

# BIOAUGMENTATION AS BIOREMEDIATION APPROACH FOR CONTAMINATED SOIL: A REVIEW

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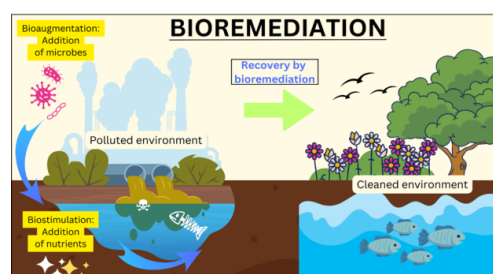
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## Graphical abstract



## Abstract

The inevitable impacts by the emerging urban developments towards the quality and function of soil have raised concerns for future environmental consequences. Pollutants that evolved from anthropogenic activities including heavy metals, pesticides, petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) tend to be persistent within the soil and hence, contribute to long-term effects towards the environment and human's health. Bioaugmentation method as a bioremediation approach is a promising strategy to rectify this issue by introducing specific microorganisms (i.e., bacteria or fungi) to degrade or eliminate targeted pollutants. This paper presents the concept and method of bioaugmentation by describing a step-by-step method as a guidance to implement this method. The identification and selection of microorganisms is a crucial element to assure the success of bioaugmentation. Additionally, there are few factors that influence the efficiency of bioaugmentation, which include pH, temperature, moisture, and type and intensity of the pollutants. The applications of bioaugmentation towards different types of pollutants as stated in this work have certified its capability of degrading, removing, and transforming pollutants into less toxic components. This review extensively focuses on recognizing and understanding the concept, the step-by-step method, and comparative studies of bioaugmentation towards different pollutants, which are heavy metals, pesticides, petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs). For future prospects, the bioaugmentation method can be improved and enhanced by employing new and advanced strategies to tackle pollutants in the long term.

**Keywords:** Bioaugmentation, bioremediation, soil contamination, heavy metals, microorganisms

## Abstrak

Kesan yang tidak dapat dielakkan oleh pembangunan bandar terhadap kualiti dan fungsi tanah telah menimbulkan kebimbangan terhadap akibat kepada alam sekitar pada masa hadapan. Bahan pencemar seperti logam berat, racun perosak, hidrokarbon petroleum dan hidrokarbon aromatik polisiklik daripada aktiviti antropogenik lazimnya kekal di dalam tanah lalu menyumbang kepada kesan jangka panjang terhadap alam sekitar dan kesihatan manusia. Bioaugmentasi sebagai pendekatan bioremediasi adalah strategi yang bijak untuk menangani isu ini dengan mengenalkan mikroorganisma tertentu (iaitu, bakteria atau kulat) untuk merendahkan atau menghapuskan pencemar bersasar. Kerja ini membentangkan tentang konsep dan cara bagi bioaugmentasi dengan menceritakan cara *step-by-*

*step* sebagai bimbingan untuk melaksanakan cara ini. Identifikasi dan seleksi mikroorganisma adalah elemen-elemen penting untuk memastikan kejayaan bioaugmentasi. Tambahan pula, terdapat beberapa faktor yang mempengaruhi kecekapan bioremediasi yang terdiri daripada pH, suhu, kadar kelembapan, dan jenis dan keamatan pencemar. Aplikasi bioaugmentasi terhadap setiap bahan pencemar yang disasarkan seperti dinyatakan dalam kerja ini mengesahkan keupayaannya bagi merendahkan, menyingkirkan dan mengubah bahan pencemar kepada komponen yang kurang toksik. Reviu ini fokus untuk mengenali dan mengakui konsep, cara *step-by-step* dan perbandingan pengajian untuk bahan-bahan pencemar berlainan. Untuk prospek pada masa hadapan, cara bioaugmentasi boleh ditambah baik dengan mengadakan strategi yang baru dan lebih maju untuk mengatasi pencemar dalam masa jangka panjang.

**Kata kunci:** Bioaugmentasi, bioremediasi, pencemaran tanah, logam berat, mikroorganisma

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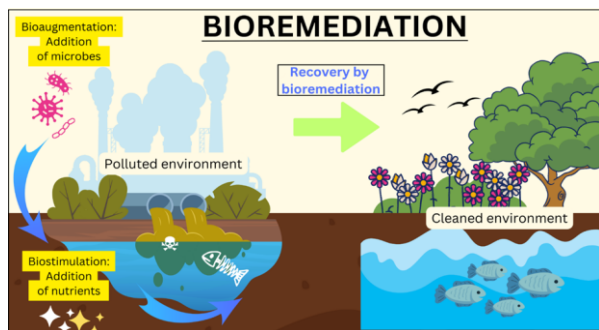
## 1.0 INTRODUCTION

In this globalization era, there are numbers of significant endeavors into developing urbans to a well-improved and more advanced variance. In order to raise these emergence developments, common activities such as industrial, agricultural, and major constructions are widely performed by humans that lasts for many years. Though these efforts and achievements contribute to a glorious progress for the urban developments, the bad impacts are often overlooked. The long-term impact of pollution caused by these activities are inevitable and cause harm to the environment. The consequences towards the soils are worrying as the morphological and physical properties as well as the soil's microorganisms' activity are significantly deteriorating and led to the decline of its self-cleaning ability [1]. Produced pollutants from those activities have brought concerns as it contaminates the soil, which are adversely dangerous to human health and as well as to the environment. The typical pollutants exist within the polluted soil are heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs) and many others. These pollutants often contaminated within the soils for a certain period with high concentration and thus, exposing its negative impacts towards the environment and humans' health [2].

There are some effective physiochemical technologies existed that may overcome this issue effectively. Examples of these technologies include solvent extraction; using soluble solvents to extract the solutes before separating the solution into two phases [3], electrokinetic remediation; utilizes driving force of electric currents to flush ions, metals, water, charged colloids, contaminants, and microorganisms within soil particles [4], and chemical oxidation; which oxidants and additives are injected into the contaminated zone to react with the contaminants [5]. Although all these methods seem promising, they are usually high in cost and not ecological friendly. This is because they employ chemicals and physical

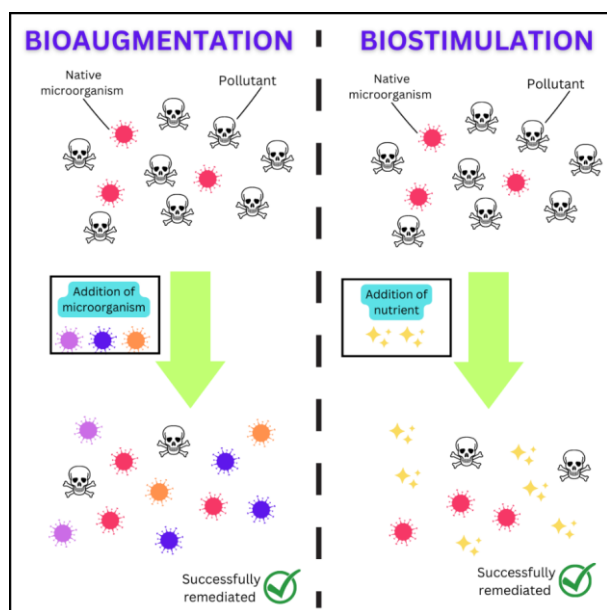
medium that considered as "stranger" or foreign materials towards these contaminants, as well as to the environment. As much as it reduces the pollution, introducing such medium will leave a non-biological trace to some extent.

Therefore, researchers are striving to find a better option to tackle these pollutants by implementing biological method instead. Biological approaches have better flexibility in the sense of performing complex reaction that are able to degrade these contaminants due to the utilization of living organisms [6]. Other than its effectiveness, biological method is more favourable because it is less expensive, less extensive and the prompt growth of the microorganism on the growth media [7]. Hence, bioremediation method is introduced to fill the eco-friendly and environment favorable gap as a promising alternative. Basically, bioremediation is a natural process utilizing biological organisms to clean up the pollutants by transforming those pollutants into non-toxic compounds by performing its metabolic process. Bioremediation is carried out by nurturing these microorganisms such as fungi, bacteria, and plants in ideal and optimum environment and nutrient to ensure and enhance its metabolic activity hence, resulting in reducing the pollutants existing within the environment. There are numerous techniques of bioremediation that has been applied globally. Currently, the common bioremediation methods that have caught persistent attentions are divided into two different significant methods which are; bioaugmentation and biostimulation [8]. Figure 1 depicts the basic understanding on how bioremediation occurs.



**Figure 1** Basic understanding on bioremediation

Both bioaugmentation and biostimulation are ideal techniques for bioremediation due to their effectiveness and methods of conducts. These two techniques share its similarity that both enhance and strengthen the removal of pollutants by adding biological organism. Fundamentally, bioaugmentation is a method of enhancing the degrading ability of microbial organism within the environment by introducing or adding new exogenous microorganism while biostimulation runs by adding nutrients in order to promote the microbial's growth [9]. Figure 2 shows how bioaugmentation and biostimulation performed generally in order to clean up the contaminants.



**Figure 2** General performance of bioaugmentation and biostimulation

Briefly, the predominant factor in selecting which bioremediation strategy to be selected on targeted pollutant is on the preliminary study or assumption on the indigenous organisms in the affected areas. Should the native organisms in the impacted site is substantially incapable to degrade the contaminant, bioaugmentation is the ideal strategy to introduce additional effective microorganism. On the other

hand, biostimulation is convenient when the indigenous organism is already adequate to degrade the pollutant in a well-loose subsurface [8]. However, biostimulation most affecting drawback is that due to the toxic contaminant, most of the time the existing microorganism within the environment is insufficient, hence adding nutrients will be defying the purpose.

The prospect key in bioaugmentation is the metabolic diversities that typically derived from the isolation source and adaptation process that contribute to a rapid degradation of contaminants [10]. The positive outcome upon implementing bioaugmentation in degrading contaminants have been done extensively by numerous researchers by utilizing different microorganisms and applying different types of bioaugmentation. It is proven that bioaugmentation is one of the most advantageous method as it is cost effective while improving polluted soil degradation capacity by allowing in-situ treatment that helped in eliminating the possibility of soil excavation [11]. Therefore, for this paper particularly, bioaugmentation is studied thoroughly to understand its mechanisms and effectiveness as a bioremediation approach towards decontaminating pollutants in the environment.

Although bioaugmentation approach has offered assurance of degrading pollutants from previous studies and researches, there are plenty of limitations that need to take into consideration for improvements and further actions. Some constraints related to bioaugmentation that are needed to grasp are: (i) bioaugmentation showed significant different effects towards different type of soils and different types of targeted pollutants [12], (ii) the complex interaction and reaction between the introduced strains and the existed indigenous microorganisms in the system that can cause possible competition [13] and (iii) the high probability of inoculated microorganisms to not survive or hardly adapting in the new environment [14].

## 2.0 THE CONCEPT OF BIOAUGMENTATION AS BIOREMEDIATION

The implementation of bioaugmentation using microorganism impart unique feature due to the mutualistic symbiosis among microbial populations in reaching the biodegradation integration of the pollutants [15]. Naturally, contaminants within the environment contained types of microorganisms that are feasible to degrade them into less toxic components. However, those existing microorganisms are either inadequate due to low supply, or do not have the catabolic pathways that enables to digest the pollutants [16]. This is where bioaugmentation plays a crucial role to clean up those contaminants by its captivating engineered bioremediation method. Basically, bioaugmentation is the process of absorbing, degrading, and removing pollutants and contaminants into less toxic components by the

injection of a specific microorganism or its respective enzyme [12]. The microorganisms injected are particularly for the targeted pollutants that will further enhance and improve the degradation capability [17]. The fundamental of bioaugmentation is influenced by the catabolic ability of the microorganism to degrade the pollution.

Although bioaugmentation has been proven of its effectiveness, the focal point that is on the question mark is how is the mechanism of the added microorganism enhanced bioaugmentation, how the enzymes are playing their roles, how to choose the optimum carbon source and other questions [18]. The mechanism performed by implementing bioaugmentation is depending on many aspects. However, the vital point is how the microorganisms identify the types of pollutants in order to effectively degrade them by biotransforming those contaminants into less toxic and more stable compound. The identification steps allow the microorganisms (i.e., *Pseudomonas* bacteria) to recognize the toxic material (i.e., heavy metals) and its surrounding as a protection and way of survival. Upon identifying, the bacteria may perform DNA multiplication to produce certain enzymes depending on its targeted elements. As example, lipase for fats, cellulose for carbons, protease for proteins and laccase for metals. In order to breathe, the bacteria will absorb oxygen, produce carbon dioxide and other gases. Furthermore, by adding glucose or source of carbon on inoculation, this would help the bacteria to survive and grow. Other than that, the respective produced enzymes (i.e., laccase) will react with metals to produce electrons which also an act of survival method by the microorganisms. These reactions will then degrade the pollutant and thus, transform those metals into less polluted element [19]. In addition, a review paper by Gao *et al.* suggested a possible pathway of PAH to degrade by bioaugmentation. PAH is firstly oxidized by the exogenous enzyme into unstable halogenated epoxide. After that, they will tend to decompose that resulting the rings to open to enter the tricarboxylic acid (TCA) cycle before being mineralized into CO<sub>2</sub> and H<sub>2</sub>O [20].

Interestingly, bioaugmentation may occur in three different approaches that varies within the origins of the inoculants provided. It is selected based on its capability and adaption towards the polluted areas. In some cases, in order to completely eliminate the pollutants, it is necessary to implement more than one form of bioaugmentation orderly. The three different approaches are as divided into autochthonous, allochthonous and gene engineered microorganisms (GEM) as depicted in Table 1 [21]. Basically, autochthonous is performed by isolating the collected microorganisms from the area before reinjected back onto the same area, while allochthonous method is performed by introducing foreign microorganisms into the polluted area, and GEM is performed by microorganisms that has been engineered [22].

The uniqueness of employing microbial communities into developing biodegradation

mechanism, the effectiveness can be enhanced by combining several bioremediation approaches to promote the microorganism's symbiosis towards removing the pollutants. As example, by cooperating bioaugmentation with other methods such as biostimulation, phytoremediation, vermiremediation and others, the removal of the pollutants can be promoted and hence, improving the health of the soil. Some researchers were showing interests in exploring these cooperative methods like studies performed by G. Lacalle *et al.* [23].

### 3.0 BIOAUGMENTATION METHOD

The step-by-step method to perform general bioaugmentation is summarized in Figure 3. Firstly, the sample collected is usually carried out at a high polluted area where the anthropogenic activities are usually done, i.e., mining, construction, landfill. Commonly, the site will be studied first to analyze its potential type of pollutants and its severance. A standard guideline of collection sample can be followed as in Hassan's work using EPA [24, 25]. For instance, soil sample usually will be collected within 0.2m-0.3m depth [26]. Upon collecting the sample, the soil is commonly will be homogenized, dried in the air and get passed through a 1.2 to 2mm sieve; this is to ensure the larger particles (i.e. stones, roots) in the soil is removed as they would be less reactive due to its mass to surface area ratio [27]. The collected soil will then be kept at 4°C [28]. Characterization of the sample is carried out to determine its physical and chemical characteristics including its pH, moisture etc. [29].

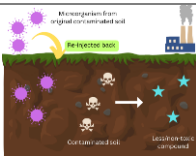
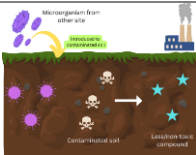
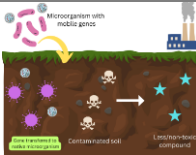
The crucial part of bioaugmentation is on the selection of the microorganism. Researchers mostly refer to previous literature to identify the potential microorganisms to be selected to tackle a targeted pollutant. However, according to Michalska *et al.*, there are step by step methods that implement the thorough order to execute into selecting the ideal microorganisms as shown in Table 2. While in other study conducted by Hassan *et al.*, they performed tolerance assay test of the isolated fungi against the particular heavy metals to analyze the potential of removal ability of the fungi and thus, screening the most optimum fungi [30]. The selection of microorganisms as the bioaugmentation method is analysed within two factors: (i) the capability to break down and clean up the targeted pollutant and (ii) the capability to survive within the nature of the environment. Examples of microorganisms that are known as the great candidate for this method are bacteria, fungi, and algae – either in single strain or in consortium media. Mrozik studied a thorough review on bioaugmentation from single and consortia strains of bacteria towards numerous types of aromatic hydrocarbons few years ago in their literature [16]. The fact that these microorganisms' microbial cell walls are made up of polysaccharides, lipids, and protein has



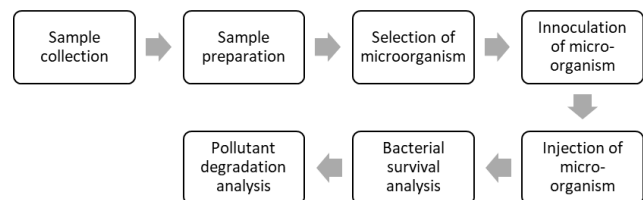
essentially contribute to the efficiency of the bioaugmentation method [31].

Upon selecting the ideal microorganism, the inoculation of the microorganisms will then be carried out to ensure the growth of the microorganism before performing bioaugmentation towards the polluted sample. Based on work by Muthukumar *et al.*, *Pseudomonas aeruginosa* bacteria was first isolated by using MSM medium which consists of  $\text{NaNO}_3$ ,  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  with glucose and yeast extract [32]. Glucose or other carbon source is commonly utilized to feed the microorganism to ensure its survival and growth. These carbon source primarily enhanced the growth and energy production by the microorganisms [33]. While when the carbon source is abundant, the presence of yeast upgrade their capability to use that substrate to maintain a favorable bioenergetic status and engage in metabolic pathways [34]. On the other hand, Melo *et al.* conducted study to degrade sulfentrazone, a type of pesticide in a contaminated soil. The bioaugmentation analysis is performed by utilizing bacterial consortium consist of *Pseudomonas putida*, *Pseudomonas lutea* and *Pseudomonas plecoglossicida*. They were cultured individually in a nutrient broth comprised of mixture of water,  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaCl}$ , meat extract and peptone at pH 6.8 for approximately 10 h at 30 °C and 150 rpm until achieving an optical density of 0.6 [35, 36]. In this case, peptone containing polypeptides and amino acids are supplied as source of nitrogen and mineral for the growth of bacteria while promoting rapid growth of cells of the culture [37]. On the other hand, Andreolli *et al.* performed bioaugmentation from fungi *Trichoderma sp. Evx1* by utilizing a lignocellulosic matrix as sources of carbon and energy [38].

**Table 1** Approaches of bioaugmentation

Approach	Method	Details
<b>Autochthonous (Indigenous)</b>	Microorganism from the polluted area will be isolated before being re-injected back onto the same area	
<b>Allochthonous (Exogenous - single strain or microbial consortium)</b>	Foreign microorganism inoculated from other site is introduced to the polluted area	
<b>Gene (Genetically engineered - GEMs)</b>	Genetically engineered microorganisms supplied with encoding respective enzymes	

Upon inoculation of microorganisms, the next step of injection of the microorganisms is performed. Two samples should be prepared where one of it will act as the control. The sample is injected with the isolated bacteria and monitored for 40 days. The pH and temperature of the soil is recorded for each 10 days. To analyze the effectiveness of bioaugmentation, two crucial parameters that needed to take into consideration are: (i) analysis of the survival of the bacteria, and (ii) analysis of the degradation of the pollutants. By monitoring the total viable count of bacteria consortia by using dilution plate technique, one should enable to identify the survival of the bacteria within the length of time. Muthukumar *et al.* discovered the total number of bacterial colonies was increased about  $1.5 \times 10^8$  CFU/g within 24 days. This has proven the effective growth of the bacteria due to its metabolic activity and the adaption of the bacteria to the new environment [39, 40]. Analytical test should be done to ensure the degradation and the removal of the pollutants. Gas chromatography mass spectrometry (GC-MS) is one of the ideal methods to analyze the removal of the pollutant by detecting the components that are still exist (i.e., hydrocarbons, heavy metals) within the samples after performing bioaugmentation. Besides that, specific test for the particular pollutants also may be performed to determine the degradation of the contaminant. As example, gravimetric method can be performed as total petroleum hydrocarbon (TPH) test by analysing the TPH concentration [41].



**Figure 3** Bioaugmentation step-by-step method

Lara Moreno *et al.* opined that only analysing the pollutant degradation is insufficient to authenticate the removal of toxic materials within the soil since it is possible that the toxic metabolites from the biodegradation process may still present. Therefore, it is highly suggested to assess the toxicity upon biodegradation to verify the success of the method [42]. By applying procedure established by Molina *et al.* to employ Microtox Test System that then translated into toxic units (TU), the toxic removal is then validated [43]. Upon implementing bioaugmentation utilising *Arthrobacter aurescens* into a trifluralin contaminated soil, TU value dropped from 7 to 0.06 which implied the absence of toxic [44]. The step-by-step method can be implemented as guidance to perform bioaugmentation method on respective pollutants. It is crucial to thoroughly select the ideal microorganism to employ and determine the conceptual approaches.

### 3.1 Factors Affecting Bioaugmentation Method

The mechanism behind bioaugmentation may be influenced by certain factors or parameters that contributed to the degradation of the pollutants. Some apparent factors that commonly identified are pH, temperature, moisture, and type and intensity of pollutant [45]. The optimum parameters complementing the microorganisms' work in biotransforming the contaminants into less toxic materials. Researchers studied the effect of these variables towards the effectiveness of bioaugmentation to obtain the ideal scheme for bioaugmentation that can be implemented and applied towards the efforts of eliminating these toxic materials productively [46].

The action of the microorganisms as the bioaugmentation agents are somewhat correlated between each other with the pH values of the soil. It is demonstrated from previous researches that for ideal growth and survival of microorganisms within soil, pH ranging from 6.5 to 7.5 is sufficient [47]. However, microorganisms may be well-adapted to acidic or alkaline condition. The influence of pH is depending on the targeted pollutants to be bioremediated as well as the microorganisms employed (i.e., bacteria or fungi). It is revealed that altering pH at polluted site would be a great effort in order to improve the bioaugmentation intensity as different pollutants may react distinctively at different pH [48]. By adjusting pH and other abiotic factors also may impacting the bacteria community structure and enzyme activity that will contribute to degradation efficiency [49].

Numbers of researchers have monitored an optimum biodegradation of pesticide-contaminated soil in alkaline environment by utilizing bacteria strains. It is witnessed that the degradation of chlorpyrifos by employing *Sphingomonas* sp. Dsp-2 reached up to 98.7% at 7<sup>th</sup> day in a pH 8 soil compared to degradation rate at only 58.1% at 7<sup>th</sup> day in a pH 4.7 [50]. Based on study by Papoola *et al.*, the optimum pH to degrade total petroleum hydrocarbon is at 4.69 with degradation rate of 77.42% by implementing both bioaugmentation and biostimulation [51]. It is monitored by Hassan that towards the finishing of bioaugmentation process towards heavy metals contaminated soil by utilizing fungi, the pH of the leachate landfill soil is declining consistently when compared to the initial value [25]. It is concluded that a reduced pH value within the process implied the increasing of the dissociation of the heavy metals. The predominant reason upon this conclusion is due to the possible metabolic by-product that may be released by the microorganisms during the bioaugmentation process [52]. These by-products typically contain organic acid (R-COOH) that resulting a higher concentration of H<sup>+</sup> in the soil due to the detachment of the H<sup>+</sup> from the carboxyl group of the acids as depicted in Eq. 1. These high concentration of H<sup>+</sup> ensued reduction of the pH within the soil [53].



where R is the alkyl group, and COOH is the carboxylic group of the acids

Other than pH, temperature also contribute significant influence towards the degradation of pollutants. As example, in cold climate area like Antarctic, natural soil degradation is slightly impossible due to the hindrance of degradation metabolic functions. It is revealed that arising of temperature correlated with the increasing of the solubility of the pollutant that resulting to the enhancement of the bioavailability of the pollutants [54]. Furthermore, it is highly favorable to achieve an optimal parameter that may enhance the microbial metabolism as well as the enzyme activity as it will help in improving the degradation of the pollutant. Increase of temperature can play an important role for this enhancement [55]. On top of that, different degradation of compound or pollutants might prefer different range of optimal temperature [56]. Interestingly, Liu *et al.* reviewed that temperature also take factor in the adsorption and desorption process of the pollutants to the degrading-microorganisms. It is reported that by increasing the temperature, the adsorption capacity and intensity also will be elevated [55]. Ideally, the range of temperature for the bioaugmentation process would be between 22-37°C [57]. According to Ferraro *et al.*, the temperature was kept constant at 37°C to provide mesophilic operation condition throughout the process and resulting in the expedition of the biodegradation kinetics onto numbers of PAHs [58].

**Table 2** Step-by-step selection of bacteria [59].

Step-by step method	Justification/Remarks
1. Identify 10 potential bacterial strain	Some are purchased and selected based on literature, while some are isolated bacteria that has been identified from the polluted area.
2. Evaluate catabolic traits of bacteria	The capability of the bacteria to degrade certain hydrocarbons are studied using Biolog plates and then calculated.
3. Analyze the toxicity of the contaminant	The toxic effect of the contaminated area to the selected bacteria were analyzed.
4. Evaluate the auto-aggregation and co-aggregation ability of the bacteria	Performed and calculated as method described by Malik <i>et al.</i> [60]. Significant to analyze the survival of inoculated strains.
5. Determine the Flocculation Activity Index (FAI)	Determined by method performed Kurane <i>et al.</i> [61]. Flocculation is important points in analyzing the survival and growth of bacteria.
6. Measure the hydrophobicity of the bacterial cell surface	Performed according method described by Kos <i>et al.</i> [60]. Correlate with the flocculation process and indication of the survival of bacteria.

Step-by step method	Justification/Remarks
7. Detect bacterial motility and chemotaxis	Performed according method by Déziel <i>et al.</i> [62]. Bacterial motility and chemotaxis indicate the survival of the strain.
8. Screen the biosurfactant production and determine the emulsification index	Biosurfactant produced using CTAB/methylene blue agar test while the emulsification index analyzed the performance of the biosurfactant produced.
9. Assess the production of siderophores	Qualitatively performed by method by [63]. To analyze the survival of the bacteria.
10. Determine the non-inhibitory concentrations of ions for the bacteria	To approximate the resistance of the bacteria to heavy metals and ammonium.

At some extent, the growth of the microbes also may be depending on the moisture content of the soil. Some microorganisms require a certain amount of water in order to operate sufficiently. Ronen *et al.* studied the effectiveness comparisons between different moisture of soil towards tribromophenol degradation, a type of pesticide. The results depicted that at 25% and 50% moisture, the degradation rate sped up, which is not the case at 10% moisture [64]. However, excessive moisture content may inhibit the degradation [65].

Moreover, decontaminations most likely vary within the types and intensity of pollutants in two different paths. First, the existence and intensity of the pollutants will necessarily influence the microorganisms. Secondly, the presence of co-contamination also will affect greatly towards this process. As example, bioaugmentation on co-contamination of heavy metals with pesticides at the same affected area may require different approach on contamination on heavy metals alone. The co-contamination may offer either impart good or bad influence towards the microbial activity depending on several factors including the soil condition [66].

## 4.0 APPLICATION OF BIOAUGMENTATION

Over the past years, bioaugmentation has proved its effectiveness through various applications done by researchers. The applications are differentiated by its tackled pollutants that vary by the regular contaminants existed in the soil. There are heavy metals, polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbon (TPH), and pesticides. The summary of the application of bioaugmentation with its respective mechanisms is tabulated in Table 3.

### 4.1 Heavy Metal

Heavy metals are metallic elements with high densities and atomic weight. Heavy metals are originated from two major sources before entering the environment which through the natural and anthropogenic sources.

Naturally, heavy metals exist through volcanic activity, metal corrosion, metal evaporation from soil and water and sediment re-suspension, soil erosion, geological weathering. Besides that, heavy metals can be obtained in polluted soil from parent soil itself – and may exist in chemical complex form such as silicates, hydroxides, sulphides, oxides, organic, phosphates and hydroxide compounds. While the latter, heavy metals may be produced from the mentioned activities which categorized as the anthropogenic sources. These also include chemical runoffs, waste-water, industrial and agricultural activities, as well as metallurgical and mining processes [67]. Heavy metals and metalloids also highly accumulated from landfill leachate that most possibly may polluted the ground and surface water bodies that caused concerns and threats to the environment [68]. Exposures of heavy metals also give negative impacts towards human's health and can be lethal, depending on its pathway and duration of exposure as well as its dosage intensity [69]. The typical heavy metals existed are chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), arsenic (As), copper (Cu), zinc (Zn), and cadmium (Cd).

According to Oziegbe, heavy metals contaminated in landfill can be remediated by using indigenous bacteria (*Pseudomonas aeruginosa*, *Klebsiella edwardsii* and *Enterobacter cloacae*) and may be enhanced by regulating its carbon source and pH value. The application has successfully remediated lead-contaminated soil up to 92.41% by using *Klebsiella edwardsii* with the enhancement of 4 g/L of peptone as its carbon source and at pH 6. At pH 5, *Klebsiella edwardsii* abled to remove cadmium contaminated soil effectively at as high as 81.56%. *Pseudomonas aeruginosa* and *Enterobacter cloacae* also exhibit significant percentage of bioremediation up to 86.38% and 85.43% respectively [70]. This implied that all the bacteria have high tolerance towards the targeted heavy metals and have high flexibility and adaption with the polluted soil [71, 72]. Njoku also reported the reduction of concentration of lead, nickel, and cadmium on media by employing three different inoculants comprise of *Bacillus megaterium*, *Rhizopus stolonifera* and a consortium of both. After 96 hours, it is recorded that *Bacillus megaterium* has degraded nickel at 73% which is the highest achievement compared to others. Upon testing the bioaugmentation on combined all three metals (lead, nickel and cadmium), *Bacillus megaterium* still successfully degraded the pollutants up until 48% [73].

In other work by Li *et al.*, bioaugmentation-assisted phytostabilization was performed to immobilize cadmium within the plant soil by using rhizobacteria *Bacillus subtilis* with the aid of ryegrass *Lolium multiflorum* L. by up to 39.1%. It demonstrated that bioaugmentation elevate the degradation efficiency compared to phytoremediation alone [74]. Heavy metals traces were often detected in leached-contaminated soil in landfill area in Malaysia. According to Emenike *et al.*, it is discovered that selection of bacteria is crucial rather than quantity or

diversity upon testing up to nine bacteria as bioaugmentation agent in landfill with heavy metal-contaminated area. It was reported that by applying combination of isolated of *Lysinibacillus* sp., *Bacillus* sp., and *Rhodococcus* sp. were affectively reduced lead, aluminum and copper by 71, 72, and 86%, respectively [75].

There are also a few studies on implementing fungi as bioaugmentation agent that effectively degraded 77% of arsenic containing soil collected from landfill by inoculating consortia fungi. The fungi utilized is autochthonous type, which isolated from the landfill and thus been identified as *Ascomycota* and *Basidiomycota* fungi [25].

## 4.2 Total Petroleum Hydrocarbon (TPH)

The wide application of petroleum as the main source of energy globally has played an important role of the production of total petroleum hydrocarbon (TPH) that mainly sourced from the well explosion, power plants and pipeline breaks which has caused adverse hazardous effects to the environment. The improper treatment and abundance of crude oil sludge that produced during storage and transportation operations for petroleum production are also the essential key for this issue [76]. Typically, hydrocarbon is an organic chemical compound that contains hydrogen, carbon, and trace elements of heavy metals. Its classification can be divided into different type of hydrocarbons which are polycyclic aromatic hydrocarbons (PAHs), total recoverable petroleum hydrocarbon (TRPH), BTEX (which include benzene, toluene, ethylbenzene, xylene), total recoverable oil and grease (TROG), total petroleum hydrocarbons (TPH), organic and inorganic [30]. TPH is related to the mixtures of compounds derived from petroleum with carbon numbers that ranging from C5 to C36. Generally, bioremediation will biodegrade longer chain hydrocarbon into shorter chain hydrocarbons hence, resulting into less toxic compounds [77]. However, degrading TPH is particularly challenging due to its low bioavailability and complex chemical composition that contribute to extensive effects on soil ecotoxicity [78]. Due to its mutagenic and carcinogenic feature, the exposure of TPH has brought concerns to human's health and also to aquatic life and terrestrial animals [79].

Lladó *et al.* has studied biodegradation of TPH by applying bioaugmentation with two different microorganisms. It is pointed that TPH is degraded at highest by 50% by ligninolytic fungus *Trametes versicolor* inoculation, along with decline of unresolved complex mixture enhancing hydrocarbon degrading microbial population [80]. Popuula *et al.* has investigated the TPH degradation efficiency of gram negative bacteria at its optimum parameter [51]. Other than single strain bacteria, a consortium of bacterial strain comprises of *Raoultella ornithinolytica*, *Serratia marcescens*, *Bacillus subtilis*, *Acinetobacter pittii*, and *Acinetobacter lwoffii* as bioaugmentation method has been conducted by Bidja Abena *et al.*

[81]. It reveals that these consortia effectively degrade TPH-contaminated soil collected from Korla, Xinjiang province that contains 15,633mg kg<sup>-1</sup> of dry soil of initial TPH concentration by up to 48.1% within 40 days. They concluded that the addition of exogenous bacteria consortia has ultimately enhanced the degradation of TPH, especially when compared to natural attenuation method [82]. Agnello *et al.* conducted study to compare the effect of bioaugmentation towards promoting phytoremediation in degrading light and heavy petroleum hydrocarbons. They discovered that applying *Pseudomonas aeruginosa* as the bioaugmentation microbial agent has complemented the utilization of *Medicago Sativa* L. or alfalfa in phytoremediation. The results concluded that by applying bioaugmentation alone, the removal of TPH is up to 59% while the bioaugmentation-assisted phytoremediation has achieved the best performance at 68% removal [83]. In an affected crude oil storage tankage area in oil and gas facility in Peninsula Malaysia, *Pseudomonas*, *Acinetobacter* and *Rhodococcus* sp. were tested both in laboratory and site field for bioaugmentation efficiency on TPH. In the field demonstration test, a successful rate of 97% of TPH is degraded in the top part of the soil when applying *Rhodococcus* sp. in slightly neutral condition with temperature of 30 to 35°C [84].

## 4.3 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs commonly existed and consequently polluting soil at industrial sites due to its industrial activities involving incomplete combustion of organic materials such as coal, petroleum and wood [55]. PAHs can be distributed into two different categories in accordance with their numbers of benzene rings which are; (1) low molecular weight (LMW-PAHs) – consist of only 2-3 rings, while (2) high molecular weight (HMW-PAHs) – consist of 4-7 rings [58]. PAHs mainly comprises of high aromatic rings and complex molecular structures that are inclined to be highly stable in the environment. This is likely because of its properties that are high hydrophobicity, high resistance to nucleophilic attack and low bioavailability [55]. Since PAHs is persistent and recalcitrant in the soil hence in the environment, the potential threat posed by PAHs-contaminated soil to human's health is the potential of its exposure to the food chain. It can cause toxic, mutagenic, and carcinogenic effects [30].

Cao *et al.* conducted study on treating PAH and heavy metal co-contaminated soil by applying bioaugmentation to enhance its phytoremediation process. It is proven that by applying bioaugmentation, 81.4% of PAH in heavier-contaminated soil is removed successfully by using *Medicago sativa* L, a fast-growing plant with extensive root system within 90 days [83, 85]. According to studies by Innemanová *et al.*, bioaugmentation was performed onto PAHs contaminated soil by carrying out three stages of inoculation as a strategy to improve the adaption of bacteria with the pollutants.



Small amount of polluted soil was inoculated with types of bacteria along with certain amount of nitrogen, phosphorus and potassium as the pre-adaptation phase before being introduced as the new inoculation onto an increased amount of soil. After 4 months upon the third inoculation, a concentration of 7399 mg/kg containing 5 and 6 aromatic rings of PAHs (benzofluoranthene, benzo(a)pyrene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene) was degraded by 72.9%. It is relatively higher when compared to biostimulation alone that only achieved at 32.2% after the same period. The highest degradation was achieved by *Acinetobacter calcoaceticus* that has also proven of its biosurfactant production by Zao *et al.* that authenticate its potential to enhance the PAHs degradation [86, 87]. In some other interesting research by Zhou *et al.*, they deployed Phen7 – a *Bacillus fimus* strain acquired through bacterial isolation method, utilizing solely phenanthrene (the targeted PAH) and nitrate as carbon resource and electron acceptor. Phenanthrene was introduced as the carbon source as a strategy and pathway for Phen7 to register its action to biodegrade phenanthrene when being applied towards the contaminated soil. This technique achieved its goal by obtaining up to 98.9% removal efficiency in 56 days under anaerobic condition [88]. To obtain the occurrence of kinetic mechanism upon the degradation process of PAHs on soil slurry by bacteria consortia, Kuppusamy studied the rate constant as well as the half-life of the PAHs. The results displayed the degradation of LMW PAHs at 80-90% while HMW PAHs at the range of 60-70%. Upon kinetic study, it depicted that the rate constant  $k$  for this method is at highest value which is around 0.024 to 0.036 day<sup>-1</sup> with the lowest half-life of 19 to 29 days. It should be emphasised that the higher the rate constant the faster the rate of the biodegradation and hence, lowering down the half-life. The consortia was developed by pooling four different strains consists of *Pseudomonas* (MTS-1, KC3), *Cupriavidus* (MTS-7) and *Bacillus* (KC5) [15].

#### 4.4 Pesticides

Pesticides are considered as the essential elements in agricultural management to prevent and eliminate pests and diseases to ensure a standard quality of crop yields. The usage of pesticides is quite common in agriculture industry but the relatively high intensity spread of this elements have led to immense effect to public health and to the environment [89]. Pesticides are complex to degrade, highly soluble in lipid, which may be consequently integrated with the food chain and are persistent in the environment. Besides the excessive amount of pesticides that may cause negative effects towards the soils, high dose of exposure to pesticides can resulting in acute intoxication and also respiratory diseases [90]. Other than providing protection towards plants or crops, the remaining spread pesticides might pass towards other pathways including sorption-desorption, flows into

surface and ground waters and also degradation [91]. The degradation of pesticides can be classified into two pathways which are abiotic degradation such as oxidation and hydrolysis, or biodegradation. However, by comparing these two pathways, biodegradation would dominantly take place within the pesticides rather than abiotic degradation. Nevertheless, the biodegradation outcome may influence by its structure molecule as some pesticides are water-insoluble or may be adsorbed tightly within the soil. Therefore, it may be confined within the soil for a long period of time and thus, contributing to the soil's contamination [92].

Cycon *et al.* has identified several types of pesticides and determined its each chemical structures to characterize its water solubility. These elements are the prospect key to discover the complementation of the degradation microorganisms towards the pesticides and also the half-life of the pesticides within the soil. The common identified types of pesticides existed in the soil and has caused the contamination are as summarized in Figure 4 [93].

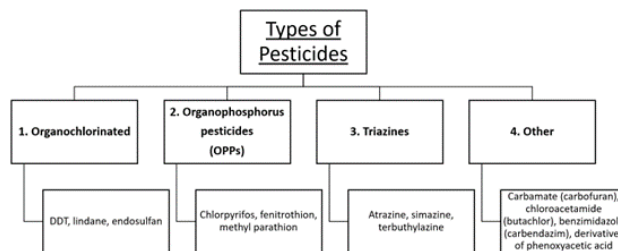


Figure 4 Types of pesticides

In an extensive study carried out by Lara-Moreno *et al.*, it is discovered that employing autochthonous *Pseudomonas brassicacearum*, *Pseudomonas guariconensis*, *Bacillus circulans* and *Arthrobacter aurescens* as bioaugmentation agents have degraded contaminated soil by 47, 48, 48 and 55% respectively in a period of 100 days [44]. Trifluralin is said to be a very persistent pollutant because it is a hydrophobic component with low water solubility with high sensitivity of adsorption onto soil and thus, increasing the retention time [94]. In other study recently, Barot *et al.* treating soil in Bardoli, India contaminated with Dimethoate, an organophosphorus type of pesticides. *Kocuria turfanensis* was identified as inoculated bacteria that successfully biodegraded dimethoate by 78.22 % which was analyzed by using GC-MS [95].

Table 3 Degradation efficiency by bioaugmentation method on pollutants

Pollutant		Microorganisms	Degradation efficiency	Reference	
Type	Compound				
<b>Heavy metal</b>	Cadmium and Lead	Bacteria	<i>Pseudomonas aeruginosa</i> , <i>Klebsiella edwardsii</i> and <i>Enterobacter cloacae</i>	Able to remove up to 86%, 92% and 85%, respectively by each bacterium.	[70]
	Arsenic, Manganese, Chromium and Copper	Fungi	Ascomycota and Basidiomycota consortium	The consortium efficiently removed the heavy metal up to 77%, 71%, 60% and 52%.	[25]
	Cadmium	Bacteria	<i>Escherichia coli</i>	97% removal of 4.63mg/L of Cadmium contaminant with the aid of 1715mg/L of chemical oxygen demand and 3% salinity	[96]
	Thallium	Bacteria	<i>Pseudomonas Putida</i>	Successfully removed up to 100 µg/L of Thallium with co-contamination with high concentrations of Iron and Manganese	[97]
<b>Pesticide</b>	Trifluralin	Bacteria	<i>Pseudomonas brassicacearum</i> , <i>Pseudomonas guariconensis</i> <i>Bacillus circulans</i> and <i>Arthrobacter aurescens</i>	Able to remove up to 47%, 48%, 48% and 55%, respectively	[44]
	Phosphorus	Bacteria	<i>Tetrasphaera</i>	85% removal in aerobic mode for 123 days with 99% of COD removal	[98]
	Pentachlorophenol	Bacteria	Bacterial strains consist of <i>Klebsiella</i> species with <i>Citrobacter</i> , <i>Staphylococcus</i> , <i>Micrococcus</i> and <i>Enterobacter</i>	71% reduction of PCP in forest soil with the aid of the bacteria	[99]
<b>TPH</b>	Contaminated soil by heavy crude oil	Bacteria	Five oil-degrading strains – not stated.	54% of TPH is removed in 90 days of incubation which is higher than natural attenuation method.	[20]
	Contaminated soil	Bacteria	Consortia bacteria	48% of TPH degradation with 41 days of half-life.	[81]
	Motor oil sludge contaminated soil	Bacteria	<i>Pseudomonas aeruginosa</i>	83% of TPH degraded efficiently in 40 days.	[39]
	Complex mixture of TPH	Fungi	<i>Rhizopus sp.</i> , <i>Penicillium funiculosum</i> and <i>Aspergillus sydowii</i>	Up to 47% of 60,600 mg/TPH kg removed in 35 days with mixed culture of fungi. Single strain only able to remove maximum of 36%	[100]
<b>PAH</b>	Complex hydrocarbons from food waste	Bacteria	<i>Bacillus subtilis</i>	81% removal of hydrocarbons. Advanced removal is observed when combined bioaugmentation with fungi	[101]
	Ibuprofen	Bacteria	Consortia bacteria including <i>Flavobacterium</i> and other bacteria	99% has removed efficiently in 21 days	[102]
	Ibuprofen	Fungi	<i>Trametes versicolor</i> and <i>Aspergillus luchuensis</i>	More than 90% removal in 3 hours using fluidized bed pelleted bioreactor	[103]
	Phenanthrene	Fungi	<i>Scedosporium aurantiacum</i> and <i>Aspergillus fumigatus</i> L	Up to 55% biodegradation efficiency with as low as 15% of relative abundance of autochthonous fungi	[104]

## 5.0 CONCLUSION

The presence of typical contaminants in soil includes heavy metal, pesticides, total petroleum hydrocarbon and polycyclic aromatic hydrocarbons are inevitable and recalcitrant to eliminate. Bioaugmentation has been widely explored and applied as an emerging solution to the polluted soil. To deduce, it is important to acknowledge the concept and method of bioaugmentation. The step-by-step method as mentioned in present paper is as guidance to exert bioaugmentation efficiently. Selection of ideal microorganisms is also crucial to ensure suitable features for respective pollutants that has high potential is determined. Furthermore, the application of bioaugmentation to each pollutant (i.e., heavy metal, pesticides, TPH and PAH) are discussed to demonstrate the extensive discovery of its efficiency. It is concurred that bioaugmentation is an excellent strategy to degrade pollutants in contaminated soil. However, limitations occur in applying this method are the competition for nutrients within the added microorganisms and native population. On top of that, not all microorganisms in soil have the degradation ability therefore, selection phase is important. Improvement on bioaugmentation method is also been explored rapidly such as co-remediation with other methods (i.e., phytoremediation, biostimulation) and optimized parameters as future prospects and development of pollutant degradations.

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## Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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