MASTER'S THESIS

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Faculty of plant protection, biotechnology and ecology

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MASTER'S QUALIFICATION PROJECT

On the topic: "Restoration of heavy metal contamination using bioremediation"

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NATIONAL UNIVERSITY OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE

Faculty of plant protection, biotechnology and ecology

APPROVE
Head of Department
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“____” _______________ **2023.**

TASK
TO PERFORM THE STUDENT'S MASTER'S QUALIFICATION PROJECT

_______________EMUMEJAKPOR KEHINDE DELE EMMANUEL_______________
(full name)

Specialty 162 “Biotechnology and Bioengineering”
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The orientation of the educational program is educational and professional
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Topic of master's qualification thesis: “Restoration of heavy metal contamination using bioremediation”

Approved by the order of the rector of NUBiP of Ukraine from 15.02.2023. №216 «C»

The deadline for submitting completed work to the department is November 1 2023.

The data for the master's qualification thesis is focused on: Environmental restoration activities. Heavy metal contamination, effect on the ecosystem and biological remediation process.

List of issues investigated:

1. Sources of heavy metal in the ecosystem
2. Environmental contamination through anthropogenic activities
3. Effect of heavy metal contamination on human and animal population
4. Exploitation of various restoration techniques
5. Removal of heavy metal using Bioremediation techniques such as; Microbial bioremediation, Phytoremediation, and Mycoremediation.

The task is dated September 1, 2022

Master's thesis supervisor ____________ ______________________
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The task is accepted for execution ____________ ______________________
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ABSTRACT

Increasing anthropogenic activities and various natural processes is rapidly and steadily increasing environmental contamination. Accumulation of heavy metals in soil and water bodies is on the raise due to uncontrolled industrial activities. Environmental contaminant especially heavy metals are non-biodegradable and so they persist and accumulate in the ecosystem by penetrating the food chain when absorbed by crop plants.

Biomagnification of toxic molecules may occur in both human and animal when the contaminated crops are consumed which may lead to severe health conditions. Therefore, to mitigate the toxic effect of heavy metals and other pollutants on human and the ecosystem, it is necessary to engage in environmental restoration projects.

This review will be focusing on bioremediation being an eco-friendly approach, that could play the central role in reduction or complete removal of heavy metals and other contaminants from terrestrial and aquatic environment in a cost-effective manner. Bioremediation may be classified into Phytoremediation, Microbial remediation and Mycoremediation. Bioremediation processes could also be improvedfor a more desirable outcome by several means including the application of genetic engineering.

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CHAPTER ONE

1.0 Introduction

Human activities such as exploration of earth natural resources through industrial activities often result into severe damage on the ecosystem. Heavy metals have been identified as environmental pollutants due to their toxic and persistence nature in the ecosystem. They accumulate in the environment and consequently contaminating the food chains.

Heavy metals are non-degradable by any biological or physical process and are therefore persistent in the soil for a long period, which poses long-term threat to the environment **(suman et al., 2018).**

Contamination of aquatic and terrestrial ecosystem with toxic heavy metals hasbecome a growing environmental problem of public health concern. Examples of these hazardous heavy metals and metalloids that are relevant in environmental contamination includes;

- Chromium (Cr)
- Nickel (Ni)
- Copper (Cu)
- Zinc (Zn)
- Cadmium (Cd)
- Lead (Pb),
- Mercury (Hg)
- Arsenic (As)

Because environmental pollution is a great threat to human and animal health; it contaminates the soil on which crops are cultivated. And thus, affecting the wildlife and sustainability of the planet earth.

To sustain life here on earth It is important to reduce to the lowest possible concentration, or remove the pollutants completely if possible. Therefore, to recover an impaired or polluted ecosystem, environmental restoration projects are required.

1.1 Meaning of Environmental Restoration

Environmental restoration is a purposeful rehabilitation of an area to recreate a functioning ecosystem. To successfully restore a habitat to its original functioning form, it is important to understand the following;

1. Species life cycles:

life cycle of all the living organisms in that habitat.

2. Hierarchical Structure:

Identification of the level and order of distribution of organisms in the habitat.

3. **Interactions:** This involves the types and extent of the interaction between organisms in the habitat.

Types of Interactions

- Interconnections and links in the food chain
- Level of competition for essentials that is necessary to sustain species populations such as; **water**, **nutrients**, **space**, and **shelter**.

1.2 Environmental restoration projects

This can be described as the structures or methods (techniques) designed to restore (rehabilitate) an environment to its natural form.

In the history of environmental restoration, several techniques have been adopted inan attempt to remove heavy metals and other contaminants from the environment, these techniques could be referred to as **conventional techniques or methods**. But these techniques have shown several challenges like;

- Problem with logistics
- Extremely high-cost of operation
- Time consumption
- Mechanical complexity
- Disruption in ecosystem distribution

CHAPTER TWO

2.0 Motivation

The limitations and challenges associated with previously used conventional methods of remediation prompted for more efficient and eco-friendly environmental remediation strategy.

2.1 CONVENTIONAL ENVIRONMENTAL RESTORATION METHODS

2.1.1 Activated carbon-based technology

In situ soil and groundwater remediation requires a method that introduces activated carbon (AC)-based amendments. Amendments commonly used include bioremediation, in situ chemical oxidation (ISCO), and in situ chemical reduction (ISCR).

2.1.2 Biowall

Another one of the natural types of environmental remediation, this permeable reactive barrier (PRB), is an in-situ method that treats groundwater using biological processes.

2.1.3 Electrokinetic-enhanced remediation

This form of environmental remediation introduces an electrical current to remove contaminants by stimulating ions to move within the subsurface.

2.1.4 Environmental dredging

If there is sediment beneath the contaminated water, it can be removed, treated, and/or moved elsewhere through environmental dredging.

2.1.5 Excavation and off-site disposal

Off-site, permitted disposal facilities can take care of contaminated material if the contaminants materials are excavated and transported to them.

Groundwater circulating wells

A circulation pattern is introduced to groundwater for this subsurface remediation method. Groundwater passes through a screened section into a well. After that, it is pumped into another screened section and then re-enters the aquifer.

2.1.6 Groundwater pump & treat

This technique starts by pumping contaminated non-aqueous phase liquid (NAPL) or groundwater out of the subsurface. Above ground, it is treated and then discharged.

2.1.7 Horizontal remediation wells (HRWs)

Using horizontal directional drilling (HDD), horizontal remediation wells (HRWs) are set up underground, either at a shallow angle or parallel to the ground surface. Obstructions at the surface may make it difficult for vertical wells to access relevant areas, so HRWs can be useful.

2.1.8 In-situ flushing

One of the more straightforward types of environmental remediation is in-situ flushing, is conducted with the injection of a liquid solution or water into a contaminated area. It uses flooding to decontaminate.

2.1.9 In situ chemical oxidation (ISCO)

This process generally uses redox (reduction/oxidation) reactions to chemically convert hazardous compounds to ones that are less toxic or nontoxic. Hydrogen peroxide and ozone are examples of oxidizing agents.

2.1.10 Landfill and soil capping

These containment methods separate the ground surface from a contamination source area or waste body by creating a barrier.

2.1.11 Permeable reactive barrier (PRB)

By blending subsurface management of fluid flow with a passive biological or chemical treatment zone, dissolved-phase contaminants in groundwater can be gotten rid of using a PRB. A dissolved contaminant plume is treated as it flows through this in situ technology.

2.1.12 Soil vapor extraction (SVE)

Typically performed in situ (but sometimes ex-situ), it rids unsaturated (vadose) zone soil of organic contaminants that are volatile and semi-volatile. The technology works by inducing controlled airflow with a vacuum.

2.1.13 Soil washing

Based on particle size, a water-based system separates bulk soil from contaminants attached to fine soil particles. To aid in the removal of heavy metals and organics, the pH of the wash water can be adjusted. Additionally, it can be augmented with a chelating agent, surfactant, or basic leaching agent.

2.1.14 Solidification/stabilization (S/S)

Solidification reduces the surface area that is vulnerable to leaching by reducing contaminant migration. The process either forms a coating over waste or creates a solid material that encapsulates it. Stabilization makes it more difficult for waste to leach by reducing solubility or immobilizing hazardous substances.

2.1.15 Solvent extraction

Soil can be separated from metal or organic contaminants by performing extraction with an organic solvent. The technology uses an extraction unit to blend contaminated soil and the solvent. The resulting solution is then sent through a separator.

2.2 BIOREMEDIATION

2.2.1 What is Bioremediation?

Bioremediation can be described as any process which involves the use of biological system such as; Plant, fungi, bacteria and Algae (living or dead) for removing any form of environmental pollutants from **air**, **water and soil** in natural or artificial settings.

Due to the natural ability of living organisms to accumulate, degrade and adsorb and common and emerging pollutants has encouraged the use of biological agents in the treatment of contaminated environment.

In comparison to the conventional treatment methods, bioremediation offers more considerable advantages because it is; scalable, cheap to run, sustainable, and eco-friendly. Most bioremediation processes are less complicated as it involving native organisms.

Various research on bioremediation methods have been strongly focused on enhancing the remediation process by introducing some organisms into the polluted site or also supplying nutrients to the existing organisms to promote their growth.

Basically, the effect of the highly toxic by-products from anthropogenic activities like agricultural process and industrialization could be mitigated by distinctive bioremediation process which in turn could prove less expensive and more sustainable than any other remediation alternatives discussed earlier. Further modification of the processes of bioremediation could lead to improved outcome.

In general, organic pollutants such as Pesticides and Hydrocarbon are more susceptible to biodegradation while inorganic pollutants and heavy metals could be less susceptible to bioremediation.

Noting that bioremediation processes include **oxidation and hydrolysis** processes.The oxidation and hydrolysis process enhances water-solubility of the organic compounds present in the contaminants, making the contaminating molecules susceptible to degradation. The continuous process of oxidation and hydrolysis further promote the susceptibility to further degradation. Example, biodegradation convertinghydrocarbons to carbon dioxide and water.

In heavy metals, Pollutants containing heavy metals can be reduced or removed with varying bioremediation techniques, whoever, the major challenge using bioremediation is in the rate at which the process will be completed. Bioremediation of heavy metal pollutant usually will take longer time.

For further emphasis, the discussion on bioremediation could be divided into 3 major types.

1. **Microbial bioremediation:**

This involves the use of microorganisms to break down contaminants by using them as a food source.

2. **Phytoremediation:**

This involves the use of plants or algae to bind, extract, and clean up pollutants suchas pesticides, petroleum hydrocarbons, metals, and chlorinated solvents.

3. **Mycoremediation:**

This involves the use of fungi’s digestive enzymes to break down contaminants such as pesticides, hydrocarbons, and heavy metals.

Bioremediation techniques can also be classified into 2 methods on the basis of removal and transport of wastes for treatment.

1) **In-situ bioremediation technique**

(Treats polluted sites directly)

2) **Ex-situ bioremediation technique**

3) (Are applied to excavated materials)

2.3 In-situ bioremediation technique

In-situ bioremediation refers to the bioremediation process that is performed directly at the original site of the contamination. In situ bioremediation concept is basically used in treatment of contaminations in soil and ground water.

However, the remediation rate and the effectiveness of the in-situ process of bioremediation depend largely on following different factors:

- Nutrient supply
- pH of the medium
- Moisture content
- Temperature
- The type of the contaminant concern
- Contaminant distribution and concentration
- Site-specific characteristics
- Concentration of other contaminants
- Microbial community of the site
- Temperature
- pH of the medium
- Moisture content
- Nutrient supply

2.3.1 Classification of in-situ bioremediation

In-situ bioremediation method can be divided into 2 forms base on manipulation of the factors listed above

Enhanced in situ bioremediation

manipulations such as aeration, adding nutrients, controlling moisture content, etc. are used to enhance the activity of organisms and increase the rate of degradation.

2.3.2 Intrinsic in-situ bioremediation

Intrinsic in-situ bioremediation allows the natural processes to happen without altering the conditions or adding amendments.

Examples of in-situ bioremediation technologies include:

2 Bioventing
3 Bioaugmentation
4 Bioslurping,
5 Natural attenuation
6 Biosparging

2.3.3 Bioventing

Bioventing is a form of in-situ bioremediation that increases the flow of oxygenor air into the unsaturated zone of the contaminated soil, this flow in turn increases the rate of natural degradation of the targeted contaminants for example.

Bioventing is an aerobic process and it is also described as a form of oxidative bioremediation process which involves oxygen. Here, oxygen act as the electron acceptor for oxidation of contaminants such as:

- Petroleum
- Polyaromatic hydrocarbons (PAHs)
- Phenols

Oxygen is preferably used as electron acceptor because it has the ability to yield higher energy and because it is majorly required by some enzyme system in other to initiate the process of degradation.

Microorganisms can degrade a wide varieties of hydrocarbon components such as; Kerosene, jet fuel, diesel, petrol/gasoline under an ideal aerobic condition. In the low-to-moderate weight **aliphatic, alicyclic and aromatic compounds** the rate of degradation can be very high because the resistance to degradation by a compound increases simultaneously as the molecular weight of the compound increases.

This translates into an increased difficulty in removal of contaminants from the environment due to the high molecular weight of the volatile compounds (contaminants).

Most bioremediation processes involve oxidation and reduction reactions in which either an electron acceptor (commonly oxygen) is added to stimulate oxidation of a reduced pollutant like; hydrocarbons, or an electron donor (commonly an organic substrate) is added to reduce oxidized pollutants such as; oxidized metals, chlorinated solvents, nitrate, perchlorate, explosives and propellants. Additional nutrients, vitamins, minerals, and pH buffers may be added to optimize conditions for the microorganisms in both these approaches

2.3.4 Bioaugmentation

This is the introduction of specialized microbial cultures into the bioremediation technique to further enhance biodegradation process.

To add oxygen below the water table involves two processes which are

1. Recirculating aerated water through the treatment zone
2. Addition of pure oxygen or peroxides, and air sparging.

2.3.5 Air sparging

is described as the process of injecting air under pressure below the water table. The pressure involved in the air injection must be great enough to overcome thehydrostatic pressure of the water and must be able to resistance air flow through thesoil.

Typically, recirculation systems is made up of a combination of injection wells and one or more recovery wells in which the groundwater is extracted and treated, also it is oxygenated, amended with nutrients and then re-injected. It is also importantto note that the amount of oxygen that can be provided by this method is limited dueto the low solubility of oxygen in water. Usually, at typical temperature it is only about8 to 10 mg/L of water is in equilibrium with air.

But greater amounts of oxygen can be made available by contacting the waterwith pure oxygen or by adding H2O2 (hydrogen peroxide) to the water in the process the hydrogen peroxide reacts very quickly, disintegrating into hydrogen and water without leaving any by-products and thus increases the amount of oxygen in water.

However, in some cases slurries of solid calcium peroxide or magnesium peroxide are injected under pressure through soil borings. The solid peroxide then react with waterto release H2O2 (hydrogen peroxide) which then decomposes and release oxygen.

In other to enhance the metabolism and growth of the microorganisms under both in-situ and ex-situ methods additional nutrients, vitamins, minerals, and pH buffers are required. Some situation will require **bio-stimulation process** which canbe achieved by the addition of specialized microbial cultures.

It is important to understand that the major end goal of bioremediation is to removeor reduce harmful compounds from the environment to improve soil and water qualityand not to further create another form of environmental disaster like some conventional methods does.

2.3.6 Bioattenuation

Bioattenuation is referred to as any of the various processes that lessen; the volume/bulk, toxicity, or the concentration of pollutants in a particular environment.

Forms of bioattenuation

1. Biological processes
2. Chemical processes
3. Physical processes

Bioattenuation processes involves the following:

- Transformation of pollutants
- Aerobic and anaerobic biodegradation
- Sorption
- Volatilization,
- Chemical or biological stabilization.

During bioattenuation, biodegradation naturally occurs with the introduction of bacteria or nutrients or both to a pollutant.

The indigenous microorganism present in the environment will determine the metabolic activity and thus act as natural attenuation.

2.3.7 Biosparging

Biosparging focuses on saturated contaminated zones, specifically related to ground water remediation.

This method is very useful for removing volatile organic chemicals. The process is achieved by slowly infusing (injecting) air into the aquifer beneath the contaminated zone. The oxygen level of the aquifer increases due to the injected, the oxygen then encourages aerobic biodegradation. Also, the introduced oxygen encourages rapid multiplication of indigenous bacteria which further accelerate the rate of destruction of the pollutants.

2.3.8 Biostimulation

In bioremediation naturally occurring bacteria plays an important role in biostimulation, and the population of these critical bacteria can be increased by addition of essential nutrients. In principle, bacteria can be used to degrade hydrocarbons. For example, in the degradation process of marine oil spillage, **phosphorus and nitrogen** is identified as essential nutrients for the bacteria involved in the biodegradation. Though the rate of bioremediation of hydrocarbons is relatively slow.

2.3.9.1 Microbial Consortium

A microbial consortium or microbial community, is two or more bacterial or microbial groups living symbiotically. Consortiums can be endosymbiotic or ectosymbiotic, or occasionally it may be both.

The role of microbial consortium or microbial community in bioremediation can be emphasized within the consortium, because the product of one bacteria species could be the substrate for another bacteria species.

2.3.9.2 Anaerobic Activities

Principally in bioremediation, anaerobic activities can be employed to treat a wide range of contaminants that has been oxidized such as:

- **Chlorinated ethylenes**
  - Perchloroethylene (PCE)
  - Trichloroethylene (TCE)
  - Dichloroethene (DCE)
  - Vinyl chloride (VC)

- **Chlorinated ethanes**
- Trichloroethane (TCA)
- Dichloroethane (DCA)
- **Chloromethanes**
- Carbon tetrachloride (CT)
- Chloroform (CF)
- **Chlorinated cyclic hydrocarbons,**
- **various energetics**
- perchlorate
- Research Department explosive (RDX)
- Trinitrotoluene (TNT)
- nitrate.

This process of treating oxidized contaminants involves:

A. Adding an electron donor to deplete background electron acceptors including nitrate, oxygen, oxidized iron and manganese and sulfate.

B. Adding an electron donor to stimulate the biological and/or chemical reduction of the oxidized pollutants.

An example is the reduction of **Hexavalent chromium (Cr[VI]) and uranium U[VI])** to a less mobile and/or less toxic forms which are **Cr[III] and U[IV]** respectively.

Another similarly example is **sulfidogenesis**; the reduction of sulfate to sulfide which can be used to precipitate metals like zinc and cadmium.

2.3.9.3 Determinants of Substrate and Injection Method

The choice of substrate and the method of injection depend on the following;

A. The nature/type of the contaminant and the distribution in the aquifer

B. The remediation objectives

C. Hydrogeology,

Using conventional well installations, substrate can be added by the following means

- By direct-push technology
- By excavation and backfill such as permeable reactive barriers (PRB) or biowalls.

In a case of slowly released products which composed of edible oils or solid substrates, they tend to remain static for an extended treatment period, while soluble substrates or soluble fermentation products of slow-release substrates can potentially migrate via advection and diffusion which thereby provide broader but shorter-lived treatment zones.

The organic substrates added are first fermented to hydrogen (H2) and volatile fatty acids (VFAs).

Carbon and Energy required for the bacterial metabolism is provided by

- The volatile fatty acids (VFAs)
- Including acetate,
- Lactate,
- PropionateButyrate

2.4 Ex-situ bioremediation technique

Ex-situ bioremediation is a technique in which the contaminants are treated away from the location where they were found. In this method of bioremediation, the contaminants are excavated or pumped out from the original contaminated site and then transferred to different environment (controlled environment) for treatment. The contaminated soils which have been excavated to a different location are placed on the surface of the ground and treated using indigenous microorganisms. This process can control and manage by providing appropriateconditions for the indigenous microorganisms.

2.4.1 Advantages of ex-situ bioremediation

- Ex-situ bioremediation techniques allow modifications of; chemical, biological, physico-chemical conditions and other parameters necessary for effective andeffficient bioremediation.
- Ex-situ bioremediation is not laborious and it is less expensive.
- Preliminary stage is short because ex-situ bioremediation does not require extensive preliminary assessment of the polluted site prior to remediation
- The great influence of soil porosity, which governs transport processes during remediation, can be reduced when polluted soils are excavated.
- As a result of depth, due to excavation processes associated with ex-situ bioremediation the pollutants are inhomogeneity.
- Generally, ex situ bioremediation techniques tend to be faster, easier to control and can be used to treat wide range of pollutants (Prokop et al. 2000).
- Non-uniform distribution and concentration, can easily be corrected by adequately optimizing some process parameters such as; temperature, pH andmixing.

2.4.2 Disadvantages of ex-situ bioremediation

- Ex-situ bioremediation methods are not suitable to be used in some sites such as under buildings, inner city and working sites (Philp and Atlas 2005).
- Moderate to extensive engineering work are required in ex-situ bioremediationtechniques. This implies that more workforce and capital are required.
- In most cases, ex-situ bioremediation techniques require large space for operation.
- The excavation features of ex-situ bioremediation tend to disrupt soil structure; as a result, the polluted site and surrounding areas are more likely to experience more disturbances.

2.4.3 Examples of ex-situ bioremediation processes

- Composting
- Soil biopiles
- Landfarming
- Slurry reactors.

In other to enhance the metabolism and growth of the microorganisms under bothin-situ and ex-situ methods additional nutrients, vitamins, minerals, and pH buffers are required. Some situation will require bio-stimulation process which can beachieved by the addition of specialized microbial cultures.

It is important to understand that the major end goal of bioremediation is to remove or reduce harmful compounds from the environment to improve soil and waterquality and not to further create any environmental disaster like some conventional methods does.

2.4.4 Biopile

Biopile which is also called bioheap is an ex-situ bioremediation technology in which excavated soil are mixed with some soil additives, formed into compost piles, and then enclosed for treatment. In biopile, heat, nutrients, pH, moisture, and oxygen, are controlled to enhance biodegradation

2.4.4.1 Components of biopile system

- Treatment bed,
- Aeration system,
- Irrigation/nutrient system
- Leachate collection system.

Soil piles can be high up to 20 feet, the irrigation/nutrient system is buried under the soil to pass air and nutrients through the soil. The pile may be covered with plastic to; promote solar heating, control runoff evaporation, and volatilization.

In a situation where volatile organic compounds (VOCs) present in the soil volatilize into the air stream, then the air leaving the soil can be treated to remove or destroy the volatile organic compounds before they are discharged into the atmosphere. The treatment time can be from a duration of 3 to 6 months. After the treatment, the excavated material is then either returned to its original location or disposed.

2.4.4.2 The limitations and Concerns of biopile

1. The machineries used in the process of excavation of the contaminated soil usually produces noise and dust, and this situation may need to be controlled.
2. It can be difficult to reduce concentration of the contaminants by more than 95

percent, or reduce contaminant levels to less than 1 part per million.

3. Treatability testing should be conducted to determine the biodegradability of contaminants, as well as appropriate oxygenation and nutrient loading rates. If there are volatile organic compounds in the soil that can volatilize into the airstream, it may then be necessary to treat the air leaving the soil to prevent discharge of this volatile compounds to the atmosphere.

4. This process may not be effective for treating chlorinated compounds and it may also not be effective in degrading the transformation products of explosives.

5. To determine; the potential degradation by-products, the potential degradation rate and the additives that best suits (promote) the microbial activities. Laboratory or field viability studies are needed.

6. Period mixing may be required because processes that involves periodic mixingmay result in more uniform treatment than static treatment processes.

7. The critical consideration of covering or containing the treatment area with an impermeable liner in other to minimize the risk of contaminants leaching into an uncontaminated surrounding soil. This must be seriously considered during the planning process.

8. To build a biopile system, a large amount of relatively flat space is required.

2.4.4.3 Application of biopile

1. Biopile treatment has been applied to the treatment of non-chlorinated volatile organic compounds and fuel-contaminated soil.

2. Pesticides, semi-volatile organic compounds (SVOCs) and even chlorinated Volatile organic compounds can also be treated, with biopile method but the process effectiveness will vary

2.4.4.4 Windrows

Comparing windrow treatment to biopile treatment, windrows showed higherlevel of hydrocarbon removal. The higher efficiency of hydrocarbon removal using the windrows was as a result of the soil type, which was reported to be more friable (Coulon et al. 2010).

Windrows is a form of ex-situ bioremediation method which rely on regular turning of piled soil (polluted) to enhance degradation activities of indigenous and/or transitory hydrocarbonoclastic bacteria that may be present in contaminated soil. The regular turning of polluted soil combined with regular addition of water leads to increase in aeration and oxygen, nutrients and microbial degradative activities, more even distribution of pollutants across the soil pile and thus speeding up the rate of bioremediation, which can be accomplished through assimilation, biotransformation and mineralization (Barr 2002).

However, windrow treatment may not be an appropriate technique to be adopt in remediating soil polluted with toxic volatiles due to the constant turning of the pile, because the emitting gas could create a secondary air contamination. Windrow treatment has been implicated in greenhouse gas such as methane. Which could be release due to development of anaerobic zone within piled polluted soil, which usually occurs following reduced aeration (Hobson et al. 2005).

2.4.5 Land farming

Generally, land farming is described as among the simplest technique of bioremediation because it requires less equipment and cost of operation. Land farmingis mostly regarded as ex-situ type of bioremediation technique, also in some other cases, it is regarded as in-situ bioremediation technique. This debate is due to the site of treatment. Pollutant depth also plays a key role as to whether land farming can be carried out ex-situ or in-situ. In land farming, one thing is common, polluted soils are usually excavated and/or tilled, but the site of treatment apparently determines the type of bioremediation.

In a situation where excavated polluted soil is treated on-site, it can be regarded as in-situ; otherwise, it is regarded ex-situ as it has more in common with other ex-situ bioremediation techniques. It has been largely reported that when pollutants lie less than 1 meter (<1 m) below ground surface, bioremediation might proceed without excavation, but when pollutants lie more than 1.7 meter (>1.7 m) below ground surface, the polluted soil needs to be excavated to the ground surface or transported to another location for bioremediation to be effectively enhanced **(Nikolopoulou et al. 2013).**

In General, polluted soils that has been excavated are placed above the ground surface on a fixed layer support to allow aerobic biodegradation of the pollutant by autochthonous microorganisms **(Philp and Atlas 2005; Paudyn et al. 2008; Volpe et al. 2012; Silva-Castro et al. 2015).**

autochthonous microorganisms enhance bioremediation during land farming, and the activities of the microorganisms can be enhanced by the following operations; tillage of contaminated soil which enhances aeration, addition of essential nutrients such as; nitrogen, phosphorus and potassium and irrigation system.

However, it has been reported that tillage and irrigation system without addition of nutrient in a soil with appropriate biological activity increased heterotrophic and

2.4.5.1 Limitations of land farming

Despite being the simplest form of bioremediation method; just like other ex- situ bioremediation techniques, land farming has some basic limitations which include: large operating space can be required, unfavourable environmental conditions can result into reduction in microbial activities, reduced efficacy in inorganic pollutant removal and it requires an additional cost for excavation. (Khan et al. 2004; Maila and Colete 2004).

Furthermore, land farming is not suitable for the treatment of soil contaminated with toxic volatile substances, this is due to the nature of pollutant removal mechanism (volatilization) associated with land farming especially in hot (tropical) climate regions.

The above stated limitations with several others limitations made land farming method of bioremediation less efficient and time demanding compared to other ex-situ bioremediation Methods.

Diesel-degrading bacterial counts and thus enhancing the rate of bioremediation; dehydrogenase activity was also observed to be a good indicator of biostimulation treatment and could be used as a biological parameter in land farming technology (Silva-Castro et al. 2015).

In a field trial reported by Paudyn et al. (2008) it was stated that more than 80% contaminant (diesel) were removed through aeration using rototilling approach in a study that lasted for over 3 years at a location in remote Canadian Arctic. This further proves that in land farming technique aeration plays crucial role in pollutant removal especially at cold regions. Usually, land farming is adopted as a remediation technique for hydrocarbon-polluted land including the polyaromatic hydrocarbons (Silva-Castro et al. 2012; Cerqueira et al. 2014)

2.5 Mechanism of Bioremediation

Volatilization (weathering) and biodegradation are the two basic remediation mechanisms involved in pollutant removal. Land farming system is expected to always complies with government regulations at all levels, and so, it can be adopted in any location and climate (Besaltatpour et al. 2011).

During bioremediation activities, leaching of pollutants into neighbouring areas can be minimized or prevented by designing suitable land farming method with impermeable liner. (da Silva et al. 2012). Moreover, land farming method of bioremediation is not difficult to design, and it is really easy to implement as it can be used to treat large volume of polluted soil with little or no environmental impact and energy requirement. It also requires low input low cost (Maila and Colete 2004).

2.6 BIOREACTOR

Bioreactors, are any specifically designed vessels in which raw materials are converted to specific product(s) after series of chemical or biological reactions. Therea different operating mode/types of a bioreactor including the following:

- Bubble column bioreactor
- Continuous stirred tank bioreactor
- Airlift bioreactor
- Fluidized bed bioreactor
- Photo bioreactor
- Packed bed bioreactor

The choice of operating mode of bioreactor depends majorly on capital expenditure and market economy. The environmental conditions in a bioreactor are expected to support and maintain the natural processes of cells present in the bioreactor by mimicking their natural environment to provide appropriate conditions for growth.

Using a bioreactor to treat polluted soil has several advantages compared to other ex-situ bioremediation methods. It is such that a dry form or slurry of the polluted samples can be fed directly into a bioreactor, in either case, the use of bioreactor system enables for proper control of all bioprocess parameters is one of the major advantages of bioreactor-based bioremediation.

These bioprocess parameters include:

- Temperature
- pH
- Agitation and aeration rates,

- Substrate and inoculum concentrations

The spectacular ability to manipulate and control the bioprocess parameters in a bioreactor system enables to enhance the biological reaction rate, and this can be effectively used to reduce bioremediation time.

It is important to note that in a bioreactor-based bioremediation system, the limiting factors of bioremediation process such as; increased pollutant bioavailability, controlled bioaugmentation, nutrient addition, and contact between pollutant and microbes. Can be established effectively in a bioreactor system thus making the system more efficient.

The applications of different bioreactors for bioremediation process have resulted in removal of wide range of pollutants. Soil or water contaminated with volatile organic compounds such as; ethylbenzene, benzene, xylenes, and toluene canbe treated using bioreactor system. Flexibility in the design nature of bioreactors hasallowed maximum biological degradation while minimizing abiotic losses (Mohan etal. 2004).

The core bacterial communities involved in bioremediation processes can be easily characterized in a long term or short-term operation of a bioreactor by tracking the changes in microbial population dynamics in a bioreactor containing slurry of crudeoil-polluted soil (Chikere et al. 2012; Zangi-Kotler et al. 2015

In addition, because bioreactor is an enclosed system, is encourage the use of genetically modified microorganisms for bioaugmentation, and after the augmentation process the genetically modified microorganisms can then be destroyed before the treated soils are returned/release for landfilling or back to the excavated site. In a bioreactor system, different types of substances such as sewage sludge can be used as bioaugmenting agent or biostimulant. The ability to effectively contain and destroy genetically modified microorganisms in a bioreactor helps to ensure that no foreign gene escapes into an environment after bioremediation. As a result of efficient mixing that is associated withbioreactor systems the role played by biosurfactants were found to be insignificant (Mustafa et al. 2015).

2.6.1 Challenges of bioreactor system

Though the efficiency of bioreactor system has been proven to be enormous due to the easy of controlling the different operating parameters. However, in other to establish best operating condition by relating all the available parameters with OFAT approach (one-factor-at-a-time); using this approach would require numerous experiments, these experiments could attract additional cost and time

However, this particular challenge can be overturn by adoption of DOE tone (design of experiment). With this design: using a set of independent variables which could be controllable and uncontrollable factors over a specified region (level) adequate information on optimal range of parameters can be extracted. (Mohan etal. 2007). More so, information about microbiological processes is of great necessity in other to optimize the processes of bioremediation (Piskonen et al. 2005).

Bioreactor system of remediation is not a common practice because bioreactor systemis an ex-situ method, if the volume of polluted soil or other substances to be treated is large, the system will require more input inform of manpower, safety measures and logistics for transporting pollutant to the bioreactor location. In general, those stated factors will amount to increase in the cost of treatment. (Philp and Atlas 2005).

Identification and design of the type of bioreactor that is most suitable for particular types of pollutants is of paramount importance. The design of a suitable mode of a bioreactor could be challenging.

Moreover, bioreactor system required several bioprocess parameters which must be properly balanced, controlled and maintain optimally, these bioprocess parameters may become limiting factors if not properly managed which can lead to reduction in microbial activities and can in turn make the bioreactor system of remediation less effective.

2.7 Agricultural practice and environmental pollution

Technological advancements in agricultural sector have been a contributing factor to the exposure of the ecosystem and humans to varieties of toxic chemical substances such as; pesticides, herbicides, insecticides, fungicides etc.

2.7.1 Pesticides

Pesticides are known to be potentially toxic to human and can result to both acute and chronic health effect. The extent of the effect also depends on the nature of the pesticide, quantity and means of exposure.

Pesticides are usually made of synthetic chemical compounds and they are used to manage pests especially in agricultural sector.

Pesticides are very important tool in integrated pest control because they are efficient, effective and economical.

However, either accidentally or inappropriate usage of pesticides can result in bioaccumulation of the toxic chemical in the ecosystem which can contaminate the food chain and thereby putting human and other non-target animals or organisms at great risk. The residual effect of pesticide is enormous as it is dangerous to the environment, human and animal with carcinogenic consequence. The residue can persist for long time in soil, in plant tissues, in water, and even in the air.

CHAPTER THREE

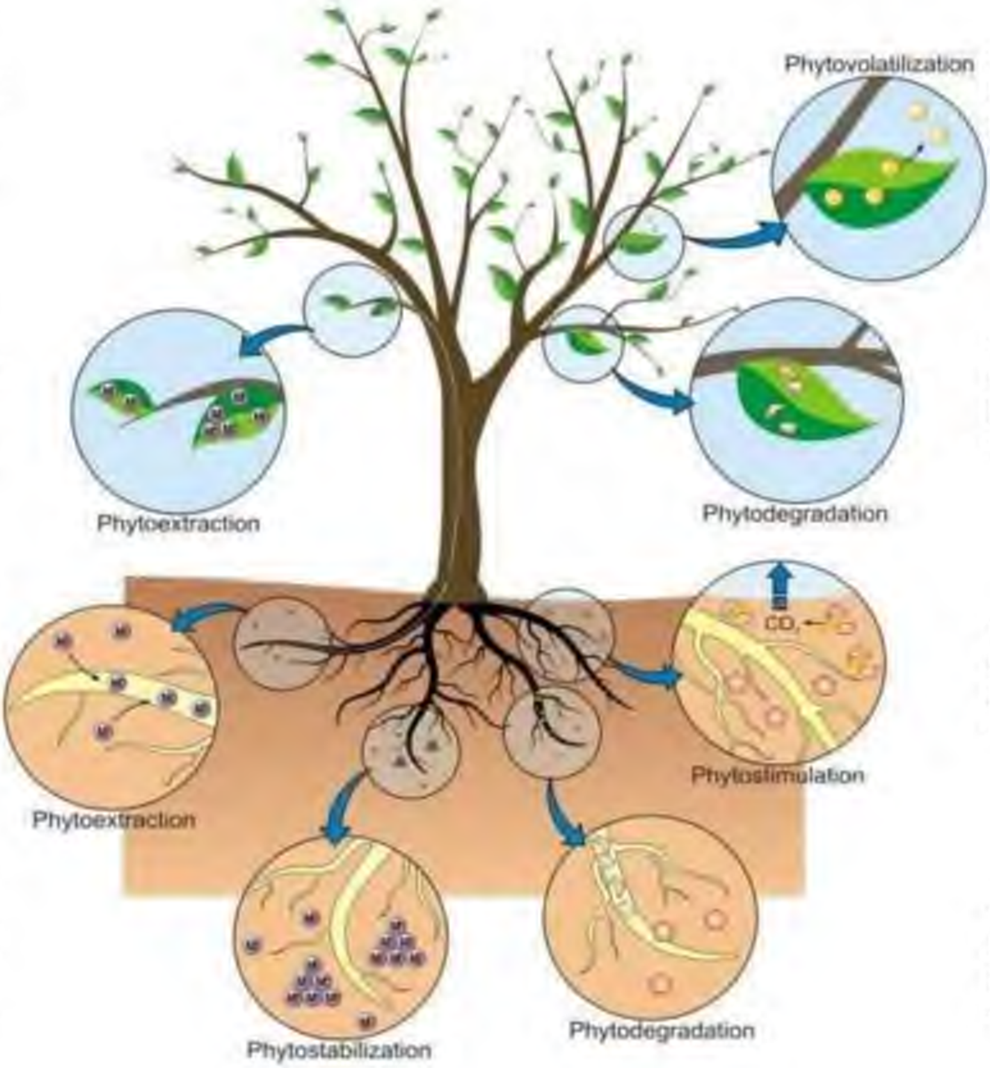
3.0 Phytoremediation

The term is derived from the Greek word phyto (plant) and Latin word remedium (restoring balance). Therefore, phytoremediation can be described as the use of plants and the associated microorganisms for the extraction, immobilization, containment, or degradation of contaminants (environmental pollutant) such as metals, Pesticides, petroleum hydrocarbons, and other chemical substances from theenvironment.

3.1 How does phytoremediation work?

Certain plants are able to remove or break down harmful chemicals from the ground when their roots take in water and nutrients from the contaminated soil, sediment, or groundwater. Plants can help clean up contaminants as deep as their roots can reach using natural processes to:

- store the contaminants in their roots, stems, or leaves.
- Convert the contaminant into less harmful chemicals within the plant or, more commonly, the root zone.
- Convert the contaminant into vapour, which are released into the air.
- Sorb (stick) contaminants onto their roots where very small organisms called "microbes" (such as bacteria) that live in the soil break down the sorbedcontaminants to less harmful chemicals.



Schematic illustration of phytotechnology mechanisms

(Source: Favas et al. (2014))

Merits and Limitations of Phytoremediation

- **3.2.1** Merits:

- It helps in maintaining soil fertility by preserving the topsoil.

- The operation cost of a phytoremediation method is lower than that of traditional processes both in-situ and ex-situ.
- Phyto remediation play an important role in improving soil health, crop yield,and in plant phytochemicals.
- Phytoremediation can benefit companies specializing in "phyto mining" as itallows for the possibility of recovery and re-use of valuable metals.
- It helps reduce the risk of metal leaching in the soil and also reduce/prevent soil erosion.

3.2.2 Limitations:

- One of the major limitations of phytoremediation is in dept of the contaminant. Phytoremediation is only limited to surface area and the soil depth reach of the plant roots.
- Without the complete removal of the contaminated soil, it is impossible to completely prevent further leaching of contaminants into the groundwater with plant-based systems of remediation
- Survivability of the plants used in the remediation process can be threatened by the toxicity of the contaminants in soil and also by other conditions such asclimate and pH.
- Bio-accumulation of contaminants (especially metals) in plant parts can contaminate consumer products such as cosmetics and foods. Therefore, safe disposal of the plant products used in phytoremediation could be challenging, but is of paramount importance to public health.
- Plant Defence and detoxification Mechanisms

Detoxification of contaminants such as heavy metal is an important mechanismin plants for the implementation of phytoremediation (Thakur et al., 2016). Plants usually adopt two defence strategies in other to adapt with the toxicity of heavy metals and other pollutants.

- Avoidance
- Tolerance

Avoidance and tolerance are the two mechanisms, plants adopt to maintain their cellular concentrations of heavy metals below the threshold levels of toxicity (Hall, 2002).

3.3.1 Avoidance:

The ability of plants to regulate/limit the uptake of contaminants and restrict the movement across plant tissues through root cells is referred to as avoidance strategy (Dalvi and Bhalerao, 2013). This is known to be the plant's first line of defence at extracellular level. This happens through a range of mechanisms such as metal ion precipitation, metal exclusion, and root sorption (Dalvi and Bhalerao, 2013).

When the plant roots come in contact with the heavy metals, the plants try to immobilize the contaminant through root sorption or by modifying metal ions, and in the process the plant roots secret varieties of root exudates, such as amino acids and organic acids, which act as heavy metal ligand to form stable heavy metal complexes in the rhizosphere (Dalvi and Bhalerao, 2013). Precipitation of heavy metals occur when some root exudates change the pH of the rhizosphere, and therefore the bioavailability of the heavy metals are limited the toxicity is lessened (Dalvi and Bhalerao, 2013).

Metal exclusion mechanism such as exclusion barriers play an interesting role in the plant avoidance strategy, this mechanism (exclusion barriers) exists between the shoot and the root system of the plant thereby restricting the access of heavy metals from the contaminated soil to within the root system; the aerial parts of the plant is protected against the harmful heavy metals by the restriction of uptake and root-to-shoot transport that occurs in the plant. Arbuscular mycorrhizas (symbiotic association that occur between plants and fungi) can restrict the entry of contaminants (heavy metals) from the soil into the root by: adsorption, absorption, or chelation of heavy metals in the rhizosphere thereby acting as an exclusion barrier for in heavy metal (Hall, 2002). Furthermore, the pectins present in the cell wall of the plant cell consist of carboxylic groups of polygalacturonic acids, these acids have negative charges and sothey are able to bind heavy metals. Therefore, cell wall functions as a cation exchangerwhich restrict the entry of free heavy metal ions into the cells (Ernst et al., 1992). Another heavy metal avoidance mechanism is embedding the heavy metals inthe plant cell (Memon and Schröder, 2009).

3.3.2 Tolerance:

Tolerance is another defence strategy adopted by plants to cope with the toxicity of heavy metals once the heavy metal ions has managed to get an entry through the exclusion barriers into the cytosol. When the excess heavy metal ions getaccumulated inside the cytosol, for the plant to survive, the plant have to detoxify them in order to minimize the toxic effects of the heavy metal ion (Manara, 2012). Tolerance strategy helps the plant to cope with the accumulated metal ion toxicity andit is described as the second line of defence for the plant, and this detoxification take place at intracellular level through varieties of mechanisms such as; chelation, compartmentalization of heavy metal ions and inactivation (Dalvi and Bhalerao, 2013).

The intracellular detoxification is mainly achieved by complexation of heavy metal ions with ligands through chelation. The concentrations of free metal ions are reduced to relatively low levels through chelation. Some of the organic compounds involved in heavy metal ion chelation include; metallothioneins, organic acids, phytochelatins, cell wall pectins/ polyphenols/proteins, and amino acids (Hall, 2002; Sharma and Dietz, 2006; Gupta et al., 2013b). There are various organic ligands andinorganic ligands in the cytoplasm which mediate heavy metal chelation. The persistence of heavy metals as free ions in the cytoplasm of plant cell is prevented by the organic acids present within the cell by reducing and complexing the bioavailability of the heavy metal ions to the plants.

Examples:

- Malate is involved in chelation of Zinc (Zn) in **Arabidopsis halleri** (Sarret et al., 2002).
- Presence of acetic and citric acids bind Cadmium (Cd) in leaves of **Solanum nigrum** (Sun et al., 2006).
- Presence of citrate mediates the chelation of Nickel (Ni) in **Thlaspi goesingense** leaves (Krämer et al., 2000),
- Heavy metals can be detoxified with certain kind of amino acids by chelating the heavy metal ions within cells and xylem sap (Rai, 2002). And heavy metal stress can induce the accumulation of this kind of amino acids that can detoxifyheavy metals.
- For instance; The production of **cysteine** can be induced with Cadmium (Cd) stress in Arabidopsis thaliana (Domínguez–Solís et al., 2004),
- Cadmium (Cd) stress, Zinc (Zn) stress, Lead (Pb) stress, Copper (Cu) stress induces high accumulation of **proline** (Roy and Bera, 2002)
- Hyperaccumulation of Nickel (Ni) stress induces **histidine** accumulation (Harper et al., 2004)

3.3.3 Chelation of heavy metals by amino acid

The presence of amino acid in plant cells is very important in detoxification of contaminants such as heavy metals in the plant system by chelating the ions of the heavy metal.

In response to high levels of heavy metals, **Metallothioneins (MTs)** and **Phytochelatins (PCs)** are also induced.

Example: In **Silene vulgaris** enhanced copper (Cu) tolerance is associated with increased expression of metallothionein (MT) gene, because the response to copper (Cu) is mediated by metallothioneins (van Hoof et al., 2001). Also, Cadmium (Cd) is chelated by phytochelatins in tobacco leaves (Vögeli-Lange and Wagner, 1990), When chelation process is complete, the complexes of ligands with heavy metals are transferred from the cytosol to compartments that are inactive such as vacuole. There, in the vacuole, the complexes are stored without toxicity (Tong et al., 2004).

Besides the vacuoles, heavy metal ions can also be sequestrated and compartmentalized in other locations in the plant such as; trichomes, the leaf petioles, and leaf sheathes (Robinson et al., 2003; Eapen and D’souza, 2005),

Efficient protection against the detrimental effects of heavy metals is achieved by sequestration and compartmentalization which isolate the toxic heavy metal ions from sensitive sites such as where respiration and cell division occurs. This process reduces the interactions between cellular metabolic processes and heavy metal ions which prevent damaging effect to cell functions (Sheoran et al., 2011).

3.3.4 Sites of sequestration and compartmentalization in plants

Besides the vacuoles, the heavy metal ion can also be transported to other places in the plant where the toxic heavy metals ions can cause less damage to the plant. Heavy metal ions can also be sequestrated and compartmentalized in other locations in the plant such as; trichomes, the leaf petioles, and leaf sheathes (Robinson et al., 2003; Eapen and D’souza, 2005).

Through translocation process, heavy metals can also be transported to old leaves which will be naturally detached from the plant during leaf shedding (Thakur et al., 2016).

One example such leaf shedding is **Plantago lanceolata** in which, zinc (Zn) is transported to the leaves just during the final week prior to leaf shedding. In this, zinc is eventually removed from the plant after the contaminated leaves fall (Ernst et al., 1992).

3.3.5 Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are free radicals, and are described as unstable molecules which contains oxygen and can easily react with other molecules in a cell. Mass build-up of reactive oxygen species in living cells may cause damage to the DNA, RNA, and proteins present in the cell which may lead to cell death.

When the level of heavy metals accumulated in the environment is high, and once the above-mentioned strategies (avoidance and tolerance) are inadequate to detoxify the plant from the detrimental effects of heavy metals, production of **reactive oxygen species (ROS)** is triggered in the plant cell due to the increased accumulation of heavy metal ions in the cytoplasm of the plant cell.

Excessive production of reactive oxygen species (ROS) results in oxidative stress, which may hinder the cell from properly carrying out all the necessary cell activities. Oxidative stress may lead to; protein oxidation, DNA damage, disruption of cell homeostasis, and inhibition of cellular processes (Huang et al., 2012; DalCorso etal., 2019).

However, in other for the plant to cope with oxidative stress that was triggered by the excessive reactive oxygen species (which was induced by heavy metal ion), the plant switch to activating the **reactive oxygen species-scavenging machineries** by inducing various antioxidant enzymes such as;

superoxide dismutase (SOD), catalase (CAT),

peroxidase (POD), glutathione reductase (GR),

Furthermore, non-enzymatic antioxidant compounds such; tocopherols, glutathione, carotenoids, flavonoids, and ascorbate are also induced (Gupta et al., 2009; Jozefczak et al., 2012; DalCorso et al., 2019). It should be noted that the anti-oxidative defence mechanisms of plants play a vital role in cellular response to the oxidative stress from heavy metal ions.

3.2 Phytoremediation processes

1. Phytoextraction
2. Phytostabilization
3. Phytotransformation
4. Rhizofiltration
5. Phytostimulation
6. Phytovolatilization
7. Phytodegradation

3.4.1 Phytoextraction

Phytoextraction is a type bioremediation in which plants are used to extract contaminants from water or soil. The contaminants are translocated and accumulatedin the aboveground biomass of the plant (Salt et al., 1995; Jacob et al., 2018). Phytoextraction has been identified as the most important phytoremediation technique for reclamation of heavy metals and metalloids from the polluted soil (Ali et al., 2013; Sarwar et al., 2017).

Phytoextraction method is a permanent solution for the removal of heavy metals from polluted environment (soil) unlike other phytoremediation methods in which plants only temporarily contain heavy metals, and these heavy metals further remain belowground. Therefore, phytoextraction is said to be more suitable for commercial application.

3.4.2 Processes of heavy metal phytoextraction

There are various steps involved in the process of heavy metal phytoextraction which include:

(i) Mobilization of heavy metals in the rhizosphere

(ii) Heavy metals uptake by plant roots,

(iii) Heavy metal ions translocation from roots to aerial parts of plant,

(iv) Sequestration and compartmentation of heavy metal ions in plant tissues (Ali et al., 2013).

In other to maximize the effectiveness and efficiency of phytoextraction remediation method, the strategies to be adopted should focused on the following factors. in light of the factors stated above, phytoextraction effectiveness and efficiency relies on factors such as;

Plant selection Plant performance

Heavy metal bioavailabilitySoil type

Rhizosphere properties.

3.4.3 Selection of plant species for phytoremediation

For effective phytoextraction, selection of the plant species that will be appropriate for the remediation of the type of contaminants involved is also vital.

3.4.3.1 Plant selection strategy

However, there are two different strategies being employed for plant selection.

1) Plant selection base on plant hyperaccumulation ability. Accumulation of heavy metals in aboveground parts to a large extent
2) Selection of plants base on high aboveground biomass production. Though these plants may have lower capacity for metal-accumulation, but the plant overall accumulation capacity of heavy metals should be averagely comparable to the capacity of hyperaccumulators (Robinson et al., 1998; Salt et al., 1998; Ali et al., 2013).

Under the same conditions, heavy metal hyperaccumulators that occurs naturally can accumulate metals in their biomass at levels 100-folds higher than common plant species which are non-hyperaccumulating (Rascio and Navari-Izzo, 2011).

Some accumulators are capable of accumulating different types of heavy metals at the same time. Plant species such as **Sedum alfredii** possess the ability to hyperaccumulate more than two metallic elements such as; Zinc (Zn), Lead (Pb), and Cadmium (Cd) (He et al., 2002; Yang et al., 2002, 2004).

3.4.3.2 Qualities of appropriate plant species for phytoextraction

Appropriate plant species for phytoextraction is expected to possess some very important qualities such as:

(i) Easy cultivation and harvest.

(ii) Ability to grow rapidly with high biomass production.

(iii) High extraction ability; to extract and accumulate high level of heavy metals and other contaminants in their aboveground biomass.

(iv) High tolerance to the toxic effects of heavy metals ions.

(v) Good adaptation to prevailing environment, strong ability to grow in poor soils.

(vi) Possession of abundant shoots and extensive root system.

(vii) High resistant to pest sand pathogens

(viii) The selected plant species should be repulsive to plant eaters such as herbivores in other to minimize the chance of heavy metals entering into the food chain, (Seth, 2012; Ali et al., 2013).

The capacity to accumulate metals in aboveground biomass is the most important characteristic and it is arguably the key factor that determine the phytoextraction potential of a plant species.

Hyperaccumulators can be described as plant species that are capable of accumulating very high level of heavy metals in their aboveground biomass without any symptom of phytotoxicity (Rascio and Navari-Izzo, 2011; van der Ent et al., 2013).

3.4.4 Quality of hyperaccumulator

Firmly, the description of hyperaccumulator should meet the following criteria:

- The heavy metal concentration of shoot-to-root ratio should be greater than 1, which is an indication of efficient ability of the plant to transport metals from roots to shoots (McGrath and Zhao, 2003; Marques et al., 2009)
- The heavy metal concentration of shoot-to-soil ratio should be greater than 1.

indicating a higher capability of the plant to take up heavy metals from the contaminated soil (McGrath and Zhao, 2003)

- Tolerance to heavy metal concentration in the shoot should be higher than 10 mg/kg for Mercury (Hg), 100 mg/kg for Cadmium (Cd) and Selenium (Se), 1,000

mg/kg for Cobalt (Co), Copper (Cu), Chromium (Cr), Nickle (Ni), and Lead (Pb), and 10,000 mg/kg for Zinc (Zn) and Manganese Mn (Baker and Brooks, 1989).
So far, several hyperaccumulators has been identified, from at least 45 angiosperm families, more than 450 plant species have been confirmed as heavy metal hyperaccumulators. (Suman et al., 2018).

3.4.5 The angiosperm families identified as hyperaccumulator

- Scrophulariaceae families

- Trees such as Fabaceae, Brassicaceae, Euphorbiaceae, Lamiaceae, And asterraceae.
- Annual herbs and perennial shrubs (Salt et al., 1998; Dushenkov, 2003)

The key and the most straight forward strategy to a successful phytoremediation of heavy metals contamination is the search for the appropriate and effective hyperaccumulator.

Table 1: List of plants species, with high capacity of heavy metal accumulation.

HEAVY METALS	SYMBOL	MAXIMUM CONCENTRATIO NIN PLANT (Mg/Kg)	PLANT SPECIES	REFERENCES
Mercury	**Hg**	13.8	Marrubium vulgare	Rodriguez et al., 2003
		3.17	Festuca rubra	Rodriguez et al., 2003
		18.275	Achillea millefolium	Weng et al., 2012
		6.45	Rumex induratus	Rodriguez et al., 2003
		2.74	Poa pratensis	Sas-Nowosielska et al., 2008
		0.2	Cicer arietinum	Wang et al., 2012
		0.315	Juncus maritimus	Zheng et al., 2011
		4.25	Silene vulgaris	Pérez-Sanz et al., 2012
		1.89	Helianthus tuberosus	Sas-Nowosielska et al., 2008
		2.35	Hordeum spp	Rodriguez et al., 2003
		0.97	Armoracia lapathifolia	Sas-Nowosielska et al., 2008
Chromium	**Cr**	20,675	Pteris vittata	Kalve et al., 2011
Arsenic	**As**	2000	Pteris biaurita	Srivastava et al., 2006

		3647	Pteris ryukyuensis	Srivastava et al., 2006
		2900	Pteris quadriaurita	Srivastava et al., 2006

		1800	Pteris cretica	Srivastava et al., 2006
		8331	Pteris vittata	Kalve et al., 2011
		2110	Corrigiola telephifolia	García-Salgado et al., 2012
		1470	Eleocharis acicularis	Sakakibara et al., 2011
Cobalt	**Co**	10,232	Haumaniastrum robertii	Marques et al., 2009
Cadmium	**Cd**	5600	Arabis gemmifera	Kubota and Takenaka, 2003
		740	Azolla pinnata	Rai, 2008
		236.2	Deschampsia cespitosa	Kucharski et al., 2005
		2075	Salsola kali	De la Rosa et al., 2004
		9000	Sedum alfredi	Xiong et al., 2004
		1140	Thlaspi caerulescens	Brown et al., 1994
		52.94 - 146.95	Turnip landraces	Li et al., 2016
		10,700	Phytolacca americana	Peng et al., 2008
		8176	Prosopis laevigata	Buendía-González et al., 2010
Copper	**Cu**	13,700	Aeolanthus biformifolis	Chaney et al., 2010
		20,200	Eleocharis acicularis	Sakakibara et al., 2011

		8356	Haumaniastrum katangense	Sheoran et al., 2009
		12,300	Ipomoea alpine	Mitch, 2002
		91.975	Pteris vittata	Wang et al., 2012
Selenium	**Se**	18,200	Lecythis ollaria	Marques et al., 2009
		14,920	Astragalus racemosus	Marques et al., 2009

Lead	**Pb**	1000	Betula occidentalis	Koptsik, 2014
		10,300	Brassica juncea	Koptsik, 2014
		9400	Brassica nigra	Koptsik, 2014
		966.5	Deschampsia cespitosa	Kucharski et al., 2005
		1138	Euphorbia cheiradenia	Chehregani and Malayeri, 2007
		5600	Helianthus annuus	Koptsik, 2014
		43,300	Medicago sativa	Koptsik, 2014
		8200	Thiaspi rotundifolium	Cunningham and Ow, 1996
Zinc	**Zn**	3614	Deschampsia cespitosa	Kucharski et al., 2005
		11,200	Eleocharis acicularis	Sakakibara et al., 2011
		51,600	Thlaspi caerulescens	Cunningham and Ow, 1996
		10,000	Thlaspi calaminare	Sheoran et al., 2009

3.4.6 Limitation of hyperaccumulator

One of the keys for efficient and safe phytoremediation of heavy metals is in the selection of the non-edible hyperaccumulators. Heavy metals can accumulate in edible parts of plants and from there the metal can enter into the food chain when such plants are consumed by animals or human. To minimize the chance of the toxic effect from heavy metals on human and animal, the use of edible crops in plant base remediation should be avoided.

The efficiency of phytoextraction can be limited by hyperaccumulators which are short-lived with low biomass production and slow growth rate. However, non-hyperaccumulators with can be useful for heavy metals phytoextraction even though non-accumulators usually accumulate lower concentrations of heavy metals in their aboveground biomass on a per mass basis, the high biomass production can compensate for the lower phytoextraction efficiency can be compensated for by their ability to produce high biomass , the total accumulation levels of heavy metals may sometime be higher than that of hyperaccumulators with low biomass (Ebbs et al., 1997; Vangronsveld et al., 2009; Vamerali et al., 2010).

Phytoextraction of heavy metals from contaminated soils have been carried out effectively with plant species producing high biomass, such as Nicotiana tabacum, Cannabis sativa, Zea mays, and Helianthus annuus. (Kayser et al., 2000; Tlustoš et al., 2006; Vangronsveld et al., 2009; Herzig et al., 2014). Phytoextraction can be carried out using grass plants because of their, high level of tolerance to abiotic stresses, high growth rate, short life cycle, and more biomass production (Malik et al.,2010).

Trifolium alexandrinum is a grass plant with; ability to grow fast with high biomass, multiple harvests in a single growth period, and high resistance capacity to pollution loads, and it has been selected as effective for phytoextraction of Cadmium(Cd), Lead (Pb), Copper (Cu), and Zinc (Zn) (Ali et al., 2012).

3.4.7 Woody plant species for phytoremediation

Woody plant species such as trees, have been identified as effective for phytoextraction Suman et al., 2018; Salt et al., 1998; Dushenkov, 200

3.4.8 Advantages of using woody plant species for phytoextraction

- High amount of biomass is produced by woody plant species and this enables the accumulation of high levels of heavy metals in their aboveground parts.
- Well-developed and deep penetrating root system of woody plants species helps to prevent the dispersal of contaminated soil in the environment, and it effective reduces soil erosion. (Suman et al., 2018)
- Due to the non-edibility nature of tree plants, they are preferred for phytoremediation, by this probability of the heavy metals entering into the foodchain via trees is lower (Burges et al., 2018).

3.3 Phytovolatilization

Phytovolatilization is a form of phytoremediation technique in which plants absorb contaminants (toxic substances) from the contaminated soil, the plant then converts the toxic substances into less toxic volatile form. These volatile and less toxic substances are then released from the plant leaves/foliage to the atmosphere throughthe process of transpiration. The plants used in the phytovolatilization of pollutants can be can be referred to as **volatilizers**

Phytovolatilization strategy can be used to volatilize heavy metals such as Selenium (Se) using plants of the **Brassicaceae family such** as **Brassica juncea** (Banuelos and Meek, 1990; Terry et al., 1992; Banuelos et al., 1993) In this process, the inorganic form of Selenium (Se) is first assimilated into two different organic forms;

selenomethionine (SeMet) and **selenoamino acids selenocysteine** (SeCys). selenomethionine (SeMet) is then biomethylated in the plant where it turns to dimethylselenide (DMSe). DMSe is less toxic compared with inorganic Selenium and it is volatile. Due to the volatility, is can be released from the plant leaves/foliageinto the atmosphere (de Souza et al., 2000; Terry et al., 2000).

Furthermore, Phytovolatilization strategy can also be applied to detoxify organic pollutants and some heavy metals like Arsenic (As) and Mercury (Hg) (Mahar et al., 2016). Mercury has high reactivity and can be easily volatilized because it remains in liquid form at room temperature in its elemental form. After released into the environment, it exists mainly as a divalent cation (Hg^{2+}) (Marques et al., 2009).

The mercury present in the environment can be absorbed by plants either withthe root or leaf, the methyl-mercury is then converted to ionic mercury, the ionic mercury is then converted into a less toxic compound which is then volatilized into theatmosphere (Bizily et al., 2000).

3.5.1 Advantage and disadvantage of phytovolatilization

3.5.2 Advantage:

One of the major advantages of phytovolatilization over other forms of plant base remediation method is that heavy metal pollutants are removed from the ground: converted into partially/less toxic forms and then dispersed as gaseous compounds into the atmosphere, **without any need for plant harvesting and disposal**.

3.5.3 Disadvantage:

Phytovolatilization strategy requires a thorough risk assessment process before its applied on the field because the process may not completely remove the contaminants from the site. However, the strategy only transfers partially/less toxic pollutants from the soil to the atmosphere, the volatile compounds released to the atmosphere will contaminate the ambient air, and in the process of precipitation the volatile compounds may be redeposited back to the soil (Vangronsveld et al., 2009).

Phytofiltration

Phytofiltration is the use of plant seedlings, shoots, or roots (rhizofiltration), shoots (caulofiltration), or seedlings (blastofiltration) to remove contaminants from contaminated waste waters or surface waters (Mesjasz-Przybylowicz et al., 2004). **Blastofiltration:** use of plant seedlings for decontamination**Caulofiltration:** use of plant shoots for decontamination **Rhizofiltration:** Use of plant roots for decontamination.

In rhizofiltration, heavy metals can either be adsorbed onto the plant roots surface or absorbed inside the plant roots. Precipitation of heavy metals on plant rootsoccurs when the pH of the rhizosphere is changed by root exudates (Javed et al., 2019),

3.6.1 Preparation of plants used for rhizofiltration:

The plants are grown hydroponically in clean water so the plant can first develop large volume of roots, after which the clean water in the hydroponics is thensubstituted with polluted water. This is done in other to make the plants acclimatize to contaminants. After the period of acclimation, the plants are then transferred to the polluted site for bioremediation task. There the roots are harvested and disposed once the plant roots become saturated (Wuana and Okieimen, 2011).

Ideally both aquatic and terrestrial plants can be adopted for rhizofiltration purpose, such plants are expected to have the following characteristics:

- High biomass production
- High tolerance to heavy metal
- Dense root system
- Fast growth

Aquatic plant species such as; Duckweed, Azolla, Hyacinth, Poplar, and Cattail are usually used for the remediation of pollution in wetland. The choice of this plants is due to their high tolerance capacity for heavy metals, high biomass production, ability to accumulate high volume of heavy metals, and fast growth (Hooda, 2007).

For rhizofiltration of terrestrial habitats, Terrestrial plants are more effective as they have shown good capacities to accumulate heavy metals. Comparing terrestrial plant with aquatic plants; Terrestrial plants such as: **Helianthus annuus** (Sunflower) and **Brassica juncea** (Indian mustard) have hairy and longer root system. (Tomé et al., 2008; Rezania et al., 2016; Dhanwal et al., 2017).

3.6.2 Advantages

Rhizofiltration operation allows for inexpensive procedure, but depending on the type of contaminant. Generally, rhizofiltration requires low cost of capital because the treatment strategy may be conducted in situ, with plants being grown directly in the contaminated water body.

After the plants may have been harvested, the entire plant biomass may be convertedto biofuel.

3.6.3 Disadvantages

The major limitation of rhizofiltration method of remediation is in the limit of the plant roots reach, pollutants that are below the depth of the plant root will not be extracted.

Also, contaminated sites may be polluted with combination of different kinds of contaminants in which treatment with rhyzofiltration alone may not be enough.

3.4 Phytostabilization

This phytoremediation strategy prevents the migration of heavy metal across ecosystem as it limits the chance of heavy metal penetration into food chain. In this strategy, plant species with high metal tolerance are used. The plants decrease the bioavailability of heavy metals by immobilizing them thereby confining the contaminants within the polluted site (Wong, 2003; Marques et al., 2009).

The use of plant in stabilization strategy of remediation does not only immobilize the metals; minimizing the possibility of leaching of the contaminant to underground water. Also, the strategy does help to minimize the possibility of wind dispersion heavy metals particles that may be present at the soil surface. (Vangronsveld et al., 2009; Mench et al., 2010). Comparing phytostabilization strategy with phytoextraction strategy, the disposal of hazardous/contaminated biomass is not required. And this is one of the major advantages of phytostabilization strategy (Wuana and Okieimen, 2011).

3.7.1 Means of phytostabilization

Phytostabilization can take place through either of the following:

Occurrence of sequestration inside root tissues, when the metal valence in the rhizosphere is reduced, adsorption of heavy metal onto root cell walls of the plant, absorption of heavy metals into plant tissues and through precipitation of heavy metal. (Ginn et al., 2008; Kumpiene et al., 2012; Gerhardt et al., 2017).

For efficient phytostabilization approach, plant should be tolerant to heavy metal and therefore, appropriate plant species selection is key as the plant roots play crucial roles in; maintaining soil structure by preventing erosion and immobilizing heavy metals through various mechanisms such as prevention of leaching, absorption and many more. Such plants should have the potential for fast growth and large production of biomass in other to achieve timely and adequate covering over the polluted soil. In given condition at the contaminated site, the covering plants should be relatively easy cultivate and maintain (Berti and Cunningham, 2000; Marques et al., 2009).

3.7.2 Introduction of soil amendments to improve phytostabilization

Soil amendment can be referred to as any substance that is intended to change the physical or chemical qualities of soil. A typical example of such substance is compost.

3.7.3 Benefit of using soil amendments

- Organic and inorganic soil amendments can be used to enhance the effectiveness of phytoremediation strategy, these added soil additives can change the redox and pH value of the soil which can confine the bioavailability of the contaminants by reducing the solubility chance of the heavy metals (Alvarenga et al., 2009; Epelde et al., 2009; Burges et al.,2018).

- The soil additives can also benefit the selected plant species as it introduces essential nutrients and organic compounds into the soil which improves the plant biochemical property. The additives can also enhance water retainabilityof the soil.

- Soil amendments promotes and encourages the growth of microorganisms found at the rhizosphere, these organisms can facilitate phytostabilization by serving as barrier filtration, preventing heavy metal ion from translocating fromplant roots to the shoot. Also, the activities of the microorganisms help in increasing depth and surface of plant root. (Göhre and Paszkowski, 2006).

- Immobilization of heavy metals and some other contaminants can be enhanced through the activities of microorganisms like bacteria and mycorrhiza living in the rhizosphere of the selected plant. The activities of the microorganisms encourage precipitation processes and production chelators. The microorganism also adsorbs metals into their cell wall thereby enhancing phytostabilization process. (Göhre and Paszkowski, 2006; Mastretta et al.,2009; Ma et al., 2011).

3.5 Phytostimulation

Phytostimulation, sometimes called rhizodegradation, rhizosphere biodegradation, or plant assisted bioremediation/degradation. Phytostimulation can be described as the enhanced breakdown of contaminant by increasing/enhancingbioactivities of microbes at the plant rhizosphere environment, the enhanced bioactivities result in increasing microbial populations.

The microbial bioactivities help in remediating the contaminants also, the contaminants can be converted into less harmful products which can serve as sourceof nutrient for soil organisms and plants.

This phenomenon can translate as a form of symbiotic relationship (at the rhizosphere) between the soil microbes and the plants; where the plants provide much needed nutrients (exudates) required for microorganisms to thrive, and in return the microorganisms create a proper soil condition that promote the growth of the plant roots and the entire plants. (Babalola, 2010)

3.8 The mechanisms of symbiotic relationship in phytostimulation

3.8.1 Plant mechanisms:

- Phytochemical exudes such as alcohols, carbohydrates, sugars, and many more; which are major sources of food (carbon) for the specific microbes thathelps in improving soil condition. However, the same (phytochemical) exudatesmay act as an allelopathic agent that is meant to help promotes it’s own growthand revegetation, they by suppressing the growth of other plants.

- The plant also helps in loosening the soil and thereby transporting oxygen and water into the rhizosphere to be used both the plant and the microbes present

3.8.2 Organismal mechanism:

The microbes help in distributing the phytochemical allelopathic agents (root exudates) thereby protecting the plant from competition and chemical build-up

Though, there may be more varieties of microorganism's population present in vegetated soil compared to unvegetated soil. However, Cyanobacteria are the most common microorganism associated with phytostimulation due their ability for nitrogen fixation.

Cyanobacteria in the rhizosphere are able to release phytohormones. When these hormones are absorbed by the plant roots, they can induce the secretion of exudates and also stimulate root growth.

3.9 Phytodegradation

Phytodegradation which is also known as phytotransformation is a bioremediation process where organic contaminants are broken down into less or non-hazardous forms by plant enzymes secreted by the root. The resulting molecules can then be absorbed by the plant and released from the leaves through the process of transpiration.

3.9.1 Plant enzymes for Phytodegradation

Plant secreted enzymes such as **dehalogenases** and **nitroreductases** are used by plants to degrade organic contaminants. (Favas et al., 2014).

Other examples of plant enzymes include: Peroxidases Phenoloxidases Esterases

Plant enzymes degrades and detoxifies organic pollutants which helps the plantto toxic effect of such contaminants.

Efficiency degradation process (phytodegradation) is achieved when there are optimal conditions of pH, and temperature. (Mukhopadhyay and Maiti, 2010)

Organic contaminants such as Explosives, pesticides, solvents and some other xenobiotic substances can be detoxified through the metabolic activities of special plants such as **Cannas**.

In phytotransformation, as a direct impact of metabolism, environmental substances are often modified chemically. When the chemical substances(contaminants) are not completely broken down into basic molecules by the plants metabolites, the resultant compound will have transformed chemical structure and qualities, this described the term phytotransformation and thus resulting in: immobilization (phytostabilization), inactivation or degradation (phytodegradation).

3.10 Application of Genetic Engineering to Bioremediation

The introduction of genetic engineering into organismal modification for bioremediation purpose has been proven to be efficient. In genetic engineering application; plants or microorganisms are modified using a foreign gene from another organism which may be animal, bacteria or even plant species. The foreign gene is inserted into the genome of a target Organism (which maybe a plant).

The targeted organism DNA will then recombine with the DNA from the insertedforeign gene. The targeted organism inherits the gene from foreign source which deposit some new specific traits to the targeted organism.

3.10.1 Advantages of genetic engineering in bioremediation

The major advantage for introducing genetic engineering into bioremediation technique is in the flexibility of generating different kind of organisms with specific characteristics or desired traits. Introduction of genetic engineering in developing

transgenic plants with desired traits in phytoremediation process is significant. It is now to transfer specific and desirable genes from hyperaccumulator to another plant species which is not sexually compatible with the hyperaccumulator (Berken et al., 2002; Marques et al., 2009).

This operation is not possible with previously used traditional system of plant breeding such as crossing.

When the modification process produces more suitable organism for bioremediation purpose, the suitability of the organism to the nature/type of the contaminant will shorten the response time of the organism for decontamination of the polluted site

3.10.2 Gene selection for genetic engineering in phytoremediation

In genetic engineering for phytoremediation purpose, the selection of genes should be based on the ability of the plant to accumulate and tolerate heavy metals.

Hyperaccumulators are naturally occurring plants that have been identified to have high tolerance and accumulation capacity for heavy metals. Increasing/enhancing **antioxidant activity** in plant helps in increasing heavy metal tolerance in the plant (Kozminska et al., 2018). And so, antioxidant activity can be enhanced by overexpression of antioxidant agents' genes.

More so, to increase heavy metal accumulation capacity with genetic engineering, it requires transfer and overexpression of the genes which are involved in; translocation, sequestration and uptake of heavy metals (Mani and Kumar, 2014; Das et al., 2016). Specifically, the genes encoding metals/metalloid transporter are transferred and overexpressed in the targeted plant.

Uptake and translocation of heavy metal can be improved with genetic engineering by overexpression of genes encoding natural chelators (Wu et al., 2010).

3.10.3 Limitations of genetic engineering in bioremediation

Despite the promising result from the introduction of genetic engineering into environmental restoration project, the approach still faces some significant setbacks.

Genetic manipulation of multiple genes involved in achieving desired traits requires a lot of effort, time and resources. Due to the complexity of the procedures involved, the process of the genetic engineering may not be successful.

Governmental approval of genetically modified plants is difficult to obtain in certain part of the world due to risk of consumption ecological safety concerns.

CHAPTER FOUR

4.0 Microbial bioremediation

Microbial remediation is the use of microorganism as biological agent for degrading or removing toxic and harmful pollutants from a contaminated site. It can also be described as: when the biological activities of microorganisms on contaminants lower the bioavailability of the toxic molecules present in the contaminants, in this process, the microorganisms convert or modify toxic contaminants (especially organic)into less toxic or non-toxic molecule which may serve as source of food and energy for the microbes. Bacteria are the major biological agent involved in microbial bioremediation. The process of microbial bioremediation can occur naturally also it can be induced artificially.

4.1 Merits of microbial bioremediation process.

There are various advantages involved in the use of microbes as remediation agent, ranging from the eco-friendliness of bacteria (microorganisms) to the valuable genetic materials that could be immeasurably useful in genetic engineering for bioremediation application.

Furthermore, due to the natural role of microorganisms in the environment: a microbe base remediation method is less labour intensive, cheap to run and simple to maintain. Also, there are limitations associated with microbial remediation.

4.2 Limitations of microbial bioremediation

Availability of microbes capable of remediation task at the polluted site is usually of great consideration. To identify an appropriate microorganism that will suit a site with complex mixtures of contaminants which are not evenly dispersed at the contaminated site may require a lot of research and complicated trials before identifying the appropriate microorganism and its optimal growth requirement.

4.3 Efficiency and optimization of a microbe base remediation

The physicochemical property of the contaminated site, chemical nature and the concentration of the pollutants can affect the microbial availability which also play a centre role in the efficiency of a microbe base remediation strategy.

The optimization and efficiency of a microbe base remediation technique is also largely own to environmental factors such as; Temperature, availability of oxygen (or any other electron accepting element), moisture content, pH, nutrient and soil type.

4.3.1 Temperature

Optimal temperature is essential for survivability of microorganism as it affects the rate of bio-chemical reactions in the microorganism. Optimal temperature required may vary with different microorganism and temperature above the optimal may result in cell death.

4.3.2 Availability of oxygen

Oxygen requirement varies with different microbes. The organisms can carry out biological degradation under an aerobic condition (oxygen is required) or anaerobic condition (oxygen is not required). Degradation of hydrocarbons happen under aerobic conditions, whereas degradation of chlorate compounds happen only in an anaerobic condition. However, oxygen supply to the soil can be increase by air sparging, soil tillage or by introduction of magnesium peroxide or hydrogen peroxide to the environment.

4.3.3 Moisture content

Water is essential for life including the life of microorganism, soil moisture content has a great influence on biological agents of biodegradation. Addition of water or moisture source helps survivability and growth of the microorganism.

4.3.4 pH

The concentration of alkalinity and acidity of the contaminated site has an impact on microbial metabolic activity which could indicate microbial growth. It is therefore essential to maintain the optimum pH requirement microbial growth.

4.3.5 Availability of nutrients

Although microbes are present even in polluted soil, the population of the microbe present may not be adequate for an effective remediation process. It is therefore necessary to stimulate the growth and activities of the microbes. Addition ofessential nutrients to the soil enhance microbial growth and reproduction.

4.3.6 Biological factors

Microbial population which are actively involved in metabolizing of contaminantand the volume of enzymes from each of their cells determines the rate of contaminant degradation. The essential microbial population can be affected by biological factors such as;

- Antagonistic interactions between microorganisms.
- Predation on microorganisms by other organism such as **bacteriophages and protozoa.**
- Interactions such as; Succession and competition.

CHAPTER FIVE

5.0 Mycoremediation

Mycoremediation is a fungi-based remediation method. This bioremediation method involves the use fungi to decontaminate, degrade or isolate contaminants form the soil. Several fungi species are hyperaccumulators, they are able to accumulate large volume of toxic molecules in their biomass for later removal.

Significantly, fungi can be used to effectively remediate heavy metal contamination. Heavy metals such; Chromium, Cadmium, Nickel, Lead, Mercury, Boron, Zink, Copper,Iron, and Arsenic can be removed from land, marine environments and from wastewater using fungi (mycoremediation) **(Joshi et al., 2011; Gazem and Nazareth 2013).**

Various types of fungi such as **Pleurotus, Aspergillus, Trichoderma** are example of fungi that has proven to be efficient in removing heavy metals from contaminated site **(Joshi et al., 2011; Gazem and Nazareth 2013).**

Owing to the ubiquitous nature of fungi, mycoremediation can be used for the treatment of; contaminated water, polluted marine environment and land pollution.

5.1 Viability of fungi in extreme environmental condition

Some species of fungi are able to survive extreme conditions like the cold weather condition in Antarctica. Fungi species such as:

Cryptococcus gilvescens Pichia caribbica Leucosporidium creatinivorum Cryptococcus victoriae

can withstand extreme cold weather and still provide efficient biodegradation of contaminants.

Due to the non-specific nature of fungi enzymes, fungi are able to break down different kind of substances such as fragrances and pharmaceuticals which may not be breakable by bacteria.

5.2 Suitability of mycoremediation

Mycoremediation is also effective in remediation of sites contaminated by contaminants such as;

- Polycyclic aromatic hydrocarbons,
- Heavy metals,
- Textile dyes,
- Organic pollutants,
- Wastewater,
- Pesticides,
- Herbicides,
- Petroleum fuels
- Leather tanning chemicals

Some fungi are capable of degrading radioactive materials and some can survive in extremely cold weather condition, thereby making them useful for remediation in cold or radioactive environment.

Rhodotorula taiwanensis is an example of fungi that can be used in the mycoremediation of radioactive waste because of its low pH and radiation resistant properties.

Mucor hiemalis can break down contaminants in wastewater such as; X-ray contrast agents, phenols, personal care products and pigments of wine distillery wastewater.

5.3 Application of mycoremediation

The ability of mushroom to accumulate metals was put to test at **VTT Technical Research Centre of Finland.** Mushroom was used to recover precious metals from a medium using mycofiltration technique, from medium with electronic waste 80% gold recovery was reported **(Salminen et al., 2015)**

5.4 Advantages of mycoremediation

Mycoremediation method does not pose any threat to the ecosystem and so itis largely considered as eco-friendly.

The ability of some fungi to extract heavy metals from a contaminated site can be useful for bioindicator purposes.

No expensive tool or equipment is required, and thus the process is generally considered as cheaper.

5.5 Disadvantages of mycoremediation

In a fungi-base remediation, not all the individual fungi of a particular fungi species are effective in the same manner of toxin accumulation. Usually, a single individual which has adapted to an older polluted environment (like wastewater or sludge) are selected, and the selection is usually done in the laboratory.

Edible mushrooms (such as **Coprinus comatus**) that are able to accumulate heavy metals in their biomass can be toxic when consumed by human or aniamls.

5.6 Limitations of bioremediation

Though bioremediation can be effectively used to mineralize pollutants of organic nature, to partially transform organic pollutants, and to alter the mobility capacity of organic pollutant. In some instances, microorganisms don’t fully mineralize the pollutant, but rather producing a more toxic compound.

Example: under anaerobic conditions, the reductive dehalogenation of **trichloroethylene** (TCE) may produce vinyl chloride (VC) and dichloroethylene (DCE) which are identified/suspected carcinogens. Though, **Dehalococcoides** (microorganism) can further reduce dichloroethylene (DCE) and vinyl chloride (VC) to the non-toxic product ethene.

However, it is impossible to biodegrade heavy metals and radionuclides elements using bioremediation, but they can be biologically transformed into less mobile forms using bioremediation. Microbial population with the metabolic capacity is required to degrade pollutants in biodegradation, therefore making the molecular pathways for bioremediation of considerable interest.

The biological processes used by these microbes in degradation are highly specific, so, various environmental factors must be considered and regulated in the process.

5.7 Application of genetic engineering

Genetic engineering can be used in bioremediation to create/design specific organisms under preliminary research. Genetically modified organisms have been designed to break down certain plastics (PET) and to treat oil spills. In the research process, two different types of genes can be inserted in the organism:

- Degradative genes encode for the proteins required for degradation of
- Pollutants, Reporter genes serves to monitor the level of pollution.

Numerous members of Pseudomonas species have been scientifically modified with lux gene for detecting polyaromatic hydrocarbon naphthalene.

Genetically modified organisms have the potential of horizontal gene transfer which has created a lot of concern regarding their released and containment in the environment. Though adequate measures have been created by scientist to address these concerns.

5.8 Measures for containing genetically modified organisms

The containment measures include modification of the organisms such thatthey can only grow and survive under specific sets of environmental conditions.

Visual identification for tracking modified organisms can be made easier with the insertion of bioluminescence genes into the modified organisms.

CHAPTER SIX

6.0 CONCLUSION

Heavy metal Contamination is a critical issue of public health concern due to the toxic effects on human and the environment. To prevent or mitigate the harmful effects of heavy metal contamination in land, water and marine environment, varieties of techniques have been developed.

Bioremediation techniques shows different advantages when compared with other physicochemical or traditional techniques. Phytoremediation has been proven to be the most efficient bioremediation technique for restoring and revegetating heavy metal polluted sites.

6.1 RECOMMENDATION

Phytoremediation method of bioremediation with the use of heavy metal hyperaccumulator has been shown to be the most promising technique for remediation of contaminated soil, and marine environment.

Phytoremediation process may take long time to achieve an acceptable result, especially in highly contaminated site. However, selection of more adaptable or genetically enhanced plant species may greatly improve plant performance and result in more efficient remediation outcome. More so, better understanding of plant defence mechanism such as tolerance and avoidance will help to design a more suitable plant species for phytoremediation of heavy metals,

Finally, the choice of phytoremediation as single strategy for remediation of heavy metals may not be practically enough, combination of other techniques such as microbial remediation or mycoremediation will provide a more desirable environmental restoration.

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