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### Bioremediation of polluted soils with pesticides using microorganisms – situation in Colombia

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**Abstract.** The analysis of the scientific literature on the subject of bioremediation in Latin America countries shows that due to the excessive and uncontrolled use of pesticides in agriculture, there are negative impacts on the environment, mainly on soil and water, as well as on human health. However, through processes such as bioremediation, which utilize the metabolic potential of microorganisms such as bacteria and fungi, it is possible to reduce the impact of pesticides on the environment and human health, which are key factors for achieving sustainable development. Colombia has a severe pesticide pollution problem, as the uncontrolled use of pesticides has affected not only the environment but also human health. Thus, bioremediation is a good alternative to the restoration of a polluted environment and the reduction of its impact on human health.

**Keywords:** bioremediation, contaminated soils, pesticides, microorganisms

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## Биоремедиация загрязненных почв пестицидами с использованием микроорганизмов – ситуация в Колумбии

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**Аннотация.** Проведенный анализ научной литературы по тематике биоремедиации в странах Латинской Америки свидетельствует, что из-за чрезмерного и бесконтрольного использования пестицидов в сельском хозяйстве возникают негативные воздействия на окружающую среду, в основном на почву и воду, а также на здоровье человека. Но благодаря таким процессам, как биоремедиация, в которых используется метаболический потенциал микроорганизмов, таких как бактерии и грибы, можно уменьшить воздействие пестицидов на окружающую среду и здоровье человека, что является одним из основных факторов для достижения устойчивого развития. В Колумбии существует серьезная проблема, связанная с загрязнением пестицидами, поскольку неконтролируемое использование пестицидов повлияло не только на окружающую среду, но и на здоровье человека. Таким образом, биоремедиация является хорошей альтернативой восстановлению загрязненной окружающей среды и уменьшению ее воздействия на здоровье человека.

**Ключевые слова:** биоремедиация, загрязненные почвы, пестициды, микроорганизмы.

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### Introduction

Pesticides are used in order to eliminate and reduce unwanted organisms. However, excessive and no controlled use of these, could have negative impacts on ecosystem, mainly in soil's organisms, creating a domino's effect, furthermore they also have impacts to the other organisms, including humans [16–19].

Certain pesticides like DDT had been used by a long time in the fight anti malaria, due to its characteristics like low cost and residual action. Moreover, due to an excessive and uncontrolled use, had occurred negative situations in human health, like intoxications, cancer, disturbances in immunological system and hematologic disorders [1]. In the case of DDT, it has a high persistence, due to its

strong adsorption by solid particles, like soil. Thus, despite in a lot of countries the use of this substance is forbidden, can find contaminated environments, even don't use DDT anymore. According to some authors, the DDT could be degraded in two years, moreover, some of them have reported that the degradation process could be 15–20 years or more. Furthermore, the continuous and indiscriminate use of pesticides, but not only organochlorides, like DDT, may affect the environment, including soils, water and air, and in some cases, could promote the pests' resistance [2–8; 12].

Nevertheless, there are methods that allow eliminate, or at least, reduce de amount of pollutants, including pesticides, one of them is bioremediation, that is a econ friendly and low cost process, that uses microorganisms' metabolic potential, in order to reduce the contamination transforming toxic substances, like pesticides, to others less toxic. In these processes may use a high variety of microorganisms, including bacterial genera, fungi and algae [9–11; 13–15; 18; 19].

In some countries, like Colombia, it's highly used pesticides in cultures, mainly corn, rice and potato cultures and also cotton, coffee, tobacco, and other fruits and vegetables. In fact, in Latin America, Colombia is the country with the highest use of pesticides for those cultures [5; 14].

In this work, we made a review about the processes of bioremediation, to achieve this goal, we have searched some of the most recent publications about this topic (papers, graduation works, books, etc.) published after 2013 year.

### **Sustainable development and environmental management related with pesticides uses**

Basically, the concept of sustainable development is based on concept of socio-economic development in line with ecological constraints; the concept of needs related with the redistribution of resources to ensure life quality for everyone; and also de possibility of usage natural resources in order to warrant quality of life for the future generations [20].

The concept is based on the three pillars of sustainability: environmental sustainability, social sustainability and economic sustainability; moreover, the concept of sustainable development is close related with ecological issues, therefore, it's most related to ecological sustainability. But in order to achieve the required ecological conditions, also need to achieve some social conditions, like tradition, culture and other social needs, like access to drinking water, employment, education, food resources, etc. [20].

Related with the soils, the Food and Agriculture Organization of the United Nations (FAO) says that most of the soils in the world will be on acceptable, poor or very poor conditions due to exposition to heavy metals and other pollutants. Hence, the disposal of toxic residues and metal's accumulation are big challenges for recovering and use of soils. Nowadays, it knows that water, air and soil's contamination is a big threat for human health, mainly for people who are involved with the environmental effects by contamination. Then, it's evident how the

environmental management, that attempt to prevent and reduce the environmental issues, carries the achievement of sustainable development [21].

It is clear that natural resources are limited, therefore they must be used judiciously in order to meet the current needs, but without compromising the future generations. This is the reason because tools like remediation and bioremediation help to issues like recycling materials, preservation of natural resources, minimization of waste and energy, and also contribute with eco-friendly processes, that help to save money, using the metabolic potential of living organisms, mainly microorganisms, despite some drawbacks, like long time treatment time, and also, in some cases could be not effective, if the pollutant is in higher concentrations that are toxic even to microorganisms existence [22; 23].

The sustainable development includes other issues, besides the environmental; in order to fulfil with the sustainability, must integrate economic and social issues that could be achieved with a process; that requires integration of environment, policies and developmental strategies in a global context. Hence the environmental management must coincide with social requirements, be viable economically and also contribute with environment's care. Since environment, society and economy are not statics, it's necessary identify, learn and study the form that socio economic aspects be included into the design criteria, in order to achieve sustainable solutions [21]. In the case of processes of recovering polluted areas with bioremediation's processes, can see that is oriented to sustainable development, since try to recover and reduce contamination with help of living organisms, that use the pollutant like energetic resource, then this is an achieve for the environmental issue, is a cheap process, therefore also is an achieve in economic issue and with the reduction of contamination helps to improve the human health, hence is also an achievement in the social issue.

In the next chapters of this review there is a description of the different techniques and processes in bioremediation, and how this works.

### **Bioremediation procedures and techniques**

Bioremediation is the process, that exploit living organisms in order to reduce or eliminate wastes in environment. In bioremediation's processes uses mainly microorganisms, like bacteria, fungi and algae. Since microorganisms possess enzymes that allow them to use environmental pollutants like food, they are a good tool to recover damaged ecosystems; therefore, the aim of bioremediation is encouraging microorganisms to work by suppling optimum level of nutrients and other essential chemicals for their metabolism in order to degrade or detoxify hazardous substances to the environment and other living beings [10; 24; 27].

There are many factors that influence the soil's adsorption of pollutants like pesticides, they are organic matter content, pH, clay content, availability of nutrients, temperature, moisture content and other chemical factors like electrical conductivity, hence, soil fumigation influence in their physicochemical characteristics [24–26].

Processes in bioremediation: Basically, the bioremediation processes utilize living organisms, mainly microorganisms like bacteria and fungi. Now there is a description of the different processes used in bioremediation.

Bacterial bioremediation: Since bacteria are easily cultivable in laboratory, this group of microorganisms has an important role in the recovering of polluted soils with pesticides. Once bacteria have adapted to the contaminated area, they often utilize the pesticide's compounds like energetic resource and promote the removal of these substances of Earth crust. The processes degradation in bacteria is achieved through enzymes, that may evolve in response to prolonged exposure to high concentration of some substances, including pesticides, that could promote new metabolic pathways in bacteria [28; 32].

But the pesticide biodegradation needs certain conditions. One of them is the interaction between the pesticide degrader and the indigenous microbial community in the polluted environment, along with the consequent competition for other substrates [32]. Sometimes in biodegradation processes is needed mixed cultures, because some bacterial genera have incomplete mechanisms, and with the addition of a bacterial consortia, can complement each other, and thus achieve degradation [29].

The bacterial degradation may be in aerobic or anaerobic conditions, both processes are needed in order to obtain mineralization of pollutants. In each process, work different enzymes, that promote degradation according to condition, be aerobic or anaerobic [30].

Bacterial genera and species that degrade pollutants: The Actinobacteria group, are present in high concentration in soils and play an important role in recycling substances, because they are capable to remove xenobiotic compounds of soils, specially pesticides, but also heavy metals and other substances, being a bacterial group that have received great global interest due to its biotechnological applications. The degradation of pollutant compounds is possible thanks to their physiological and metabolic properties, like production of extracellular enzymes and formation of other secondary metabolites [29].

But there are other bacterial genera and species that degrade compounds like pesticides: *Bacillus thuringiensis*, *Pseudomonas*, *Enterobacter*, *Alcaligenes faecalis*, *Alcaligenes eutrophus*, *Lactobacillus plantarum*, *Brevundimonas*, *Mycobacterium*, *Bacillus* sp., *Arthrobacter*, *Acetenobacter*, *Photobacterium*, *Rhodococcus*, *Streptomyces aureus*, *Streptomyces* sp., *Janibacter*, *Frankia alni*, *Frankia* sp., *Gordonia*, *Aerobacter*, *Moraxella*, *Flavobacteria*, *Klebsiella* sp. [1; 24; 26; 29; 30].

Also there are some bacterial strains that help to degrade pesticides, including DDT, like *Xanthomonas* sp., *Arthrobacter citreus*, *Ralstonia eutrophus* A5, *Pseudomonas acidovorans*, *Eubacterium limosum*, *Pseudoxanthomonas* sp. And specially *Chryseobacterium* sp. PYR2 that degrade DDT in high contaminated soils [31].

According to some authors like Betancur [1], bacterial species like *Alcaligenes eutrophus* A5, *Serratia marcescens* DT-1P, *Micrococcus varians*,

*Lactobacillus plantarum* and *Pseudomonas* sp. Can degrade DDT in aerobic conditions. In anaerobic conditions, DDT degradation could be achieved with *Staphylococcus haemolyticus*, *Synecococcus* sp. and *Klebsiella pneumoniae*.

Fungal bioremediation: Fungi are unique organisms due to their morphological, physiological and genetic features; they play key roles in the environmental equilibrium, since they are able to colonize all matrices, like soils, water and air. Furthermore, almost every natural organic compound can be degraded by fungi, because they have a diverse variety of enzymes like amylases, lipases and proteases that allow them to use substrates as fats, starches, proteins, and also pectines, cellulose, and hemicellulose like carbon resources, and furthermore, degrade pollutants like polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and herbicides [35].

Filamentous fungi, yeasts and mushrooms may degrade pollutants in soil, certain species like lignolytic fungi may achieve this purpose, thanks to cellular enzymes like lactase, that work in dechlorination of chlorophenolic compounds. Furthermore, mycelium of ectomycorrhizal fungi, that is surrounded by silts in hyphae, can kidnap pollutants like DDT and allow reduction in toxicity of their cells; therefore, fungal species have high resistance to those compounds, evidenced by the adaptive processes that have fungal species in order to assimilate new carbon resources [1], and in the degradative process, they release pollutants to soil into non-toxic substances [33]. Also, fungi secrete exoenzymes to supply the energetic necessities for growing hyphae, the xenobiotic-metabolizing enzymes grant the metabolism of distinct compounds, hence they tolerate a wide range of natural and anthropogenic substances, including aromatic amines and their derivatives [34]. They induce structural changes to the pesticides, rendering them into non-toxic compounds that are released to soils. Sometimes, those non-toxic compounds may be degraded by bacteria [36].

Fungi have advantages over bacteria in bioremediation processes, since bacteria needs pre-exposition to the specific pollutant that will be degraded, in order to stimulate enzyme expression, because levels of pollutant limit the enzyme expression. Furthermore, fungi present a great potential to degrade high molecular weight hydrocarbons and other recalcitrant organic compounds, therefore, they are a powerful biotechnological tool due to their potential biologic control [34].

The fungal genera and species often utilized in bioremediation processes are: *Flammulina velupites*, *Stereum hirsutum*, *Coriolus versicolor*, *Dichomitus squalens*, *Hypholoma fasciculare*, *Auricularia auricula*, *Pleurotus ostreatus*, *Agrocybe semiorbicularis*, *Aspergillus* sp., *Trichoderma* sp., *Agrocybe semiorbicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velupites*, *Hypholoma fasciculare*, *Pleurotus ostreatus*, *Stereum hirsutum*, *Avatha discolor* [33; 34; 36].

Bioremediation techniques: there are different techniques that can be used in order to do a process of bioremediation, they are mainly biostimulation and bioaugmentation.

**Biostimulation:** this technique modifies the environment in order to stimulate microorganisms, mainly bacteria, capable of bioremediation. In order to achieve this, biostimulation do it with addition of nutrients and chemical compounds that work like electron acceptors, like phosphorus, nitrogen or carbon. Basically intent to stimulate indigenous microflora, although not only needs addition of nutrients, but also control other environmental requirements, like pH, temperature and oxygen [24; 37; 38; 40].

The processes of biostimulation are often used in degradation of organic pollutants, due to improve microbial activity, but sometimes is used too in degradation of heavy metals [39]. Therefore, this is a proficient way to degrade compounds like pesticides.

**Bioaugmentation:** this process consists in the addition of microorganisms different to the indigenous microflora, that could be natural, exotic or engineered microorganisms, in order to increase and enhance the biodegradative capacity of indigenous microbial population on the contaminated area. This process is used mainly when de indigenous microflora in the contaminated area is low [24; 37; 38].

In order to do a proficient process of bioremediation through bioaugmentation, it's necessary to select the appropriate strains, regarding these characteristics: a high potential of contaminant degradation, fast growth, ease of cultivation, the capacity to withstand high concentration pollutants and be able to survive in a high variety of environmental conditions [41].

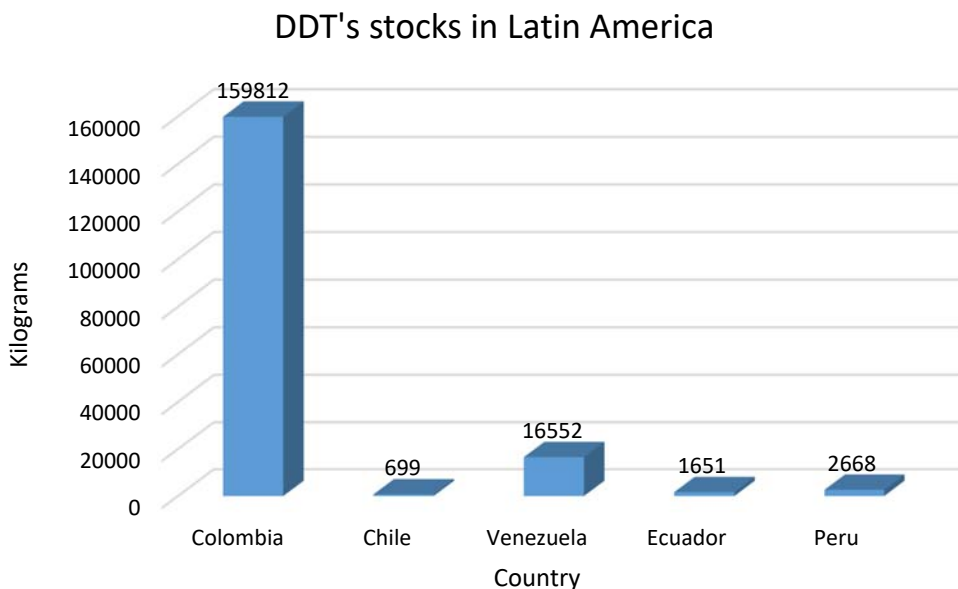
### **Pesticides highly used in Colombia**

Colombia is one of the countries with highest use of pesticides, mainly for their use in cultures of cotton, potato and rice. Mainly in Colombia are used pesticides like organophosphates (OP) [8]. In fact, in the 2000 year, Colombia was the country with the highest prevalence of intoxications by pesticides [44].

Due to the increase of demand of these cultures, in the 1970's Colombia began to produce DDT, toxaphen and aldin, but their use was suspended in order to fulfil national and international rules and laws related with the use of those substances, like Stockholm Convention for Persistent Organic Pollutants [1; 5]. But despite nowadays the use of some pesticides like DDT or toxaphen are forbidden not only in Colombia, but also worldwide, in Colombia have been stored stocks of obsolete pesticides, including toxaphen and DDT, that have been stored underground without protection, and do negative effects to soil where they were stored [3; 14; 42].

The Figure 1 shows the existences of DDT in Latin America.

Despite the organochlored pesticides, like DDT inhibit growth of microorganisms, and therefore is difficult their degradation, after long time permanence of these substance in soil, microorganisms may develop adaptation to them and use them like resource of primary carbon [3]. Sometimes the effects of pesticides on microbiota not always are negative, since after the application of an herbicide, for example, could increase the size of some group of microorganisms [43].



**Figure 1. Stocks DDT stored in Latin America [5]**

Impacts in health related with the use of pesticides in Colombia: in Colombia were reported 3 cases of accidental massive intoxication with pesticides. The reports were in the towns of Chiquinquirá (500 persons), Pasto (300 persons) and Puerto López (190 persons), but according to information of National Health Institute of Colombia, there is not a proficient system for the register of these situations [45]. Furthermore, in 2014 was reported 9167 cases for intoxication with pesticides [46], and during 1998–2011 were 4835 deaths related to intoxications with pesticides, on average, 345 deaths each year. 58.9% of these deaths related with pesticides were in men. In these cases, 553 (11.4%) was not determined intoxication and 224 (4.6%) related to accidental intoxication [47]. These cases could be related with exposition to pesticides during culture processes.

Certain pesticides used in floriculture industry had reported adverse effect in health, mainly in reproductive health. According to Arias et al. [42], in some female workers in greenhouses near to Bogota, had spontaneous abortions, prematurity and congenital malformations in pregnancies that occur after to start to work in those industries and the children born of these workers were medically examined and it was found an increased risk of birthmarks and hemangiomas.

In some regions of Colombia exists the problem related to fight against the drug traffic and one of the strategies of Colombian government is the fumigation of illegal farming of cocaine and poppy plantations, hence at the south of Colombia there are a high amount of denouncements related to fumigations with glyphosate, since some people there, have problems like serious eyes' and skin irritation, abscesses, gastrointestinal diseases, acute respiratory infections and conjunctivitis



[48], and also other diseases, like liver toxicity, nephrotoxicity and cardiovascular affections [49].

Bioremediation processes in Colombia: in Colombia have developed some studies related with bioremediation soils using microorganisms. Oviedo et al., (2017) [50] demonstrated that bacterial genera like *Streptomyces* sp., *Pseudomonas* sp., and *Arthrobacter* sp. had the bests growths in samples with Atrazine, tolerating concentrations of this substance to 1000 ppm. Of these microorganisms, *Pseudomonas* sp. had not only the highest tolerance to atrazine, but can degrade it to concentrations lower to 0.1 mg/L after 6 days. The investigation was developed in the department of Cordoba (north of Colombia).

But to degrade atrazine also is efficient the genera *Streptomyces* sp. That can tolerate high temperatures (near 40 °C), and also may degrade atrazine as levels as *Pseudomonas* sp. do. *Streptomyces* sp. [50]. Since in the north of Colombia is a zone with high temperatures, then this is a genera with good potential for bioremediation processes in this region, and others with warm climate.

Jaramillo-Colorado & Bermúdez-Tobón (2016) [8] also report the efficiency of *Pseudomonas* sp. To degrade substances like organophosphates. The investigation was made near the city of Cartagena, also at the north of Colombia. In a region at the north of Colombia, where are cotton's cultures, Kopytko et al. (2017) [3] shown in their research, that bacterial genera like *Pseudomonas*, *Burkholderia*, *Aeromonas* and *Bacillus* are proficient in processes bioaugmentation in order to obtain degradation of DDT.

Marín & Jaramillo (2015) [51] in their research shown the potential of species *Bacillus* sp. and *Pantoea aggloremans* to degrade organophosphates in soil. The authors say that these species can degrade this substance in soil in proportions of 73.5 and 68.67% respectively. Also say about the importance of the bioremediation treatments to degrade pollution of pesticides and avoid contamination of other food products, like milk, since in the studies' area were cows and the milk they produce, have some traces of organophosphates.

## Conclusion

Bioremediation is an eco-friendly method that allow recover polluted environments like soils, thanks to the metabolic action of organisms, but mainly microorganisms like bacteria and fungi. Furthermore, is a resource that may be used in order to fulfil some of the goals in sustainable development, because contribute to recover polluted environments, that warrant not only environmental sustainability, but also social sustainability, since also may contribute to human health and access to food and drinking water.

According to the review, one of the most effective microorganisms to do bioremediation processes is *Pseudomonas* sp., because proofs pollutants to high concentrations, (to 1000 ppm) and may degrade pollutants. Furthermore, is a proficient microorganism used in bioaugmentation's processes, that may help to the indigenous microflora soil to recover environment.

In Colombia, there is a problem with the non-controlled use of pesticides, since these substances have had adverse effects in the environment, mainly soils and also in human health, since have been people with health problems like abscesses, respiratory, diseases in skin, eyes and gastrointestinal problems, and also some congenital malformation during pregnancy in some women. Hence is necessary to found alternatives that allow protect the cultures, recover environments and search other alternatives different to fumigations, for eliminate illegal farming in order to fight against illegal drugs traffic.

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