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# BIOREMEDIATION OF HEAVY METALS FROM SOIL: AN OVERVIEW OF PRINCIPLES AND CRITERIA OF USING

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*Фактори забруднення, що виникають у нашому середовищі, впливають на якість життя людей, а також на життєдіяльність рослинного і тваринного світу. Забруднення навколишнього середовища відбувається в різних його складових, таких як: повітря, вода, ґрунт. Такий негативний вплив може бути комплексним і відбуватися одночасно у повітряному, водному і едафічному середовищах. Його можна виявити за рівнем хронічного ефекту забруднення, рівень гострої токсичності якого буде проявлятися внаслідок акумуляції. Небезпечна концентрація полютанта визначається його типом і токсичними властивостями. Хоча відомо, що деякі органічні забруднювачі можуть мати токсичні та канцерогенні ефекти у мінімальній концентрації, і діяти на клітинному рівні, оскільки біохімічна деградація органічної речовини відбувається досить повільно. Іони важких металів потрапляють у ланцюги живлення з ґрунту і рослин, досягаючи гострих токсичних рівнів у метаболізмі людини та тварин. З цієї причини дуже важливо видаляти з ґрунту сполуки та іони важких металів методом біоремедіації, крім звичайних методів, оскільки останні є досить ефективними. У цьому дослідженні узагальнено методи, що застосовуються для біоремедіації, та проаналізовано доцільність їх використання для видалення деяких важких металів з ґрунту. Також проаналізовано визначення рівня токсичності важких металів у рослинах, які використовуються для фіторемедіації. У статті наводяться сучасні методи фіторемедіації, які можуть бути застосовані для очищення ґрунтів та ефективність використання з цією метою певних видів рослин.*

***Ключові слова:*** *мікроорганізми, фітотоксичність, рослина, ґрунт, важкі метали.*

## INTRODUCTION

Recently, there have been significant developments in biological treatment methods in order to provide an efficient, effective environmentally friendly waste treatment application. Microorganisms used in many activities help in removing the pollution, which is suitable for dangerous contamination of the soil and its biological activities in the soil, which plays an important role in most of the problems related to the biodegradation or biological transformation / recovery of soil pollutants [1]. It is accepted as an environmentally friendly application and it is considered to be more advantageous to use biological methods, which are financially economical compared to chemical and physical treatment methods. To date, really well accepted methods have been developed for biological treatment, with particular emphasis on pollutant breakdown problem processes. However, the biodegradation method has been used interchangeably with bio-improvement; the first one is a disposition process applied in the transaction. In this current review study, it is defined as a natural process based on biological treatment mechanisms to inactivate (mineralize, degrade or transform) the soil by reducing the concentration of pollutants through biological improvement. The process of removing the contaminant from the medium depends on the contaminant nature that the medium may contain: agrochemicals, highly chlorinated complex organic compounds, synthetic dyes, organic hazardous wastes such as gases, heavy and toxic metals, complex hydrocarbons, nuclear working waste, high complex plastics and stable sewage waste. Apparently, biological treatment techniques can be applied by categorizing them as laboratory trials or large scale by taking into consideration the classical application methods. The characteristics of the pollutants to be treated, the potential of pollution and the size of the pollution, the location of the pollutant, its environmental characteristics, and the environmental policies of the region and the economic value of the treatment are considered as the main criteria to be considered when choosing any biological treatment technique [2; 3].

Biological pollution treatment studies cause serious contamination of soil or groundwater, such as hydrocarbon compounds, as it is a special pollutant such as toxicity [2–6]. In addition, when it comes to the reason for cleaning areas contaminated with other important pollutants besides hydrocarbon compounds, it is possible to consider together other treatment techniques that can be applied economically, more environmentally and effectively for treatment. In addition, given the nature of the activities that lead to crude oil refining pollution, it should be taken into account that it is easy to prevent and control environmental environment contaminants other than hydrocarbon compounds. In addition, dependence on oil and other related products as a major source of energy appears to be a contributor to the increase in pollution from this class of pollutants [7; 8]. The main purpose of this review study is to provide comprehensive information about the biological treatment technique that provides limitations, principles, advantages and possible solution methods related to the methods used as biological treatment. In this study, the possibilities of biological treatment methods were discussed in order to investigate what can be done to disseminate practices.

## BIOLOGICAL TREATMENT STUDIES FOR WASTE REMOVAL IN THE AREA

These techniques include removing pollutants from pollution areas and then moving them to another area for removal. Removal from the area, bioremediation techniques are generally based on: treatment cost, depth of pollution, type of pollutant, degree of pollution, geographical location and geology of the contaminated area. It has been defined as performance criteria that also determine the choice of bioremediation techniques applied away from the field [9].

**Short-term technology.** Short-term technology-mediated bioremediation involves collecting the excavated dirty soil from the ground, followed by aeration to increase nutritional change, and sometimes bioremediation, mainly by increasing microbial activities. The main components of this technique are: ventilation, irrigation, nutrient collection system and a treatment bed. The use of the treatment technique away from this particular area is increasingly considered due to its constructive features, including the economics that enable effective bioremediation provided that food, temperature and ventilation are adequately controlled [10].

**Separation technique.** As one of *ex situ* bioremediation techniques, windrows rely on periodic turning of piled polluted soil to enhance bioremediation by increasing degradation activities of indigenous and/or transient hydrocarbonoclastic bacteria present in polluted soil. Windows, one of the *ex situ* bioremediation techniques, are based on periodic rotation of piled dirty soil biological improvement by increasing the degradation activities of domestic and/or temporary hydrocarbonoclastic bacteria found in dirty soil [11]. Windrow treatment compared with biological treatment showed higher rate of hydrocarbon removal; however, the high efficiency of the wind type for the removal of hydrocarbon is a result of the soil type reported to be more friable [12]. However, due to periodic turning due to wind fighting application, it may not be the best option to adopt in the improvement of soil contaminated with toxic volatile substances. The use of dressing plays a role in the release of $CH_4$ (greenhouse gas) due to the development of the anaerobic zone in piled soiled soil.

**Bioreactors.** A bioreactor, as the name implies, is a system in which raw materials are converted into specific products after a series of biological reactions. A bioreactor has different types of operating modes, including: piecewise, various feeding, sequencing batch, continuous and multi-stage. Choosing the mode of work mostly depends on the market's economy and capital expenditure. Conditions in the bioreactor support the natural process of cells by imitating and protecting their natural environment to ensure optimal development conditions. Examples of contaminated areas can be given to a bioreactor as dry matter or slurry; in both cases, the use of the bioreactor in the treatment of contaminated soil has several advantages over other *ex situ* biological improvement techniques. Optimum control of bioprocess parameters (temperature, pH, agitation and ventilation rates, substrate and vaccine values) are some of the most important advantages of bioreactor based biological treatment. The ability to control and manipulate operating process parameters in a bioreactor indicates that biological reactions within the rector can be enhanced to effectively reduce the time of bioremediation. An important feature of the bioremediation process is the controlled limiting factors, such as controlled bio-magnification, nutrient addition, increased pollutant bioavailability and mass transfer (contact between pollutants and microbes) can be created effectively, making bioreactor-based biological treatment more efficient.

## FIELD STUDIES

Land farming is amongst the simplest bioremediation techniques owing to its low cost and less equipment requirement for operation. In most cases, *ex situ* studies are considered bioremediation, in some cases *in situ* studies are also considered bioremediation technique. This discussion stems from the purification field; the pollutant intensity plays an important role as to whether the fieldwork will be carried out on site or elsewhere. A subject is quite common in field work, dirty soils are often excavated and/or cultivated, but the treatment site apparently determines the type of bioremediation. When the excavated dirt is processed at the site, it can be thought to be in place; otherwise, it is *ex situ*, as it is more common with other *ex situ* bioremediation techniques. Although the bioremediation technique is simple, field studies, like other *ex situ* bioremediation techniques, have some limitations, including: large work area reduced microbial activities due to adverse environmental conditions, additional cost due to excavation, and reduced effectiveness in removing inorganic pollutants [13; 14]. In addition, it is not suitable for the treatment of soil contaminated with toxic volatile substances (evaporation), especially in hot climatic regions (tropical) due to its pollutant removal design and mechanism. Such limitations and others make field-based biological treatment less time-consuming and less efficient than other *ex situ* biological treatment techniques.

## IMPROVED ON-SITE BIOLOGICAL IMPROVEMENT

**Stimulating the natural *in situ* biodegradation.** This technique involves controlled stimulation of the air flow by increasing the activities of natural microbes by supplying oxygen to the unsaturated area to increase bioremediation. In stimulating natural *in situ* biodegradation, changes are made by adding nutrients and moisture in order to increase the biological conversion of the pollutants to a negative state, with the ultimate aim [9]. With this technique, it has gained popularity among other biological treatment techniques, especially in the restoration of contaminated oil waste and contaminated sites [15]. In a scientific study conducted by Sui and Li [4], the effect of air injection rate on evaporation, biodegradation and biotransformation of the area contaminated with toluene through biological measures was been modeled.

**Saturated zone bioremediation.** With this technique, bio-discovery is very similar to injecting air into the soil surface to stimulate microbial activities to ensure removal of contaminants from contaminated land areas. However, unlike bioventing, air is injected at the saturated zone, which can cause upward movement of volatile organic compounds to the unsaturated zone to promote biodegradation. Bioremoval effectiveness depends on two important factors: soil permeability and pollutant biodegradability, which determines pollutant bioavailability to microorganisms [9].

As with bioventing and soil vapour extraction (SVE) methods, biosparing is similar in operation to a technique known as *in situ* air scattering (IAS), occurring in high air flow conditions to ensure evaporation of the pollutant, in operation with a technique closely related to biodegradation. Likewise, the mechanism for removing both similar pollutants is similar for both methods. Biosparging is widely used in the treatment of petroleum products, especially diesel and kerosene derivatives and soil aquifers. Kao et al. [16] reported that biodegradation of aquifer soils contaminated with toluene, ethylbenzene, benzene, and xylene (BTEX) led to a shift from anaerobic to aerobic conditions; this has been proven to be with dissolved oxygen, redox potentials, nitrate, sulphate and heterotrophs, which are cultures of total microorganism, with a corresponding reduction in dissolved iron, sulphur, methane and total anaerobes and methanogens. It demonstrates that all reduction in BTEX reduction (70%) and also biosparging can be used to treat BTEX contaminated groundwater. However, with large limitation, it is possible to estimate the direction of the air flow.

**Phytoremediation.** This method is based on the use of plant interactions (physical, biochemical, biological, chemical and microbiological) in polluted areas to reduce the biotoxic effects of pollutants. There are various mechanisms (accumulation or extraction, degradation, filtration, stabilization and evaporation) processes involved in plant development, depending on the type of contaminant (inorganic or organic). Inorganic pollutants (toxic heavy metals and radionuclide) are often removed by extraction, transformation and sequestration.

On the other hand, organic types of pollutants (hydrocarbons and their chlorinated compounds) are intensely removed by decomposition, rhizo-remediation, stabilization and evaporation. Mineralization is possible when certain plant species such as willow and clover are used [17; 18]. Important factors to consider when choosing a plant as a biological remover are: the root system, which may be fibrous or long, depending on the depth of the pollutant, the above ground biomass shape that should not be available for animal feed consumption, the pollination of the pollutant, the plant's survival and adaptation to the existing environmental conditions, plant growth rate is the time required to monitor in the field and, above all, to achieve the desired level of cleaning. In addition, the plant must be resistant to diseases and pests [19]. Investigation allowed to determine which crops are capable to accumulate lead. According ability to accumulate lead crops were in the following range: clover > sugar beet > corn > sunflower > winter wheat [20]. In some contaminated soil environments, the removal of contaminants by plant species has been reported to include the following processes: uptake by largely passive treatment, translocation from the roots carried out by the flow of flux and accumulation in the shoot. In addition, translocation and substance accumulation are due to sweating and accumulation between xylem stem and adjacent tissues, respectively. However, the process is likely to be different depending on the nature of the contaminant and other factors such as plant variety. It makes sense to select most of the plants growing in any pollution zone from good phyto-remediators. Therefore, for the success of any phytoremediation process approach, it is primarily dependent on optimizing the potential of native plant species growing in contaminated areas to be biogrowthed or improved by endogenous or exogenous plant rhizobacteria. It has been reported that the use of rhizobacteria (PGPR) from plant cultivation and cultivation can play an important role in the phememediation process, because the PGPR biomass production process and the tendency of plants to increase tolerance to heavy metals and other adverse soil (edaphic) conditions should be known [21; 22].

**Internal bioremediation.** Internal bioremediation, also known as natural slimming, is an *in situ* bioremediation technique involving the indirect treatment of dirty areas without any external force (human intervention). This process relies on microbial, aerobic and anaerobic processes to biologically treat contaminants, including contaminants. Ignoring external factors shows that this technique is cheaper than other techniques. With this feature, the purification process should be monitored to ensure that bioremediation is an ongoing and sustainable method, in monitored natural attenuation (MNA) must be followed. Further, MNA is often used to represent a more holistic approach to intrinsic bioremediation. According to the United States National Research Council (US NRC), there are three important criteria that must be met in internal biological treatment, and they include the following items: demonstration of contaminant reduction from contaminated sites, isolated species detection based on laboratory analysis showing the natural presence of microorganisms isolated from contaminated sites. Evidence of biological degradation or conversion potential of pollutants present in the contaminated area they are obtained from and the potential for biodegradation potential in this area [9]. In line with these criteria, M'rassi et al. [23] first isolated hydrocarbon disintegrating bacteria from soil contaminated with refining oils, and demonstrated the biodegradation potentials of isolates by growing their capacity to reduce saturated and unsaturated hydrocarbon substrates as mineral sources, and also by reducing their capacity to reduce hydrocarbon concentrations.

**Bioremediation promises.** As can be understood from the above, bioremediation process techniques are diverse and proved to be effective in the improvement of soil sites contaminated with different types of contaminants. Microorganisms play a very important role in the bioremediation process; therefore, it provides important information on the future of any biological improvement technique, when pollutant diversity, amount, and organism community structure in contaminated environments are provided with other environmental factors that can prevent microbial activities from being kept within the optimal range. Molecular techniques such as 'Omics' (genomic, metabolomic, proteomic, and transcriptomic) have contributed to better understanding of microbial identification, functions, metabolic and catabolic pathways, thereby eliminating limitations related to microbial culture-related methods. Nutrient limitation, low population of the organism or lack of degrading microbes and pollutant bioavailability are among the important pitfalls that can prevent the success of bioremediation. Since the bioremediation is completely dependent on the microbial process, there are two main approaches to increase the speed of microbial activities in contaminated areas: bio-stimulation and bio-magnification.

Bio-stimulation involves the addition of nutrients or substrates to a polluted sample in order to stimulate the activities of autochthonous microbes. It is clear that, since microorganisms are found in all environments, pollutants are naturally present in contaminated areas, and that organism numbers and metabolic activities may increase or decrease in response to the concentration of contaminants. Thus, the use of agricultural and industrial wastes with appropriate nutrient composition, especially nitrogen, phosphorus and potassium, will help solve the problem of food restriction in most polluted areas [24]. However, it has also been reported that the addition of excessive stimulants leads to inhibited microbial metabolic activity and diversity.

## CONCLUSION

The most important step for a successful bioremediation is the workplace characterization that helps to create the most suitable and applicable type of bioremediation technique (*ex situ* or *in situ*). For *ex situ* bioremediation techniques, it tends to be a more expensive method due to additional costs for field excavation and soil transportation. However, they can be used for a controlled treatment of a wide variety of pollutants.

In contrast, on-site techniques do not have an additional cost for field excavations; however, the study can disable biological treatment techniques in some areas, with the difficulty of the equipment's field setup being unable to effectively visualize and control the bottom surface of the contaminated area. Ultimately, the cost of soil improvement is not the main factor that must determine the biological treatment technique to be applied to any contaminated area. Topics such as the type of soil studied, depth reached by the pollutant and the type of pollutant, area location related to human settlement in the region, and determination of the most appropriate and efficient method for the effective processing of contaminated areas, including the geological characteristics of the contaminated areas, including the performance characteristics of each bioremediation technique should be included in research.

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