galdames et al., 2017; Leal et al., 2017; Aggangan et al., 2017).

3 Problems associated with cadmium and lead

Recent research on contamination with lead and cadmium, and other heavy metals in food (Al-Hossainy et al., 2017; Perryman et al., 2017; Si-riangkhwut et al., 2017), and its consequences on human health as conditions with lesions in pregnancy, gastrointestinal irritation, nausea, vomiting,

kidney damage, emphysema, and lung cancer (Antoine, Fung and Grant, 2017; AbuShady et al., 2017; Shakir, Zahraw and Al-Obaidy, 2017; Amadi, Igweze and Orisakwe, 2017) clearly show the reasons about the concern that has been awakened worldwide on this subject and especially due to the progressive accumulation of these metals in the tissues of humans and animals which results in damage at the molecular genetic level (Abarshi, Dantala and Mada, 2017; Al-Gburi, Al-Tawash and Al-Lafta, 2017; Skiba, Kobylecka and Wolf, 2017; Nkansah et al., 2016). Regarding the entry of Cadmium in the body (Díaz García and Arceo, 2017) determines that it is done via gastric with a mechanism of damage that includes: dysfunction by formation of free radicals, apoptosis or activation via the caspases, protein denaturation and by decrease in trans epithelial resistance. Regarding the entry of lead into the body, this same author, determines that it is via gastric, respiratory and contact, with various mechanisms that include: bone storage, mitochondrial damage and formation of free radicals, apoptosis, inflammation by activation FN-k β , RAAS and attraction of macrophages and decrease in NO production.

Chumbipuma, in 2016, describes that with respect to lead, its systemic effects are nonspecific, including adynamia, sleep disorders, headache, pain in bones and muscles, digestive symptoms (constipation), abdominal pain, nausea, vomiting and decreased appetite. He also reports that acute lead to-

xicity occurs after respiratory exposure at high concentrations with encephalopathy, renal failure and gastrointestinal symptoms, and finally concludes that workers exposed for a long time and without personal protection measures may present with a peripheral polyneuropathy, that predominantly affects the upper limbs, the extensor muscles that the flexors and more the dominant side, what has been called the "painter's hand" because it was presented in these workers by the use of paints with a high content of lead.

Anderson, in 2016 showed that the increase in blood lead concentrations of young university students decreases the vitality and fast linear mobility in their sperm. According to Londño Franco, Londño Muñoz and Muñoz García (2016), the World Health Organization mentions with respect to cadmium that the presentation and severity of signs, symptoms and alterations in the organism is related to the amounts, the exposure time and the metal entry route; chronic exposure shows anemia, renal dysfunction, kidney stones, osteoporosis, osteomalacia, respiratory disorders, hypertension, nervous disorders (headache, vertigo, sleep disturbance, tremors, sweating, paresis, involuntary muscle contractions), weight and appetite loss, prostate and lung cancer; in acute poisoning there is pneumonitis and pulmonary edema, gastroenteritis, nausea, vomiting, abdominal pain, diarrhea, kidney failure, and finally chromosomal aberrations, teratogenic and congenital effects and that in the kidney (renal tubules) can accumulate for up to thirty years.

Contaminants in cocoa

The metallic contaminants of cocoa can be defined as those metals, not intentionally added, that are present in cocoa as a result of production (Chavez et al., 2015; Ramtahal et al., 2016), manufacturing, elaboration, preparation (Moreno et al., 2017), treatment, packaging, transport, storage or as a product of environmental contaminations with the potential to present risks to people's health (CODEX Alimentarius, 2015; Londño Franco, Londño Muñoz and Muñoz García, 2016). These metals can be found in soils naturally or as a result of anthropogenic activity, be absorbed by plants, concentrated in seeds and taken from them by humans, which constitutes a potential risk to health (Amadi, Igweze and Orisakwe, 2017; Paul, 2017; El-Amier, Elnaggar and El-Alfy, 2017).

Various investigations have shown that during the process of cocoa benefit, there are variations

in the physicochemical characteristics of the grain, depending on the type of fermenter used and the drying time, which can affect metal concentrations, as well as the quality and safety of the final product (Uyttendaele, Boeck and Jacxsens, 2016; Laires Amaiz et al., 2013).

Within this framework, it is clear that the source of contamination with lead and/or cadmium from cocoa is diverse, the translocation from the soil to the different parts of the plant is complex and little studied and the manifestation of its presence has consequences that are observed not only for plants but also for human beings and, therefore, it is necessary to update the available techniques that exist on the management of this problem in different crops to have a frame of reference that can potentially be applied in cocoa, and thus be able to select those that are most appropriate for the environmental and natural conditions in which this crop is developed within Colombia or anywhere in the world.

4 Phytoremediation of heavy metals

The ideal plant species for phytoextraction are those that have the capacity to accumulate and tolerate high concentrations of metals in harvestable tissue (Tariq and Ashraf, 2016) and this is possible since they have the power to eliminate contaminants that persist in the environment through various mechanisms such as phyllofiltration, phytostabilization, phytoextraction, phytovolatilization and phytotransformation (da Conceição Gomes et al., 2016), in addition to the containment and degradation of metals, phenolic compounds and various dyes, as well as other organic and inorganic contaminants (Tahir, Yasmin and Khan, 2016). Also, there are investigations in phytoremediation that have potential to be applied in the cultivation of cocoa, given the availability of the material with which it was worked.

Tariq and Ashraf (2016) pointed out that hyperaccumulators have different accumulation potentials for different metals. The study developed by them at the laboratory level showed that corn (*Zea mays*) is a hyperaccumulator for Co and Cr after the application of a chelator such as EDTA, while sunflower (*Helianthus annuus*) proved to be a hyperaccumulator for Cd under similar conditions, with a removal of 56.03%. The turnip plant (*Brassica campestris*) exhibited hyperaccumulative properties for

Cr. On the other hand, pea (*Pisum sativum*) was found to be the best accumulator of Pb without application of the EDTA chelate with a removal efficiency of 96.23% (Ojoawo, Udayakumar and Naik, 2015; Tariq and Ashraf, 2016).

It has been proven that the ability of soybean (*Glycine max*) to accumulate heavy metals present in the soil is low compared with its natural predisposition to tolerate them (Ibiang, Mitsumoto and Sakamoto, 2017); however, it was evidenced that its accumulating power can be potentiated when sowing it together with material from the *Melastoma* genus (Syam et al., 2016). Another work has proven the effectiveness of soy as a cadmium hyperaccumulator, since adding up to 300 mg/Kg of nano particles of TiO₂ in the soil gives up to an additional 400% of µg of cadmium/plant (Singh and Lee, 2016).

The bark and leaves of *Moringa oleifera* can be used as an alternative absorbent for the removal of heavy metals from contaminated waters through a technique of obtaining modified citric acid (CAMOB and CAMOL) and its use in soils shows potential for research (George et al., 2016). At ground level, applications of foliar extract have been used to concentrate the lead contents of the soil where beans are grown (*Phaseolus vulgaris*) achieving a remarkable improvement in the stress caused by this element through the activation of the antioxidant system of the roots of beans (Howladar, 2014).

A study developed on the India-Pakistan border in an area cultivated with sugarcane and sorghum, where large amounts of phosphorus fertilizers are used, which are reported as sources of heavy metals in the soils, resulted in reports of the potential use of these plants as phytoextractors of lead, cadmium and copper because their Metal Bioaccumulation Factors (BAF) were around 1, which turns out to be a very good indicator (Singh and Lee, 2016). In the United States, sorghum sown in soils contaminated by heavy metals also showed its phytoextracting ability, either alone or in a mixture with arbuscular mycorrhizas of the *Azotobacter chroococcum* type (Dhawi, Datta and Ramakrishna, 2016).

The humus extracted from composted waste materials from the palm oil extraction *Elaeis guineensis*, mainly the empty bunches, improved with the addition of NPK-based fertilizers were evaluated in their fertilizing and heavy-metal phytoextraction, especially copper (Cu) in cucumber plants (*Cucurbita pepo*). The results indicate that the nutritional

content of empty clusters is low, but the potassium intake is the most significant. Regarding its effect on heavy metals, it was concluded that with the addition of Nitrogen copper extraction is improved, as it had been in other works developed by Moreira et al in 2011 (Winarso, Pandutama and Purwanto, 2016).

An equally promising crop for the extraction of heavy metals is rapeseed or canola (*Brassica napus*), due to several advantages: 1. It has a great capacity to accumulate metals (2000 mg/K for cadmium), 2. It produces enough biomass, it is easy to cultivate and has great climatic adaptability and 3. It can be used in the industry.

In China, a study was carried out showing that there are varieties that accumulate cadmium in their roots or seeds and for this reason if the variety hyperaccumulates in the seed it should be used for the production of biodiesel; but if the hyperaccumulation, that the Hyperaccumulation occurs in the roots, it must be used in the manufacture of edible vegetable oils (Fu et al., 2016). Another investigation proved that this plant has a hyperaccumulating power of zinc and that it develops very well in soils contaminated with this metal (Belouchrani et al., 2016).

In order to remove lead and cadmium from wastewater and with potential use in soils irrigated with these waters, plants of the species *Eichhornia sp.*, *Lemna sp.*, *Spirodella sp.*, *Azolla sp.*, *Pistia sp.* were used. It is concluded that the use of *Lemna sp.* is promising because of its excellent hyperaccumulation of the two metals, regardless of the pH of the treated water (Verma and Suthar, 2015).

It was worked in Egypt with *Solanum nigrum* and it was proven that it has a great power of accumulation of zinc in its roots and leaves, of lead in its stems and fruits, of cobalt in its roots and fruits. It is highlighted that the levels found in their tissues were often higher than those found in the soil studied (Saad-Allah and Elhaak, 2017). It has been found that this plant is associated with arbuscular mycorrhizae of *Phylum Ascomycota*; therefore its excellent role as a cadmium hyperaccumulator (Khan et al., 2017).

In Australia, the phytoextraction produced by *Acacia pycnantha* and *Eucalyptus camaldulensis* was confirmed, concluding that *E.camaldulensis* presents in its leaves 1 500% more copper than the levels present in the soil; that the average of bioconcentration factors are higher in *E.camaldulensis* and are very close to one (1) for copper, zinc, cadmium

and lead, and also the translocation factor of zinc and cadmium is higher in *Acacia pynantha* (Nirola et al., 2015). In Italy with the application during four years of an Integrated System of Phytoremediation (IPS), which includes *Acacia saligna*, *E. camaldulensis*, rhizobacteria and mycorrhizae, it was proved that phytostabilization in the soil of lead, cadmium and zinc can be produced (Guarino and Sciarrillo, 2017).

The ability of *Marsilea crenata* to accumulate lead in its roots and transfer it to its leaves and sprouts was proven by a laboratory-level experiment in tomato plants. The evaluations were made through the technique of measuring their bioelectrical responses and it was established that this plant can be used as a bioindicator of lead contamination in commercial rice or tomato crops, as its leaves turn yellow when there is excessive presence in the soil of this metal (Nurhayati, Hariadi and Lestari, 2015).

In a study developed under greenhouse conditions in Tunisia, the role of *Medicago sativa* alfalfa was evaluated, and it was found that its bioconcentration factor (the relationship between the content of metals in the soil and the content in the foliage) demonstrates its potential use in any crop taking advantage of the wide range of adaptation present in this legume (Elouear et al., 2016). These results are similar to the ones found by (Flores-Cáceres, 2013); (Coyago and Bonilla, 2016).

5 Bioremediation of heavy metals

Bioremediation uses biological agents (microorganisms) for the complete elimination of pollutants and/or toxic substances from the environment, while the transformation of toxic pollutants into harmless forms through chemical modifications carried out by living organisms (bacteria and fungi) is called biotransformation (Dzionek, Wojcieszynska and Guzik, 2016). In this regards, research works have been developed in recent years that have been quite successful and that show possible bioremediation mechanisms of contaminated soils (Benyahia and Embaby, 2016), and that have the potential to be applied in the cocoa crop, given the ease of obtaining the biological material with which it was worked.

One of the difficulties that is granted to bioremediation is the long time that has to pass to see the effects; however, in works developed in different parts of the world it has been proven that the

endophytic bacteria associated with hyperaccumulative plant species favor the efficiency of the bioremediation process and increase the production of vegetable biomass through three mechanisms: (1) increase in the surface of the root and the production of root hairs, (2) increase in the availability of metals, (3) increase in the transfer of soluble metals from the rhizosphere to the plant (Ahemad, 2015). Some bacteria species used to improve the extraction of heavy metals and hydrocarbons are: *Burkholderia sp.* (Yang et al., 2016), *Scirpus triqueter* (Chen et al., 2017), *Pseudomonas sp.* J4AJ (Di Martino, 2015), *Bacillus subtilis* (Oyetibo et al., 2017), *Microbacterium sp.* SUCR140 (Soni et al., 2013), *Delftia sp.* JD2 (Ahemad, 2015).

In Brazil, a rehabilitation system was designed that included the planting of *E. camaldulensis* in furrows and *Brachiaria decumbens* between the intermediate furrows, accompanied by the sowing of arbuscular mycorrhizas of the *Glomus sp.* type, which has already been used in the rehabilitation program of contaminated areas by heavy metals (Liu et al., 2017). A great power of extraction was found in this combination of materials in the *Brachiaria*, with an order of accumulation efficiency in its biomass of Zn, Cu, Cd and Pb. In addition, this work concludes that knowledge of the dynamics of these fungi in their role as rehabilitators of contaminated soils contributes to the revegetation and establishment of new plant materials in highly polluted areas (Leal et al., 2016; Nirola et al., 2015). It has also been worked with soil incorporation of arbuscular mycorrhizas *Glomus mosseae*, *Glomus intraradices*, *Glomus etunicatum* for minimization of cadmium in the soil and it was found that *Bassia indica* can be used in mixture with these fungi to decrease the dispersion of cadmium in the soil (Hashem et al., 2016).

The application of *Conocarpus biochar* in the soil at the time of sowing significantly reduced the concentrations of heavy metals in corn plants, showing a great immobilization power: 60.5% in manganese, 28% in zinc, 60% in copper, 47% copper (Al-Wabel et al., 2015).

In a laboratory test in Austria, in maize plants inoculated with the bacterium *Burkholderia phytofirmans* PsJN mixed with gravel sludge was found that it significantly improves the fixation of heavy metals in the soil, preventing them from contaminating the vegetative material through immobilization and stabilization processes (Touceda-González et al., 2015; Yang et al., 2016).

In India, the natural ability of native bacteria to reduce and detoxify lead, cadmium and chromium from industrial tannery effluents deposited in rivers and lands adjacent to factories was investigated. After making a biochemical characterization, it was found that *Micrococcus sp.* and *Hafnia sp.* have great potential for bioremediation of the aforementioned metals (Marzan et al., 2017).

The bacterium *Microbacterium oleivorans* has been studied for its ability to decompose polycyclic aromatic hydrocarbons, such as petroleum, and has also been evaluated to decontaminate land contaminated with heavy metals. Even in small dosages its results are quite encouraging to continue testing it in different conditions and crops (Avramov et al., 2016; Xia et al., 2015).

Microorganisms have ample metabolic capacities that allow them to use different types of substrates with the objective of obtaining energy and in many cases transforming them, heavy metals are substrates that can be immobilized or transformed by these organisms using different strategies which can affect their bioavailability. This situation has allowed bioremediation techniques to be implemented in the use of fungi and bacteria in order to reduce the pollutant load of different environments (Beltrán-Pineda and Gómez-Rodríguez, 2016). The use of genetic engineering techniques has allowed the manipulation of microbial strains that naturally exhibit good bioremediation capacities to generate microorganisms with enhanced capacities that show promising results in *in vitro* and field level studies; however, the best results have been obtained when using together the capacities of plants and microorganisms in a mechanism known as symbiotic (Baghour et al., 2001). The hyperaccumulating plants (metalophytic) have the capacity to remove, reduce, transform, mineralize, degrade, volatilize or stabilize heavy metals thanks to its high capacity of accumulation in the roots and translocation different vegetative organs reaching levels of removal of up to 100% (Buta et al., 2014). Physiological and biochemical adaptations include the development of complex structures called metal-proteins or metallothioneins, which allow controlling the accumulation of Cd, Cr and Hg, which also provide protection to the cell from toxic effects (Paz-Ferreiro et al., 2014). On the other hand, thanks to advances in genetic engineering many genes that generate resistance to heavy metals have been introduced into plant cells as is the case of the transgenic plant spe-

cies that express the proteins organomercurial lyase (MerB), and MerA (mercury reductase), have a greater tolerance to organic Hg complexes and reduce Hg (II) to Hg (0). Microremediation and phytoremediation are considered promising technologies in the treatment of heavy metal contamination, their use in the field and laboratory show the biotechnological potential in the recovery of affected environments (Swain, Adhikari and Mohanty, 2013).

6 Conclusion

There are numerous organisms with the ability to fix or absorb heavy metals, most of them anthropic, which are present in the soil solution and then end up contaminating the food consumed. For the cultivation of cocoa it is necessary to develop comprehensive remediation programs, including phytoremediation techniques (*B. campestris*, *M. sativa* or *E.camaldulensis*) and bioremediation (*arbuscular mycorrhizae* of the genus *Glomus*, and bacteria such as *M. oleivorans* and *B. phytofirmans*) and although the best results are achieved in relatively long time, it is necessary to start now to offer a chocolate with good flavor and aroma and that complies with the current regulations on lead and cadmium contents.

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