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Experimental laboratory activities preparatory to the remediation of contaminated marine sediments

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DEDICATION

I would like to dedicate my thesis

TO

My PARENTS, for their endless love and support.

My BROTHER, Eng. Sami, and to my SISTERS, Dr. Aisha and Dr. Sayida for their consistence encouragement to accomplish this work.

Everyone who participated in this study, helped me in any way.

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THANK you all…

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EXPERIMENTAL LABORATORY ACTIVITIES PREPARATORY TO THE REMEDIATION OF CONTAMINATED MARINE SEDIMENTS

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ABSTRAC

Marine ecosystems around the world are threatened by human activities. Human activities can directly or indirectly change their biodiversity and environmental quality, endanger valuable goods and services, and ultimately endanger human health. height. Especially in coastal areas, pollution is the cause of habitat changes. The impact of such activities on all biological components and the physical and chemical conditions of the environment may vary depending on the intensity, spatial and temporal scope, and complex interactions between different pressure sources, for example. The Mediterranean Sea has changed a lot. It is one of the most severely affected marine areas in the world in history. The current impact is exacerbated by climate change.

The present thesis work aims to evaluate the effect of bioremediation treatment of contaminated marine sediments from Bagnoli area on the geotechnical properties (deformability and hydraulic conductivity). In marine biology laboratory, the sediments were treated using different microorganisms (i.e., bacteria, fungi and mixed of bacteria and fungi) and monitored until 87 days. In the geotechnical laboratory, both compressibility and hydraulic conductivity were evaluated by means of one-dimensional incremental load compression tests and permeability tests respectively. Tests were carried out on the untreated sample and on treated samples after 28 days of treatment. Additionally, the capability of the samples of leaching inorganic compounds was assessed.

According to the results, there is a remarkable degradation of PAHs after 87 days of bioremediation using the specified organisms; the mix of bacteria and fungi is the most efficient type of treatment,

showing good degradation ability also after 28 days of treatment. The samples subjected to geotechnical tests showed no significant differences between contaminated and biotreated samples, highlighting low compressibility and a high permeability, typical of sandy soils. Zinc, Nickel and Manganese were detected in the leachate of the tested samples but the treatment by bacteria and fungi seems not to affect the mobility/bioavalilability of the metals.

Chapter One: Overview and Aim of The Thesis

1. Soil importance: environmental point view

The soil, by definition, is a mixture of different materials that covers the uppermost layer of the earth's crust. It is mainly made up of mineral particles, water, air, living organisms, and some organic matter. This varying combination of organic and mineral matters, indeed, gives the soil its high distinctive value to function as an essential components of earth ecosystem. Overall, soil could be described as an extremely complex, variable, and living medium. However, since the development process of the soil is immensely slow, it is normally considered as a non-renewable natural resource. Obviously, the soil interfaces between earth, air and water, and therefore has inherent capability to perform the following vital functions: 1) food and other biomass production, 2) storage, 3) filtration, and 4) transformation of many substances including water, carbon, nitrogen. Other critical functions of the soil is serving as a habitat and gene container, as a platform for human activities, landscape and heritage and acting as a provider of raw materials. Moreover, it contains around twice the amount of carbon in the atmosphere and three times the amount to be found in vegetation. Importantly, all the mentioned functions are worthy of protection because of their socio-economic as well as environmental values.

1.1 Definition of contamination and pollution

Pollutant refers to a substance or ingredient of any product that is capable to cause a long- or short-term damage for both human health and the ecosystem. The presence of these compounds behind some limits could strongly affect the human amenities, comfort, health, or property values. Generally speaking, pollutants are produced as a result of human activities including fuel combustion, head and power generation, industrial facilities and municipal/ agricultural wastes. Even though, there are two main sources of pollution, including local or regional sources, under

certain conditions, sediments can travel long distance thereby affecting different places and different species.

A contaminant, however, is regarded as a pollutant but the concentration of these substance are not above natural (baseline) levels. It is important to mention that a contaminant is an undesired material, though it does not necessarily cause harm. Thus, a contaminated soil is a soil whose chemical state deviates from the normal composition but does not have a detrimental effect on the organisms (Kabata-Pendias, 2011). In other words, all pollutants are contaminants, but not all contaminants are pollutants. Of particular concern is that the substances introduced into the environment differs in their bioavailability to the organisms, disregard they are pollutant or contaminant. Accordingly, determining when contamination has resulted in pollution requires not only chemical but also biological measurements (Chapman, 2007). In addition, assessing the capability of a contaminant to create harm to a given target, by migration through environmental media, is the task of the environmental geotechnics that is the discipline that applies the principles of geotechnical engineering in effectively solving several environmental problems. (JOHNSTON *et al.,* 2014).

1.2 The particular case of sediments

The name "sediments" refers to an accumulated material that either has entirely formed within the wetland (autochthonous, intrabasinal sediment, i.e., that formed inside the basin), or has been transported into the wetland (allochthonous, extrabasinal sediment, i.e., that formed outside the basin) (Semeniuk and Semeniuk, 2004). The sediment is also pointed out as the deposits of eroded products that already lost their kinetic energy and created a layered structure.

In contrast, soil is a material that is located near or at the surface of any pre-existing sediment or rock body and directly interfaces with earth's atmosphere. Additionally, under extant conditions,

the soil is subjected to biological, chemical, or physical modifications. (Jackson, 1997). Of note, one of the driven process in soil formation is the erosion process, which includes chip away the rock, and converts them into tiny fragments. The erosion process also involves in moving of the rock or the soil from one place to another.

1.3 Generalities of soil pollution

Soil pollution generally arises as a result of anthropogenic activities. The direct discharge of industrial wastes to soil, the accidental spillages of chemicals, the application of agricultural chemicals (pesticides) to soils, the percolation of contaminated surface water to subsurface stratum or improper disposal of wastes (e.g., leaching of wastes from landfills) are just few examples causing soil pollution with a variety of inorganic and organic pollutants (Mirsal, 2004). Altogether, soil pollution is a build-up of toxic compounds and disease-causing agents that ultimately has an adverse effect the human health and ecosystem. Among the most significant soil chemical pollutants are Petroleum HydroCarbons (PHCs), Polycyclic Aromatic Hydrocarbons (PAHs) (e.g., naphthalene and benzo(a)pyrene), solvents, pesticides, lead, and other heavy metals. Contamination has been found to correlate with the degree of industrialization and intensity of chemical compounds. Concern is highly growing that contaminated soil may pose serious threat to human health. Human exposure to the soil contaminations may occur either through direct contact with the contaminated soil or through migration of contaminants across the environmental media (i.e. soil, groundwater, air and surface water) reaching the human target as vapors from the contaminants, or from secondary contamination of water supplies within and underlying the soil. It should be emphasized that the soil pollutants with the greatest concern for human health are PHCs solvents, pesticides, lead, and other heavy metals.

With regard to the soil pollution sources, these primarily can be classified into two main sources, point source and non-point source (diffuser source). Point source pollution means that the

pollutants are released from discrete conveyances, i.e., a discharge pipe. The main point source dischargers are factories and sewage treatment plants. Collectively, point source pollution does have a single source which is often early to trace and identified. In contrast, Non-Point Source Pollution (NPSP) describes the discharge of pollutants from several places or widespread area rather than from specific identifiable primary sources such as discharge pipes. There are several mechanisms by which non-point source pollution could occur, including runoff, precipitation, atmospheric deposition, drainage, and seepage.

Without a doubt, the presence of pollutants does not cause a certain and immediate threat to the surrounding inhabitances (human, animals, or plants), because their impacts depend on the nature, the concentration and the distribution of these pollutants themselves. Other important parameters that also play a key role in determining the risk to the inhabitants is the exposure parameters, which are time dependent.

The exposure of human targets to the soil contaminants is typically categorized into direct and indirect exposure. Direct exposure can occur by intentional ingestion (e.g., PICA or GEOPHAGY), incidental ingestion (e.g., from hand-to-mouth contact), or via dermal contact. People may also be indirectly exposed to soil pollutants as a result of migration of contaminants (e.g., uptake from soil into food crops and subsequent ingestion, inhalation of contaminated vapors from soil).

In order to take an action against the soil pollution or, at least, to reduce this problem, there are an approach composed by two main stages that should be followed. Firstly, an assessment of the potential risk that pollutants can pose to various inhabitants and human health, should be performed. Secondly, the remediation of the soils should be carried out, considering two main

possible techniques: *in-situ* (without any soil removal) and *ex-situ* (removal of contaminated soil to be treated on or off the site).

Existing remediation technologies can be classified, as a function of the type of remediation process, into four major types:

a) chemical and physical methods

b) biological methods

c) fixation methods

d) thermal destruction

In general, the selection of remediation technology is based on several parameters such as the concentration of pollutants, the risk engendered by the pollution, the available financial resources and time restrictions (Mirsal, 2004).

1.3.1 Overview of Contaminated sites and soils in Italy

Currently, most of the contamination of soil is produced by human activities. In European countries that has grown wealthier, several anthropogenic primary sources of contamination have been reported such as waste disposal, industrial and commercial activities, military activities, and storages and transport spills on land of different fuels or chemicals. Additionally, an inappropriate disposal of nuclear waste or releases of radioactive material either accidently or by other means are important sources of soil contamination in Europe (van Liedekerke et al., 2014). According to a recent European Commission Report (2014), in Europe alone there is a total number of 2,500,000 of potentially contaminated sites and 342,000 contaminated sites are likely to require an immediate remediation.

Italy certainly has many polluted sites that has been the subject of several geographical epidemiological studies indicating the presence of high risk for human health. These sites,

identified basing on site characteristics, on the quantity and toxicity of the contaminants, on the effects to the surrounding areas (in terms of health and environmental risk) and on the possible detrimental effects on Cultural and Environmental Heritage, were recognized as Sites of National Interest (SINs). They are characterized by high complexity and diversity of contaminations. Therefore, the Italian government adopted a national program (D.M. 471/99) to protect human health, terrestrial, and marine environment from hazardous substances released in the most impacted areas (Ausili, et. al., 2020). Based on last report of ISPRA (2019) there are about 39 sites that could be classified as SINs, and 17 of them are located along the coast and partly in the marine area (Figure 1.1). These marine contaminated sites were subjected to a combination of industrial activities (e.g., chemical, petrochemical, metallurgical, steel, mechanical, pharmaceutical, cement, thermal, or thermoelectric plants). (European Environment Agency, 2018).

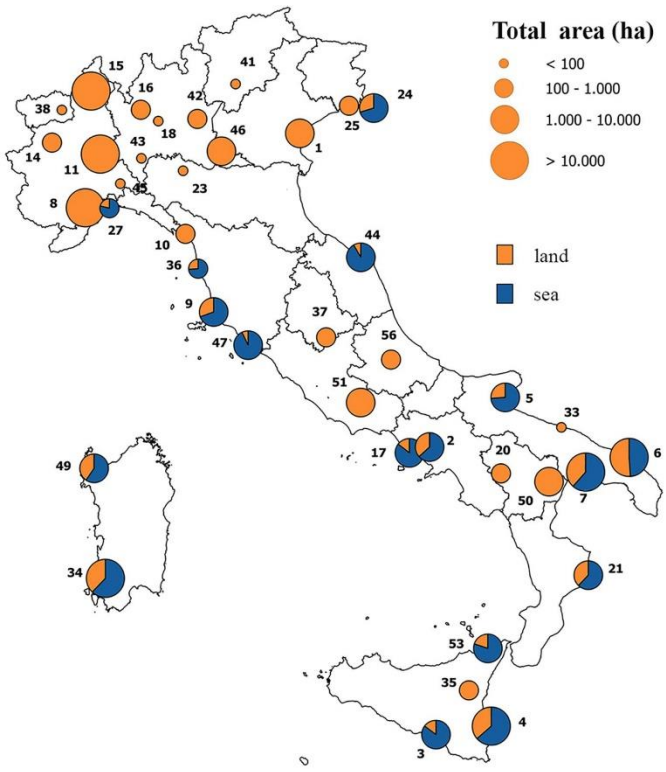

Figure (1. 1): Location of SINs in Italy (from ISPRA, 2019, modified).

1.4 Pollutants of concern

Contaminants are introduced into the marine environment through a variety of sources, and eventually they can be dissolved in water, stored in sediments or ingested by animals. Overall, some of these substances are naturally produced at low concentrations, while the others are produced by human activities. Contaminants that are toxic to plants and animals are accumulated through the food web. In Europe, the most ubiquitous contaminants in the soil are heavy metals (35%), mineral oil (24%), polycyclic aromatic hydrocarbons (PAHs) (11%), another Aromatic hydrocarbon (Benzene, Toluene, Ethylbenzene, and Xylene) (BTEX) (10%) and chlorinated hydrocarbons (CHC) (8%). However, the contribution of phenols and cyanides is negligible considering that both account to 1% (van Liedekerke et al., 2014). In Italy, the main reported marine contaminants are metals and trace elements, heavy hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), organochlorine pesticides, dioxins and furans, chlorinated organic solvents, and organotin compounds (TBTs). Based on European Water Framework Directive (2000/60/EC), all of these contaminants are priority contaminants that require immediate removal (Ausili, et. al., 2020).

1.4.1. Heavy metals

Heavy metal is a term refers to any metals with relatively high densities, atomic weights, or atomic numbers, and that is usually toxic even at low concentration. The earliest discovered metals were Iron, Copper, and Tin, and precious metals are Silver, Gold, and Platinum. With respect to light metals such as Magnesium, Aluminum, and Titanium, they were discovered in the last century. Other group of metals includes Gallium, Thallium, and Hafnium.

Some heavy metals such as Iron, Cobalt, and Zinc, are essential nutrients that are required in several biological and biochemical processes in body, while others are relatively harmless such as

Ruthenium, Silver and Indium. Of note, the existence of a large amount of these metals will be toxic, while presence of Cadmium, Mercury, and Lead even in minute quantity is highly poisonous. The main potential sources of heavy metal poisoning include mining, tailings, industrial waste, agricultural runoff, occupational exposure, paints and treated timber (Kabata-Pendias, 2011).

In soils, metals are distributed mainly between two phases: the soil solution and the soil solid phases. Metals in the soil solution phase may exist as free ions, or form inorganic and organic complexes, suspended colloids of clay, and sesquioxide's (Gobran et al., 2000). Conversely, the soil solid phases contain metals exchangeable bound to charged surfaces, complexed with organic matter, in hydrated oxides of Fe and Mn, as precipitates (carbonates, phosphates, sulfides) or as structural components in minerals (Gobran et al., 2000). The behavior and fate of heavy metals in soils depends on numerous physicochemical processes (e.g., dissolution, sorption, complexation, migration, precipitation, occlusion, diffusion into minerals, binding by organic substances, absorption and sorption by microbiota and volatilization). These processes are further affected by soil properties, such as cation exchange capacity (CEC), pH, redox potential and texture (Agneelo, 2014). Of these factors, pH is generally considered as the most influencing factor.

Unlike organic pollutants that might be oxidized to carbons and being harmless, the heavy metals are characterized by their non-biodegradability, persistence for long time and toxicity. Of particular concern is that a high concentration of heavy metals has a direct and significant impact on the water quality, with long-term implications on ecosystem and human health. Therefore, the European Parliament has included heavy metals among the descriptors of quality status of European seas (Descriptor 8 in the EU Directive 2008/56/EC, i.e. MSFD: Marine Strategy Framework Directive) (Fonti, et al, 2015).

The Table 1.1 illustrates the standard global concentrations of main heavy metals. Without a doubt, presence of heavy metals at high concentration results in adverse effects on the microbial community of the soil, plants and human. One example of these dramatic influence of such high concentration is inhibition the microbial activities and enzymatic actions (e.g., decomposition of organic matter and nutrient cycles) (Su, et. al., 2014).

Table (1. 1): Average heavy metal (mg kg-1) in urban soils from different cities in the world. (adapted from Manta etal., 2002) concentrations

City	Hg	Pb	Zn	Cu	Cd	Cr	Co	Ni	V	Sb	Mn	References
Rome		330.8			0.31							Angelone et al. (1995)
Pittsburg	0.51	398			1.2							Carey et al. (1980)
Boston		800										Spittler and Feder (1979)
Warsaw		57	166	31	0.073	32	5.1	12			337	Czarnowska (1980)
Humburg		218.2	516	146.6	2	95.4		62.5			750	Lux(1986)
Salamanca		53.1			0.53							Sa´nchez-Camazano et al. (1994)
Coruna		309	206	60	0.3	39	11	28		3		Cal-Prieto et al. (2001)
Center Madrid		621										Pellicer (1985)
Madrid		161	210	71.7		74.7	6.42	14.1	30		437	De Miguel et al. (1998)
Bankok		47.8	118	41.7	0.29	26.4		24.8			340	Wilcke et al. (1998)
Aberdeen		94.4	58.4	27		23.9	6.4	14.9			286	Paterson et al. (1996)
Birmingham		570										Department of the Environment (1982)
Glasgow		216	207	97	0.53							Gibson and Farmer (1986)
Central London		647										Rundle and Duggan (1980)
Greater London		250										Rundle and Duggan (1980)
Outer London		322										Davies et al. (1979)
London boroughs		294	183	49	1							Culbard et al. (1988)
London		294	183	73	1							Thornton (1991)
Hong Kong		93.4	168	24.8	2.18							Li et al. (2001)
Hong Kong		100	93.9	27.5	1.89							Wong et al. (1996)
Hong Kong		89.9	58.8	16.1	0.94							Chen et al. (1997)
Manila		213.6	440	98.7	0.57	114		20.9			1999	Pfeiffer et al. (1988)

1.4.2. Total petroleum hydrocarbons and Polyaromatic Hydrocarbon

Total petroleum hydrocarbon (TPH) is a term used to describe a large family of heterogeneous chemical compounds which are mainly made from hydrogen and carbon, originated from crude oil. TPHs can also be defined as mixtures of hundreds of TPHs that vary in the structure (e.g., alkanes, alkenes, cycloalkanes, and aromatics) and size (e.g., 6 to more than 35 carbon atoms in a molecule). More specifically, these TPHs include the Aliphatics (consisting of Alkanes, Cycloalkanes, and Alkenes), Aromatics (consisting of Lower-Molecular-Weight compounds i.e., Monoaromatics, as well as Higher-Molecular-Weight i.e., Polyaromatics), and some petroleum-based hydrocarbon molecules with different composition and axial orientations (McIntosh 2014). Figure (1.2) shows that TPHs can basically be divided into four major structural groups Alkanes, Cycloalkanes, Alkenes and Aromatics (Figure below)

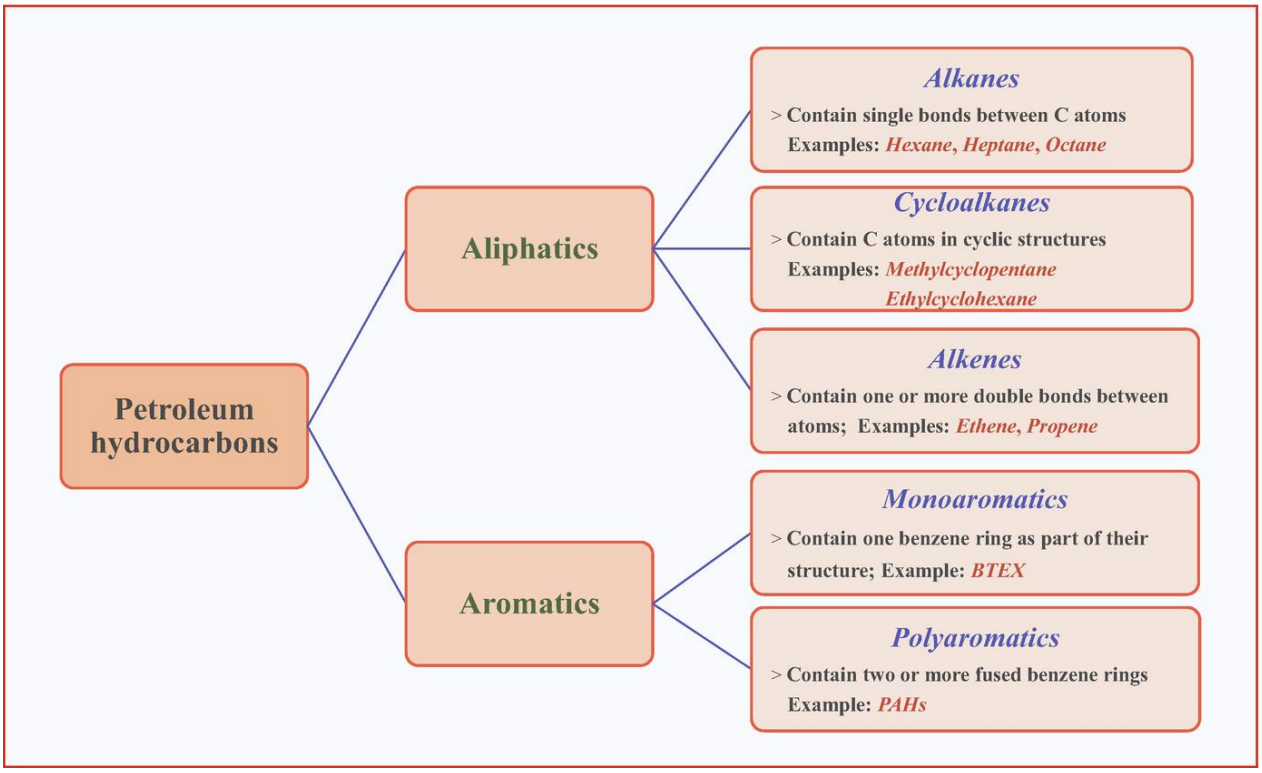

Figure (1. 2): Classification of Petroleum Hydrocarbons (Adapted from Kang et al. 2010)

Of note, around 90% of TPH and PAHs in the environment reside in soil surfaces (Wild and Jones 1995), and these compounds ultimately lead to dramatic modifications of several physico-chemical properties of the soil. The main consequences of such changes are the wildlife and human health can be negatively affected through direct or indirect contact to contaminated sites. Plants grown in polluted sites, for instance, can absorb the TPH, including PAHs, thereby leading to a possible entry to the human and animal populations through the food web (Kang et al. 2010). It is known that some of TPH compounds could release to the soil, and then evaporate into the air, whilst others move downwards, dissolve into the groundwater and move away from the release area (Teng et al., 2013). The TPH compounds may also attach to particles in the soil staying for a long period of time. Importantly, in water bodies when TPH is released, the light TPH fractions usually float forming thin surface films, whereas the heavier TPH fractions accumulate in the sediment at the bottom of the water (Ou et al., 2004).

1.5 Remediation technology:

Many environmental agencies such as U.S. Environmental Protection Agency (US EPA) and European Environment Agency (EEA) intent to create standards and laws promoting the health of the human beings and the environment. Additionally, the main goal of such agencies is to improve the health of the humans by researching the effects of the pollutants, determine limits on their use and determine the safe tolerance levels for chemicals and other pollutants in food, animal feed, and water. Moreover, US EPA and EEA are responsible for detecting and preventing environmental crimes, monitoring pollution levels, and setting standards for the handling of hazardous chemicals and waste. In addition, they are responsible for the remediation of the most contaminated land and respond to environmental emergencies in case of oil spills or natural

disasters. Generally speaking, the best approaches in responding to an emergency disaster is to start the assessment of the potential risk followed by remediation.

Over the last decades, many physical, chemical and biological approaches were developed in order to remove the pollutants that are already dispersed to the soil. Although, physical and chemical approaches are more effective than biological approach, they are expensive and non-environmentally friendly (i.e., high energy demand and high chemical consumption) (Hamdi et al., 2007). Unlike chemical and physical approaches, the bioremediation approach is dependent on the living organisms to break down the organic substances by their enzymes and eventually help in removal of the pollutants.

As mentioned earlier, there are several technologies available to remove the soil contaminants. However, the selection of a proper method is based on many factors which include the following: 1) cost, 2) long-term effectiveness/persistence, 3) commercial availability, 4) general acceptance, 5) the abundance of target metal concentrations, 6) media type (heavy metals and organics), and 7) physicochemical properties (toxicity, mobility, volume, etc.) (Nejad et al., 2017). As said before, remediation technologies is broadly classified into two main groups: *in-situ* and *ex-situ* treatment techniques. *In-situ* remediation strategy aims to remove contaminants from the soil or sediment without moving the soil or sediment itself, therefore the treatment of pollutants is performed at the place of origin. On contrary, in the *ex-situ* technologies, the excavation of the soil is necessary, and the treatment of the soil or sediments takes place either outside the contaminated site (off-site treatment) (Song, et al., 2017) or on the same site (on-site treatment).

Notably, there is another classification for the possible techniques that can be used for remediation and is dependent on the applied process (Figure 1.3):

1- Physical: it means removal of the contaminates physically (e.g., Capping, Vapor Extraction and Electrokinetic Remediation/or Electrical Adsorption)

2- Chemical: in this approach, certain method can be used depending on a chemical reaction (e.g., Solidification, Soil Washing and Nanotechnology)

3- Thermal: it involves supplying heat to the soil which itself leads to volatilize or destruct the contaminants. The technique might be achieved by vitrification or thermal desorption.

4- Biological: it means employing a living thing (e.g., animal, plant or microorganism) in order to decontaminate the pollutants. There are many methods that can be used to reach this target. Therefore, the most widespread techniques are:

   a- Bioventing: Bioventing is a process of stimulating the microorganisms in the soil by providing air or oxygen to existing soil microorganisms, thus the presence of oxygen increases the microbial metabolism of organic contaminants. It should be noted that this technique is dependent on the ability of the air to penetrate through the soil, so the particle size and permeability are essential considerations for the proper functioning of this technique.

   b- Bioaugmentation: this method is used to accelerate and enhance the biodegradability of contaminants (in soil, sediment or groundwater) by adding a specific organism that are either selective microorganisms or genetically modified in order to reach the maximum removal. It is worth noting that the selection of the microorganisms is based on the physiology and the metabolic capacity of these organisms (Hu, et.al., 2011).

   c- Biostimulation: this aims to stimulate the native organisms by modifying some environmental parameters, such as nutrients (nitrogen, phosphorus and potassium),

adding biosurfactants or biopolymers, increasing the electron acceptors with the addition of oxygen, or even controlling temperature and humidity (for the *ex-situ* degradation process), thus providing the optimal conditions for the bacteria activity thus for the deterioration of the microorganisms (Abed, et.al., 2015).

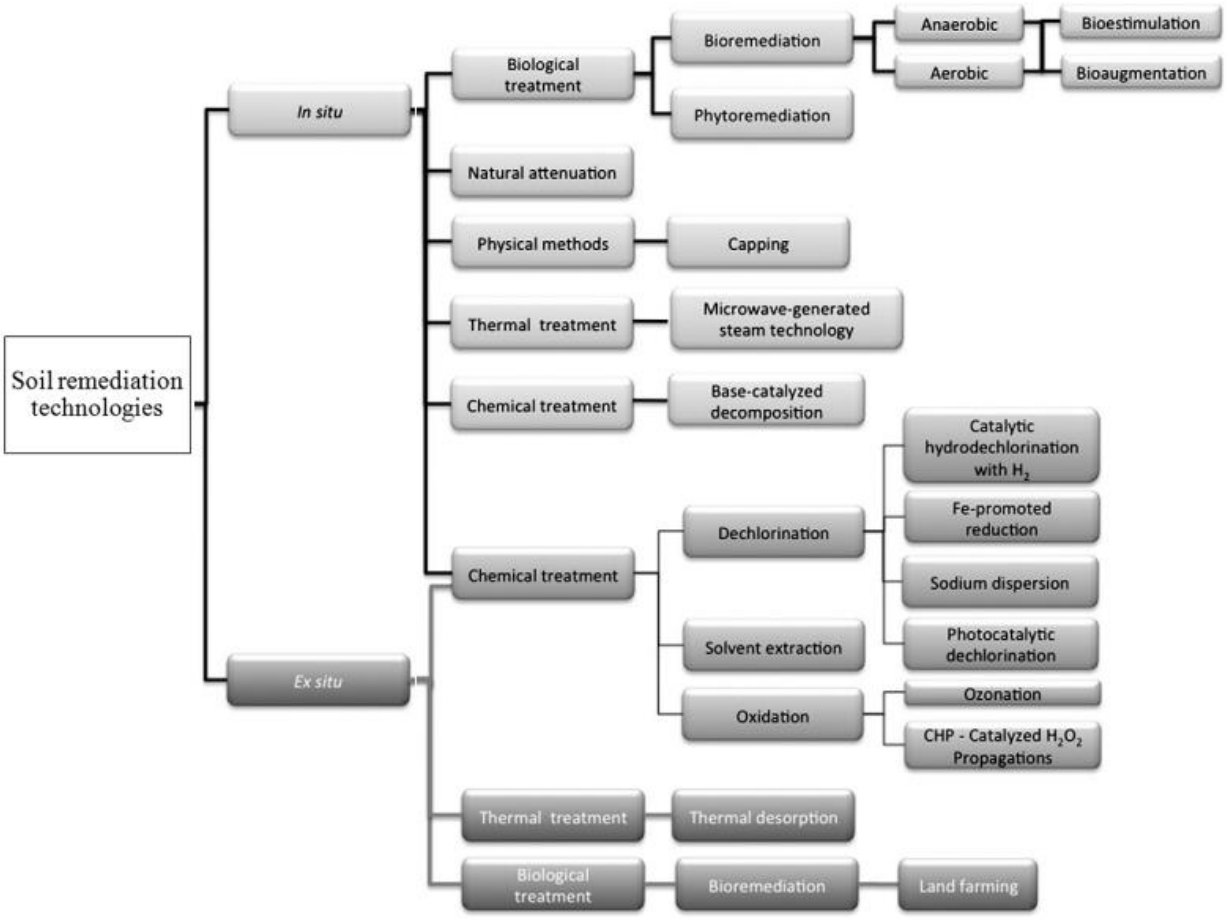

Figure (1. 3): Soil Remediation Technologies (Adopted from Gomes et al.,2012)

The differences between bioaugmentation and biostimulation, especially with respect to addition of selected species and the presence of indigenous microorganisms, are depicted in Figure 1.4 (BioRangers,2020). It is also important to note that, although bioaugmentation and biostimulation

are classified as in-situ bioremediation strategies, both can be applied in ex situ configuration as well (Bodor, et.al., 2020)

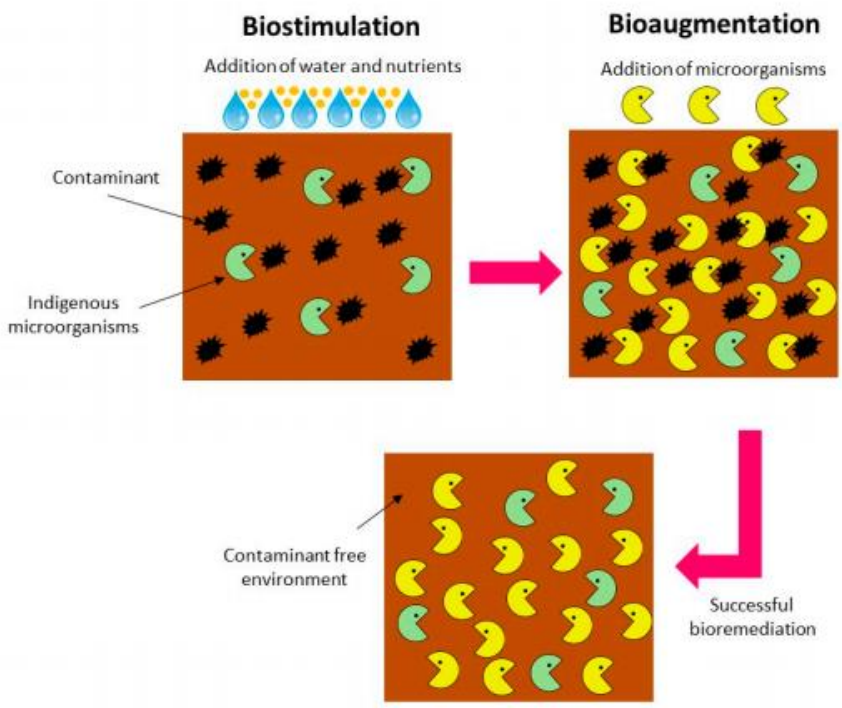

Figure (1. 4): Differences between Biougamntation and biostimulation (Adopted from BioRangers,2020)

1.6 Thesis work and its aims

The present thesis work focused on studying the possibility of the bioremediation and the subsequent further reuse of the contaminated marine sediments located in the marine portion of the Bagnoli area, (Naples, Southern Italy). The area is included in the Bagnoli-Coroglio SIN. The Bagnoli industrial activities included an important steel plant started in 1910 (Figure 1.5), thus the environmental impact of this activity was huge, especially on the marine ecology. Years later, in 1964, two long piers were built for unloading raw materials and loading finished products. Mistakenly, contaminated materials were placed between the two piers. Consequently, without any

isolation barriers, the piers zone was converted to a source of contamination to the nearby area. Many investigations have reported that Bagnoli coastline became a high contaminated site, therefore, the steel production was suspended in 1990, the industrial facilities were totally dismantled at the beginning of 2000s (Romano et al., 2004).

Regarding the geological framework, Bagnoli belongs to the Campi Flegrei volcano-tectonic system and represents the northern margin of a volcanic caldera. The intensity of volcanic activity is very high, also the underwater gas emission and Bradyseismic movement were reported to be high as well (Romano et al., 2004). As a result of tectonic and volcanic activities, 24 caldera and hydrothermal spring were created and most of them are submerged along the Bagnoli shoreline (Sharp and Nardi, 1987). In 2001, Celico et al. reported high levels for some chemical elements, such as (As, Fe and Mn) in the groundwater, which could be interpretated as the result of the interaction between the groundwater and deep flow along some faults and fractures in the volcanic rock.

Furthermore, the wastewater treatment plants (WWTP) in the area discharged their effluent directly to the Bagnoli coastline. Thus, the accommodation of pollutants in that zone caused an increase in the magnitude of the marine environment contamination. To emphasis, around eight WWTP's discharged their effluent to the shoreline, while the other two discharged offshore, using submarine pipes (30 m depth). These ten WWTP's are treating domestic, industrial and runoff water, however the chemical composition and flow characteristics could not be identified yet (Bertocci ,et al. 2019).

In this specified area, a previous study has been conducted and high concentrations of heavy metals (Cu, Fe, Hg, Mn, Pb and Zn), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) (Damiani et al., 1987) were reported. Similarly, earlier studies reported high concentrations

of Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn in the sediments between the two piers (Sharp and Nardi, 1987). Romano (2004) also reported that Cd, Pb, Zn, Fe and Mn are presented in the area. Besides, PAH's were found in the sediments with concentration of several orders of magnitude higher than those reported from several marine benthic ecosystems worldwide (Arienzo et al., 2017). More recently, Sprovieri et al. (2019), studied all the available environmental data (from 1999 until 2013) with the integration geomorphology of the seabed, and found that there was a continuous supply of the contaminations occurred after the suspension of the industrial activities.

For the previously mentioned reasons, Bagnoli-Coroglio site was specified as Site of National Interest (SIN) and therefore it represents one of the priority areas for habitat restoration, selected at a national level.

Aims of the present thesis work is the feasibility study of the bioremediation treatment of the marine sediments from Bagnoli area and the assessment of the geotechnical properties (deformability and hydraulic characteristics) of the sediments after the bioremediation treatment.

In fact, beside the fact that the elimination of any contamination that may cause a threat to human and ecological system is an issue of a pivotal importance there is a considerable interest in reusing the contaminated materials (i. e., sediments) rather than dispose them. Approaching the remediation issue with this double aim offers the possibility of a sustainable management of the contaminated materials.

Hence, assessing the deformability properties of the sediments after the treatment is necessary for predicting their behavior both if a capping layer will be necessary to isolate the contaminated portion of sediments during the biological in-situ treatment (i.e., application of a load) and if they

will be stored in a coastal hydraulic fill (land reclamation) or reused in various geotechnical applications (e.g., roads construction, embankments, and construction of artificial islands).

The selection of one of the previous alternatives is a function of the effectiveness of bioremediation in removing the contaminants.

Aims of the present work can be summarized in the answer to the following specific questions:

1- What is the efficiency of bioremediation for the sediments of the Bagnoli harbor?

2- What is the difference (if any) between the properties of the marine sediments of Bagnoli harbor before and after the treatment?

3- Is there any correlation between mechanical and hydraulic properties with the type of biological remediation, and the concentration of pollutant and type of pollutant?

In order to answer to these questions, an experimental study was carried out on a lot of sediments from the Bagnoli harbor.

From a biological point of view, three types of bioremediation treatment were applied and studied: (1) with bacteria, (2) with fungi and (3) with a mix of bacteria and fungi. Concentration of contaminants and their bioavailability were monitored during the treatment. This part of the experimental study was carried out in the biology laboratory of the Department of Life and Environmental Sciences DISVA.

From a geotechnical point of view, the deformability characteristics of the sediments were studied both on the untreated sediments and on the sediments treated with the three cited pools. To this scope incremental load one-dimensional compression tests were carried out from 6.25 kPa to 800 kPa of applied pressure. Also, the hydraulic conductivity and the leaching capability of sediments

before and after the treatment were studied by means of permeability tests with consolidometers; the use of bladder accumulators allowed for the permeation with seawater and for the sampling of the outlet water to assess the possible leaching of contaminants. Geotechnical tests were carried out in the Environmental Geotechnics laboratory of the Department of Materials, Environmental Sciences and Urban Planning, SIMAU.

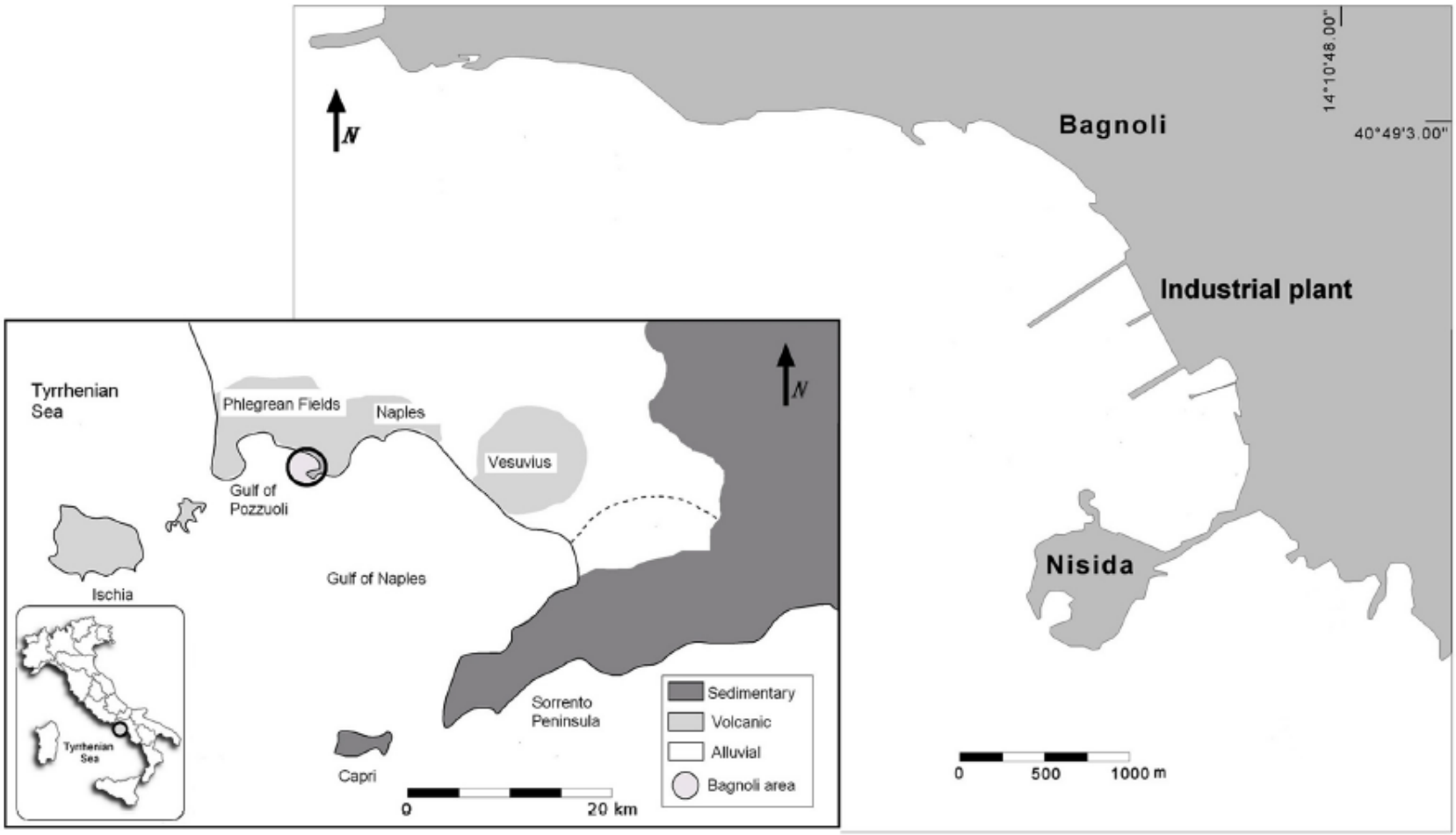

Figure (1. 5): Location of study area and sampling stations (adopted from Romano et al, 2009)

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Chapter Two: Contamination and Remediation of sediments

2.1 Sources of pollution in the marine environment

Ocean or marine pollution generally results from human and natural activities that directly or indirectly have harmful effects on the marine ecosystem. Different sources of ocean pollution (Figure 2.1; Figure 2.2) have been identified and broadly classified into nonpoint source and point source. Nonpoint source (NPS) pollution often represents the biggest source of ocean pollution because it can come from many sources. This type of pollution is mainly caused by land runoff, wind-blown debris, and atmospheric deposition. In contrast, point source pollution (PS) is a single identifiable source of pollution from which pollutants are released, such as industrial zone or oil/chemical spills. Even though PS pollution events might have large impacts, fortunately, they occur less often. Discharge from faulty or damaged factories or water treatment systems is also considered point source pollution.

In particular, approximately 80% of ocean pollution originates from land-based human activities. Human activities produce different types of pollutants such as nutrients, sediments, and pathogens (disease organisms), as well as potentially toxic chemicals including metals, pesticides, industrial products, and pharmaceuticals. Importantly, the birth of industrial revolution has been identified as the key driver of ocean pollution, which allowed emitting a significant amount of material from industries, sewage treatment plants, and agriculture. These materials eventually have reached marine ecosystems and negatively affected it. It is worth mentioning that land-based activities and sources are not the exclusive source of ocean pollution, where in the air could also be an important source of marine pollution. For decades, ocean pollution, a crucial problem that is threating nature and human beings, has been overlooked and largely neglected by policy makers, governments, and people themselves. However, in recent years, public have witnessed several highly visible events

that subsequently have raised their awareness of marine pollution. Two examples of those events are the Exxon Valdez oil spill in Alaska, and the Deepwater Horizon gusher in the Gulf of Mexico which have polluted the seas with oil from ships (Weis, 2015).

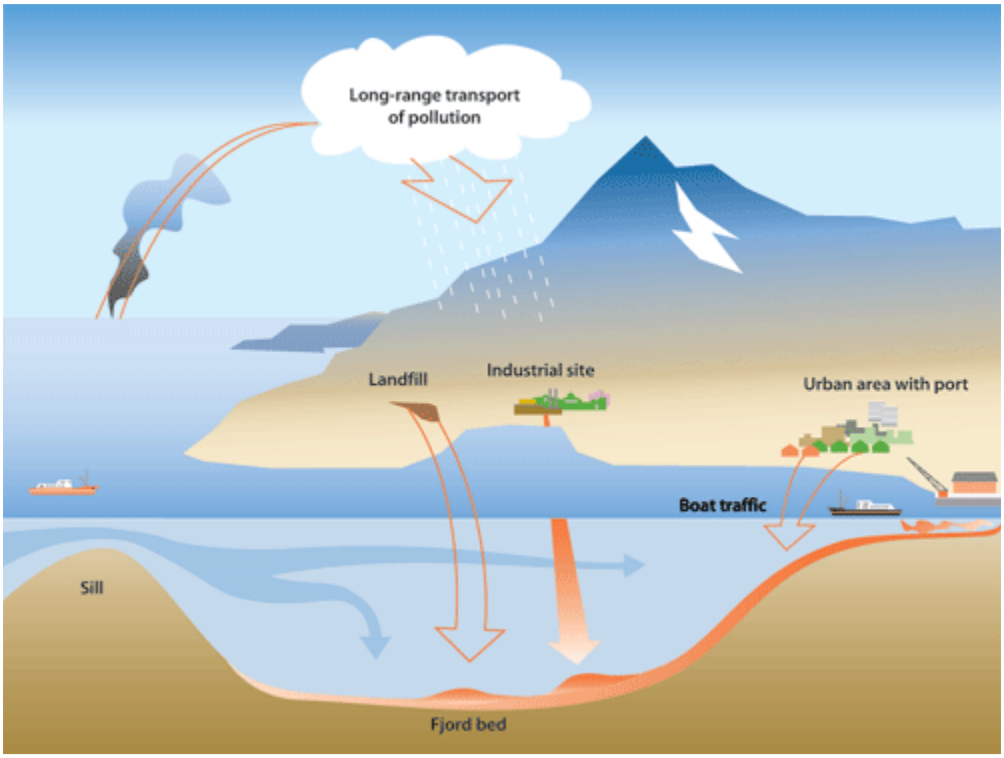


Figure (2. 1): Marine Contamination Sources

The coastal environments are among the most complex and heterogenous environments because of their interconnection between terrestrial and marine environments. In fact, wide range of definitions for the coastal environment have been suggested, some are based on geography, while others are based on ecosystem functionalities. In general, the coastal environment refers to any piece of land next to, bothering or adjourning the seashore, which is characterized with variable width based on the object of the context within which it is being defined. Simply, sometimes it points out to the narrow linear corridor of shoreline separating the continental shelf from the oceanic land mass. At other times it may be considered to extend both largely inwards, towards

the continental shelf and outwards, farther away from the shoreline towards the terrestrial land mass (Ralph,1993). For the purposes of evaluating marine pollution sources and its effects on the coastal environment, the coastal environment can be said to transcend the shoreline up to the exclusive economic zone seaward and across the estuarine and intertidal zones to the lower reaches of the freshwater tributaries, sandy beaches and sometimes even arid continental land masses landwards where such bounds the world Seas and Oceans (Ralph,1993).

Generally speaking, marine pollutant might be classified into physical, chemical, biological and radioactive pollutants. However, there are further classifications that are based on the environment of occurrence, source of the pollutant or even mode of impact.

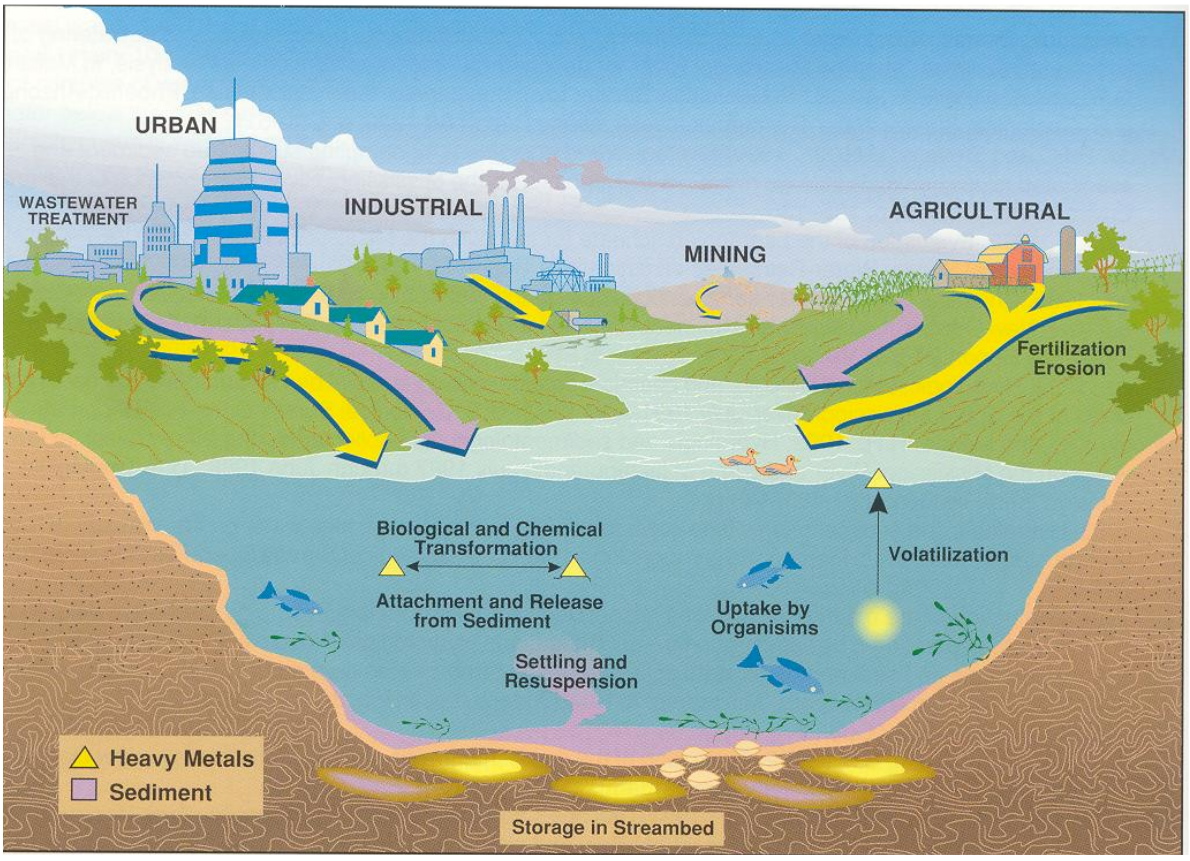


Figure (2. 2): Contamination Source and possible modification/migration of pollutants

Marine sediments are deposited under a wide variety of depositional environments and overall subdivided into two main categories, named pelagic and terrigenous. Pelagic deposits are usually found in deep-sea environment, whereas terrigenous deposits are confined to nearshore environment. Herein, it is convenient to make a fundamental distinction between nearshore and deep-sea deposits, a distinction that recognizes the importance of the shelf break in dividing two very different oceanic depositional regimes.

Nearshore sediments have been defined as the deposits that are mainly found on the shelf regions under a wide variety of regimes and are strongly influenced by the adjacent land masses. The nearshore is a highly dynamic environment due to the movements of the tides and currents. As a result, physical, chemical and biological conditions in nearshore areas are much more variable than in deep-sea ones. Nearshore depositional environments include estuaries, fjords, bays, lagoons, deltas, tidal flats, the continental terrace and marginal basins.

Deep-sea sediments are usually referred to the deposited that are in a depth > 500 m of water, though no consensus on the minimum depth of sediments to be considered as "deep". In reality, multiple factors such as remoteness from the land-mass sources, reactivity between particulate and dissolved components within the oceanic water column, and the presence of a distinctive biomass led to the setting up of a deep-sea environment that is unique on the planet. Because of this, deep-sea sediments, which cover more than 50% of the earth's surface, have very different characteristics from those found in continental or nearshore environments. Two of the most distinctive characteristics of these deep-sea sediments are (a) the particle size and (b) the rate of accumulation of their land derived components (Chester, 2000).

It should be mentioned that land-derived material is accumulating in deep-sea sediments at a rate of the order of a few millimeters per 1000 years. In contrast, those materials deposited in the

nearshore areas have non-carbonate fractions that can accumulate at greater rates than a few millimeters per year (Chester, 2000).

In general, marine pollutants might be of high concern in case they cause significant adverse effects on human or marine ecosystem and are not under control. However, the marine constituents that pose little risks may be considered of low concern, especially if they are under control. The table below illustrates some of main pollutant types. However, each environmental agency has its own priorities depends on some factors which may differ at the local and regional level depending on site-specific circumstances.

Table (2. 1): Type of contaminations and classifications

Priority	Pollutant Group	Example
high	Nutrients Nitrogen	Nutrients Nitrogen
	Pathogens Enteric viruses	Pathogens Enteric viruses
	Toxic organic chemicals PAHs	Toxic organic chemicals PAHs
intermediate	Selected trace metals Lead	Selected trace metals Lead
	Other hazardous materials Oil, chlorine	Other hazardous materials Oil, chlorine
	Plastics and floatables Beach trash, oil, and grease	Plastics and floatables Beach trash, oil, and grease
Low	Biochemical oxygen demand (BOD)	
	Solids	

2.2 Fate of Sediments in Marine Environment

The aquatic environments are well-known to be highly energetic environments. Hence, the fate and transport of contaminants in sediments are largely controlled by numerous, sometimes simultaneous processes (e.g., diffusion, advection, bioturbation, and degradation) that have been found to occur in or near the sedimentation zone. It is worth mentioning that the outcomes of such

processes depend basically on the physical/chemical properties of the contaminants and sediments, as well as the type of water body the sediments rest in. In aquatic environments, many toxic chemicals, including PCBs, PAHs, metals and munitions constituents, which are less likely to biodegraded, have the ability to coat the fine-grained particles and concentrate in bottom sediments.

Remarkably, the main chemical process governing the possible retention of pollutants during transportation in the soil is the sorption. The sorption is a process in which the solutes accumulate at the surfaces and interfaces (e.g., adsorption) or from one phase to another (e.g., partitioning). This process is known to directly influence the transport and reduce chemical and biological reactivity of relatively hydrophobic organic chemicals (HOCs) such as polynuclear aromatic hydrocarbons (PAHs) and chlorinated aliphatic, aromatic compounds in surface aquatic and groundwater systems. Consequently, the molecules that are highly hydrophobic (higher octanol water partition coefficient (K_{ow})), less polar, and larger in organic contaminant molecule, are the most likely to bioaccumulate and sediment organic carbon matter (EPA 2000). In the late 1970s and early 1980s, researchers found that HOC sorption by soils and sediments is driven by hydrophobic interactions, including the entropic effects of aqueous phase and non-specific interactions of the HOCs with soil/sediment organic matter (SOM).

Soils and sediments often contain a wide spectrum of physically and chemically different organic materials ranging from biopolymers such as polysaccharides, lipids, proteins and lignin, humic substances derived from biopolymers, to diagenetically matured kerogen and combustion-related black carbon or char materials (Aiken et al., 1985, Stevenson, 1994, Song et al., 2002). The lipid, in particular, because of their hydrophobicity, are strong sorbents for HOCs, but their contents in many soils and sediments are often very low and may play an insignificant role in the overall

sorption process. Other biopolymers have low affinities for HOCs (Grathwohl, 1990, Xing et al., 1994), and thus are not considered as dominant sportive phases in soils and sediments (Weber et al., 2001)., The tendency to sorb some of the dissolved contaminants has been noticed in those sediments that are characterized with high clay or organic carbon content. The sorptive contaminants are then released from the sources and continue to be source of contamination even after depletion the main source.

For optimum model accuracy, both adsorption and advection should be taken into account as well (Zoppou 2001). With reference to metals, they could be part of the sediment mass through precipitation (e.g., carbonates, sulfides, phosphates, hydroxl complexes) or adsorption (e.g., clay, sediment organic matter). Of note, a radical change in the sediment geochemistry such as falling pH or change in redox seems to facilitate their resuspension as ions. Hence, some of the metals (e.g., arsenic, cadmium, lead, selenium, zinc) may bioaccumulate, while others do not (e.g., copper, nickel). A metal of particular concern is the atmospheric mercury, which can deposited into surface water and can undergo a series of bio transformations in sediments to transform in the highly bio accumulative and toxic methylmercury.

The transport of contaminants associated with sediments is influenced by several factors, including the type of surface water and the source of contamination. The sediment transport in fluvial systems can happen either in a suspended load or bed load, in which the current velocity has a direct impact on both loads. The suspended load is comprised of small particles such as very fine sands, silts, clays and associated organic matter, while the bed load is made up of larger particles. Suspended sediments, which account for the greatest contaminant mass, are more likely to move across large distances before settling out of the water column. Usually, settlement occurs in deposition areas of streams and rivers when water energy levels fall. It also occurs in the deltas of

streams and rivers of a lacustrine or marine environment when water meets its discharge point (NAVFAC 2004).

The transport of contaminated sediments in near-shore lacustrine and marine areas is driven by many factors, including for instance wave energy flux, tidal energy flux, wind forced currents, subsurface currents, and the topography found on the water body floor (NAVFAC 2004 and USEPA 2005).

One of the major challenges associated to contaminated sediments is their ability to affect not only the quality of groundwater but also surface water. It seems likely that water from a water body in a losing system passes through the sediments picking up contaminants dissolved in the sediment pore water and potentially depositing them into groundwater. Whilst on the other hand groundwater passing through sediments in a gaining water body can carry contaminants existed in the sediment pore water up into the bodies of water.

Currently, analytical models are extensively applied in order to improve understanding transport mechanisms and predicting solute transport. In these models, the exact solutions of the model equation is often used to describe the fate and transport of contaminants, and are simplified to produce such solutions. It is important to emphasize that such models function as a starting point in describing contaminant migration before moving on to more sophisticated numerical models (Liu and Ball, 1998). Nonetheless, they are useful tools for validating numerical approaches and afford a fast and computationally efficient approach for estimating contaminant migration. With the assistance of a spreadsheet or a calculator, the determination of the exact solution of the analytical model as well as numerous parameter correlations (e.g., C/Co) and variations can be rapidly and easily performed. Another important obstacle in using the analytical models, is the that characteristically applicable merely to simple contaminant transport systems as there is a necessity

to produce exact solutions. Additionally, analysis of spatial and temporal variability (e.g., non-linear sorption mechanisms) cannot often be accommodated as the transport equation, in particular it can become extremely difficult to solve. Finally, the use of simplifying assumptions requires further detailed arguments to support the modelling approach adopted. (Go et al., 2009)

Apart from analytic models, numerical models for fate and transport of contaminants in porous media allow addressing complex processes that might reflect the realistic environmental conditions. The complexities associated with temporal and spatial variations (e.g., porosity as a function of depth, non-linear sorption mechanism) give clear example of the challenges that can be accommodated precisely by a numerical approach. It has been observed that diffusive transport of organic contaminants was progressively modelled from a simple Fickian process (Goldberg and Koide, 1962) to a spatially explicit transport mechanism affected by sediment physical (Boudreau, 1996) and organic matter content heterogeneity (Xia and Pignatello, 2001). Furthermore, numerical models have been found to be beneficial in studying the impact of a diverse benthic community on the fate and transport of organic contaminants in bed-sediments as well (Choi et al., 2002). Nevertheless, numerical approaches have several drawbacks that may limit its use. It could, for instance, produce numerical dispersion or unrealistic results if model parameters were ‘‘fitted” with values outside the typical range found in the literature, to reduce relative errors between the numerical results and the actual data. (Go et al., 2009). Additionally, their use is relatively time consuming and costly.

2.3 Biological treatments

The presence of contaminated sediments in seabed at high concentration is a serious threat for both humans (threaten the health of people) and marine ecosystem. Recently, many countries have recognized and acknowledged the growing danger from contaminated sediments and prepared a

list of hazardous materials that required an urgent treatment (Kadali et al. 2012). Therefore, different methods of treatment have been developed and applied, including chemical and physical technologies together with biological treatments (Sarkar et al. 2005). Chemical and physical approaches have included solidification, stabilization with additives such as magnetite, solvent extraction, ultrasonic treatment, thermal desorption and incineration (Gallego et al. 2007). However, all these approaches have either environmental or economic disadvantages or both of them. For example, treating one cubic meter of curd contaminated soil by disposal into a landfill may cost $880, while incinerating one cubic meter costs about $700; thermal desorption costs $260 per cubic meter (Makadia et al. 2011; Rahman et al. 2003). Additionally, some of the chemical treatment methods leave behind unwanted toxic chemicals such as solvents that themselves have potential deleterious impact on humans, soil and microbial communities. Overall, these approaches are neither safe, nor environmentally friendly. Moreover, they offer only temporary solutions (Das et al. 2012). Consequently, there is the unmet need to find fast, inexpensive, safe and environmentally friendly approaches to solve contamination of sediments in the marine environment.

Bioremediation is a safe, cost effective, efficient and eco-friendly approach in restoring the polluted marine compared to chemical and physical treatment approaches (Mansur et al. 2015; Zhang et al. 2010). Bioremediation of soil contaminated with hydrocarbons has initiated in the middle of last century when Davis (1967) summarized the early work and concluded that specific microorganisms showed the potential to degrade petroleum hydrocarbons and utilize them as a main carbon source for energy and growth (Kumar et al. 2011). Later studies showed that indigenous isolates from soil and water have the ability to degrade a wide range of contaminants

in the environment including different hydrocarbons (Hazen et al. 2010; Jain et al. 2012; Mansur et al. 2014).

Bioremediation is defined as a process which points out to the utilization of microorganisms such as bacteria, fungi and yeast to minimize (detoxify, degrade, and mineralize) concentration of pollutants or converting them to harmless products (Das et al. 2012; Kumar et al. 2011; Sarkar et al. 2005; Surridge et al. 2009). Basically, the removal of the pollutants is highly dependent on the mechanisms of enzymatic attack and on the activity of the living organisms (Kumar et al. 2011). Effective bioremediation can only be achieved when environmental conditions permit and enhance soil microorganism's growth and activity and when potential degraders are present. (Mansur et al. 2015).

In terms of bioremediation approaches, three main techniques can be used. The first, is enhancing bioremediation by supplying nutrients, named biostimulation (BS) (Adetutu et al. 2011; El Fantroussi & Agathos 2005). BS can improve the degradation rate of the pollutants by optimizing the environmental conditions of the microbial community, including addition of nutrients, aeration, controlling pH and temperature (Margesin et al. 2000). BS is primarily a process which focuses on supplying nutrients to increase the population of indigenous contaminant degraders (Sarkar et al. 2005). In hydrocarbon degradation, for example, nutrient supply traditionally focuses on the addition of N and P in various nutrient sources such as urea, inorganic fertilizers, compost, sawdust, biosolids and manure (Cho & Kende 1997; Namkoong et al. 2002; Walworth & Reynolds 1995). Prior studies have been conducted to explore the effectiveness of BS on soils contaminated with different types of pollutants. For example, Yu and co-authors found that BS enhanced the bioremediation of the soils contaminated with heavy hydrocarbon. The authors found that, after 140 days incubation, the degradation rate was increased significantly in the BS treatment, with a

TPH removal efficiency of 30.80 %) compared to 9.2 % in non-amended setup (control). Under aerobic conditions (i.e., in the presence of oxygen). The microorganisms utilize the hydrocarbon contaminants to generate carbon dioxide, water and microbial cell mass (biomass) which could be described by the following reaction (IMO 2001):

$$1\ \text{kg HC} * + 2.6\ \text{kg O2} + 0.07\ \text{kg N} + 0.007\ \text{kg P} \rightarrow 1.6\ \text{kg CO2} + 1\ \text{kg H2O} + 1\ \text{kg biomass}$$

Where * is Hydrocarbon contaminants

The second bioremediation approach that aims to enhance the degradation capacity of the soil microbial community is bioaugmentation (BA), which involves introducing specific microbial strains or consortia, to the contaminated sites. The success of this approach can be assessed by exploring the changes occurring in the biotic factors (the increase in microbial biomass, degradative enzyme activity and survival) following the addition of pollutant degrading microorganisms (Mrozik & Piotrowska-Seget 2010; Thompson et al. 2005). Another strategy to measure effectiveness of BA approach is to assess the chemical structure and physicochemical properties of the contaminated soil and the concentration of pollutants (Tyagi et al. 2011). A study by Teng et al observed that the bioremediation was highly effective in removing polyaromatic hydrocarbons (PAHs) from polluted soil samples. After 28 days incubation, the BA treatment showed a 23.2% decrease in PAH concentration compared to 3.5 % in control (untreated) soil. Moreover, a study was conducted to compare efficiency of BA and BS on removal of hydrocarbon from polluted soils when applied to biopiles. After 140 days of incubation, bioaugmentation resulted in a reduction between 64% and 68% in contaminant concentration compared to a 0.0 % reduction with biostimulation (Liu et al. 2011). Of particular concern, Sun et al (2012) observed that combining both approaches (BS and BA) led to synergistic effects and a higher reduction in low molecular weight and high molecular weight of PAHs.

The third approach which is commonly used in bioremediation is the natural attenuation (NA). NA involves a reduction in toxicity of contaminants naturally in the absence of physio-chemical or biological processes (Scow & Hicks 2005). Because NA basically relies only on natural degrading processes, it often requires a longer time to bring the contaminants to a lower concentration. This approach, in fact, may raise concerns and objections from local communities as there are no outward signs of bioremediation (Bento et al. 2005). Even though the longtime is a major limitation of this approach, NA has been used routinely at remediation sites of soil contaminated with petroleum hydrocarbon. In a study by Bento et al. (2005), NA was able to reduce the concentration of diesel polluted Long Beach in California-USA by 36% compared to 59 % and 68 % reduction obtained by BA and BS, respectively.

2.3.1 Factors influencing hydrocarbon biodegradation.

As for the other remediation techniques, bioremediation has some limitations and barriers affecting the contaminant degradation rate. Firstly, one key factor influencing the rate of bioremediation is the concentration of contaminants. Kumar et al. (2011) study found that compounds such as aromatic hydrocarbons, residual oils and sludges and chlorinated organic compounds were resistant to microbial attack at high concentrations because of their toxicity. In addition, the presence of additional inhibitory substances (e.g. heavy metals) may further limit the activities of the hydrocarbon degraders (Adetutu et al. 2011). Secondly, a lack of an appropriate enzyme may prevent the degradation of the pollutants e.g laccase, an enzyme involved in PAH degradation (Peixoto et al. 2011; Thapa et al. 2012). This is often the case for xenobiotic compounds such as chlorinated hydrocarbons. Thirdly, a lack of nutrients such as nitrogen and phosphorus may significantly reduce biodegradation by preventing the native microbial communities achieving active growth and degradation of the contaminant (Röling & Van Verseveld 2002). Inappropriate

soil physiochemical characteristics (pH and temperature) also influence the activity and diversity of the soil microbial community and thus may result in an inadequate removal of pollutants (Hamamura et al. 2006; Molina-Barahona et al. 2004; Zhang et al. 2012). Finally, oxygen limitation and a lack of moisture are further factors which may limit the degradative activities of the hydrocarbonoclastic microorganisms (Kabelitz et al. 2009). Some studies on bioremediation have reported a rapid loss of the soil moisture content in a range between 50-80% (Calvo et al. 2009) during treatment. Taken together, to achieve a successful bioremediation outcome, all the above-mentioned factors are potentially important and need to be optimized before commencing any bioremediation strategy.

Table (2. 2): Factors affecting the bioremediation potential of a contaminated site (adopted from Mansur, 2015)

Factor	Effect	Reference
High concentration of hydrocarbons	Resist microbial attack	(Kumar et al. 2011)
Presence of inhibitory substances such as heavy metals	Inhibit the growth and activity hydrocarbon degraders	(Adetutu et al. 2011)
Lack of appropriate enzymes	Prevents hydrocarbon degradation	(Thapa et al. 2012)
Lack of nutrients	Prevents microbial growth and degradation	(Röling & Van Verseveld 2002)
Soil pH and temperature	Influence the activity and diversity of microbes	(Hamamura et al. 2006)
Lack of soil moisture and oxygen	Limits the degradative activity of the microorganisms	(Calvo et al. 2009)

2.3.2 Microorganisms used in bioremediation.

One of the most significant factors that restricts biodegradation of oil pollutants in the environment is their limited availability to microorganisms (Providenti et al., 1995). The difference in

susceptibility of hydrocarbons to microbial enzymatic has been observed and generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkanes (Rosenberg and Ron, 1996). Of interest, a pollutant such as the high molecular weight ones (PAHs), may not be degraded at all. Microbial degradation is considered as the major and ultimate natural mechanism aims in cleaning up the hydrocarbon pollutants from the environment (Iliya, 2008).

Importantly, the science of classifying organisms is called Taxonomy. Classification is a critical step in understanding the diversity and how past and present life on Earth evolve. All modern classification systems have their roots in the Linnaean classification system, which, in turn, was developed by Swedish botanist Carolus Linnaeus in the 1700s. That classification was called Linnaean system and included only all living things that were known at his time. In that Linnaean system, the obvious physical traits, such as number of legs or shape of leaves was the major factor ruled grouping the organisms.

Years later, the biochemistry of organism was developed, and new species have been discovered. Therefore, a new classification was required in order to include all of the species, a new Taxon was developed and called Domain which includes all microbial kingdoms. Currently, most biologists agree that there are three domains of life in nature: Bacteria, Archaea, and Eukaryota (see Figure below). Both Bacteria and Archaea consist of single-celled prokaryotes. Eukaryota consists of all eukaryotes, from single-celled protists to humans. This domain includes the Animalia (animals), Plantae (plants), Fungi (fungi), and Protista (protists) kingdoms.

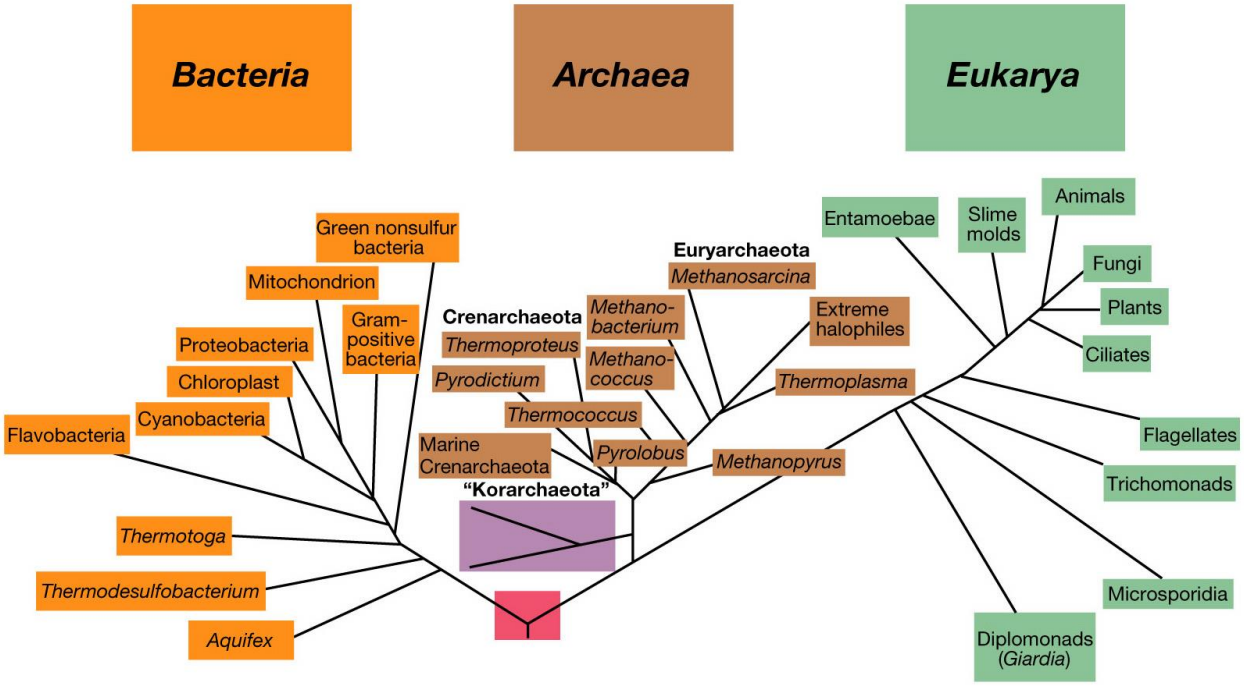


Figure (2. 3): Phylogenetic classification of organisms in domains, based on the rRNA sequence.

This phylogenetic tree is based on comparisons of ribosomal RNA base sequences among living organisms. The tree divides all organisms into three domains: Bacteria, Archaea, and Eukarya. Humans and other animals belong to the Eukarya domain. From this tree, organisms that make up the domain Eukarya appear to have shared a more recent common ancestor with Archaea than Bacteria.

2.4 Bioremediation and Microorganisms

Bioremediation is a managed process involved in degrading, transforming, or detoxifying soils contaminants to less toxic or non-toxic forms, through the action of various living microorganisms. Microorganisms act properly against pollutants when nutrients and carbon are sufficient to provide them the energy needed for their growth and survival. Degradation of natural substances in the marine ecosystems provides the necessary food for the development of microbial populations. Bioremediation technologies harness these natural processes by promoting the enzymatic production and microbial growth necessary to convert the target contaminants to non-toxic end products (Atlas, 1995).

For a successful bioremediation process, the microorganisms, or their enzymes, need to be in physical contact with the organic contaminant. Both properties of the soil and the type of the contaminant determine bioavailability and bioaccessibility of the contaminant in the soil (Harms, 2011). Previously the terms bioaccessible and bioavailable were not differentiated and both features were referred as bioavailable. However, the current usage of these terms is more explicit. Bioavailability represents the fraction that is taken up by the cells, followed by toxic effects or biodegradation by intracellular mechanisms. The term bioaccessibility, often also called environmental availability, considers the fraction that is potentially available for biota in soils. From the risk assessment point of view, bioaccessibility is more important than the total concentration, because toxic effects can be attributed to a contaminant only when it is accessible (Cavanēarová et al., 2013).

2.4.1 Degradation Kinetics

The Monod equation is commonly used to model substrate degradation and microbial growth (Saberiyan et al. 1996). The Monod equation assumes that a single substrate and single type of

microorganism are involved. In reality, there are usually multiple substrates and multiple microorganisms involved. However, the Monod equation is usually selected for ease in analyzing data, and it offers adequate accuracy. The Monod model takes advantage of the fact that the biodegradation rate is a function of substrate concentration. The Monod equation takes the form in equation 2.1, when substrate concentration (C) is small compared to K:

$$\frac{dC}{dt} = -k_m * \frac{X}{K_s} * C \qquad (2.1)$$

Where:

C= contamination concentration at time t (mg/kg)

K_m=maximum substrate utilization rate (day^{-1})

K_s= half-velocity coefficient (substrate concentration at one-half the maximum growth rate (mg/kg)

X= microbial concentration (mg/kg)

T=time (days)

Assuming

$$K = k_m * \frac{X}{K_s} \qquad (2.2)$$

Where K= degradation rate constant, and k_m, X and K_s are constants for the system,

Substitute (2.2) in (2.1),

$$\frac{dC}{dt} = -k_m * \frac{X}{K_m * \frac{X}{K}} * C$$

$$\frac{dC}{dt} = -K * C \qquad (2.3)$$

$$\frac{dC}{C} = -K\,dt \qquad (2.4)$$

$$\ln C = -K\,t + C_1 \qquad (2.5)$$

If $C = C_0$ at t=0, then $\ln C_0 = C_1$ and

$$\text{Ln } C = -K\,t + \ln C_0 \qquad (2.6)$$

It can be written:

$\ln C - \ln C_0 = -Kt$ (2.7)

$$\ln \frac{C}{C_0} = -Kt \quad (2.8)$$

The value K is measured from a empirically study by plotting the and log of C/C0 vs time and performing a regression analysis. The degradation rate constant can then be used in Equation (2.8) to calculate the length of time required to degrade a specific waste to half of its initial concentration. This is commonly referred to as the half-life of the contaminant.

2.4.2 Bacteria

Today, the use of microorganisms such as bacteria to act as a significant removal tool for environmental pollutants has become a promising technology because of its cost saving, eco-friendly and sustainable nature (Guerra et al., 2018). The continuous development and improvement of microbial remediation technology has also provided a new method for the remediation of petroleum hydrocarbon pollution, which has attracted much attention (Dombrowski et al., 2016; Dvořák et al., 2017). Moreover, there were many applications utilizing bacteria in order to degrade waste products by food, agriculture, chemical and pharmaceutical industries. (Guerra et al., 2018).

Degrading Hydrocarbon Contaminants

The degradation of hydrocarbons by microorganisms is mainly caused by the catalysis of intracellular enzymes. The process of microbial degradation of hydrocarbons has four main steps: First, pollutants are emulsified by surfactants secreted by microorganisms. Next, the emulsified hydrocarbon is adsorbed by the surface of the microorganism. Then, the hydrocarbon adsorbed on the surface of the cell membrane enters the cell membrane through active transport or passive transport, endocytosis. Finally, the hydrocarbon entering the cell undergoes an enzymatic reaction

with the corresponding enzyme to achieve the purpose of degrading the pollutant. (Li et al.,2019). To summarize these steps, figure (2.4) illustrates these biodegradation processes and their methods.

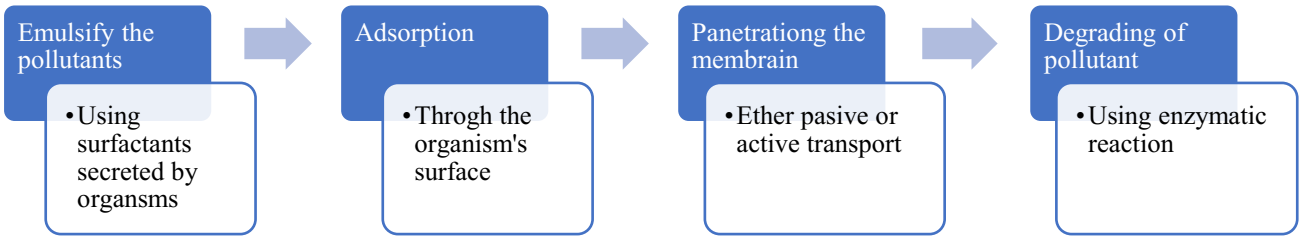


Figure (2. 4): Hydrocarbon degradation mechanism by microorganisms

Microbial degradation is the essential and ultimate natural mechanism by which one can clean up the hydrocarbon pollutants from the environment (Lal and Khanna, 1996). The recognition of biodegraded hydrocarbons in marine sediments was reported by Jones et al., 1983. They studied the biodegradation of alkyl aromatics in marine sediments which occurred beforehand the detectable biodegradation of n-alkane profile of the crude oil. The main microorganisms, namely, Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus had been observed in alkylaromatic degradation processes. Furthermore, Adebusoye et al., (2007) studied the microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria. They observed nine bacterial strains, namely, Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium sp. which could degrade crude oil.

It is worth mentioning that biodegradation of hydrocarbons is a complex process, thus many factors have been reported by Cooney et al., (1985). One of the important factors that limit biodegradation

of oil pollutants in the environment is their limited availability to microorganisms. Firstly, the composition of pollutant is play an important role to understand the availability of used the correct treatment. Another factor is temperature, which has a strong effect chemical reaction of pollutants. In fact, temperature has a strong impact on the enzymatic activity of microorganisms and, therefore, the degradation rate of hydrocarbon will be affected (Figure 2.5). Other factors are alkaline or acidic pH, lack of nutrients and competitive action with indigenous microorganisms. To improve the bacterial density and competitive advantage of exogenous bacteria, many studies developed effective approaches to immobilize the hydrocarbon degrading bacteria on high biomass, high metabolic activity and strong resistance to toxic chemicals (Zhang et al., 2019).

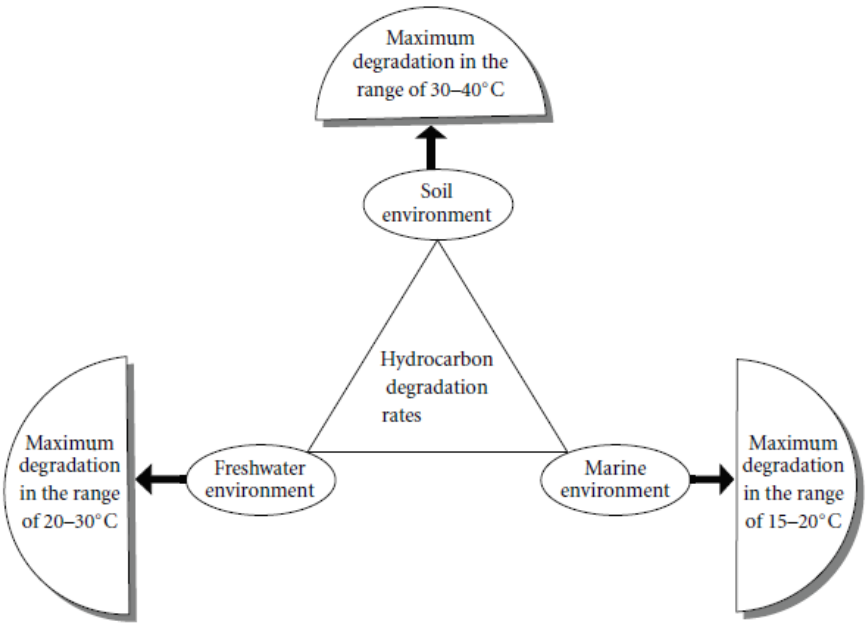


Figure (2. 5): Hydrocarbon degradation rates in soil, fresh water, and marine environments (Adopted from Das and Chandran, 2011).

Degrading Heavy metals

High levels of heavy metals in environmental is becoming a serious threat to living organisms in an ecosystem (Siddiquee et al., 2015). Metal toxicity is of great environmental concern because of their bioaccumulation and nonbiodegradability in nature (Gautam et al., 2014). Several inorganic metals like magnesium (Mg), nickel (Ni), chromium (Cr3+), copper (Cu), calcium (Ca), manganese (Mn), and sodium (Na) as well as zinc (Zn) are vital compounds where small quantities are needed for metabolic and redox functions. Additionally, heavy metals such as aluminium (Al), lead (Pb), cadmium (Cd), gold (Au), mercury (Hg), and silver (Ag) are not needed for the biological processes and with high concentrations they can cause harmfulness to living organisms (Lakherwal, 2014). The toxic effects of heavy metals on living organisms are summarized in Table (2.3)

Toxicity of heavy metals is the ability of a metal to cause detrimental effects on microorganisms, and it depends on the bioavailability of heavy metal and the absorbed dose (Rasmussen et al., 2000). Heavy metal toxicity involves several mechanisms such as breaking fatal enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein (Gauthier et al, 2014). Thus, the physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals.

Table (2. 3): Toxicity of heavy metals to microorganisms (Adopted from Igiri et al., 2018)

Metal	Side effect on organisms
Cadmium (Cd)	1- Damage and denaturation of microorganisms 2- Weakening the bioremediation capacity of microbes.
Chromium Cr (III)	Change the structure and activity of enzymes
Intracellular cationic Chromium Cr (III)	Negatively charged phosphate groups of DNA which leads (affect transcription, replication, and cause mutagenesis)

Copper (Cu (I) and Cu (II))	Interrupt the ROS which cause severe injury to cytoplasmic molecules, DNA, lipids, and other proteins
Aluminum (Al)	could stabilize superoxide radicals, which is responsible for DNA damage
Lead (Pb)	Damage cell membranes and destroy the structure of DNA.
Mercury	Denature protein, inhibit enzyme function, disrupt cell membrane
Nickel	Upset cell membrane, hinder enzyme activities and oxidative stress
Zinc	Death, decrease in biomass, inhibits growth

Generally, the mechanisms of microbial degradation of heavy metals are mainly a) biosorption, b) bioaccumulation, c) biotransformation, d) bioleaching, e) biomineralization, f) co-metabolism. However, the biodegradation of inorganic metals that interact with microorganisms, bacteria specifically, has different mechanisms which allowed the microbes to survive metal toxicity.

1- Bio Sorption Mechanism

2- Intracellular Sequestration

3- Extracellular Sequestration

4- Extracellular Barrier of Preventing Metal Entry into Microbial Cell

5- Methylation of Metals.

6- Reduction of Heavy Metal Ions by Microbial Cell

Bacteria possess many genetic systems for maintaining the resistance against toxic metals or maintaining intracellular homeostasis of metal ions (Chudobova et al., 2015). In bacteria the most well-known genetic mechanisms of metal resistance are the presence of metal binding proteins (Hobman and Crossman 2014) and heavy metal efflux systems (MoraledaMun˜oz et al., 2010). On other words, the bacterial genes are involved in specific metal binding, transport and resistance.

Certain bacteria have evolved the necessary genetic components that confer resistance mechanisms, allowing them to survive and grow in environments containing high levels of arsenic that would be toxic to most other organisms (Rahman et al., 2015b).

2.4.3 Fungi

The use of fungi as a method of bioremediation provides another option to clean up the pollutions in the environment. The bioremediation with help of fungi has drawn little interest in the past two decades since most bioremediation research has focused mainly on the use of bacteria. Recently, fungi have received considerable attention for their bioremediation efficiency which is attributed to the enzymes they produce. In addition, Husaini et al., (2008) reported that fungi have some advantages over bacteria such as fungal hyphae that can penetrate contaminated soil to reach the polluted area. Moreover, filamentous fungi showed some advantages in the transport or translocation of essential substances, including nutrients and water, and the pollutant itself, over significant distances (Boswell et al. 2003; Furuno et al. 2012; Boswell et al. 2002; Harms et al. 2011; Jacobs et al. 2002; Worrich et al. 2018). More interestingly, fungal mycelia could be act as "highways" in accelerating the transport of pollutant-degrading bacteria over distance in soil which can enhance bioremediation (Banitz et al. 2013; Kohlmeier et al. 2005; Wick et al. 2007).

Hydrocarbon Degradation

Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. Most rot fungi produce high redox potential enzymes such as manganese peroxidase (MnP), laccases (Lac), and lignin peroxidases (LiP) for the oxidation of lignin. These enzymes are not generally substrate-specific as they can oxidize a wide range of xenobiotics, including pesticides, plastics, and hydrocarbons (Asemoloye et al., 2020). On other word, the biodegradation of hydrocarbons by fungi has traditionally been considered co-metabolic process.

Filamentous fungi, e.g., Aspergillus and Penicillium spp., have been investigated for the degradation of aliphatic hydrocarbons, chlorophenols, and polycyclic aromatic hydrocarbons, with the organic pollutants serving as carbon and energy sources (Harms et al. 2011; Hofrichter et al. 1994; Pinedo-Rivilla et al. 2009). The reported efficiency of biodegradation ranged from 6% (Jones et al., 1970) to 82% (Pinholt et al., 1979) for soil fungi.

2.4.4 Degrading Heavy metals Mechanisms

Biosorption and Bioaccumulation

Biosorption and bioaccumulation are processes by which the microorganisms, or biomass, bind to and concentrate heavy metals and contaminants from the environment (Joutey et al., 2015). It should be noted that both biosorption and bioaccumulation work in different mechanisms. In particular, during biosorption processes contaminants are adsorbed onto the sorbent's cellular surface in amounts that depend on the composition and kinetic equilibrium of the cellular surface. Thus, it is a passive metabolic process that does not require energy/respiration (Velásquez and Dussan, 2009). On the other hand, bioaccumulation is an active metabolic process that needs energy and requires respiration (Vijayaraghavan and Yun, 2008; Velásquez and Dussan, 2009). Since contaminants (such as heavy metals) bind to the cellular surface of microorganisms during biosorption, it is a revisable process. In contrast, bioaccumulation is only partially reversible. Biosorption was also shown to be faster and to produce a greater number of concentrations (Velásquez and Dussan, 2009).

Biosorption

Biosorption is a promising method that became available in two decades ago. This method has an outstanding potential such as cost-efficient method and high efficiency of reducing heavy metal pollution that comes from industrial and agricultural sources (Fomina and Gadd, 2014; Javanbakht

et al., 2014). This method depends on the sequestration of toxic heavy metals by the moieties of bio sorbent cell surfaces such as those found in fungi/yeast, bacteria, and algae (Nilanjana et al., 2008). The applications of biosorption in bioremediation from soil, landfill leachates and water that include heavy metal were reported by Fomina and Gadd, (2014) and Tran et al., (2015).

The potential bio sorbents of several living organisms have been tested. This includes several microorganisms, in particularly bacteria such as Bacillus subtilis and Magnetospirillum gryphiswaldense, fungi such as Rhizopus arrhizus, yeast such as Saccharomyces cerevisiae and algae such as Chaetomorpha linum and marine microalgae (seaweed) (Romera et al., 2006; Vijayaraghavan and Yun, 2008; Wang and Chen, 2008; Zhou et al., 2012). Furthermore, biomasses were proposed and investigated as a potential lower-priced and economical means of treating effluents charged with toxic heavy metals. Biomasses such as industrial wastes (waste biomass of Saccharomyces cerevisiae from fermentation and the food industry), agricultural wastes (corn core) and other polysaccharide materials, were investigated and reviewed (Vijayaraghavan and Yun, 2008; Wang and Chen, 2008). It is reported that bacteria, compared with other organisms, are considered outstanding bio sorbents due to their high surface-to-volume ratios as well as several potential active chemosorption sites in their cell wall such as teichoic acid (Beveridge, 1989). More surprisingly, dead bacterial strains are also proposed as potential bio sorbents with biosorption capacities that outperform living cells of the same strains. The biosorption capacity of chromium ions in the dead Bacillus sphaericus was increased by 13–20% in comparison with living cells of the same strain (Velásquez and Dussan, 2009).

2.5 Geotechnical overview

Natural processes like erosion or man-made processes such as excavations can cause the soil to be unloaded. On the contrary, the slow process of sedimentation will result in an increase in effective stress in the soil due to the increase in overburden pressure. Therefore, the consolidation history is very important to understand the real behavior of that soil (Sällfors, 2013).

A soil that has been exposed to a higher stress level than the current stress, and has been consolidated at that stress level, is referred to being over-consolidated. The highest stress level that the soil has experienced in its geotechnical history is called the pre-consolidation stress. If the current stress level is the highest that the soil has been exposed to and the pore water pressure is stationary, the soil is referred to as "normally consolidated". A soil can also be under-consolidated if primary consolidation is still occurring in the soil (Sveriges Geotekniska Förening, 2016). The relationship between the pre-consolidation pressure and the current effective vertical stress in the soil is called over-consolidation ratio (OCR)

$$OCR = \frac{\sigma_c'}{\sigma'}$$

Where:

σ_c' = Pre-consolidation pressure [kPa]

σ' = Vertical effective in-situ stress [kPa]

OCR>1 (over consolidation)

OCR=1 (normal consolidation)

OCR<1 (under consolidation)

It should be noted that soil deformation is usually higher in normally consolidated soil than it is in over consolidated soil. Regarding this point, to understand the reason for the above it is important to understand the concept of consolidation in soils mechanics.

2.5.1 Consolidation Theory (Terzaghi, 1925)

When a saturated stratum of soil is subjected to an increment of stress, the pore water pressure is increased accordingly in a zone of influence (Figure 2.6). The increase in pore pressure leads to the creation of a hydraulic gradient that cause the water to flow out of the zone of influence, through the voids of the soil (draining process). In case of sandy soil, high permeability will cause a rapid drainage, while in clay soil the water drainage may take longer time due to the relatively low permeability. The excess pore water pressure is reduced when the water dissipates, and the same amount of the stress is transmitted to the soil skeleton as an effective stress increase (Terzaghi, 1943). This process is called the "consolidation process".

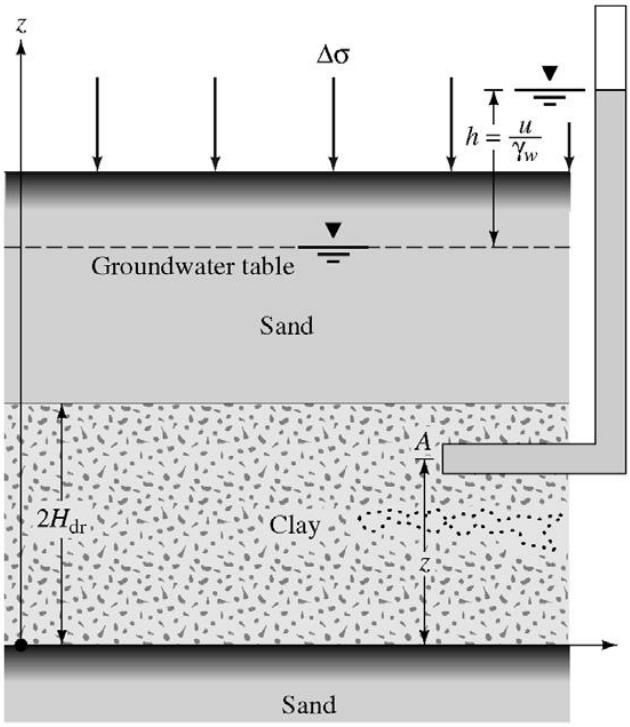


Figure (2. 6): Stresses on clay layer

The consolidation is a time-dependent process that involves a reduction in total volume via expulsion of water from the void space of saturated soils due to excess pore water pressure (Terzaghi 1925; Taylor 1948). As said before, the process can be very slow for soil with low

permeability, like clays and silty clays, while the process can be considered almost instantaneous in coarser soils. One-dimensional consolidation theory, also known as small-strain consolidation, was firstly proposed by Terzaghi (1925) with the following assumptions:

- The soil is fully saturated.
- The soil is homogeneous.
- Both the water and the soil particles are uncompressible.
- The coefficient of permeability is constant during the consolidation process.
- The volume decrease in the soil depends entirely on the increase of the effective stress.
- The deformation develops in the vertical direction(z) only.
- Darcy's law is valid.

It should be noted that, the consolidation theory is uncoupled into two stages: (1). at the instant of load application, there is no volume change, and the excess pore water pressure is equal to applied load. (2). at any time t, the excess pore water pressure will be dissipated. The volume change of the soil is equal to the volume of water flowing out. The problem has an initial condition expressed as followings:

$$U_{w0} = P$$

Where U_{w0} = the excess pore water pressure, and P = the applied load.

Based on the mentioned assumption, the differential equation for the one-dimensional consolidation of saturated is

$$\frac{\partial u}{\partial t} - \frac{\partial \sigma}{\partial t} = \frac{k}{\gamma_w * m_v} \frac{\partial^2 u}{\partial z^2}$$

where:

M_v = Compression modulus [kPa]

k = Coefficient of permeability [m/s]

t = Time [s]

z = Depth below the ground surface [m]

σ= Total stress

In many consolidation problems in which the total stress σ remains constant throughout consolidation, therefore the equation could be written:

$$\frac{\partial u}{\partial t} = \frac{k}{\gamma_w * m_v} \frac{\partial^2 u}{\partial z^2}$$

The value of $\frac{k}{\gamma_w * m_v}$ represent the coefficient of consolidation C_v.

$$\frac{\partial u}{\partial t} = C_v \frac{\partial^2 u}{\partial z^2}$$

, To solve the basic differential equation of 1D consolidation theory this equation the following assumption were considered:

u=0 at Z=0, u=0 at Z=$2H_{dr}$, u=u_0 at t=0

$$u = \sum_{m=0}^{m=\infty} \left[\frac{2u_0}{M} \sin\left(\frac{Mz}{H_{dr}}\right)\right] e^{-M^2 T_v}$$

Where:

u = pore pressure (excess hydrostatic) at particular values of depth (z) and time (t)

u_0= initial value of excess hydrostatic pore pressure

$M = \frac{\pi}{2}(2m+1)$

H = thickness of a singly drained layer

T = dimensionless time factor $T_v = \frac{c_v t}{H_{dr}^2}$

Int the laboratory, the deformation rate of the soil that can be measured using the oedometer test with vertical flow of water is only applicable to one dimensional consolidation problems. In this test (details at §3.5.1), the soil specimen is placed inside a metal ring with two porous stones, one at the top of the specimen and the another at the bottom. The load on the specimen is applied, and compression is measured. The specimen is kept under water during the test. Each load usually is kept for 24 hours (Das, Sobhan ,2018). The load usually is doubled, which doubles the pressure on the specimen, and the compression measurement is continued. When the maximum load is reached, an unloading stage is introduced that may be conducted in one or multiple steps. When the test is completed, the final height of the sample, the water content and the dry weight are measured.

The evaluation of data measured during the consolidation test is presented graphically using some parameters (e.g., time, settlement or stress). Figure (2.7), shows the relationship between deformation of the specimen against time for a given load increment, sometimes called Rate of Consolidation curve. Therefore, it can be observed three distinct stages: a) Stage I: Initial compression, which is caused mostly by preloading, b) Stage II: Primary consolidation, during which excess pore water pressure gradually is transferred into effective stress because of the expulsion of pore water. c) Stage III: Secondary consolidation, which occurs after complete dissipation of the excess pore water pressure, when some deformation of the specimen takes place because of the plastic readjustment of soil fabric (Das, Sobhan ,2018).

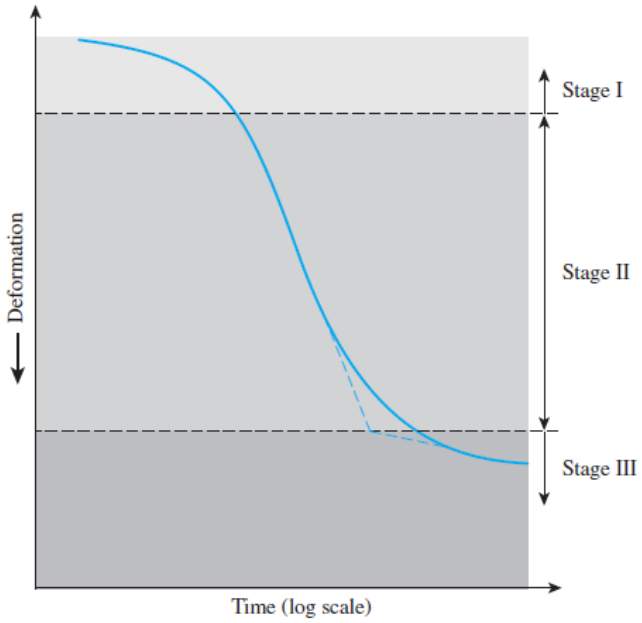


Figure (2. 7): Time–deformation plot during consolidation (Adopted from Das and Sobhan,2018)

Another interesting relation that can be observed form oedometer test is the change of void ratioof the sample by increasing the applied stress. In fact, when the applied stress is increase, there is a decrease in the volume of voids, the curve is usually called “compressibility curve” (Figure 2.8).

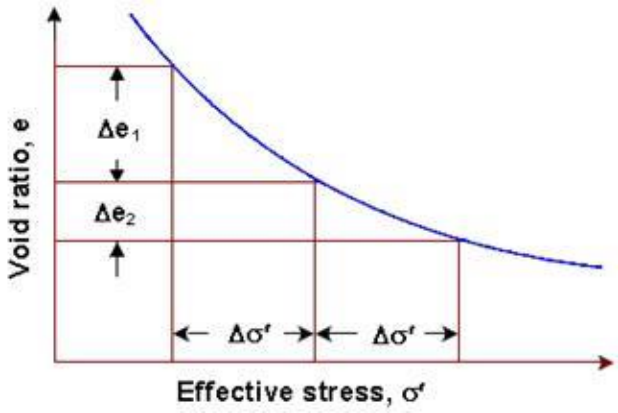


Figure (2. 8): Void Ratio-Stress vs stress

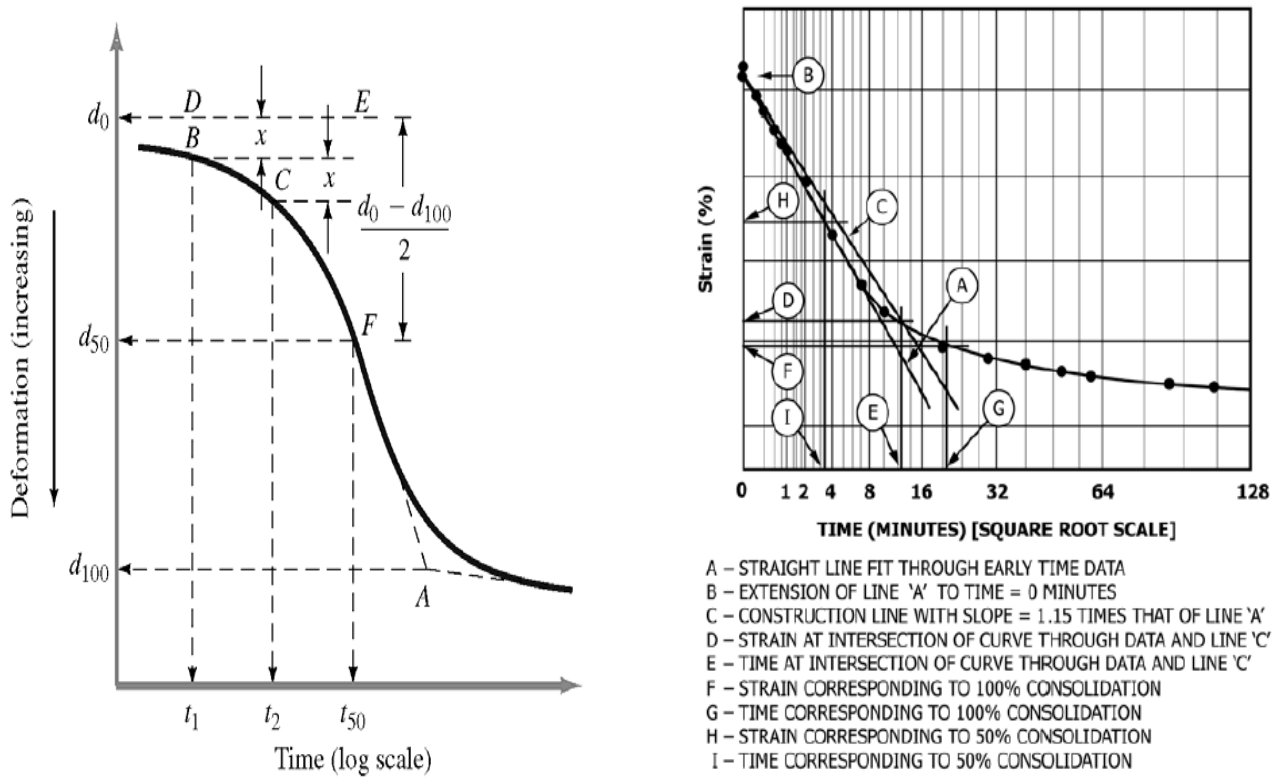
More practically, the time rate of settlement of soil can be estimated using the coefficient of consolidation, C_v which mainly depends on the boundary condition of that stratum. In the literature, there are two common methods that can be used to determine the coefficient of

consolidation directly from the oedometer test. The first method called the logarithm of time fitting method or called Casagrande (1940) method. The coefficient of consolidation, C_v, is determined by estimating the time at 50% consolidation (t_{50}), as shown in the figure (2.9 a). Then, C_V can be estimated as:

$$C_v = \frac{0.197\ H_{dr}^2}{t_{50}}$$

the second possibility is represented by the square root of time fitting method or Taylor method (Taylor 1948, ASTM D2435), the coefficient of consolidation, C_V, is determined by estimating the time at 90% consolidation (t_{90}), through multiplication of 1.15 times the in the abscissa of initial value. Figure (2.9b) illustrates the procedures. The value of C_v, can be estimated using the following formula:

$$C_v = \frac{0.848\ H_{dr}^2}{t_{90}}$$



A) Casagrande Method

B) Taylor Method

Figure (2. 9): A) Casagrande method to calculate consolidation coefficient and B) Taylor Square Root of Time Fitting Method (Adopted from ASTM D2435).

At the end of primary consolidation (after complete dissipation of excess pore water pressure), some settlement is observed because of the plastic adjustment of soil fabrics. This stage of consolidation is called secondary consolidation (Das, Sobhan ,2018). In other words, secondary compression settlement results from the time-dependent rearrangement of soil particles under constant effective stress conditions. For highly compressible soils, such as soft clays and peats, secondary compression is important whenever there is a net increase in C_v due to surface loading. In most cases, secondary compression settlement can be predicted using the secondary compression index, C_α.

In case of compressibility curve (void ratio-stress plot), we can use the following formula:

$$C_\alpha = \frac{\Delta e}{\Delta log t}$$

While in case of settlement-stress plot (Figure 2.10), ε_α is used to specify the total definition.

$$\varepsilon_\alpha = \frac{\Delta \varepsilon}{\Delta log t}$$

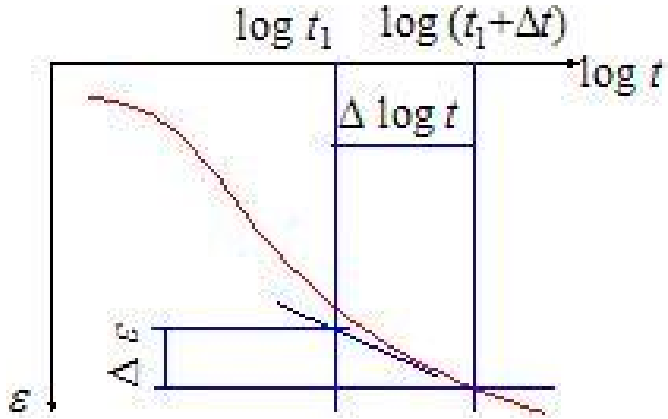


Figure (2. 10): Secondary settlement coefficient

2.5.2 Hydraulic conductivity

The hydraulic conductivity (also permeability) is the measure of the quantity of water that flow through permeable materials. The study of the permeability to water through permeable soil media is important in soil mechanical filed. In engineering applications, permeability is necessary for estimating the quantity of underground seepage under various hydraulic conditions, for investigating problems involving the pumping of water for underground construction, and for making stability analyses of earth dams and earth-retaining structures that are subject to seepage forces (Das, Sobhan ,2018). On the other hand, groundwater and surface water interact in different topographic and climate environment, and they are the main water sources that support aquatic ecosystems. From an ecological point of view, permeability is a very important parameter because nutrients and pollutants can also be transported across the interface the adjusted soil layer. Regarding the river systems, two types of interactions between groundwater and surface water are commonly known: the 'gaining stream' through which groundwater flows into surface water, and the 'losing stream' through which surface water flows into groundwater. Thus, if the groundwater or surface water becomes contaminated, the contamination might spread in both as a consequence. Therefore, understanding the relationship between groundwater and surface water is essential in order to effectively manage water resources.

In the literature, number of methods have been investigated intensely in order to understand the interactions between groundwater and surface water. These methods include hydraulic, numerical, thermal, isotopic, biological, and hydrogeochemical approaches. In fact, hydraulic conductivity relies on the hydraulic gradient. The hydraulic conductivity of soils is linked with Darcy's law (Darcy, 1856). Darcy's law states that a proportional relationship exists between hydraulic flux and hydraulic gradient. Darcy's law is stated as:

$$Q = kAi$$

Where:

Q = Water flux (m^3/sec)

k= Hydraulic Conductivity of the soil (m/sec)

A= Cross-sectional area

i= Hydraulic gradient =- Δh/L

The soil texture play an important role in permeability and water infiltration. For example, soils with sandy textures have large and interconnected pore spaces that allow water, thus contamination, to drain very quickly through the soil (high permeability). In contrast, clay textured soils have small pore spaces and particle geometry that cause water to drain slowly through the soil (low permeability). The goal of the low permeability barriers used for waste containment systems is to minimize the amount of liquid filtration by (1) accomplishing a sufficient low saturated hydraulic conductivity for the barrier material and (2) limit the amount of liquid that are located on top of the barrier. For a hazardous waste facility, the EPA requires that the compacted soil liner be at least 0.9 m thick and have a hydraulic conductivity < 10^{-7} cm/s. Moreover, drainage layers are typically required to have a hydraulic conductivity > 1 cm/s (Daniel, 1997)

Hydraulic conductivity is a characteristic that reflects the relative ease of fluid flow through porous media and is one of the most variable material parameters among the geotechnical properties. Approximate ranges of hydraulic conductivities values useful for geo environmental application are provided in Table 2.4.

Table (2. 4): The values of hydraulic conductivity of different type of soil (Adopted from Shackelford, 2013)

Material	**Saturated hydraulic conductivity (ms^{-1})**	**Comments**
Gravel	$10^{-2} – 10^{-3}$	Values based on "clean" soils;
Sand	$10^{-3} – 10^{-5}$	variation in k_{sat} based on particle size distribution
Silt	$10^{-5}–10^{-8}$	Variation in k_{sat} based on mineralogical composition of silt particles
Clay	$10^{-8}–10^{-12}$	Variation in k_{sat} based on mineralogical composition of clay particles
Geosynthetic clay liner	$10^{-10}–10^{-11}$	Values based on sodium bentonite sandwiched between two geotextiles
Sand–bentonite mixture	$10^{-9}–10^{-10}$	Values based on a mixture of clean sand (w/o fines) and 4–10% (w/w) sodium bentonite

Many techniques and methods have been developed and reported in previous studies to measure the hydraulic conductivity of soils in both filed and laboratory. Moreover, hydraulic conductivity of soils can be measured by direct or indirect methods. Figure below illustrates different methods that can be used to determine the hydraulic conductivity of soil.

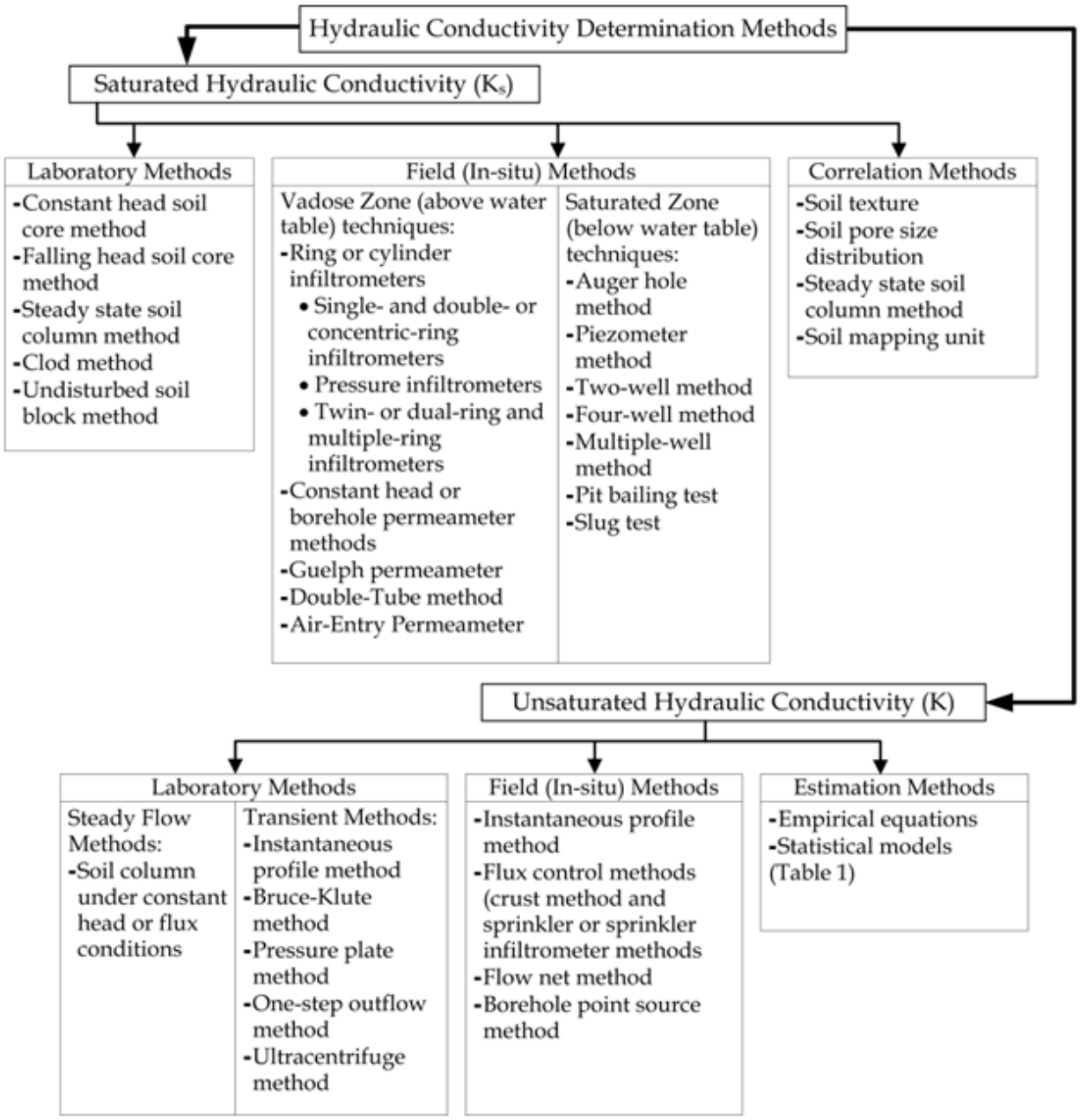


Figure (2. 11): Methods and tests used to determine the hydraulic conductivity (Adopted from Deb and Shukla, 2012)

In this study, falling head test and rigid wall permeameter were used to evaluate the hydraulic conductivity of marine sediments.

2.6 Leaching of contaminants

Due to the freshwater scarcity in certain places, groundwater becomes one of the main sources, thus it has a ubiquitous influence on human life. On the other hand, groundwater might be contaminated due to the leaching of pollutant (e.g., hydrocarbons, heavy metals, etc.) and it may become harmful for living beings and for the environment (Hossain, et, al, 2019). In Bagnoli harbor, the accumulation of contaminated sediments with elevated concentrations of different pollutants such as hydrocarbons, heavy metals, phosphorus, and organic toxicants has a severe concern due to the high toxicity, persistence, and abundance of such pollutants. Unfortunately, there are many examples of the carcinogenic effects of these compounds.

Highly polluted sediments pose a threat to the surrounding environment mainly due to the leaching of metallic contaminants that provokes negative environmental impacts on surface and groundwater (Chen et, at., 2016). Moreover, damage will be caused to the benthic organisms, flora and fauna, and to the aquatic systems. Additionally, the heavy metals that accumulate in living organisms may enter the food chain, which in turn poses a direct threat to human health. For instance, high concentrations of cadmium and zinc were found in the leaves of plants growing on dredged sediments landfill and elevated concentrations of cadmium were found in small mammals in the area (Hashim et. al., 2018).

Contaminant leaching from secondary contamination sources in soil is the process of extracting substances from soil with either an organic or inorganic agent, releasing them from the solid phase into the liquid phase via dissolution, desorption, and complexation (Ali, et. al., 2018). In the United States, the Toxicity Characteristic Leaching Procedure (TCLP) is used to determine whether or not a waste product (including harbor sediment) is considered a hazardous waste (U.S. EPA, 1992; Hardaway et al., 1999). Leaching of contaminants from sediments is influenced by element

chemistry, pH, redox potential, complexation, liquid-to-solid ratio (L/S), contact time, and biological activity (van der Sloot et al., 1996).

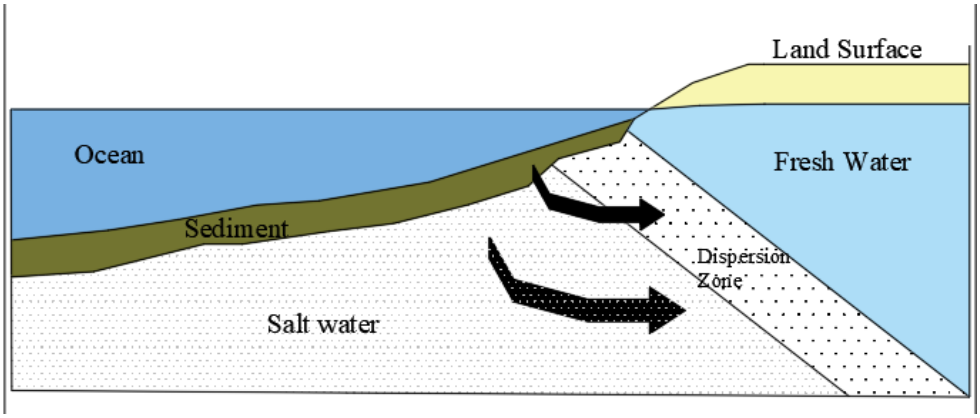


Figure (2. 12): An example of leaching of contaminant to groundwater and flow into surface water.

Leaching tests have been widely used to determine the potential of organic and inorganic wastes to leach dangerous concentrations of heavy metals into the environment, for example, to measure the leachability of heavy metals from contaminated sediments and sludge. Moreover, this test has been widely used to measure the leachability of heavy metals from wastes that were used in asphalt, stone matrix asphalt, soil stabilization, and paving blocks.

These leaching tests performed are Static diffusion test (tank test), Dynamic diffusion test (modified tank test), Batch leaching test for crushed material, up-flow percolation test (column test), batch leaching test for monolithic samples, and leaching test of monoliths with magnetic agitation. The batch leaching test is simple to set up, easy to perform, and has the shortest duration. As such, the batch leaching test was considered suitable for a quick first screening when selecting the appropriate S/S mix design. For the present thesis work the column test was selected to study the leaching properties of sediments. The column test is a longer test than the batch test, but it fully represents the real mechanisms of the leaching process, in which the cause of leaching is the

percolation through the contaminated porous media (schemes in Figure2.13). The test is performed by means of permeameters (flexible of rigid wall) by periodically sampling the outlet fluid e determining the concentration in the leachate.

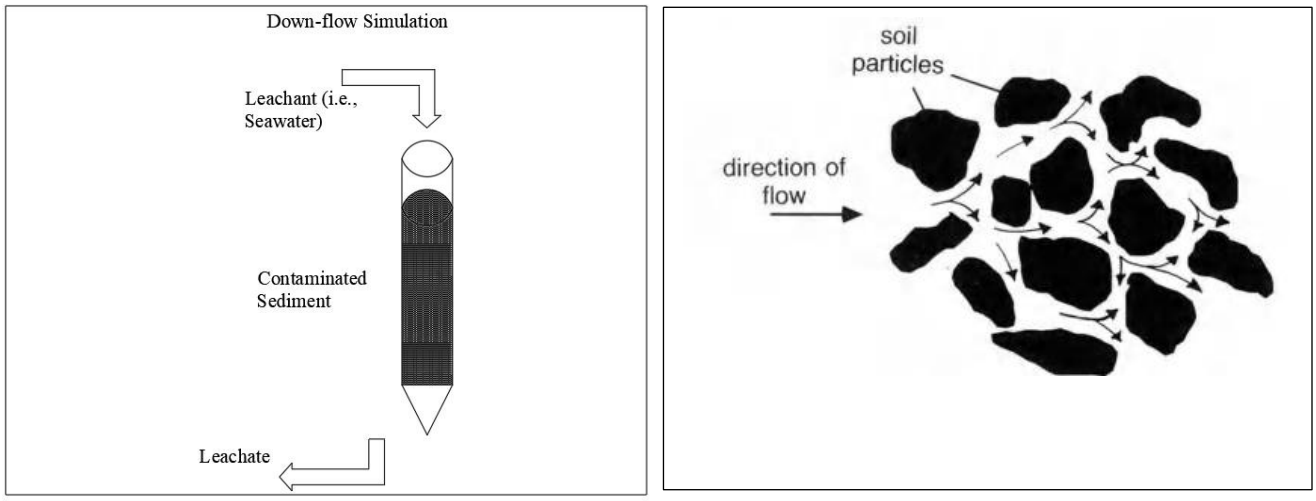


Figure (2. 13): leaching test and leachate flow through particles.

The objectives of the leaching tests are:

- Compare the leaching capability of sediments before and after three different treatments of bioremediation with reference to heavy metals.
- Assess, if any, the effect on leaching of different applied pressure during the tests.

In a broader view, the leaching process should be evaluated in order (1) to consider the possibility of realizing a permeable capping treatment on the contaminated sediments in the seabed and (2) to evaluate the possibility to locate them in a hydraulic fill for land reclamation. In both cases, considering that bioremediation treatment is effective mainly for organic contaminants, it is important to verify that there would not be a huge release of inorganic compounds (i.e., heavy metals) during and/or after the treatment and that the bioremediation treatment would not enhance the leaching capability of sediments.

2.7 Sediment management

Sediments are considered highly dynamic part of a river or marine system: they are created by loose particles of sand, clay, silt, organic matter, solid particles that are released from wastewater treatment plants, and also by decomposing plants, animals and other living organisms (Bortone, 2007). Sediments originate typically in river basins through erosion mechanisms and are then transported to the coast, where they settle to the bottom of oceans that constitute their final repository (Salomons and Brils, 2004). Topography, climate, hydrology, geology, but also the land use exert an influence on sediments' formation and movement: materials can be temporarily deposited in the river bed, then they can be transported again; dams, which are often present in regulated rivers, may artificially trap sediments, hence reducing their supply downstream (Salomons and Brils, 2004); sediments can also be deposited in floodplain areas or lake beds (Bortone, 2007). Due to the action of wind, water and ice, transport of particles is not restricted to a single area of the river basin (Bortone, 2007); therefore, downstream areas such as deltas, wetlands and harbours may be highly impacted by sediment movement (Salomons and Brils, 2004).

Large volumes of sediments are dredged from the seabed of harbor zones for the purpose of maintenance of ship pathway and/or for sediment remediation. The global dredging processes produce about 600,000,000 m^3/year of sediments (Said et. al., 2015). In Europe only, approximation 200 million of m^3 of sediments are dredged each year, more than half of them are contaminated and the cost of disposal is high (SedNet, 2011). In the region of Nord-Pas de Calais in France more than 1,000,000 m^3 of sediment is dredged yearly and more than 30% of this massive amount is polluted (Sabra, et. al., 2012); about 8,000,000 m^3/ year is dredged in Tunisia (Said et.

al., 2015). Additionally, about 4,000,000 m^3/ year is removed from the Grand Canal in Hangzhou (Chen et. al., 2003).

Contaminated sediments are considered waste, and currently less than 5% of sediments are processed for ultimate valuable material. Until the early 1990s, most of the dredged sediments were transferred to the deep sea or deposited on land (the cheapest method). Fortunately, this tendency has been changing in recent years. A convention for the protection of the marine environment and some new European waste legislations have been passed to establish guidelines for the correct disposal of dredged sediments at sea, and to avoid the traditional concept of treating these contaminated materials as typical waste because they are regarded as commercially reusable resources. In fact, the London protocol (IMO, 2009), for marine disposal, and the European Waste Framework Directives for onshore disposal (EU Directive, 2008), required sustainable management in order to minimize the sediments and waste (Todaro et al., 2016).

On the other hand, strategies of dealing with contaminated sediments can include different technologies of dredging or excavation, transport, pre-treatment, treatment and/or dispose sediments and treatment residues (Lofrano et al. 2016). Pollutants are extracted from dredged sediments through a number of chemicals, physical, biological or thermal methods in a specially designed reactors or in line-processes.

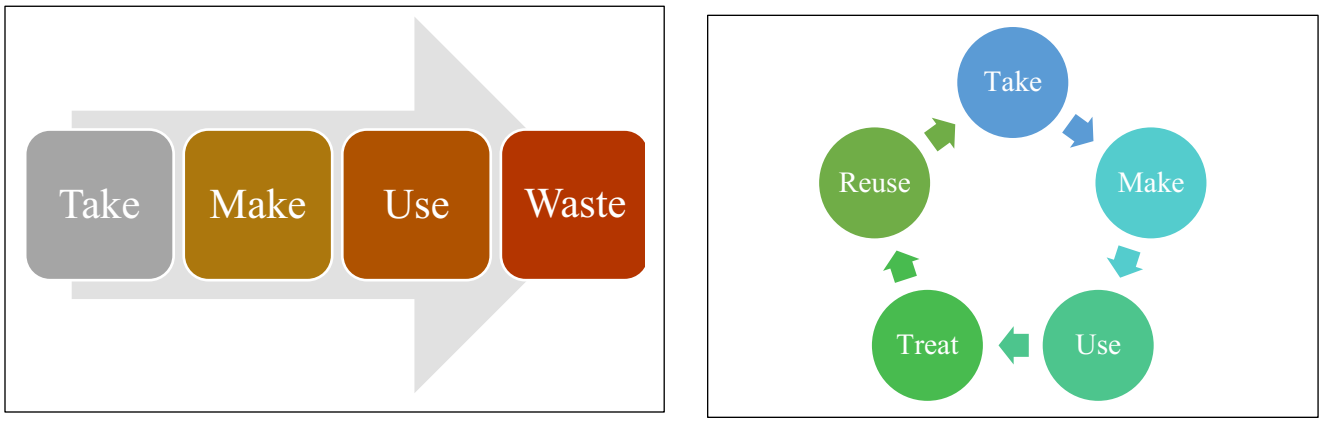


Figure (2. 14): Sediment management strategies e.g., a) linear process and B) circular process

In the past, the linear processes used as a main economic strategy based on traditional model "Take, Make, Use, Waste". Later, this strategy was developed to circular processes which replaced the linear processes, not only in the economic sector but in many sectors such as business, social and the environment (Figure 2.15). Practically, the main goal of creating strategies to treat contaminated sediments is to accomplish of high limits of sustainability and environmental safety. In fact, the circular process is a tool that promoted the sustainability. Therefore, thinking about reuse possibility, the treated sediments can be recycled as aggregates for road construction (Wang et al. 2012), cemented mortars (Couvidat et al. 2016), fill material and blocks (Wang et al. 2015), raw materials in brick production (Cappuyns et al. 2015; Messina et al. 2017) or can be disposed of in hydraulic fills for land reclamation purposes or, as a final alternative, in sanitary landfills for not hazardous waste. Another application of decontaminated sediments could relocate them inside the original water bodies after dredging (Olsen et al., 2019). The approval of relocated the sediments in water bodies without using an active barrier was implemented in EU countries by Waste Framework Directive 2006.

2.8 Effect of contamination on soil characteristics. State of the art.

There have been numerous studies to investigate the effect of contaminations on the mechanical properties of soil. In the following, a review of these studies is presented.

In 1992, Evgin and Das studied the shear strength of motor oil contaminated quartz sand via triaxial tests, and they found a significant decrease in internal friction angle (ϕ) and a substantial increase in the volumetric strain of loose and dense sands. Additionally, Al-Sanad et al. (1995) reported that due to oil pollution, the strength and permeability of Kuwaiti sand decreased slightly, while the compressibility increased. They found that the influence of crude oil on the strength parameters of soil is greater than the effects of light gas oil and benzene. Das (1999) performed several experimental studies to investigate the shear strength of poorly-graded sand that is polluted by crude oil. He concluded that a slight reduction in the friction angle was observed. This drop in the value of the friction angle might cause a dramatic reduction of the bearing capacity of the soil used as foundation soil.

Another study conducted by Khosravi et al. in 2013 focused on soil mechanical properties with increasing of gas oil level from 2 to 20 wt%. Significant observations were the raising of the value of cohesion (c) and a reduction in the internal friction angle, in addition to an increase in kaolinite compressibility. Interestingly, changing on gas content did not change the shear strength of the soil. Moreover, Kermani and Ebadi (2012) studied fine-grained CL soil taken from fields of Tehran petroleum refinery. They conversely found that, in this case, oil contamination increased the internal friction angle, maximum dry density MDD, compression index, and Atterberg limits. On the contrary, a decreased Optimum Moisture content (OMC) and a decreased cohesion of soil were observed. Similar results were obtained by Soltani-Jigheh et al. (2018) on silty sand collected from the vicinity of crude oil storage tanks in Tabriz oil refinery site in Iran. Furthermore, Estabragh et

al. (2014) investigated the consolidation behavior of soils contaminated with water-soluble glycerol and ethanol. They observed that the increasing in organic fluid caused increasing on pre-consolidation pressure, although a decreasing on compression index was noticed too. Likewise, Safehian et al, (2018) reported that the contamination of diesel caused a reduction on MDD, cohesion, internal friction angle, and Unconfined Compressive Strength (UCS) of illite soil. On the other hand, they stated an increase in OMC value.

In 2020, Negahdar and Nikghalbpour investigated the effects of various concentrations of lead nitrate $Pb(NO_3)$ and zinc nitrate hexahydrate $Zn(NO_3)$ heavy metals on sandy mixtures. Therefore, one-dimensional consolidation tests, direct shear tests, unconfined compression tests, consistency tests, sediment tests, adsorption tests, and X-ray diffraction tests were conducted. Noticeably, the presence of Pb(II) and Zn(II) would decrease the initial void ratio and sedimentation of samples and this was attributed to the noticeable changes in soil microstructure. Moreover, due to the presence of Zn, the unconfined compression tests show that there is a noticeable reduction of the strength up to 52%.

Moreover, Heris et al. (2020) investigated the effects of lead and gasoline on the geotechnical properties of three types of contaminated soil. The selected amount of lead contents was (1000, 2000, 5000, 10000, 20000 ppm) while the gasoline percentages were (the 3%, 6% and 9% weight of dried soil) with the 4 days. They performed several tests including compaction, permeability, direct shear test, unconfined compressive strength, X-ray diffraction, and SEM analysis. The main findings were an increment in maximum dry density and internal friction angle with an increase in the amount of lead and gasoline in all types of samples. Also, increasing lead content caused increase in the values of cohesion and hydraulic conductivity while a reverse trend was observed with the presence of gasoline.

In addition, in 2019, Joshi et al., studied the effect of present of zinc contamination on the engineering properties of clay soil. Thy used different amount of zinc (5,10 and 15 ppm) as zinc nitrate solution in different curing times 7,14 and 21 day. Later on, Atterberg's limit, Standard Proctor, unconfined compression, (CBR) and direct shear tests were evaluated for both contaminated and uncontaminated samples. They found that dry density and plasticity index were increased at lower value of zinc concentrations. Moreover, Unconfined compressive strength, California bearing ratio and cohesion value increases with increasing zinc concentration and curing period.

Furthermore, Soorya and Rani (2015) investigated the geotechnical properties of high plasticity clay contaminated with lead and iron from industrial waste. Therefore, liquid limit, plastic limit, free swelling and strength properties were studied. They reported that at low concentrations of lead and iron, soil strength and shear resistance were not affected while the opposite situation is detected at high concentrations.

Regarding the contamination of sediments with crude oil, some studies reported that the oil contamination led to elevate the compression coefficient significantly. Noticeably, the shear strength parameters did not change dramatically (Jia et al., 2011).

The review above indicates that the remediation is important for the possible reuse of contaminated sediments. Moreover, the previous cited studies have almost exclusively studied the mechanical properties of contaminated soil, but no research studied were carried out on the effects of a remediation treatment. Hence, this study attempts to explore if and, if yes how, the bioremediation is able to affect the mechanical properties of contaminated sediment.

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Chapter Three Materials and Methods

3.1 General

The initial investigations of the Bagnoli site by the Italian government have shown that the site is potentially contaminated because the measured concentrations are higher than the screening values (Italian CSC Concentrazione Soglia di Contaminazione) defined by the environmental regulations (Legislative Decree n. 152/06). These results recommended the necessity of a deepen study and/or a remediation action to be undertaken.

3.2 Characterization of the sample

Sediments from Bagnoli (Naples) in South Italy, supplied by Next Geosolutions Europe S.p.A., were used for experimental study reported in the present thesis.

A bulk sample of the contaminated sediment was collected on November 27, 2017. The sample was collected at a depth between 0 and 1.82 m from the seabed. The bulk sample was put in a plastic container, which was then closed and labeled. Finally, the bulk sample was stored in a dark room at $4C^{o.}$ (Figure 3.1a).

Before starting our experiments, it was necessary to assess the initial concentration and type of the contamination in the specified sample. Therefore, the sample collected from the site was characterized and quantified. Tables 3.1, 3.2, and 3.3 present the physical and the chemical properties of the collected sample.

Table (3. 1): The physical properties of the collected sample

General Info.	Site Name	20
	Level	0-50cm
	Latitude	4518176.73
	Longitude	429548.18
	Area	Bagnoli (Coroglio)
	Date of Sampling	11/23/2017
	Organic substance	2.65
	Water content (%)	31.7
	Classification	Sand
Main classes (%)	gravel	3.98
	sand	90.95
	silt	3.84
	clay	1.23

Table (3. 2): The chemical properties of the sample (inorganic compounds)

Chemical Compounds (metals)	Al	mg/kg	61653
	As	mg/kg	67
	Cd	mg/kg	1.4
	Cr	mg/kg	23
	Cu	mg/kg	16
	Fe	mg/kg	118413.2
	Hg	mg/kg	0.3
	Ni	mg/kg	10
	Pb	mg/kg	252
	V	mg/kg	69
	Zn	mg/kg	4.81E+02

Table (3. 3): The chemical properties of the collected sample (organic compounds)

Organic Compounds	Hydrocarbons C> 12	µg/Kg	2.73E+05
	Naphthalene	µg/Kg	924
	Anthracene	µg/Kg	9654
	Phenanthrene	µg/Kg	14870
	Acenaphthylene	µg/Kg	1757
	Acenaphthene	µg/Kg	2011
	Fluorene	µg/Kg	1238

	Fluoranthene	µg/Kg	44701
	Pyrene	µg/Kg	38131
	Benzo (a) anthrax	µg/Kg	14964
	Chrysene	µg/Kg	12412
	Benzo (b) Fluoranene	µg/Kg	13713
	Benzo (a) pyrene	µg/Kg	18286
	Benzo (k) fluoranthene	µg/Kg	7351
	Indeno (1,2,3, c, d) pyrene	µg/Kg	8.89E+03
	Benzo (g, h, i) perylene	µg/Kg	9957.36
	Dibenzo (a, h) anthracene	µg/Kg	2426
	benzo (j) fluoranthene		7079
	benzo (and) pyrene		13131
	Sum_IPA_16	µg/Kg	2.01E+05

The threshold/screening values of concentration defined by Italian regulations for each specific compound are shown in Table 3.4.

Table (3. 4): Intervention limits for residential and commercial/industrial land use according to the law 152/2006 and natural background values

Parameters	DLgs 152/2006 residential use (mg/kg)	DLgs 152/2006 industrial and commercial use (mg/kg)	Background (mg/kg)
pH			
Electrical conductivity (mS/cm)			
Sulfides			
Sulfates			
Fluorides			
Cyanides	1	100	
Complex cyanides			
S			
As	20	50	37
Ba			
Be	2	10	12
Cd	2	15	2
Co	20	250	130
Cr Total	150	800	150
Cr VI	2	15	1

Hg	1	5	1
Mo			
Mn			
Ni	120	500	120
Pb	100	1000	112
Cu	120	600	120
V	90	250	110
Zn	150	1500	158
Phenols	1	60	
Benzene	0.1	2	
Toluene	0.5	50	
Xylene	0.5	50	
Total hydrocarbons	50	750	105
Monochlorinated benzene	0.5	50	
2-Chlorinated phenols	0.5	25	
2,4-Dichlorinated phenols	0.5	50	
2,4,6-Trichlorinated phenols	0.01	5	
Pentachlorinated phenols	0.01	5	
1,2-Dichlorinated ethane	0.2	5	
1,1,2-Trichlorinated ethane	0.5	15	
Pyrene	5	50	
Benzo(a)anthracene	0.5	10	
Chrysene	5	50	
Benzo(b)fluoranthene	0.5	10	
Benzo(k)fluoranthene	0.5	10	
Benzo(a)pyrene	0.1	10	
Dibenzo(a,h)anthracene	0.1	10	
Benzo(g,h,i)perylene	0.1	10	
Indeno pyrene	0.1		
Dibenzo(a,i)pyrene	0.1	10	
PAH total	10	100	
PCB	0.06	5	

At the biology laboratory, the sample that was kept in a plastic tube was broken down into smaller pieces (subsamples) in a plastic tray in order to homogenize the sample (Figure 3.2 b) and the sediments were

manually mixed. Then the sediments were sieved through a sieve of 2mm openings to ensure a complete separation of the coarser and finer particles of the sediments.

A)

B)

Figure (3. 1): The sample was kept in a sealed plastic tube (a), and to ensure a complete homogenous sample, the sample was mixed in a plastic tray (b).

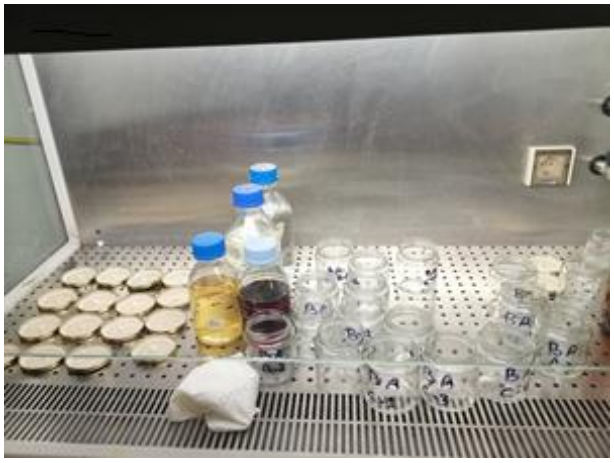
The samples were prepared for bioremediation systems in 16 cylindrical glass jars of 15 cm in diameter and 20 cm in height. Each cylinder has a top metal cap containing a hole of 4mm in diameter in order to ensure the aeration of the samples. The oxygen is mandatory to the system, but to prevent or at least to minimize any contamination from the air, a cotton wool plug was placed on the top hole of the metal cap. At this stage, we had 16 different samples that were ready to be placed in the reactors[1] where the biological reaction of degradation of the chemicals would take place. The reactor jars were labeled based on the classification described in Table 3.5.

1 Reactor: refers to the system designed to support microorganisms' activities (i.e., growth and productivity of organisms)

Table (3. 5): Samples classification.

	Sample Code	Type of Treatment
Specified for Biological Tests	BA-CTR-1	Sample Control (No Treatment)
Specified for Biological Tests	BA-CTR-2	Sample Control (No Treatment)
Specified for Geotechnical Tests	BA-CTR-3	Sample Control (No Treatment)
Specified for Geotechnical Tests	BA-CTR-4	Sample Control (No Treatment)
Specified for Biological Tests	BA-A-1	Using Bacteria
Specified for Biological Tests	BA-A-2	Using Bacteria
Specified for Geotechnical Tests	BA-A-3	Using Bacteria
Specified for Geotechnical Tests	BA-A-4	Using Bacteria
Specified for Biological Tests	BA-B-1	Using Fungi
Specified for Biological Tests	BA-B-2	Using Fungi
Specified for Geotechnical Tests	BA-B-3	Using Fungi
Specified for Geotechnical Tests	BA-B-4	Using Fungi
Specified for Biological Tests	BA-C-1	Using Bacteria +Fungi
Specified for Biological Tests	BA-C-2	Using Bacteria +Fungi
Specified for Geotechnical Tests	BA-C-3	Using Bacteria +Fungi
Specified for Geotechnical Tests	BA-C-4	Using Bacteria +Fungi

A) Jars before the labeling



B) Jars after labeling

Figure (3. 2): System with labeled codes

Figure (3. 3): Jars filled with samples.

In order to have a complete simulation of the bioaugmentation, a specific microorganism (i.e., bacteria and fungi) were added to the jars with certain quantities. Table (3.6) showed the strains of microorganisms used to utilize in bioaugmentation. Additionally, table (3.7) displayed the quantity of both microorganisms and seawater were added to each jar.

Table (3. 6): Taxonomic details of the bacterial and fungal pools utilized in the bioaugmentation treatments.

Bacterial pool (n. strains used for each taxon)	Fungal pool (n. strains used for each taxon)
Aeromonadaceae (5)	Eurotiales (1)
Alteromonadales (2)	Filobasidiales (2)
Bacillales (22)	Glomerellales (1)
Enterobacterales (3)	Hypocreales (1)
Pseudomonadales (8)	Microascales (1)
Rhizobiales (9)	Onygenales (1)
Rhodobacterales (1)	Pleosporales (1)

Table (3. 7): The quantities of sea water and microorganisms for each sample

	Sample Code	Sea Water (mL)	Bacteria (mL)	Fungi (mL)
Specified for Biological Tests	BA-CTR-1	75	-	-
Specified for Biological Tests	BA-CTR-2	75	-	-
Specified for Geotechnical Tests	BA-CTR-3	75	-	-
Specified for Geotechnical Tests	BA-CTR-4	75	-	-
Specified for Biological Tests	BA-A-1	73	2	-
Specified for Biological Tests	BA-A-2	73	2	-
Specified for Geotechnical Tests	BA-A-3	73	2	-
Specified for Geotechnical Tests	BA-A-4	73	2	-
Specified for Biological Tests	BA-B-1	73	-	2
Specified for Biological Tests	BA-B-2	73	-	2
Specified for Geotechnical Tests	BA-B-3	73	-	2
Specified for Geotechnical Tests	BA-B-4	73	-	2
Specified for Biological Tests	BA-C-1	73	1	1
Specified for Biological Tests	BA-C-2	73	1	1
Specified for Geotechnical Tests	BA-C-3	73	1	1
Specified for Geotechnical Tests	BA-C-4	73	1	1

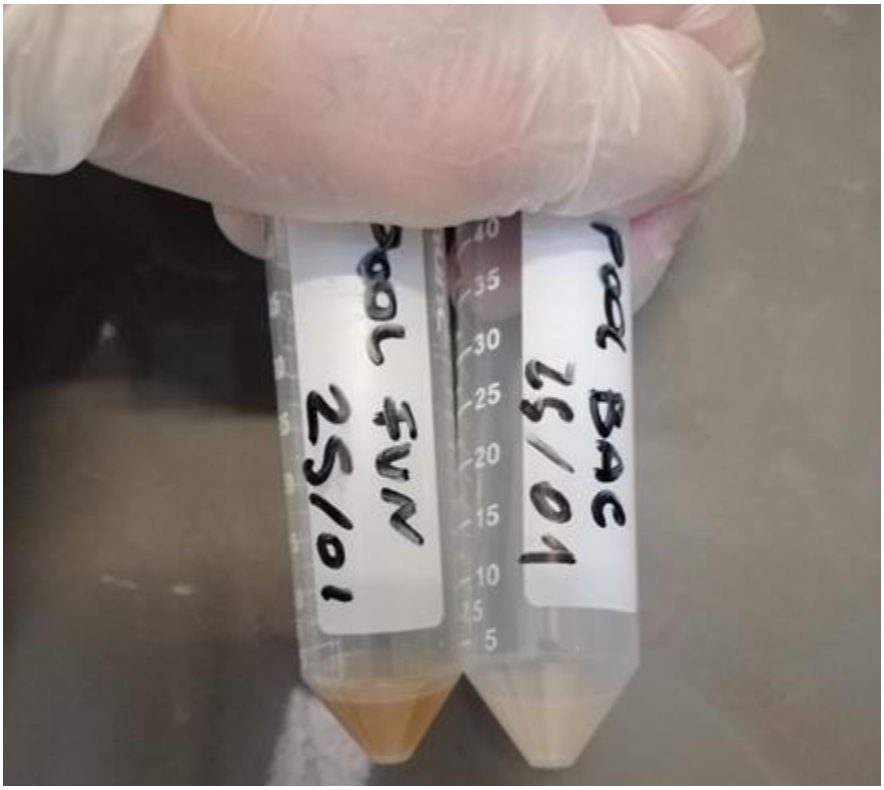


Figure (3. 4): Bacteria and Fungi Solutions

3.3 Monitoring

Temperature, pH and the growth of microorganisms were monitored at various frequencies throughout the experiment. In this study, pH and temperature were measured at three different time points: at baseline (T0), after two weeks (T1) and after 4 weeks (T2). The following subsections describe the monitoring and the sampling that was carried out throughout the experiment.

Figure (3. 5): pH and temperature measurements in the lab

Temperature Monitoring

Temperature was measured within the contaminated soil mass in each reactor using an electrical thermometer. As mentioned above, the measurements were performed at three time-points (T0, T1 and T2).

Table 3.8 shows the measurements of temperature and pH. It should be noted that sample CTR3 and CTR4 were note involved, because they were already in use for the geotechnical tests.

pH Monitoring

The pH measurement was determined with GLP 21 pH meter. Prior to measurement, the pH meter was calibrated using specific buffer solutions at different pH values (e.g., 4.0, 7.0 and 9.2) and

each value of the soil solution was recorded carefully. Next, the electrode was washed with deionized water and immersed in the sample.

Table (3. 8): Measurements of pH and temperature for all jars

		T0		T1	
	Sample Code	**Temperature (°C)**	**pH**	**Temperature (°C)**	**pH**
Specified for Biological Tests	BA-CTR-1	23.5	6.256	21.9	6.51
Specified for Biological Tests	BA-CTR-2	23.3	6.181	21.3	6.59
Specified for Geotechnical Tests	BA-CTR-3	23.9	6.06		
Specified for Geotechnical Tests	BA-CTR-4	24	6		
Specified for Biological Tests	BA-A-1	23.4	6.021	21.5	6.64
Specified for Biological Tests	BA-A-2	23.6	6.08	21.2	6.66
Specified for Geotechnical Tests	BA-A-3	23.9	5.963	20.9	6.6
Specified for Geotechnical Tests	BA-A-4	24	5.953	21	6.6
Specified for Biological Tests	BA-B-1	23.3	6.01	21.6	6.76
Specified for Biological Tests	BA-B-2	23.4	6.038	21.7	6.74
Specified for Geotechnical Tests	BA-B-3	23.7	5.807	21.1	6.6
Specified for Geotechnical Tests	BA-B-4	23.9	5.783	21.5	6.57
Specified for Biological Tests	BA-C-1	23.4	5.858	22	6.68
Specified for Biological Tests	BA-C-2	23.5	5.857	22	6.73
Specified for Geotechnical Tests	BA-C-3	23.6	5.661	21.6	6.63
Specified for Geotechnical Tests	BA-C-4	23.7	5.618	21.9	6.59

3.4 Biological Analysis of the Microorganisms community

For the purpose of biological investigations (e.g., presence of hydrocarbons, presence of heavy metals, DNA extractions, etc.) it was necessary to prepare multiple subsamples from the reactor system (figure3.6) with a proper quantity. In this study, the quantity of 36 mg was taken from each jar to perform the required tests, In Table 3.8 some details are summarized. Next, all samples were kept at -20˚C in a freezer located in the laboratory for downstream tests.

Table (3. 9): Subsample's quantities

Test	Quantity (mg)
Hydrocarbons	5
Heavy metals	20
DNA extraction	5
Abundance of Bacteria	3
Abundance of Fungi	3

Figure (3. 6): preparing for subsamples.

Figure (3. 7): samples after subsampling from the main jars.

A biological analysis was conducted on the samples to specify microorganisms and investigate the community composition of both bacteria and fungi. Also, this analysis was conducted at three-time interval (T0, T1 and T2) for each sample to detect any potential changes in the biodegrading community. In term of biological analysis, DNA extraction was carried out to isolate DNA from the bacterial community in each sample, and then spectrophotometer was used to measure the concentration and purity of the extracted DNA. The following parts explain the procedures in detail.

3.4.1 DNA Extraction from Soil Samples

To limit any potential source of contamination, all containers and tools that were in contact with the sample (s) during the experiment were washed thoroughly, dried, and autoclaved before use. Cabinet was also disinfected using 90% concentration of alcohol. Additionally, gloves were worn at all times when handling soil and extracted DNA samples because of the sensitivity of PCR and the high-risk of contamination by human cells.

The soil samples were purified using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA). The kit contains 50 PowerBead Tubes, PowerSoil™ Solution C1, C2, C3, C4, C5, and C6. Also, it includes PowerSoil™ Spin filters and 200 Collection Tubes.

The manufacturer's recommendations procedure was employed for DNA extraction and amplification as described in the PowerSoil® DNA Isolation Protocol figure (3.8). Using 0.25 gm of soil, the sample was vortexed in a PowerBead Tube provided in the kit. The PowerBead Tubes contain garnet beads and a guanidine thiocyanate buffer that can disperse the soil and dissolve humic acids, while prevent DNA degradation. Sixty µl of C1 solution, which contains anionic detergents to break down fatty acids and lipids integral to organismal cell membranes was added to each PowerBead Tube mixture. The samples were gently vortexed for approximately 10 minutes to ensure homogenous mixing and complete cell lysis. After centrifuging at 10,000 x g for 30 sec at room temperature, 500 µl of supernatant was transferred to a new 2 ml collection tube. Next, the sample was treated with 250 µl of a aqueous lysis reagent (solution C2) to remove organic and inorganic substances, incubated at 4˚C for 5 minutes, and then centrifuged at 10,000 x g for 30 sec at room temperature. Six hundred µl of supernatant was transferred to clean 2 ml collection tubes, 200 µl of a second inhibitor removal solution was added to continue the breakdown of non-DNA contaminating materials found in soils and sediments. After incubation for 5 minutes at 4˚C and centrifugation, no more than 750 µl of the supernatant was transferred to a new tube. A small amount (1.2 ml) of a salt solution was added to encourage DNA binding to the silica matrix of the purification column. For each sample, approximately 675 µl of the supernatant was loaded onto a spin filter, centrifuged for 1 minute, and the flow through discarded three times to process the entire volume of each sample through its individual column. An ethanol-based wash was added to remove residual salt and contaminants before centrifugation for 30 seconds at 10,000 x g. The

wash flow-through was discarded and the spin filter was carefully transferred to a new 2 ml microcentrifuge tube, after which 100µl of sterile elution buffer was added to the filter membrane of the spin filter and the samples were centrifuged for 30 seconds at 10,000 x g.

The spin filters were then discarded, and the tubes of purified DNA were stored at -20˚C prior to further analysis.

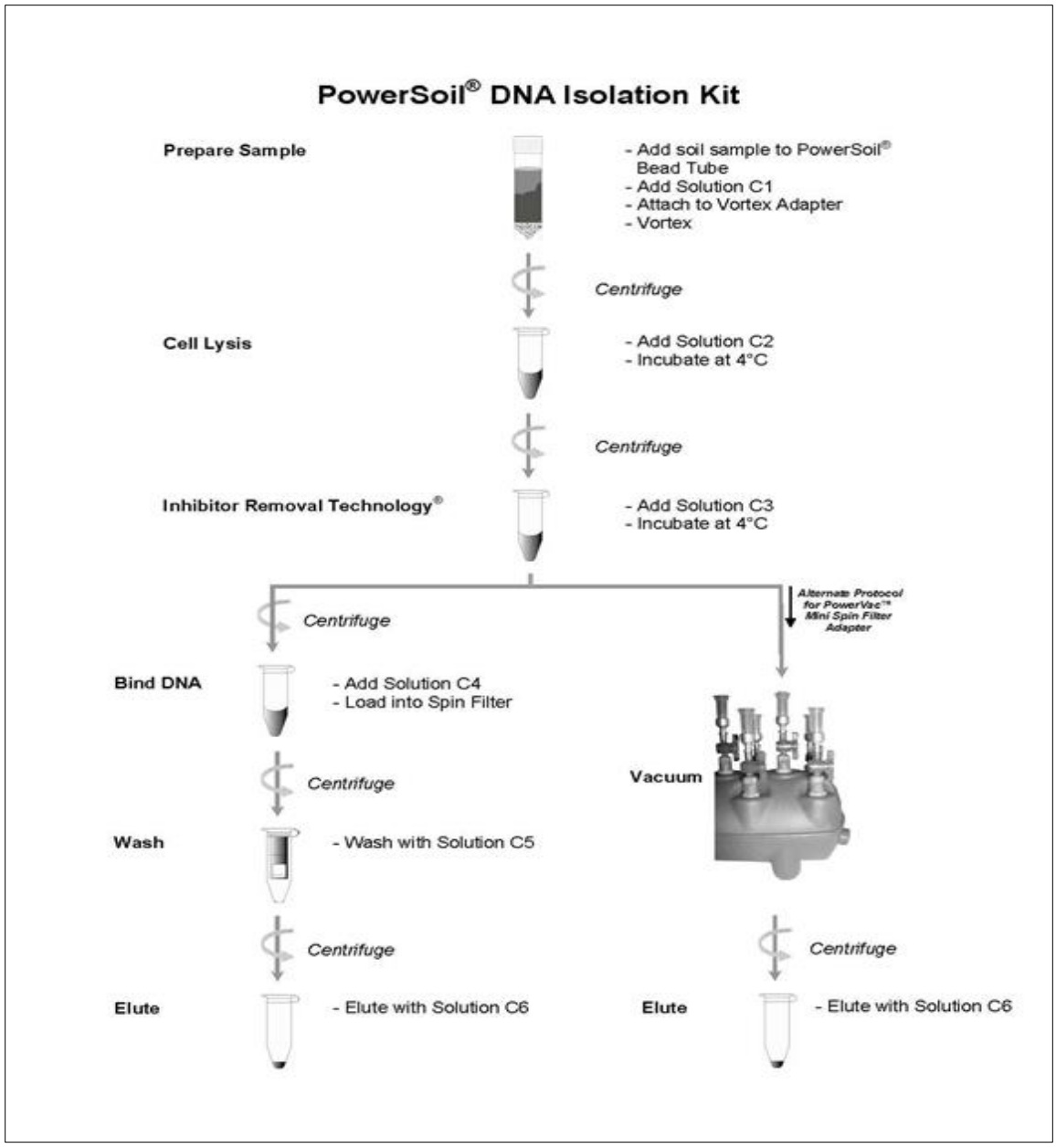
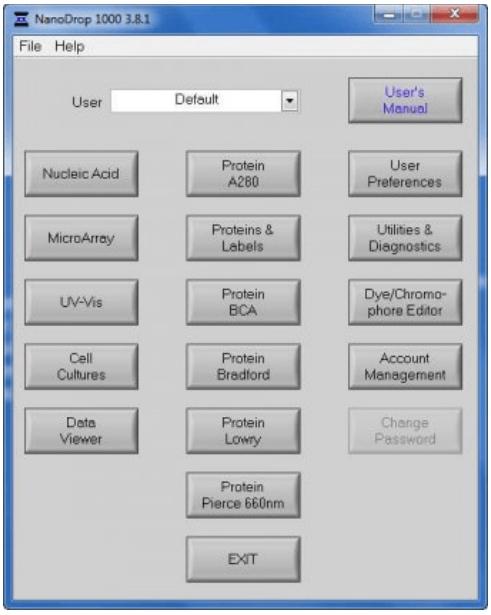


Figure (3. 8): Details steps of DNA extraction, figure adapted from powersoil®DNA isolation kit catalog no: 12888.50 & 12888.100

3.4.2 Quantify Extracted DNA

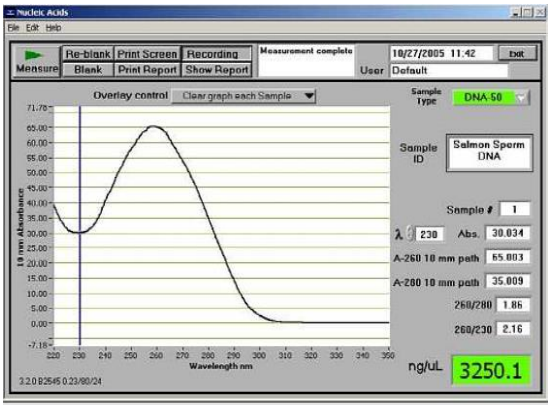
After isolating DNA, it is necessary to figure out how much DNA, or RNA it has, and how much this genetic material is pure. This is done classically by UV-visible spectrophotometer (figure 3.9a). The most common technique to determine DNA yield and purity is also the easiest method.

A) Nanodrop device



B) software screen

C) cleaning nanodrop pedestal



D) Reading the results

Figure (3. 9): Quantifying DNA instruments

Procedure for DNA quantification by Nanodrop technique

1. Open and turn on the computer attached to the NanoDrop.

2. Wash the NanoDrop pedestal.

    a. There should be a lab wipe in the pedestal from the previous user. Lift the upper arm of the NanoDrop and remove the wipe.

    b. Add 4-5 µl of purified water to the lower pedestal, then lower the arm.

    c. Wait 30-60 seconds.

    d. Lift the upper arm and use the wipe to vigorously scrub both the upper and lower pedestals.

3. Open the NanoDrop software on the computer by double-clicking the "ND-1000" icon that looks a bit like an hourglass.

4. Initialize the NanoDrop (figure 3.9b).

    a. Click on the "Nucleic Acid" button in the NanoDrop software. This will bring up a dialog box. DO NOT click "Okay" until you've added water.

    b. Add 1-2 µl of purified water to the lower pedestal, then lower the upper arm (figure 3,b).

    c. Click "Okay" on the computer and wait ~20 seconds while the NanoDrop initializes.

    d. When it's done, lift the upper arm and dry the pedestal with a wipe

5. Blank the NanoDrop.

    a. Add 1-2 µl of the buffer your sample is in. If you resuspended a DNA pellet using TE, for example, blank now with TE.

b. Lower the upper arm of the NanoDrop and click the “Blank” button on the software.

c. Wait ~20 seconds for the blank measurement to be made (figure 3.9c).

d. When it’s done, lift the upper arm and dry the pedestal with a wipe.

6. Measure your sample.

a. Add 2 µl of your sample to the lower pedestal, then lower the upper arm.

b. In the “Sample ID” box, type in the name of your sample.

c. Click the “Measure” button on the software and wait ~20 seconds for measurement (figure 3.9d).

d. When it’s done, lift the upper arm and dry the pedestal OR lift the upper arm and carefully pipet up as much of the sample as you can to retain it for further use (see final Helpful Tip, above).

7. Collect your data.

a. Write down any measurements you’re interested in. You can move the cursor to check the absorbance number at various wavelengths.

b. Click the “Print Screen” button to print the complete spectrum, if desired.

c. When finished making all measurements, click “Print Report” to get a table of all data.

8. Clean the pedestal.

a. Add 4-5 µl of purified water to the lower pedestal, then lower the arm.

b. Wait 30-60 seconds.

c. Lift the upper arm and use a wipe to vigorously scrub both the upper and lower pedestals.

d. Place a new folded lab wipe on the lower pedestal and close the upper arm.

3.5 Geotechnical tests

As mentioned in the methodology, after the biological setting of the samples, some of them was subjected to geotechnical tests. Therefore, when the samples were delivered to the geotechnical laboratory, they were stored in a chemical cabinet to prevent any air contamination. However, by that time, the container jars contained plenty of water therefore some water separated at the top surface of the sample. The excess water was carefully removed using a syringe because otherwise the sample couldn't be handled for the geotechnical tests.

It should be noted that ASTM standards was the main guideline to perform the geotechnical tests. The moisture content of the samples was immediately measured upon the sample arrival at the lab. According to ASTM D2216-10 method, water content is measured oven-drying a representative portion of the sample. The water content (%) is calculated from the sample weight before and after the drying stage.

The equipment's used in moisture content measurement:

- Microwave oven
- A balance readable and accurate to 0.01 g
- Numbered glass weighing container.

The procedures are:

- Clean and dry the glass container and weigh it (W1).
- Take a specimen of the sample in the container and weigh it (W2).
- Place the container in the microwave oven, arrange power in order to have a temperature ranging from 150 to 180 C and dry for 15 minutes.

- After the necessary number of intervals, record the final constant weight (W3) of the container with the dried soil sample.

Calculate the water content of the soil as a percentage of the dry soil weight, using the following equation:

$$MC(\%) = \frac{W_2 - W_3}{W_3 - W_1}$$

Where:

W1 = Weight of container (g)

W2 = Weight of moist soil + container (g)

W3 = Weight of dried soil + container (g)

3.5.1 One-dimensional consolidation Test Procedure

Consolidation Test is used to determine the rate and magnitude of settlement in soils. The settlement values obtained by this test are usually mainly due to primary consolidation. The results of consolidation test are used to determine all the consolidation parameters including Compressibility Coefficient (a_v), Coefficient of Volume (m_v), Compression Index (C_c) and Coefficient of Consolidation C_v.

Equipment's used for Consolidation Test:

- oedometer
    - Consolidation ring
    - Two porous stones
    - Two filter papers

- Loading pad
- Equivalent loads to final stresses (6.25, 12.5, 25, 50,100, 200, 400, 800)kPa

- Dial gauge (accuracy of 0.002mm)
- Stopwatch.
- Knife or spatula or fine metal wires
- Weighing balance (accuracy of 0.01g)
- Vernier calipers
- Oven
- Seawater reservoir

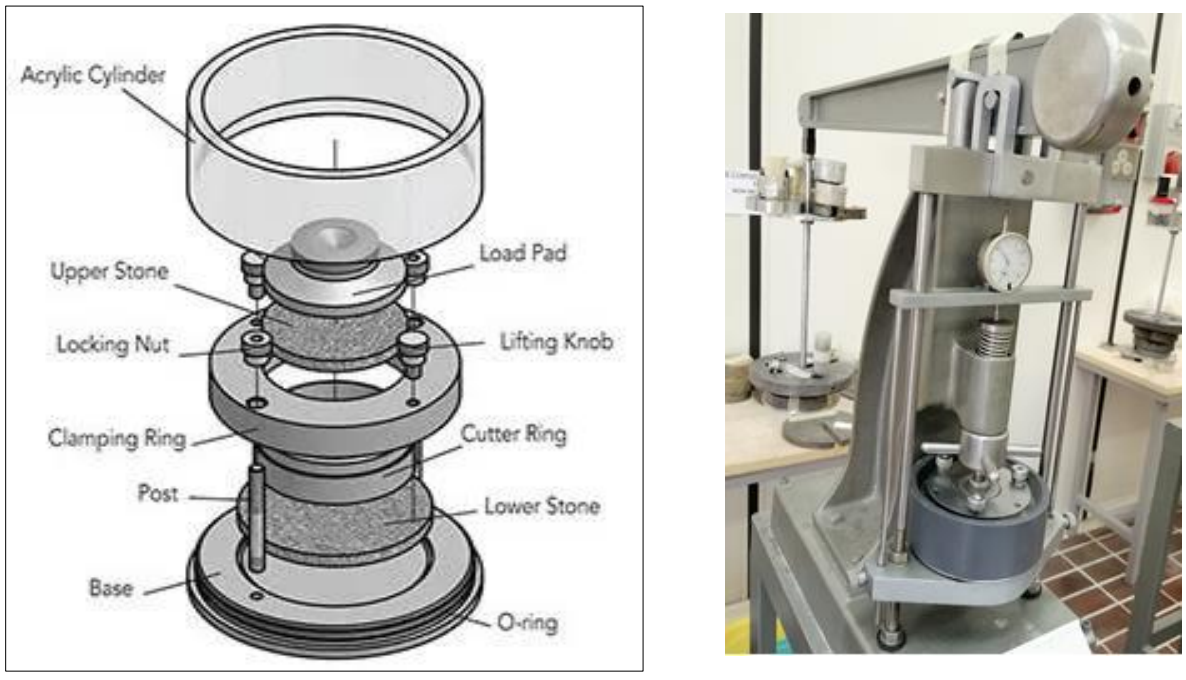


Figure (3. 10): Odoemeter tests equipment

The Steps that were followed to perform the consolidation test are described in the following

- The cutter ring is cleaned, dried, and weighted, and the inner diameter and height are measured using weighing balance and calipers, respectively.
- Measure the initial weight of the base, bottom porose stone and bottom filter paper, then put the ring and fill it with the distributed sample. (Both porose stone and filter paper are wet)
- Make sure that the ring should not contain any soil on its outer part and weight the metal ring with specimen again.
- Assemble the bottom part of the oedometer: bottom porous stone, filter paper, specimen ring, and fill the ring with the sediments with the help of a spoon and of a spatula in order to reach the accurate density.
- Put the other filter paper and the other porous stone on the top of the sample.
- Place the loading pad on the top porous stone and lock the oedometer using metal screws provided.
- check the applied load is vertical and on the center of specimen.
- Arrange the dial gauge in a position in such a way that it should allow sufficient space for swelling of soil specimen.
- Water reservoir is filled with seawater and the specimen should be submerged.
- Now apply the initial load (equivalent to water pressure) which should not allow any swelling in the sediment.

- Leave the load until there is no change in dial gauge reading. write down the final reading of dial gauge for initial load.
- First load increment of 6.25 kPa is applied and start the stopwatch immediately and note down the readings of dial gauge at various time intervals. In general, readings are taken at 0.15, 0.30, 1, 2, 4, 8, 15, 30 minutes, 1, 2, 4, 24 hrs.
- In general, primary consolidation of soil (90% of consolidation) is reached within 24 hours. Hence readings are noted up to 24 hours.
- Next apply the second load increment of 12.5 kPa and repeat same procedures.
- Similarly apply the load increments 25, 50, 100, 200 and 400 kPa and repeat the same procedure and note down the readings.
- When values of last load increment are noted, now reduce the load gradually from 400, 100, 25, 6.25 kPa. At every point note down the final gauge readings.
- Next apply the second cycle of incremental loads 25,100, 400 reaching 800 kPa with same procedures.
- Now remove the assembly from loading frame and dismantle it.
- Take out the specimen ring and wipe out the excess water and weigh the specimen ring and note down.
- Finally measure the moisture content and send part of the sample to be scanned using electron microscope.

A)

B)

C)

D)

Figure (3. 11): a and b illustrate the oedometer equipment before and after the test. C and d show the sample preparation for moisture content after oedometer test.

3.5.2 Permeability test

Determine the coefficient of permeability of the given soil is very useful in solving some problems such as seepage of water through soil. Thus, constant head and falling head methods are commonly used to measure the permeability of soil. The falling head method of is preferable for soil with low discharge, whereas the constant head permeability test is used for coarse-grained soils with a reasonable discharge in a given time. For very fine-grained soil, capillarity permeability test is recommended (Mitchell and Madsen, 1987).

On the other hand, there are different types of permeameter which can be classified into rigid wall and flexible wall cell. For rigid-wall cells used as permeameters, Daniel et al. (1985) identified the major advantages as lower cost, simplicity, greater adaptability to testing compacted soils, compatibility with a wide range of chemicals used as permeant liquids, and the lack of need to apply high confining pressures. The major disadvantages of rigid-wall cells were also identified by Daniel et al. (1985)

Equipment used for Permeability Test (Figure 3.12):

- Permeameter with its accessories (Panel Boards, Bladder Accumulators and Rigid Wall Cell, water tank supplier, water tank drained)
- soil specimen.
- Seawater
- Weighing balance (accuracy of 0.01g)
- Stopwatch.
- Knife or spatula or fine metal wires

- Vernier calipers
- Two porose stones
- Filter paper

The sequence for the permeability/leaching test is described in the following.

- Clean, dry, and wax to the cell wall to prevent any fraction between the sample and the wall.
- Mix the sample in the jar to reach a homogeneous media.
- Add a sufficient amount of the sample in the cell and note down the weight.
- Using the caliper, measure the height of the sample in mold.
- Assemble the cell, from bottom to top in the order, bottom porous stone, filter paper, specimen, filter paper and top porous stone.
- Then the piston should be placed and the pressure chamber is filled with water and when the soil sample is saturated, both the top and the bottom outlets are closed.
- Fill the inlet bladder accumulator with seawater and keep refilling during the test.
- Connect the cell with bladders and the pressure panel as well.
- Set the required pressure from the panel.
- Open the valves and note down both time, temperature and the head losses for inlet, outlet and cell pipes. (figure)
- From effluent bladder accumulator, take a sample 10 ml of leached water for chemical test.

- Once the test is complete, measure and record the final height, diameter, and total mass of the specimen. Then determine the final moisture content.
- Calculate the hydraulic conductivity, k, as follows:

$$k = \frac{aL}{2At} \ln\left(\frac{h_1}{h_2}\right)$$

Where:

k = hydraulic conductivity, m/s,

a = cross-sectional area of the reservoir containing the influent liquid, m2,

L = length of the specimen, m,

A = cross-sectional area of the specimen, m2,

t = elapsed time between determination of h1 and h2, s,

h1 = head loss across the specimen at time t1, m, and

h2 = head loss across the specimen at time t2, m.

- Correct the hydraulic conductivity to that for 20°C (68°F), k20, by multiplying k by the ratio of the viscosity of water at test temperature, T, to the viscosity of water at 20°C

$$k_{20} = R_T k_{measured}$$

Where:

K_{20} = hydraulic conductivity corrected

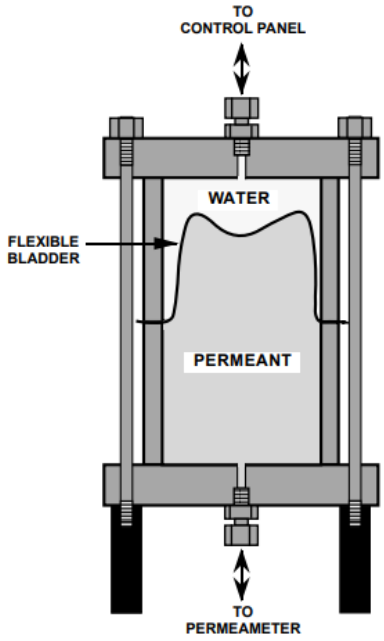
R_T = Correction Factor

$K_{measured}$ = actual hydraulic conductivity

The use of bladder accumulators (Figure 3.12 B) in both the inlet and the outlet of the permeameter was necessary because:

- The inlet bladder allow to permeate the specimen with the seawater instead of the distilled water
- The outlet bladder allow the sampling of the water flowing out from the specimen in order to determine the concentration of the chemicals for assessing the leaching capability of the specimen.

A)



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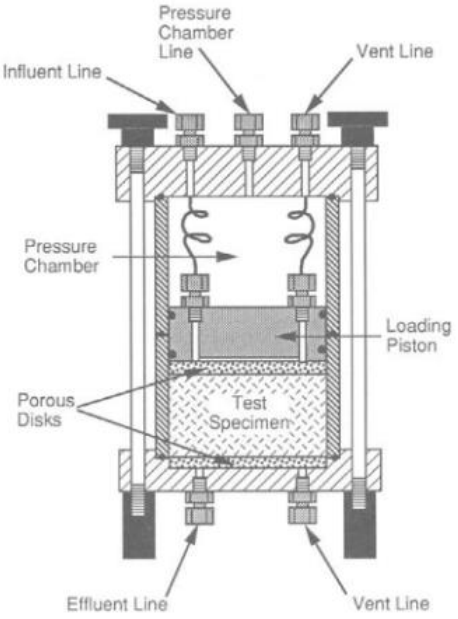


C)

Figure (3. 12): Rigid wall permeameter (adopted from Trautwein Soil Testing Equipment Co.). a) control panel, b) blender accumulator and c) Rigid wall permeameter

Chapter Four: Experimental results and discussion

4.1. introduction

In the present chapter, experimental results of biological and geotechnical tests on the contaminated marine sediments from Bagnoli basin are presented and discussed. The biological lab results will be presented first, followed by the geotechnical results.

4.2 Biological Results

The biological treatment of the sediment was conducted using different types of microorganisms e.g., bacteria and fungi. These species have shown unique capacity in their metabolic activities, which consequently enhances the efficiency of contaminants degradation (Figure 4.1).

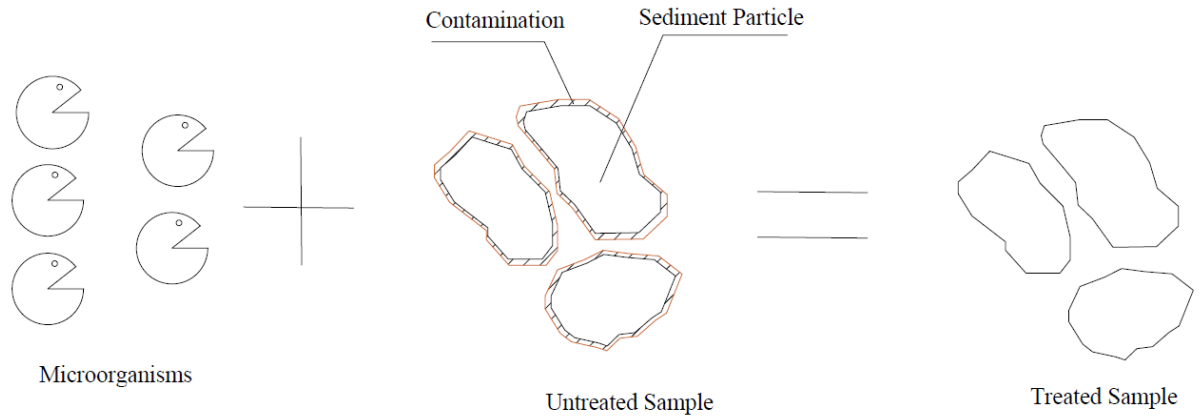


Figure (4. 1): Schematic diagram of biological treatment

As mentioned in the methodology section, the biological assessment of microorganisms was carried out after 0, 14, 28 and 87 days of incubation. During the indicated time points, the concentrations of various types of PAH (e.g., total PAHs, High-molecular weight PAHs and Low-molecular weight PAHs) were investigated. Table (4.1) summarizes the average values of

concentration of PAHs at the previously cited time points, while the trend of the residual PAHs concentrations in sediments as a function of the treatment time points are presented in figure (4.2).

Table (4. 1): PAHs concentrations with time

	Treatment	**Average values**			Variation of PAHs from first day (%)		
Days		0	14	28	0	14	28
TOTAL PAHs (µg kg^{-1})	Untreated	**3.16E+05**	**2.00E+05**	**3.77E+05**	100.00	36.56	-88.17
	Treated with Bacteria	**2.88E+05**	**3.04E+05**	**3.62E+05**	100.00	-5.78	-18.99
	Treated with Fungi	**3.41E+05**	**2.26E+05**	**3.99E+05**	100.00	33.66	-76.30
	Treated with Mixed (BAC + FUG)	**3.32E+05**	**2.46E+05**	**1.90E+05**	100.00	26.04	22.83
Low-molecular weight PAHs (µg kg-1)	Untreated	**1.02E+04**	**7.21E+03**	**1.25E+04**	100.00	29.17	-72.89
	Treated with Bacteria	**9.57E+03**	**1.04E+04**	**1.19E+04**	100.00	-8.89	-14.11
	Treated with Fungi	**1.24E+04**	**8.07E+03**	**1.35E+04**	100.00	35.10	-67.27
	Treated with Mixed (BAC + FUG)	**1.24E+04**	**8.70E+03**	**6.98E+03**	100.00	30.06	19.78
High-molecular weight PAHs (µg kg-1)	Untreated	**3.05E+05**	**1.93E+05**	**3.64E+05**	100.00	36.80	-88.74
	Treated with Bacteria	**2.78E+05**	**2.94E+05**	**3.50E+05**	100.00	-5.68	-19.16
	Treated with Fungi	**3.29E+05**	**2.18E+05**	**3.86E+05**	100.00	33.61	-76.63
	Treated with Mixed (BAC + FUG)	**3.20E+05**	**2.37E+05**	**1.83E+05**	100.00	25.88	22.94

In the maximum considered time (87 days) all the experimental treatment seems to be effective in reducing the concentration of both low and high molecular weight poliyciclic aromatic hydrocarbons (LMW PAHs and HMW PAHs, respectively). The treatment with mixed species (Bacteria and fungi) showed the lowest PAHs concentration at the control times of 14, 28 and 87 days compared to day zero and seems to be the only mix that is effective since the first 14 days.

The removal efficiency of total PAHs in 14 and 28 days for the treated samples with the fungi and mixed species (bacteria and fungi) were 66 % and 73 %, respectively. On the other hand, for the samples that were treated with bacteria only, an increase in the total PAHs was observed with 5 % and 25 % after 14 and 28 days, respectively. This scenario may occur for some reasons, for example, the high concentration of PAHs could be explained by microorganism's decomposition.

The most likely causes of the microorganisms' decomposition is the oxygen availability, that might turn out and cause a severe decomposition of microorganisms, accordingly.

The CTR sample, in figure (4.4), showed also degradation of HMW-PAHs only for dibenzo[(a,e)/(a,h)/(a,j)/(a,l)]pyrenes, indeno[1,2,3-cd]pyrene and Benzo(g,h,i)prelene. More interestingly, bioaugmentation (especially BAC+FUN) increased the degradation of all HMW-PAHs (figure 4.4) and LMW-PAHs (figure 4.2) especially (Acenaphthylene, Anthracene, Phenanthrene and Fluorene).

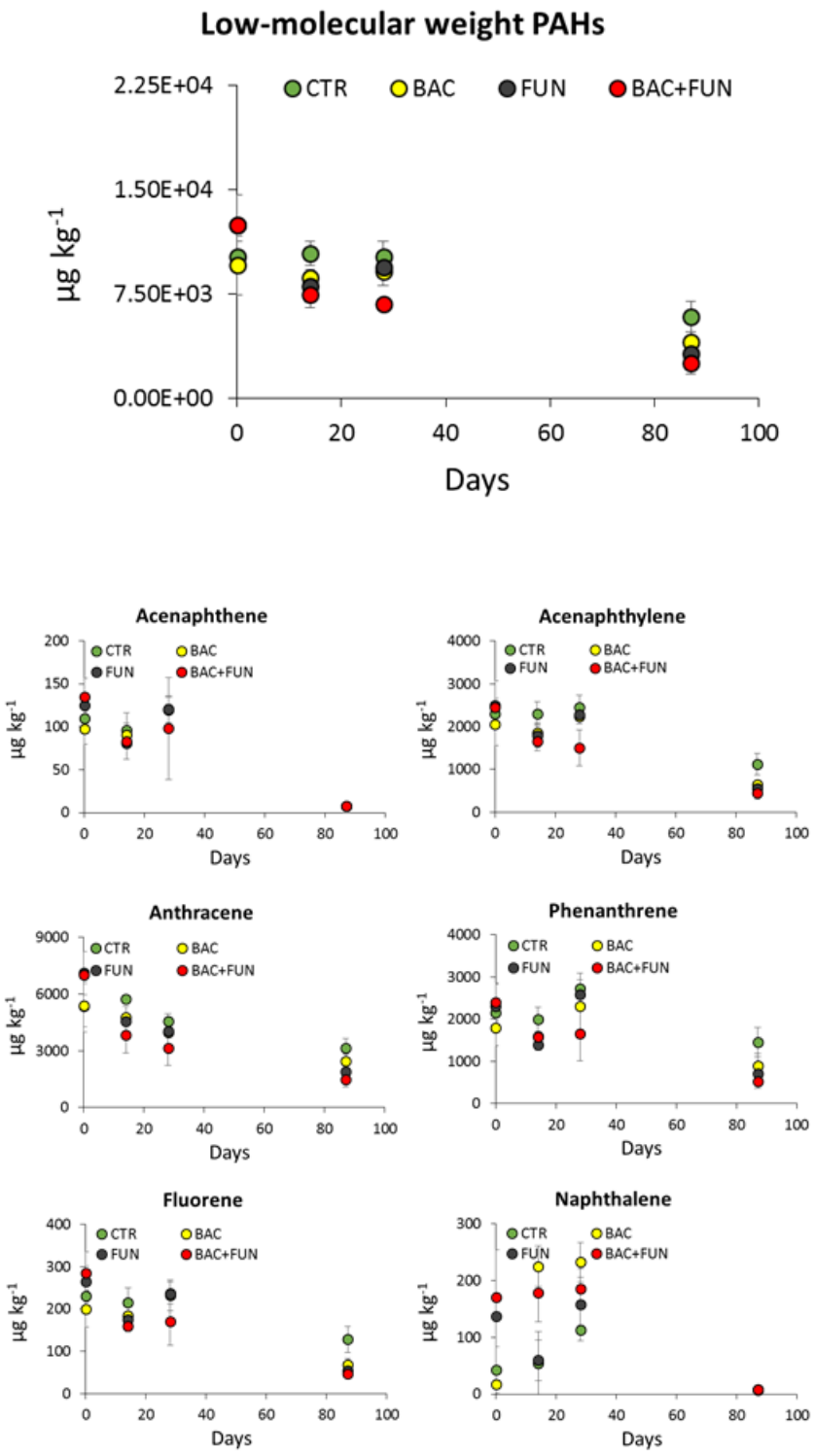


Figure (4. 2): LMW PAHs concentrations in the untreated control sample (CTR) and in the three experimental treatments (BAC= bacteria; FUN= fungi; BAC+FUN= combined treatment bacteria + fungi).

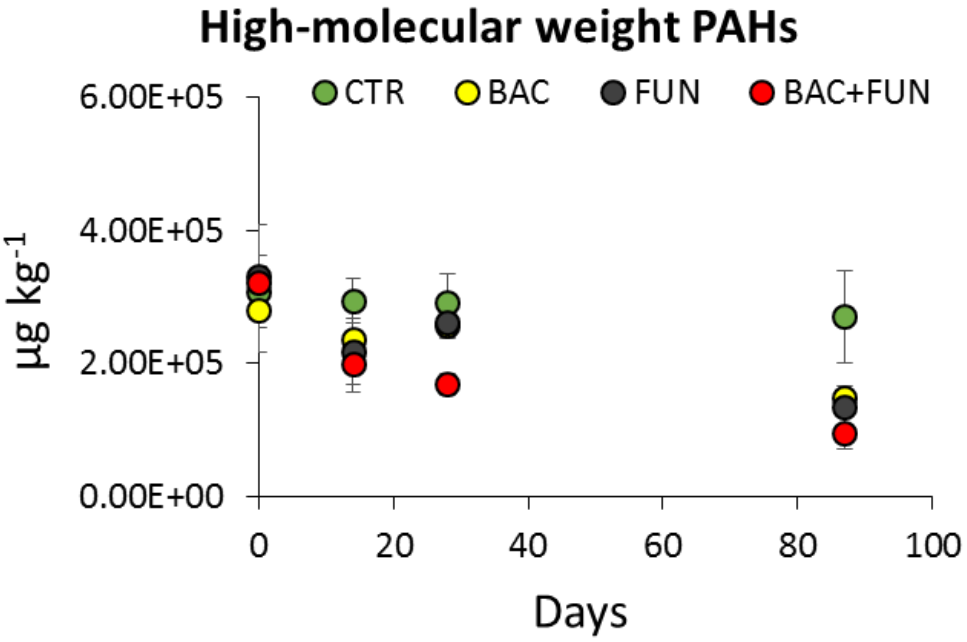


Figure (4. 3): The averaged concentrations of HMW PAHs in the untreated control sample (CTR) and in the three experimental treatments (BAC= bacteria; FUN= fungi; BAC+FUN= combined treatment bacteria +fungi).

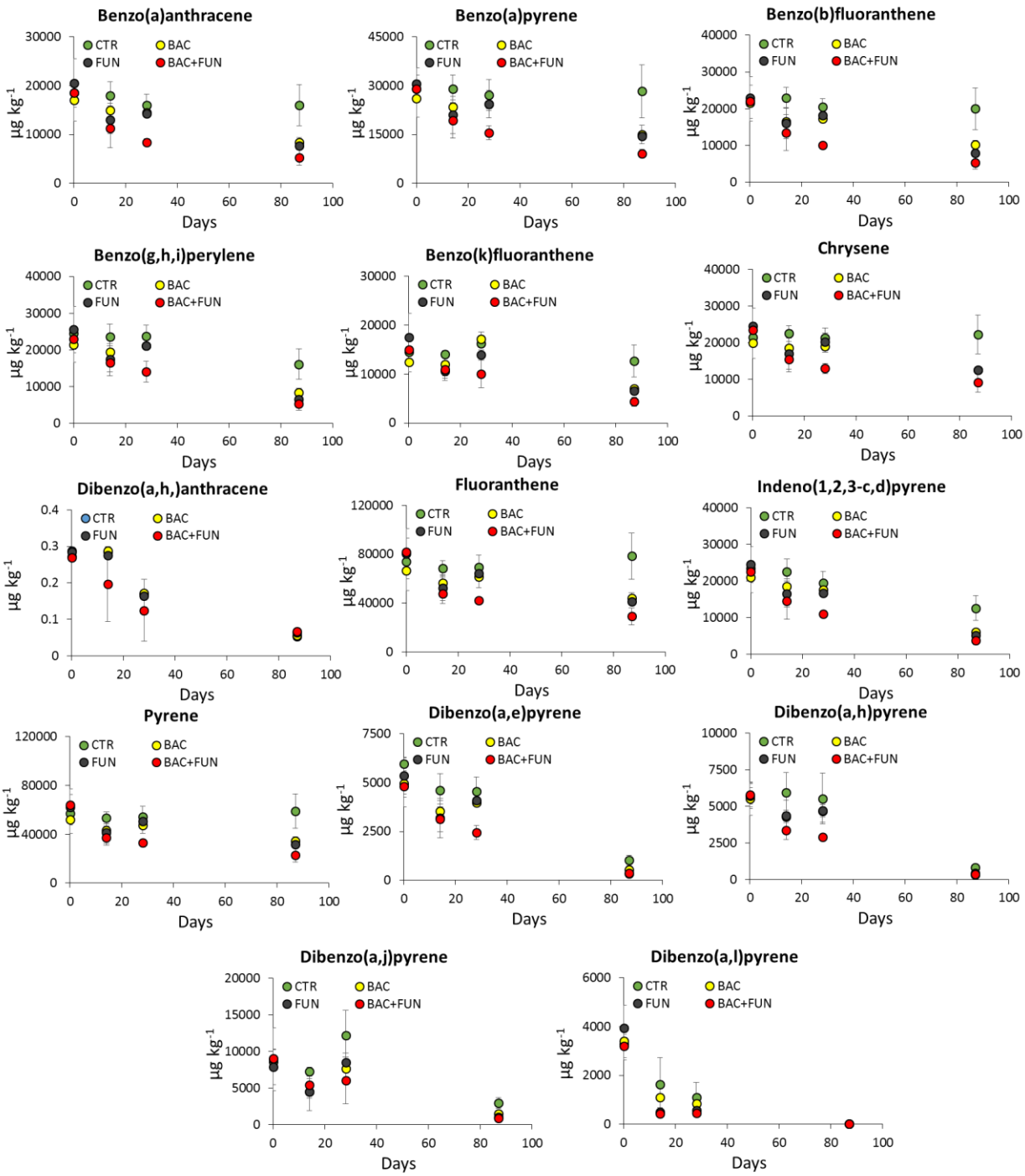


Figure (4. 4): HMW PAHs concentrations in the untreated control sample (CTR) and in the three experimental treatments (BAC= bacteria; FUN= fungi; BAC+FUN= combined treatment bacteria+fungi).

Lastly, after 87 days of treatment, sample (BAC+FUN) showed a high efficiency to degrade hydrocarbons. However, the same sample was subjected to heavy metals analysis to understand the possibility of degradation or partitioning of heavy metals. Results in figure (4.5) indicated that (BAC+FUN), which is the most effective treatment, dose not effect or increase the bioavailability fraction of the metals. Hence, the mobility of heavy metals is countless consequently. On other words, the environmental impact will not be affected by the fate of heavy metals during the bioremediation using (BAC+FUN).

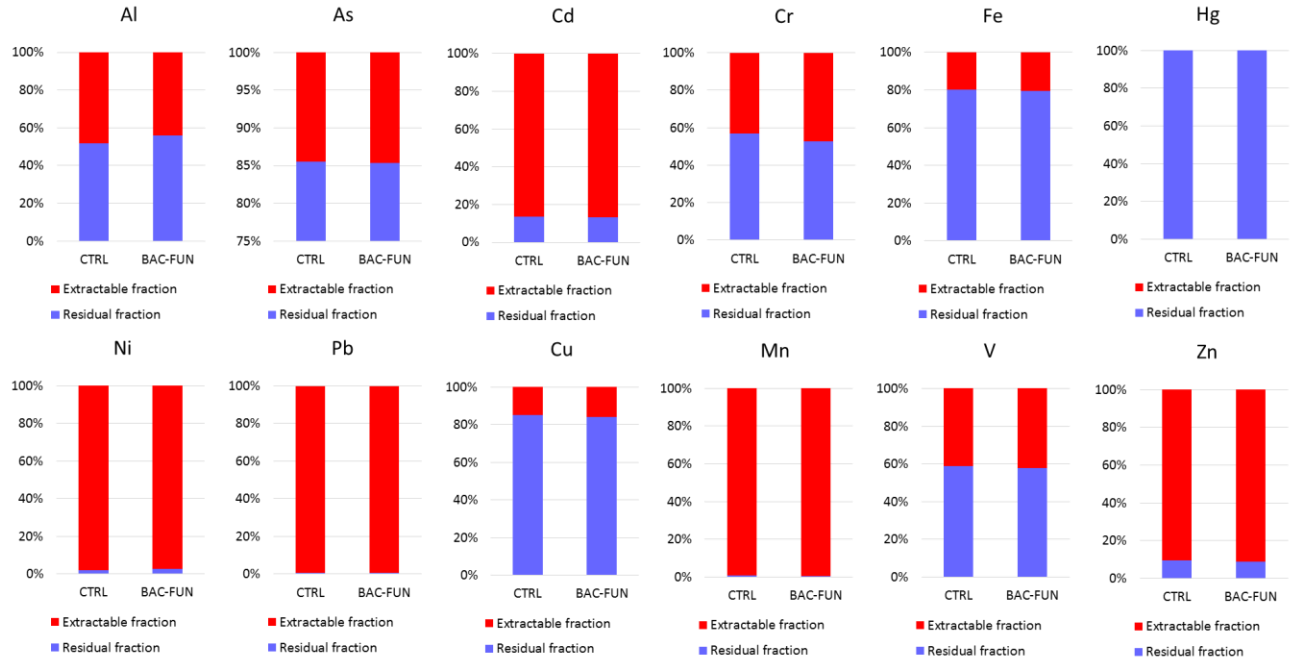


Figure (4. 5): Heavy metals fractionation after bioremediation from sample (BAC+FUN).

4.3 Geotechnical Results

. The presence of specific contaminants derived by the marked increase in the human activities can cause fundamental modifications of soil properties. These modification in the soil properties can be the cause various geotechnical problems such as structural cracks, ground settlement, heaving of structures, instability of slopes, depletion of strength and deformation characteristics, and changes in compaction characteristics. In this work, the experimental investigations include chemical, biological, and geotechnical tests of the contaminated and treated sediment samples to measure both effects of the presence of contaminants and of the biological treatment.

4.3.1 Physical properties

Soils and sediments normally contain a finite amount of water, which can be expressed as the "water content." The soils hold moisture within the pore spaces either between or within the soil aggregates. It should be noted that this pore space is usually not only occupied by water, but also by air. In case all of the pores are occupied by air, the soil is totally dry, whilst if all of the pores are filled with water, the soil is saturated. Scientifically, the moisture parameter is widely used in different fields e.g., hydrology, agricultural, geology and engineering fields. Ina addition, biologically, moisture content of the soils has been found to be an important factor in determining growth and development of microorganisms and their physiological diversity. Skopp et.al (1990) reported that the maximum microbial activity in soil occurs when the water content of the soil reaches 60%. From an engineering point view, a reliable measurement of moisture content is extremely important because it gives indirect information on some soil's mechanical parameters such as compaction, dry density, settlement.

In this work, the water content of samples was determined based on ASTM D2216. This method was used for both contaminated and treated samples. The measured water contents are presented in the table blow.

Table (4. 2): Water Contents

Sample	CTR	BAC	FUN	BAC+FUN
Water Content (%)	61.2	50.5	52.3	50.0

From table (4.2), it can be observed that the moisture content of the contaminated sample (CTR) was the highest value while the others are similar (about 50%). All the values are sufficiently high to ensure the possible microbial activity.

The grain size composition of the tested sediments is reported in Table (4.3). They are mainly composed by the sand fraction that represents more than the 90% of the sample.

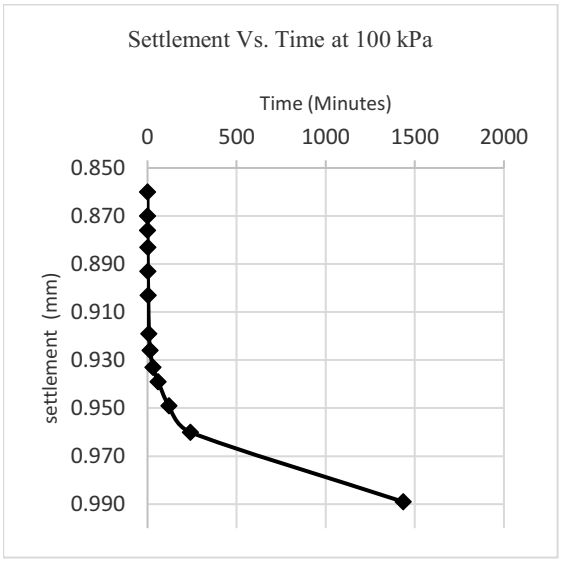
Table (4. 3): Grain size composition of the tested sediments

Main Classes (%)			
gravel	sand	silt	clay
3.98	90.95	3.84	1.23

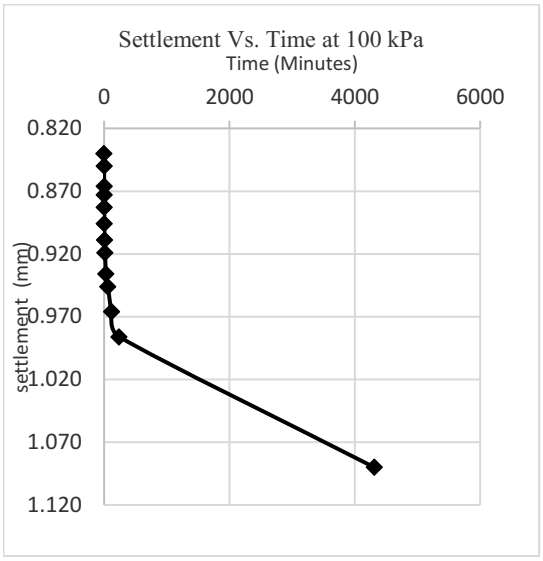
4.3.2 Compressibility

. In this work, one of the main goals was to examine the primary consolidation of both treated and untreated samples of sediments. In the geotechnical laboratory, four oedometric tests were carried out on: (1) untreated sediments, (2) sediments treated with bacteria only, (3) sediments treated with fungi only, (4) sediments treated with the mixed of bacteria and fungi. The treated sediments

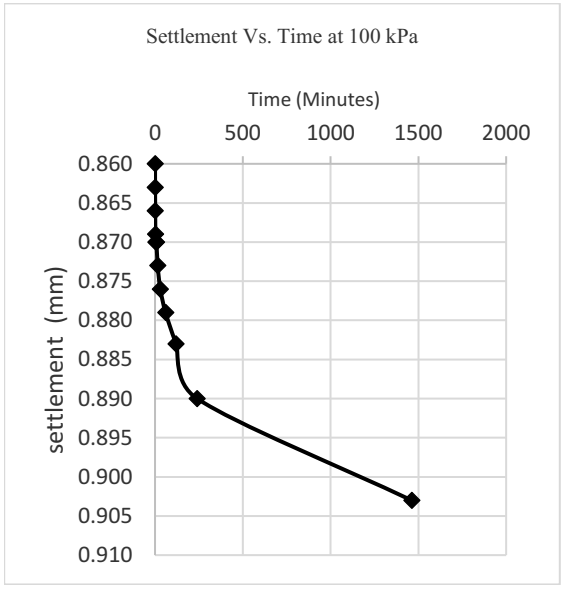
were tested after 28 days of biological treatment. The reading of the settlements with time may be plotted for each stress increment. Figure (4.6) illustrates the trend of settlements versus time, with reference to 100 kPa of applied pressure. The results revealed that BAC+GUN sample had the lowest settlement value if compared to the other samples. However, the sample treated with bacteria only showed a higher settlement value among all other samples, and the increase in settlement was 10 % if compared to the contaminated sample. The common trend in the settlement vs time curves is characterized by an initial sharp increase in settlements, typical of sandy soils followed by a final part that doesn't lean on a horizontal line, meaning that stationary values are not reached and suggesting the occurrence of a not neglectable secondary compression of the samples.

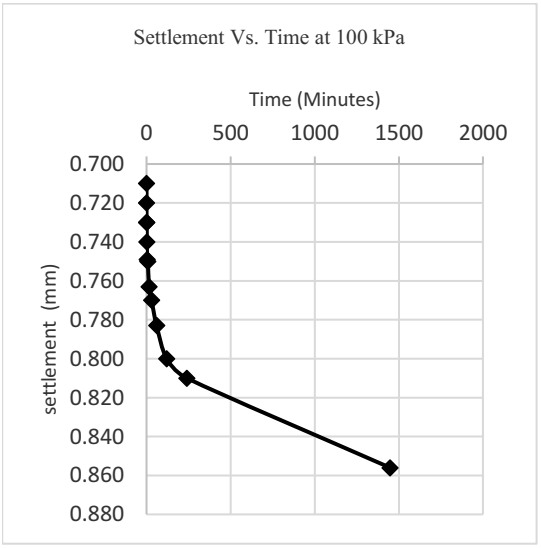


(A) Contaminated Sample



(B) Sample Treated with Bacteria





(D) Sample Treated with Mixed (BAC+FUN)

Figure (4. 6): Settlements of the samples with time due to an applied pressure of 100 kPa

Additionally, it is well-known that compression mainly occurs as a result of a rearrangement of the particles that cause a decrease in voids of the soil that are commonly represented in the term of change in the void ratio. Therefore, the final stress-strain relationships, can be presented in a graphical form by plotting the void ratio as a function of the applied stress, with each point on the curve related to the final condition of each pressure increment (Figure 4.7).

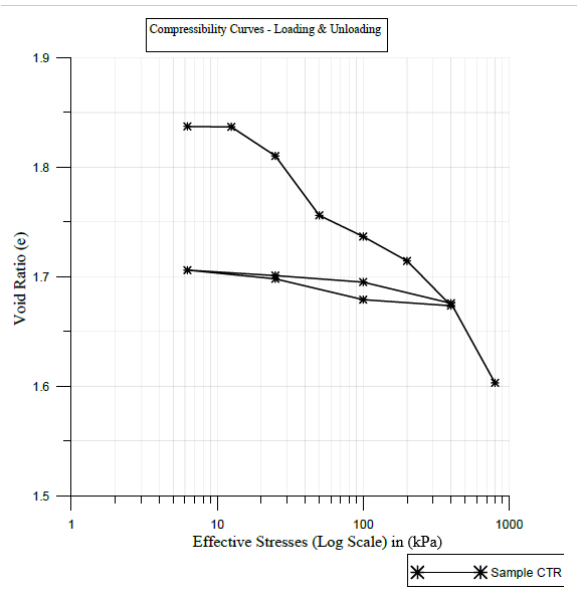
The specimens were prepared in order to start from the same void ratio (about 1.85) in order to isolate the contribution of the type of treatment only. The minimum reduction of void ratio in the considered pressure range was found in the specimen treated with fungi only. Anyway, the variations in void ratio with respect to entire stress levels were similar for the untreated and the treated samples. In light of these results, the test process used in this study is consistent and reproducible. Figure (4.7) also demonstrates that, with reference to the first 28 days of treatment, the bioremediation is not able to affect the compressibility behavior of sediments e.g., (the change in void ratio for the treated samples is similar to that of the untreated sample).

We also observed that the consolidation of sediments occurred very fast, and this indeed means the sediments were able to drain both water and air rapidly, this is fully justified by the dominant sandy fraction in the sample.

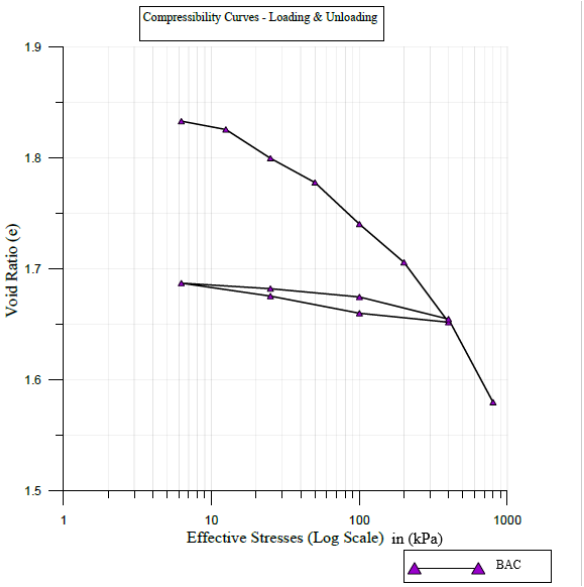
We should acknowledge that in the early stage of the first loading steps, the readings of the untreated sample were not very accurate because of the untouched dial gage with the sample. However, this technical problem was fixed for the rest of the samples.

To have a further better understanding of the significant effect of each treatment with different species of microorganism, each treated sample was compared with untreated one in graphs (4.8, a, b, c). Moreover, figure (4.8 d) shows the compression curves for untreated sample versus all treated

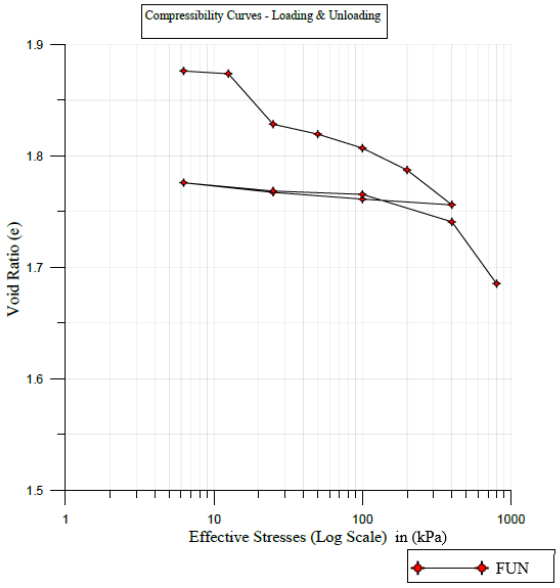
samples. To sum up, the compressibility behavior of both contaminated and biotreated sediments indicated a similar trend, therefore it might be argued that the biological treatment does not compromise the performance of the sediments in terms of compressibility. Further investigation for different types of sediments (e.g., mainly clayey or silty) is recommended.



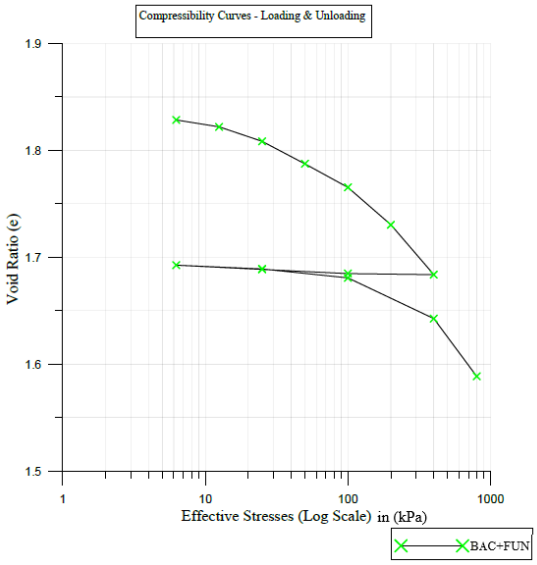
(A) Contaminated Sample



(B) Sample treated with bacteria

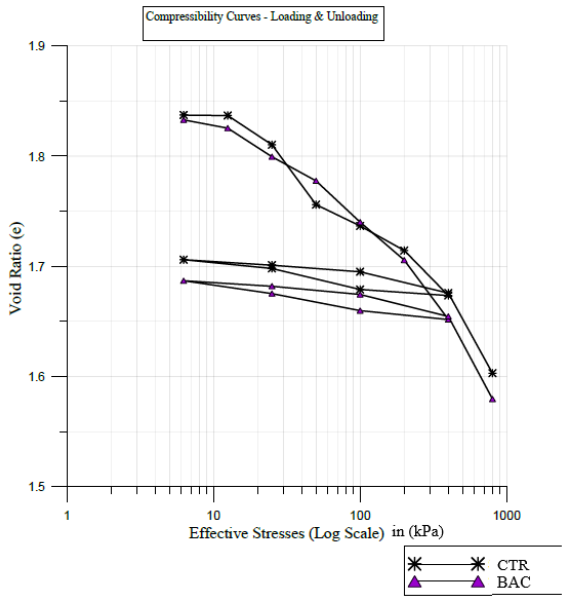


(C) Sample treated with fungi

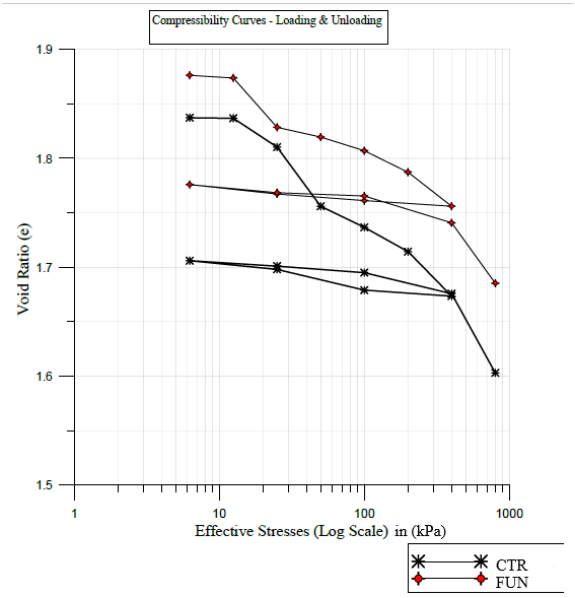


(D) Sample treated with mixed (BAC+FUN)

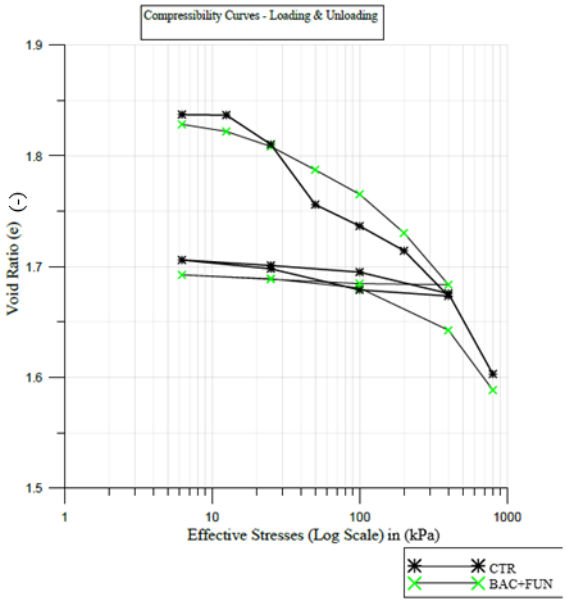
Figure (4. 7): Compressibility Curves



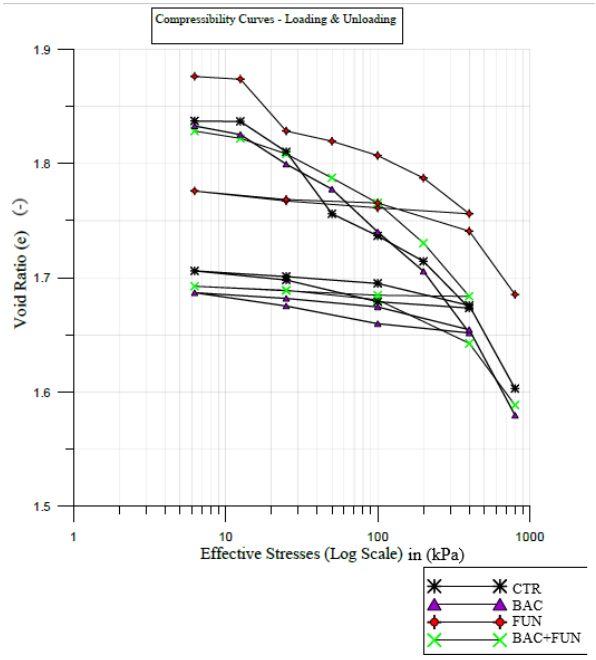
(A) Compressibility curves of untreated and treated with bacteria.



(B) Compressibility curves of untreated and treated with fungi



(C) Compressibility curves of untreated and treated with mixed (BAC+FUN)



(D) Compressibility curves of all sample

Figure (4. 8): Comparison in compressibility curves of the contaminated and treated samples.

Compression index (Cc) of cohesive soils gives a direct indication of its tendency to settle under the applied load. Higher compression index indicates a higher tendency to settle which ultimately leads to structural destress. The variation in compression index of the samples under the influence of contamination and bioremediation are summarized in table (4.4) and depicted in Figure (4.10).

Table (4. 4): Compression index values

	Compression Index Cc						
Applied stress (kPa)	Untreated	Treated with Bacteria (BAC)		Treated with Fungi (FUN)		Treated with Mix (BAC+FUN)	
0	0.009406	0.018833	Increase	0.00438	Decrease	0.039528	Increase
6.25	0.001492	0.024896	Increase	0.008056	Increase	0.021501	Increase
12.5	0.088036	0.08614	Decrease	0.150545	Increase	0.045002	Decrease
25	0.180549	0.072696	Decrease	0.029706	Decrease	0.070004	Decrease
50	0.064162	0.12448	Increase	0.04179	Decrease	0.073004	Increase
100	0.07411	0.114522	Increase	0.065454	Decrease	0.116506	Increase
200	0.135785	0.179251	Increase	0.10372	Decrease	0.155009	Increase
400	0.241726	0.24896	Increase	0.184279	Decrease	0.17951	Decrease
800	0.009406	0.018833	Increase	0.00438	Decrease	0.039528	Increase

As shown in the table above, the compression index Cc has different tendency at different stress levels, whereby the treated samples with bacteria and with the Mix (BAC+FUN) had higher Cc if compared with the untreated sample. In contrast, the sample treated with fungi showed lower Cc than what was noticed for untread sample. The reduction of Cc values indicates that the biological treatment using fungi seems to improve the compressibility behavior of the specified sediments although not to a great extent. Similar results were reported by Canakci et al. study (2015) that used bacteria calcium carbonate to treat soil contaminated with organic matter. Nonetheless, the presence of hydrocarbons contaminants has been found to have a lubricating effect causing a decrease in the particle friction and, eventually, an increase in the compression index Cc.

From figure (4.9) is observable that the compression index curve for sample treated bacteria has a steep climb curve comparing to the other curves, while the sample treated with fungi showed a

smother compression index curve. (CTR, BAC and BAC+FUN) have a sharp increase in Cc. While sample BAC+FUN indicates a lower Cc after 100 kPa trend among all samples. Overall, the figures indicated that the compression indexes for all tested samples have a similar tendency.

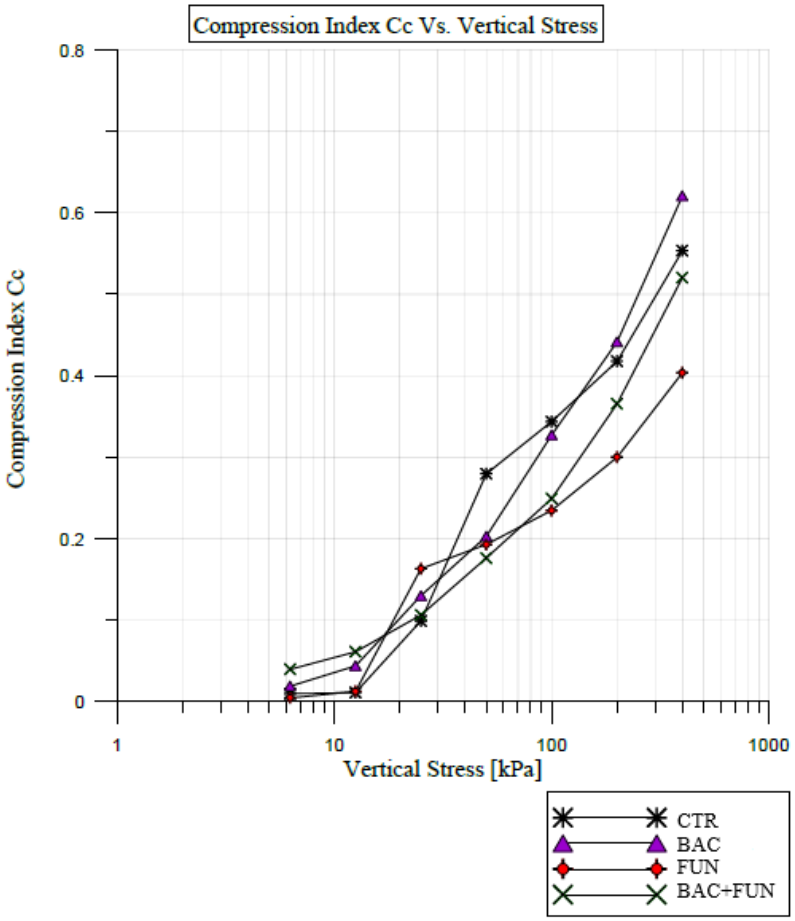
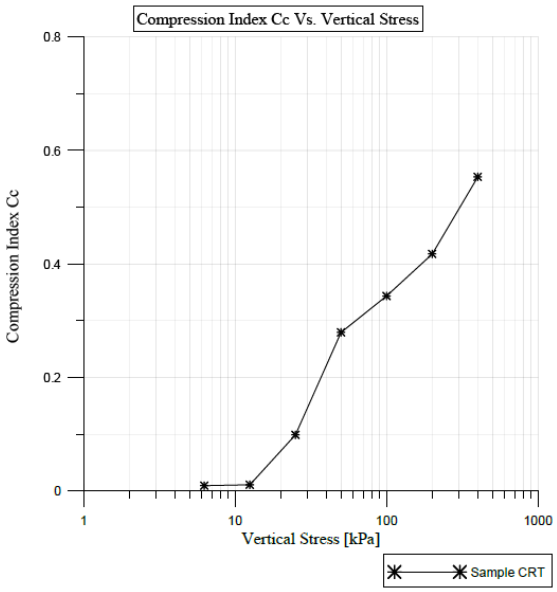
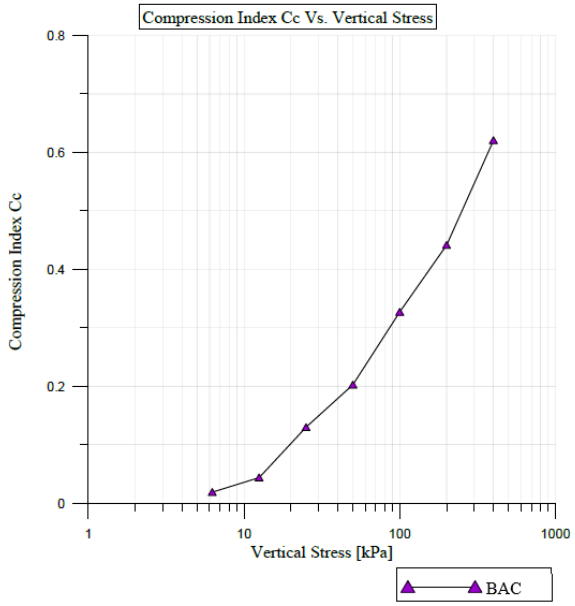


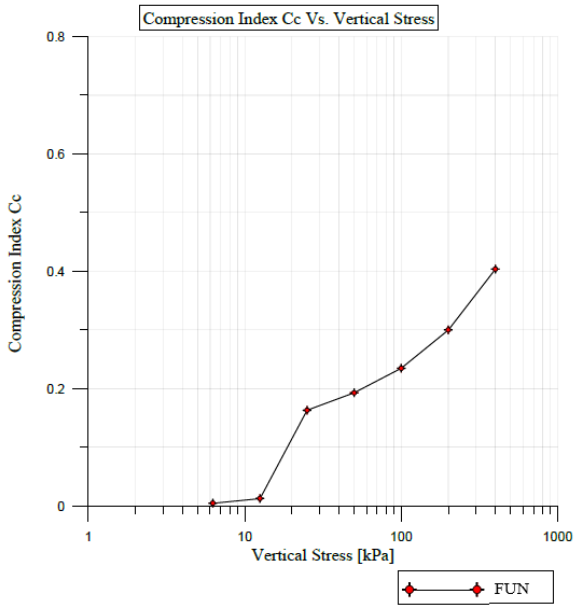
Figure (4. 9): Comparison in compression index for all the tested samples



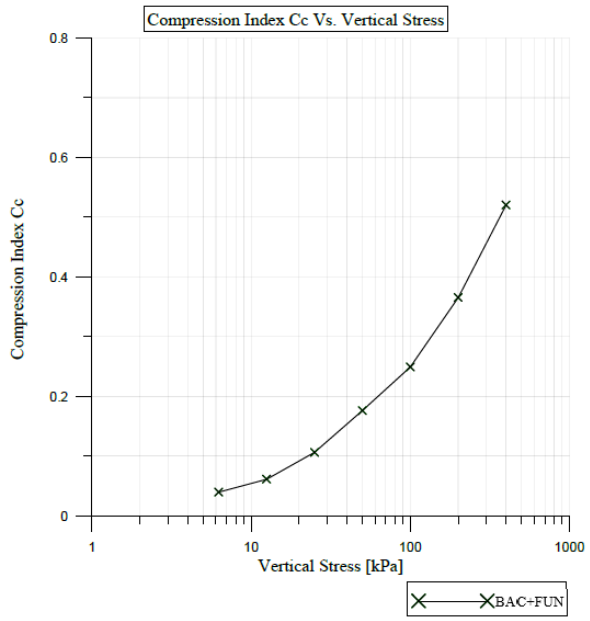
(A) Compression index vs. stress for untreated sample



(B) Compression index vs. stress for treated with bacteria sample



(C) Compression index vs. stress for treated with



(C) Compression index vs. stress for treated with

Figure (4. 10): Compression index for all the tested samples

The coefficient of consolidation (C_v) is one of the most important parameters obtained from the consolidation test, gaining particular importance in the preloading technique for ground improvement (Sridharan and Nagaraj 2012). In the present study, C_v was calculated based on the log time (Taylor) method. Figure (4.11) shows the measured variation in C_v with consolidation pressure. The sample BAC+FUN leads to the highest initial values of C_v. Notably, at initial stage, the untreated sample showed a very low value of C_v, while the treated samples had very high values. However, when the applied stresses increased, the treated samples tended to have lower C_v values. The C_v value of untreated sample showed the lowest value among all other tested samples, similar trend was observed in soil contaminated with crude oil (Ijimdiy and Igboro, 2012). On the contrary, as expected from the previous results, the treated sample with fungi showed the highest values of the coefficient of consolidation for all load ranges.

The available literature shows that C_v is not a constant, but it varies with differences in consolidation pressure. The mechanical and physico-chemical properties of bentonite (e.g., type of bentonite, nature of pore fluids and exchangeable cations) have been suggested to influence the compressibility behavior of the soil (Olson and Mersi, 1970). Furthermore, Sridharan and Jayadeva (1982) stated that the compressibility of soil is affected by the mineral particles as well through the diffuse double layers. On the contrary, for cement-stabilized treatments, the C_v values was found to increase quickly as consolidation pressure applied, then C_v is dropping with maximum pressure (Hebib and Farrell, 2000). Altogether, the opposite trend of correlation between C_v and consolidation pressure can be attributed to the mechanism that controls the compressibility behaviour of the samples i.e. mechanical forces or physico-chemical forces (Sobti and Singh, 2017).

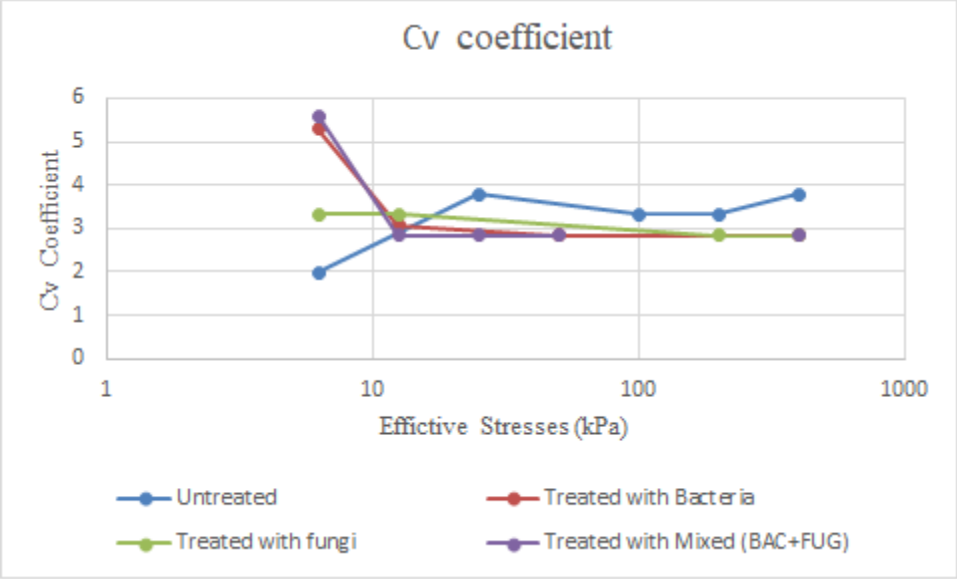


Figure (4. 11): Consolidation coefficient

As the applied normal pressure rises during consolidation, the plastic deformation of the sediment increases, and ultimately leads to markedly climbing values of coefficient of secondary consolidation, Cα. Thus, the plastic deformations are responsible for the rearrangements of the particles in the soil/sediments. The calculations related to Cα are clarified in the appendix.

From Figure (4.12) many remarkable findings should be considered. From 50 kPa onwards, we noted that the Cα constantly increased. In the treated samples with fungi only, Cα showed the lowest climbing slope, but then revealed an improvement in the secondary consolidation as compared to the untreaded sample. In the samples treated with bacteria only or treated with mixed microorganisms (BAC+FUN), Cα was constantly greater than the untreated sample.

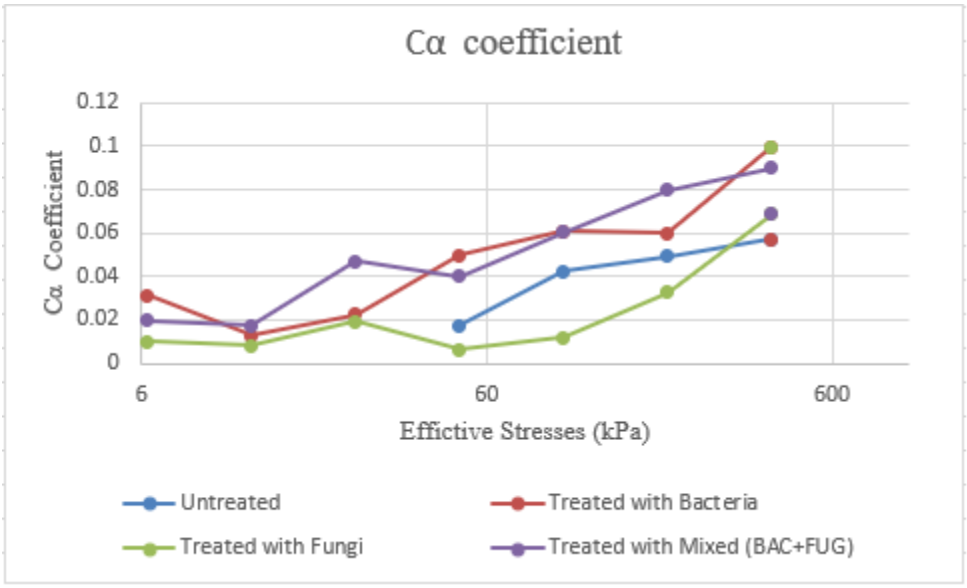


Figure (4. 12): Secondary consolidation coefficient

4.4 Hydraulic Conductivity

One of the most important properties of the soil is its permeability, as it provides a clear insight about seepage, stability and settlement of the soil. There are many factors capable to affect soil permeability, such as the shape and size of the particles, void ratio, and physicochemical properties of the permeated liquids.

Permeability tests were carried out on contaminated and biotreated soil samples (after 28 days of treatment) to study the hydraulic conductivity and the rigid wall permeameter was equipped with two bladder accumulators:

- The inlet bladder in order to permeate the sample with seawater.
- The outlet bladder in order to collect sample of the effluent to be analyzed for inorganic compounds with the aim to assess the leaching capability of the contaminated and treated samples.

Samples were saturated by allowing seawater to enter in the sample from the influent bladder. Saturation of the soil sample was ensured under steady state flow conditions as well.

At the beginning, a confining pressure of 20 psi was applied at the sample cell, while the applied pressures were 19 psi at both inflow and outflow. In this case, we called the difference between inflow and outflow pressure Zero pressure, because the only hydraulic gradient was realized by the difference in the head of the filtering burettes. Later on, only the outflow pressure was reduced gradually to 17 and to 15 psi, and we designated them as 2 and 4 psi pressure stages, respectively.

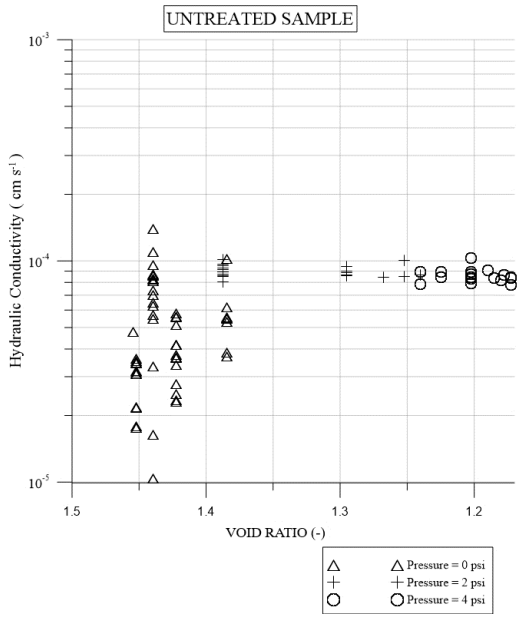
In this study, ASTM D5856 Standard Test Method for Measurement of Hydraulic Conductivity Using a Rigid-Wall was followed, where the hydraulic conductivity values were calculated using the following equation.

$$k = \frac{aL}{At}\, ln\left(\frac{h_1}{h_2}\right)$$

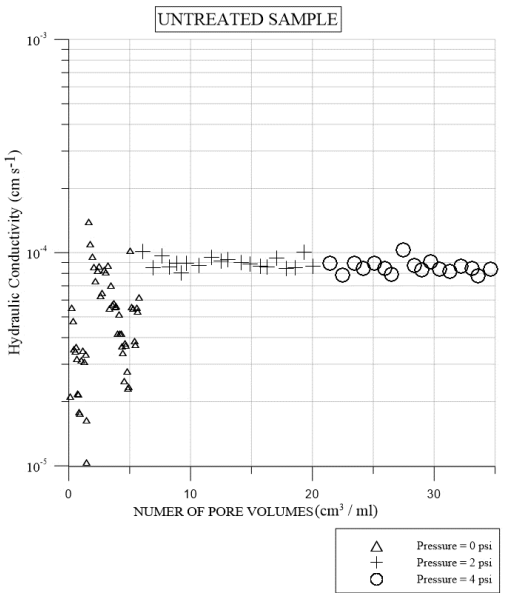
The results of the permeability test with fallen head on both contaminated and biotreated sediments were shown as hydraulic conductivity, k, in figures from 4.13 to 4.16. It is noticeable that at zero psi pressure, the hydraulic conductivity values randomly fluctuated, and this might be attributed to the idea that the zero psi pressure couldn't provide an effective confinement condition for the sample. Overall, we noticed that with the increase in pressures (e.g., 2 and 4 psi), more stable values of hydraulic conductivity coefficient were obtained. Therefore, and to provide a clear comparison, only the values of hydraulic conductivity at 2 and 4 psi were considered for the comments.

In k-e plots shown in Figures (4.13,14,15 and 16) the hydraulic conductivity values as a function of void ratio can be observed. The results indicate that at 0 psi pressure, the hydraulic conductivity

values are not a stable value at the same void ratio. The results obtained from these tests seem to be consistent with studies in literature. One example is Yu and Li (2004) study that investigated the void ratio and the stress level on clay hydraulic conductivity in contact with pore fluid. The authors found that a variation in permeability at the same void ratio could be explained as a result of particle re-arrangement. Additionally, in the same graphs, the relationship between hydraulic conductivity and NPV was reported.

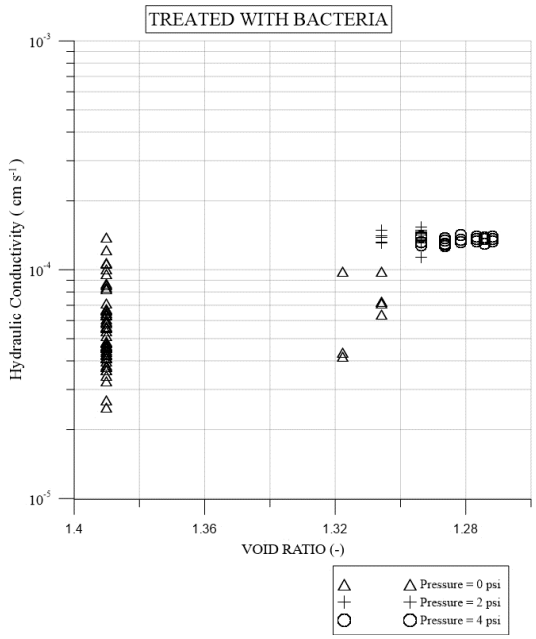


(A) k vs. Void Ratio

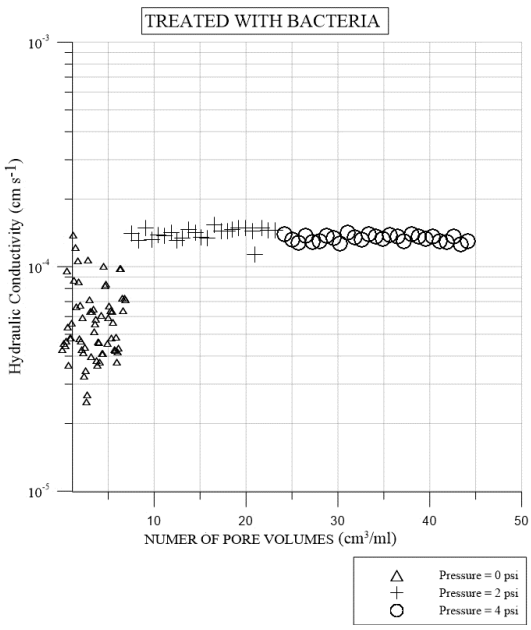


(B) k vs. Number of Pore Volumes

Figure (4. 13): The hydraulic conductivity of Untreated sample.

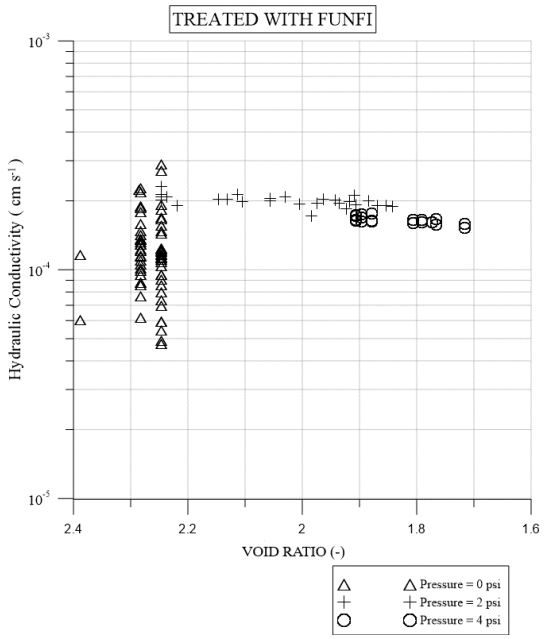


(A) k vs. Void Ration

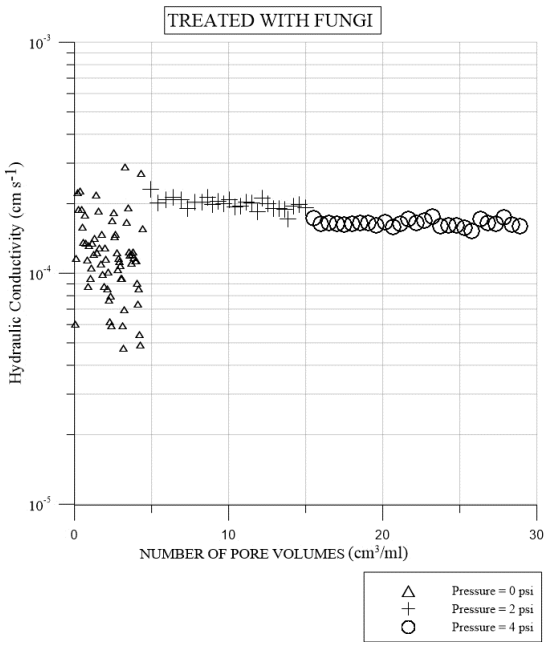


(B) k vs. Number of Pore Volumes

Figure (4. 14): Hydraulic conductivity of sample treated with bacteria,

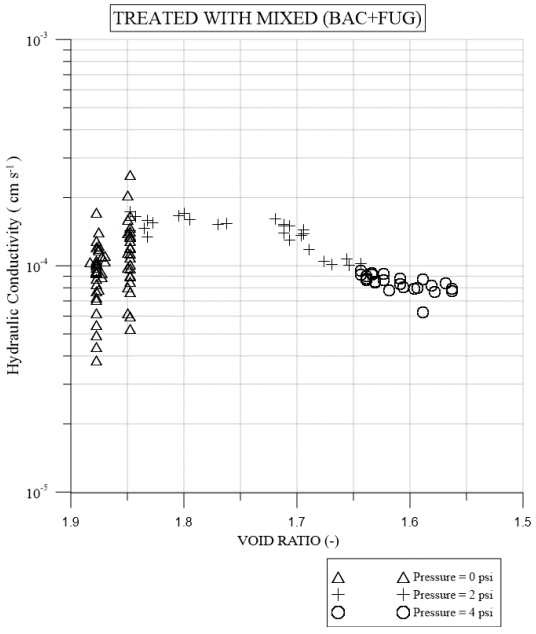


(A) k vs. Void Ratio

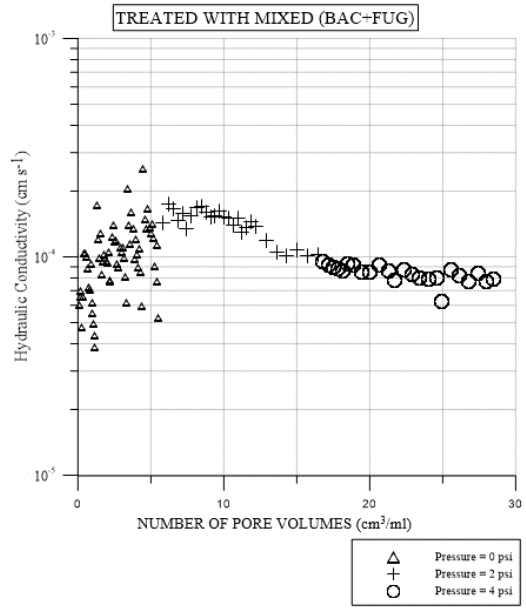


(B) k vs. Number of Pore Volumes

Figure (4. 15): Hydraulic conductivity of sample treated with fungi.



(A) k vs. Void Ratio



(B) k vs. Number of Pore Volumes

Figure (4. 16): Hydraulic conductivity of sample treated with both bacteria and fungi.,

The averaged values of hydraulic conductivity coefficients for each pressure step were determined for the contaminated and the treated samples and presented in Table (4.5) and in Figure (4.15). Obviously, the hydraulic conductivity values decreased when increasing the effective confining pressure. The increase in the confinement pressure to 4 psi caused a rearrangement of particles which consequently decreased the hydraulic conductivity.

Even though, reached the pressure stage of 4psi changes in hydraulic conductivity values among the four tested samples are not significant The hydraulic conductivity of both contaminated and biotreated samples are within the same order of magnitude and similar: at the pressure stage of 4 psi the minimum value was 8.4E-05, registered for the sample treated with the mix BAC+FUN, and the maximum value is 1.6E-04, registered for the sample treated with fungi (FUN). It is worth noticing that the initial void ratio is not the same for the tested samples.

Table (4. 5): Averaged hydraulic conductivities from direct test (fallen head test)

	Hydraulic conductivity (cm/s)			
Sample name / Pressure (psi)	CTR	BAC	FUN	BAC+FUN
Zero psi	5.0E-05	6.0E-05	1.3E-04	1.0E-04
Two psi	9.0E-05	1.4E-04	2.0E-04	1.4E-04
Four psi	8.6E-05	1.3E-04	1.6E-04	8.4E-05

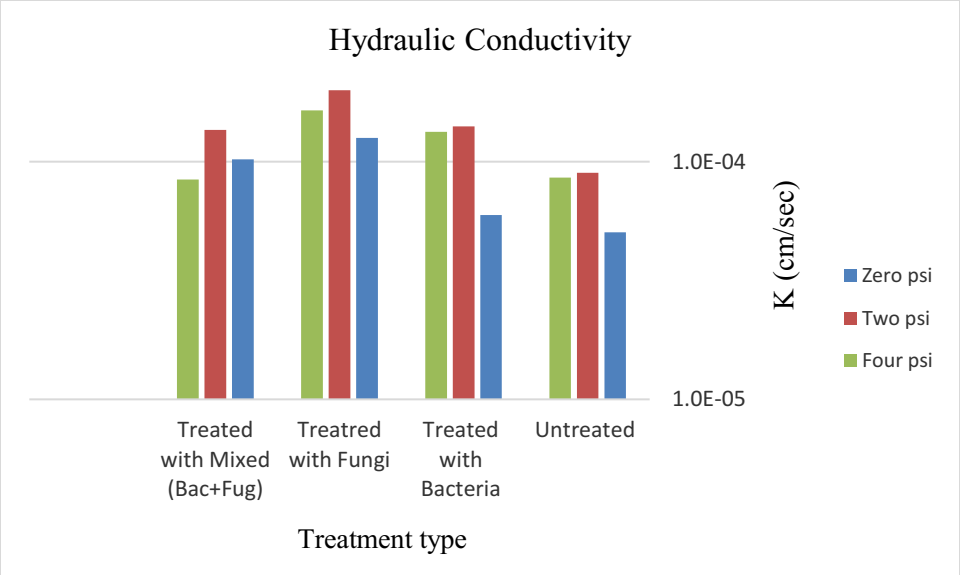


Figure (4. 17): Comparison between average hydraulic conductivity and confinement pressures

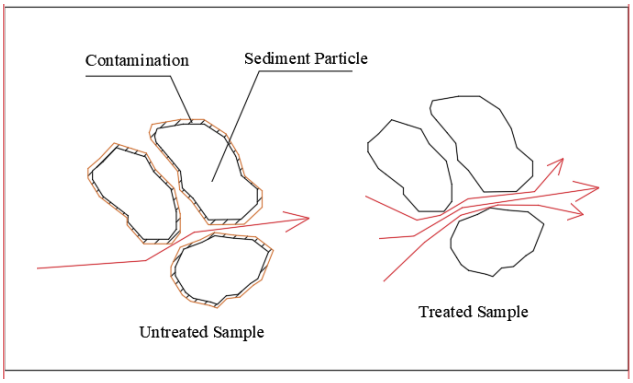


Figure (4. 18): The effect of desorption of contaminations on hydraulic conductivity.

In the literature, Al-Sanad, Eid, and Ismael (1995) observed a constant decrease in the permeability of poorly-graded sand due to oil contamination. Likewise, Meegoda and Rajapakse (1993) explored the variation in hydraulic conductivity of saturated clays at both short-term and long-term exposure to organic fluids. While no changes in the permeability for short-term exposure was found, long-term exposure resulted in an increase in the intrinsic permeability, in particular. Hence, the study concluded that the type, the amount and the viscosity of chemicals in pore fluids affect the compressibility of contaminated soils.

Further, Khamehchiyan and co-authors (2007) studied the permeability of soil with oil contamination, and found a decrease in the permeability of poorly-graded sand and silty sand. They justified the decrease in the permeability coefficient of the sandy sample as a consequence of the reduction in volume of soil porosity due to the presence of crude oil. Similarly, Singh et al. (2009) study investigated that the permeability of both low plasticity clay (CL) and high plasticity clay (CH) in the presence of 9% of oil contamination. It has been found that the permeability of CL increased from 2.26 × 10−8 to 2.87 × 10−8 m/s, while in CH the permeability increased from 2.86 × 10−10 to 4.46 × 10−10 m/s. In line with that, Chew and Lee (2010) investigated the effect of palm biodiesel on permeability of poorly graded sandy soil using the constant head test. They have reported that the soil permeability decreases when the oil content increases. The reason beyond that was the soil pores were filled with palm biodiesel which strongly limited the water flow. Later on, Akinwumi et al. (2014a) studied the effect of engine oil with different percentage (0, 2, 4, 6, 8, and 10%). They found that the permeability of clayey soil reduced from 8.24 × 10−6 to 5.2 × 10−6 cm/s when content of oil was increasing from 0 to 10 wt%.

Table (4.6) in below summarizes the effect of hydrocarbons contamination on the permeability of different soils. The data revealed that the permeability values mainly depends on soil and contaminant types (Khodary et al., 2018).

Table (4. 6):Effect of hydrocarbons contamination on permeability (Adopted from Khodary et al., 2018)

Reference	Soil type	Oil type	K values		Mechanism
			Natural soil	Contaminated soil	
Nazir (2011)	Clay	Motor oil		K increased three times	The K value increased only for contaminated clay soil due to (1) the contraction of the double layers and enlargement of clay pores and (2) the reduction of dielectric constant
Singh et al. (2009)	CL	Used motor oil (9%)	2.26 × 10−8 m/s	2.87 × 10−8 m/s	
	CH		2.86 × 10−10 m/s	4.46 × 10−10 m/s	

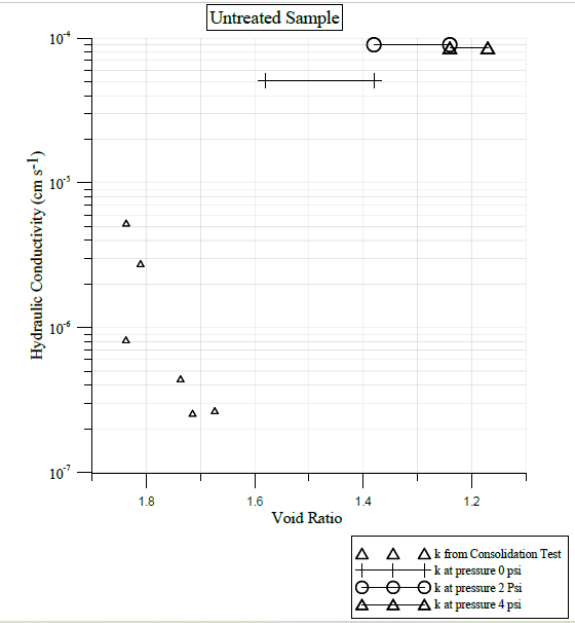
					of oil which decreases the thickness of DDL and the soil structure tends to be flocculated resulting an increase of K value of the contaminated soil
Al-Sanad et al. (1995)	SP	Heavy crude oil (6%)	1.72 × 10−5 m/s	1.38 × 10−5 m/s	The K value was decreased for sand and lateritic clay soil due to the reduction of pore volume and the increasing of the kinematic viscosity of oil
Shin and Das (2000)	SP	Engine oil, Oman crude oil, and lamp oil		K decreased by a value of 75%	
Rojas et al. (2003)	SM, ML, and CL	Gear, engine, and crude oils		K decreased by values of 24 and 98%	
Chew and Lee (2010)	SP	Palm biodiesel		K decreased by a value of 37%	
Akinwumi et al. (2014a)	Lateritic clay	Crude oil (10%)	8.24 × 10−6 cm/s	0.9 × 10−6 cm/s	

4.5 Comparison between permeability obtained from oedometric and falling head tests

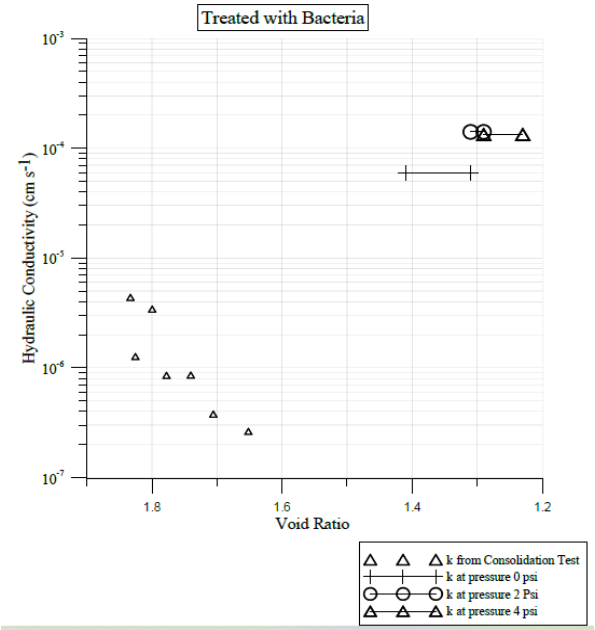
The hydraulic conductivity is usually measured by two approaches using either the constant-head or falling-head. The former is more convenient for coarse-grained soils, while the latter is recommended for fine-grained soils and is independent of the hydraulic gradient (i), making effective the calculation of k based on Darcy's law (Assaad and Harb, 2013). Regarding the gravelly soils, because of the presence of oversized gravel particles with a wide range in size between 2 mm and 20 mm, special sampling tools and large-scale testing apparatus are required to measure the hydraulic conductivity.

The hydraulic conductivity data versus void ratios of the contaminated soil calculated from the oedometric test are presented in the figures below together with those determined by the permeability tests. It can be observed in figure (4.19) that the hydraulic conductivity values obtained from the oedometric tests were lower than values from the falling head test. The hydraulic conductivity values determined from the oedometric tests range from to 9 x 10^{-7} (cm/s) to5x 10^{-5} (cm/s) for void ratios varying from 1.66 to 1.88. Values of hydraulic conductivity determined by

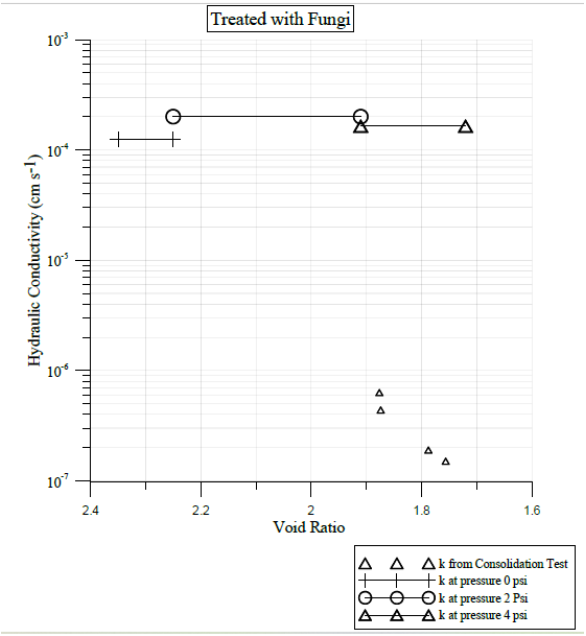
permeability tests are about 1 x 10^{-4} (cm/s). These great differences in hydraulic conductivity values obtained from consolidation tests and permeability tests (about 2 orders of magnitude) allow us to state that the hydraulic conductivity derived from consolidation test are not suitable for this type of materials.



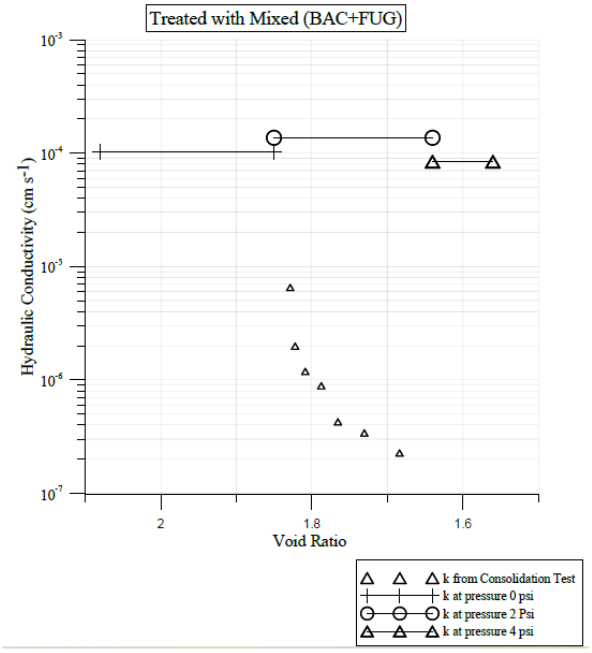
(A) Hydraulic conductivity from Odeometer and falling head test for contaminated



(B) Hydraulic conductivity from Odeometer and falling head test for treated sample with Bacteria.



(C) Hydraulic conductivity from Odeometer and falling head test for treated sample with Fungi.



(D) Hydraulic conductivity from Odeometer and falling head test for treated sample with mixed (BAC+ FUG).

Figure (4. 19): Comparison between hydraulic conductivity from oedometric tests and permeability tests for all samples.

4.6 Heavy Metals Analysis

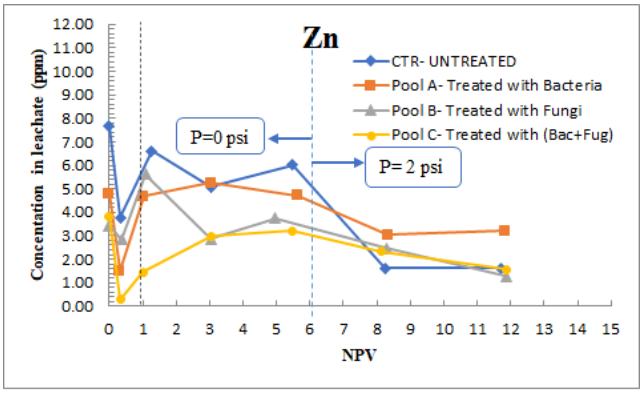
4.6.1 Effluent leachate composition

Leaching the liquids from the contaminated sites which contains various heavy metals can contaminate the soil beneath the secondary source of contamination, groundwater or surface water. Many studies have investigated the health problems associated with contaminated site. It has been reported that leachate containing heavy metals poses serious threats through the contaminated food chain. The levels of contamination of metals that are leached from solid could be determined by various leaching tests, such as the Toxicity Characteristic Leaching Procedure (TCLP) (USEPA Method 1311), the Synthetic Precipitation-Leaching Procedure, or SPLP test (USEPA Method 1312). In case of TCLP, a solution of high buffer but mild acidity condition (i.e., acetic acid) is required to separate metals from solids, while a dilute and unbuffered solution of sulfuric and nitric acid can be used in SPLP.

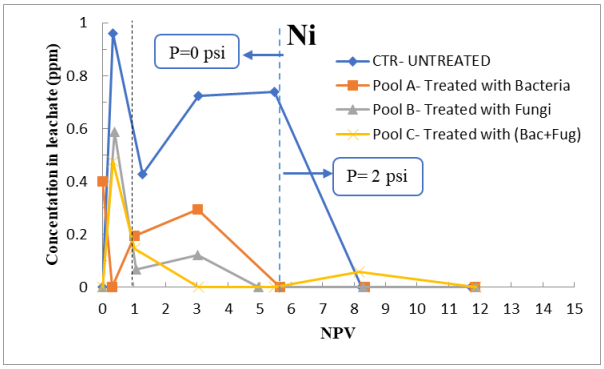
Another purpose of this study was to detect the metals that can leach from the sample of the sediments from Bagnoli harbor, therefore the leachate that accumulated in outlet blender in hydraulic conductivity test was analysed for inorganic compounds. Then, in SIMAU department, the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) test was performed. Many elements were detected but only Ni, Zn and Mn are the metal present with significant concentration values.

Similar studies have investigated Bagnoli site and shown the concentrations of Fe, Mn and Pb were considerably high (Damiani et al., 1987). Additionally, Romano et, al., (2009) reported that Ni, Pb, Zn and PAHs have exceeded by several times the reference values, indicating high degree of diffused contamination, with the highest concentrations in the stations located close to the industrial plant.

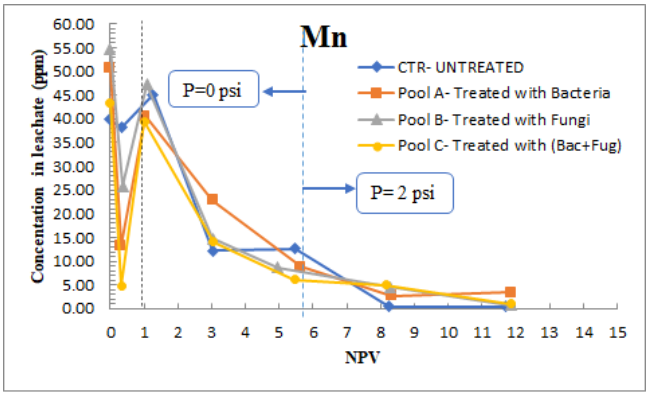
Results below illustrate the concentration of each metal in the effluent fluid from column test as a function of the Number of Pore Volume (NPV). In fact, Pore Volume is defined as the total volume of pores in a bed of sediment particles. Hence, NPV refers to the number of times that void volume are full of liquid. Due to its definition, the first pore volume should not be considered in evaluating the leaching capability of the sediments because it surely contains a significant portion of the contact water (i.e., the water already presents in the pores of the sample) that would be displaced by the influent seawater.



B) Concentrations of leached Zinc.

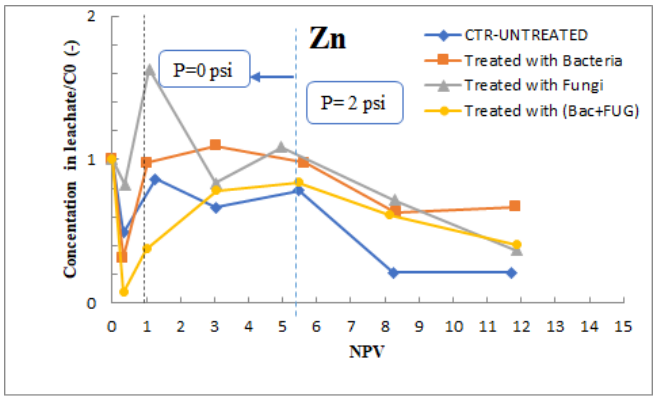


A) Concentrations of leached Nickel

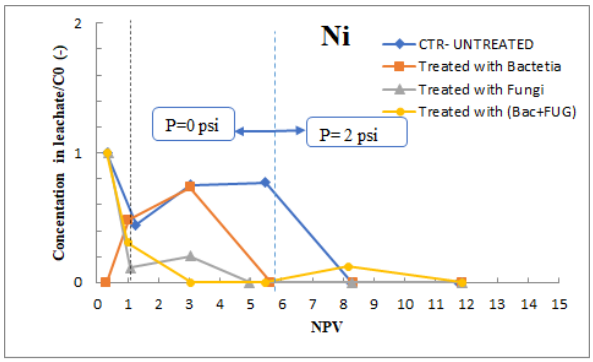


C) Concentrations of leached Manganese.

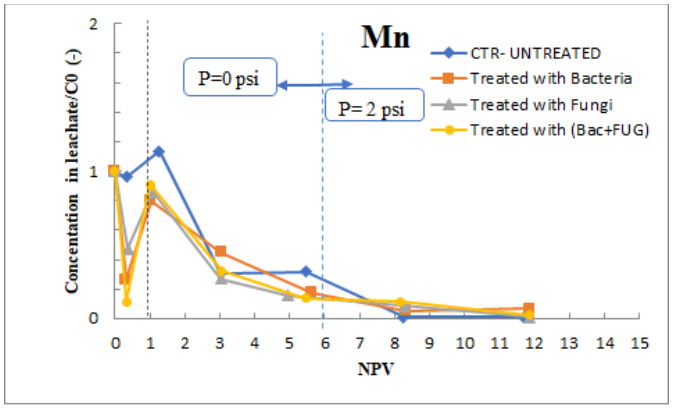
Figure (4. 20): Leached concentrations of a) Zinc, b) Nickle and c) Manganese



A) Normalized concentrations of leached Zinc.



B) Normalized concentrations of leached Nickle.



c) Normalized concentrations of leached Manganese.

Figure (4. 21): Normalized leachate concentrations of a) Zinc, b) Nickle and c) Manganese

The graphs in figures (4.20) and (4.21) show the concentration and the normalized concentrations of Ni, Zn and Mn in each sample with respect to initial concentration. It is clear that both the concentrations of Zn and Mn have similar trends. For the treated samples with microorganisms, we observed an increase in the leachability of Zinc in comparison to untreated samples. On the other hand, the graph (4.20 b) shows a remarked difference in leached of Ni for the examined samples, for which the untreated samples revealed the highest nickel concentration in the leachate.

In figure (4.20 c), Mn showed a uniform correlation with NPV, thus, in the control sample, the Mn concentration in leachate was 45 μg/L (the highest level during whole test period) at 1 NPV. This level was four times greater than the national limit of groundwater quality of Italy (10 μg/L) (D.Lgs 02 febbraio 2001, n.31). The concentration at 6 NPV reduced to 10 μg/L which is the national limit.

The leached mass of heavy metals can be calculated using the following:

Leched mass = TEM- IM

Where:
TEM= Total Effluent Mass

IM= Initial mass present in the pore volume

It should be noted that TEM can be calculated using:

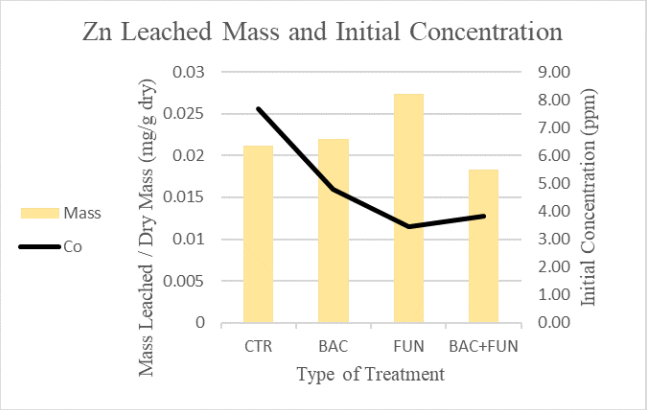
TEM = Averaged NPV* Void Volume* Concentration in the leachate

IM= Void Volume * Initial Concentration (i.e., concentration in the contact-pore water)

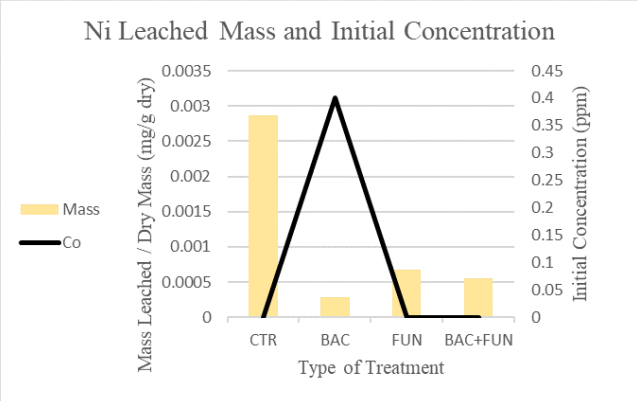
Table (4.7) illustrates the mass balance of Zinc for the untreated sample as an example. The rest of the calculations are attached in the appendix.

Table (4.7): Mass balance calculation

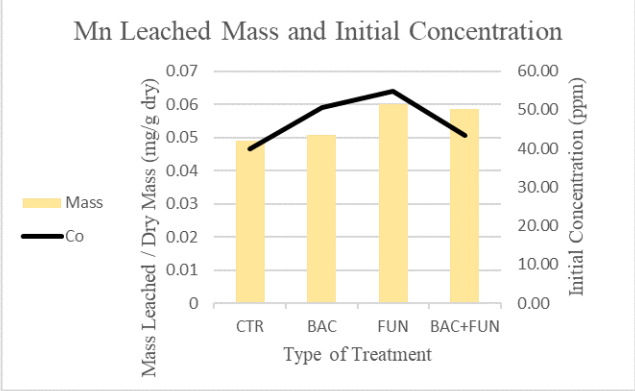
	NPV	Age. NPV	Zn conce.	TEM	IM	Leached Mass
CTR	0		7.659581	0	0.484385	2.241986843
CTR2	0.337	0.16843	3.77	0		
CTR5	1.263	0.79993	6.61	0.564543		
CTR11	3.040	2.1515	5.08	0.676733		
CTR19	5.474	4.256789	6.01	0.990074		
CTR23	8.250	6.861789	1.61	0.318181		
CTR30	11.724	9.986879	1.61	0.17684		
				2.726372		



A) Normalized leached mass of Zinc.



Normalized leached mass of Nickle.



Normalized leached mass of Manganese.

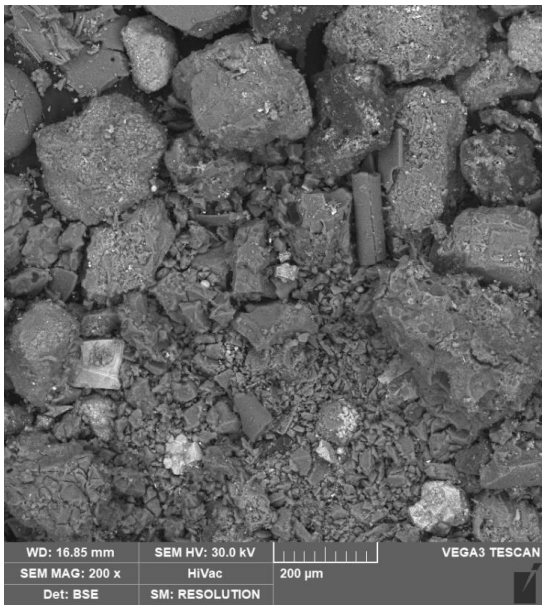
Figure (4. 22): Normalized leached mass with initial concentration for : a) Zinc, b) Nickel and c) Manganese.

In Figure (4.22) the initial concentrations and normalized mass leached with respect to dry masses for CTR, BAC, FUN and BAC+FUN are presented. It can be noticed that Mn showed a correlation between initial concentration and normalized masses and no significant differences can be noticed in the leaching capability among the four tested samples. Also, Zn doesn't show significant difference in the leached mass among the four samples. On the contrary, Ni showed a different tendency: the mass leached by the untreated sample is one order of magnitude higher than that leached in the treated samples, in this case, seems that the treatment is able to limit the release of this compound, especially that using bacteria.

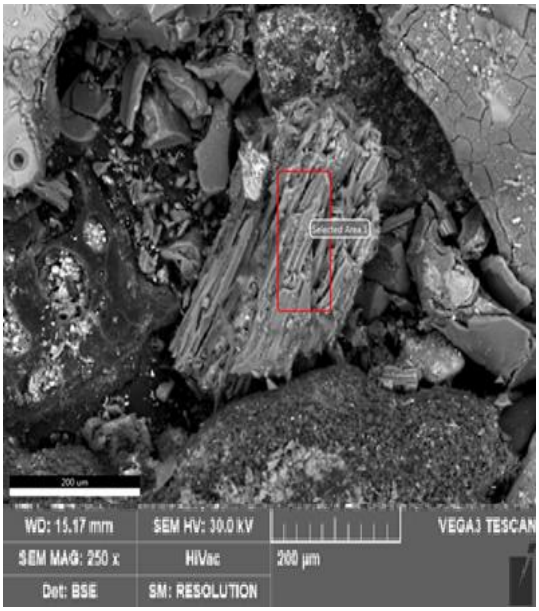
4.3.2 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS)

The Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-Ray Spectroscopy (EDS) test was performed in SIMAU department. The samples derived from both contaminated (untreated) sediments and sediments treated with BAC+FUN were observed and analyzed: SEM images and spectra are reported in figures from 4.23 to figure 4.28 together with composition in term of weight percentage.

- CTR Sample



A

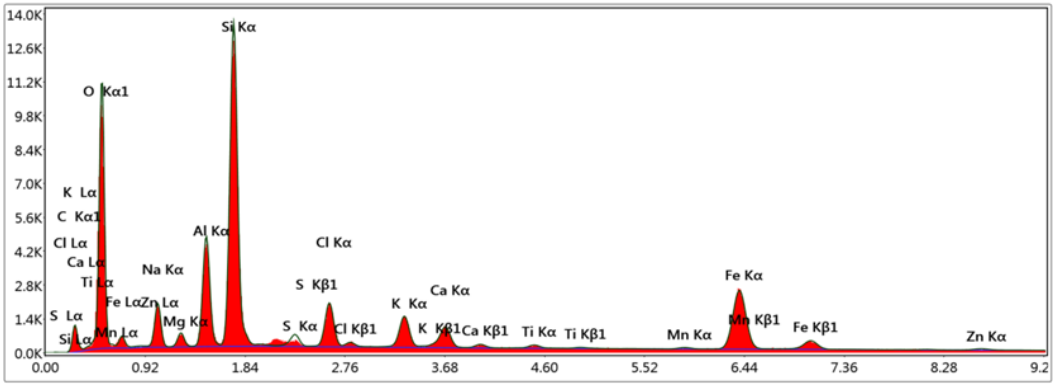


B

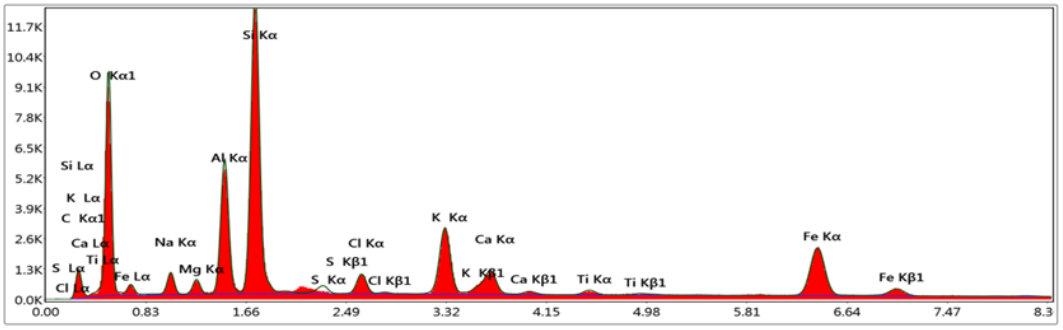
C)

D)

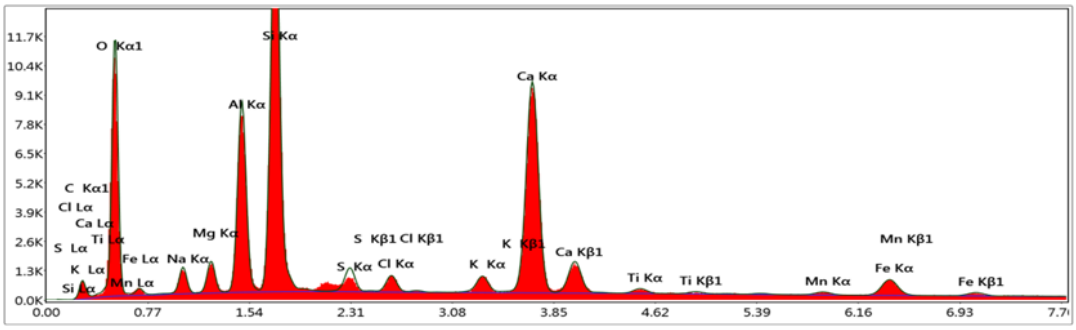
Figure (4. 23): SEM images for a)CTR sample , B) CTR sample for area 1 , c) CTR sample for area 2, , and D)CTR sample for area 3



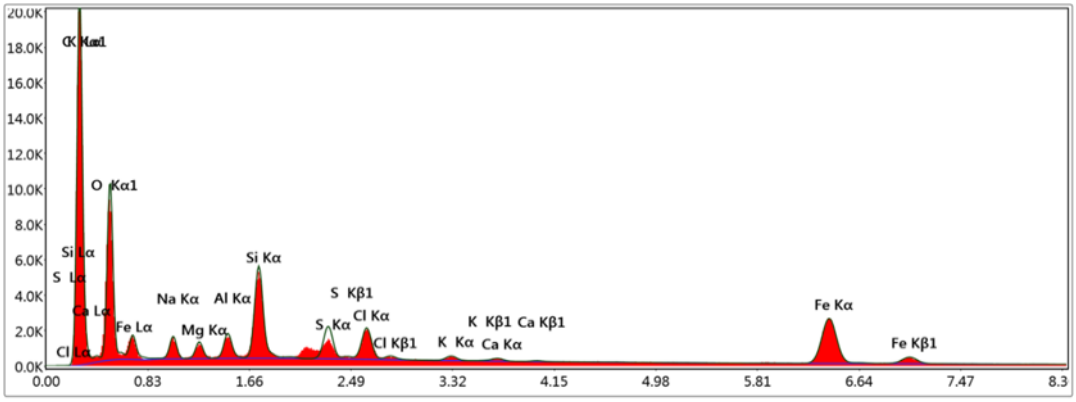
A)



B



C



D

Figure (4. 24): Spectra of elements of a) CTR sample, B) CTR sample for area 1 , c) CTR sample for area 2, and D) CTR sample for area 3.

CTR 1

Element	Weight %	Atomic %	Error %
C	10.87	17.6	10.94
O	43.91	53.37	8.93
Na	6.31	5.34	9.86
Mg	1.18	0.94	10.75
Al	6.89	4.97	7.16
Si	17.03	11.79	6.07
S	0.66	0.4	11.35
Cl	2.62	1.44	5.11
K	1.9	0.95	4.76
Ca	1.42	0.69	5.12
Ti	0.24	0.1	18.8
Mn	0.14	0.05	34.62
Fe	6.6	2.3	1.98
Zn	0.24	0.07	12.93

A

CTR 2

Element	Weight %	Atomic %	Error %
C	11.79	19	10.64
O	43.9	53.11	9.15
Na	3.41	2.87	10.99
Mg	1.27	1.01	9.84
Al	8.59	6.17	6.78
Si	16.94	11.67	6.04
S	0.44	0.27	14.45
Cl	1.28	0.7	7.26
K	4.35	2.15	3.54
Ca	1.76	0.85	4.77
Ti	0.39	0.16	11.3
Fe	5.87	2.04	2.06

B

Element	Weight %	Atomic %	Error %
C K	5.64	9.57	11.49
O K	45.15	57.5	9.32
NaK	3.12	2.76	10.58
MgK	2.06	1.73	9.15
AlK	9.39	7.09	6.58
SiK	17.52	12.71	6.01
S K	1.19	0.76	9.39
ClK	0.84	0.48	8.79
K K	0.81	0.42	8.28
CaK	12.18	6.19	2.26
TiK	0.32	0.14	11.53
MnK	0.26	0.1	20.7
FeK	1.5	0.55	3.99

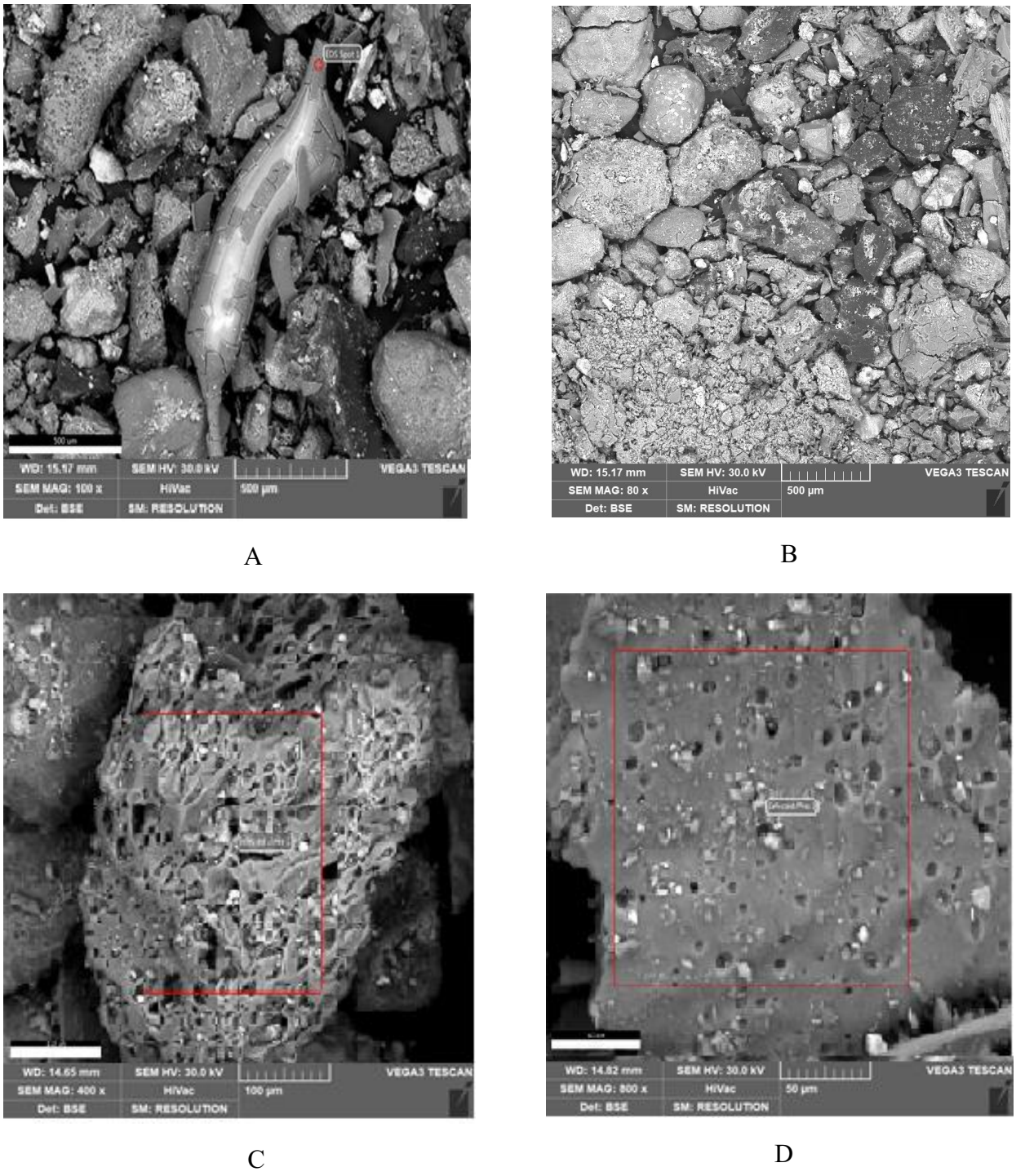
C

Element	Weight %	Atomic %	Error %
C K	55.07	65.26	7.22
O K	33.11	29.46	9.96
NaK	2.1	1.3	10.52
MgK	0.82	0.48	9.34
AlK	0.9	0.47	7.52
SiK	2.56	1.3	4.86
S K	0.91	0.4	3.73
ClK	0.98	0.39	3.43
K K	0.15	0.06	11.62
CaK	0.11	0.04	21.58
FeK	3.3	0.84	2.62

D

Figure (4. 25): Quantification of elements of a) CTR sample , B) CTR sample for area 1 , c) CTR sample for area 2, and D) CTR sample for area 3.

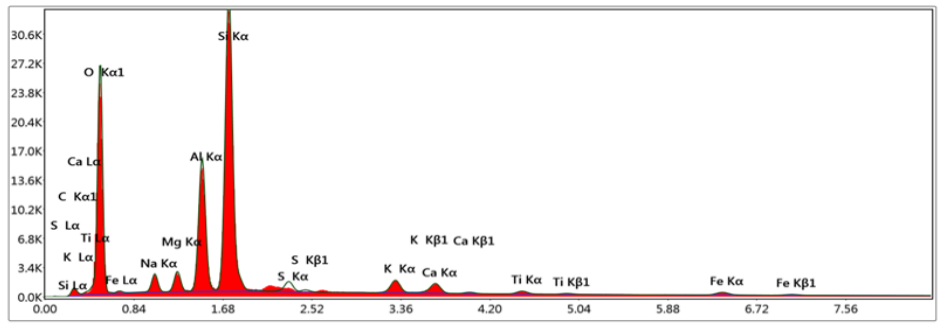
- Sample Treated with BAC+FUN



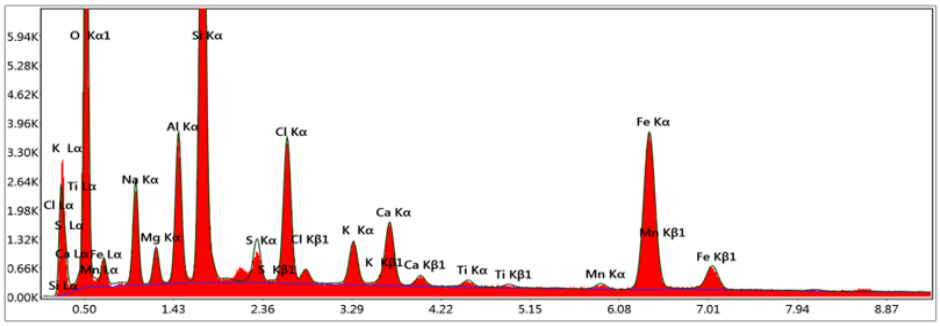
A B

C D

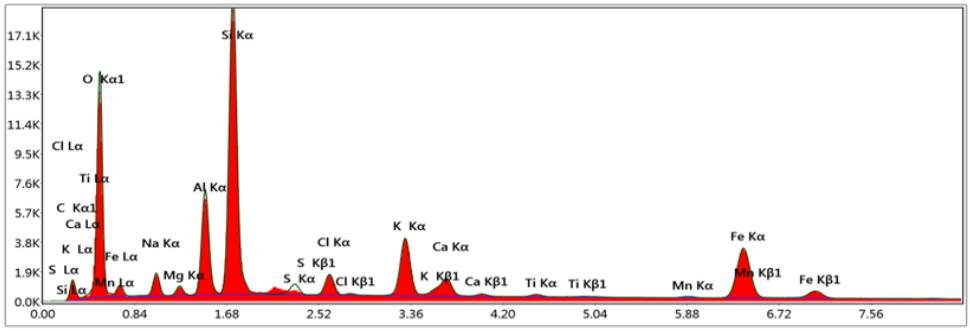
Figure (4. 26): SEM images of a) BAC+FUN sample, B) BAC+FUN sample for area 1 , c) BAC+FUN sample for area 2, and D) BAC+FUN sample for area 3



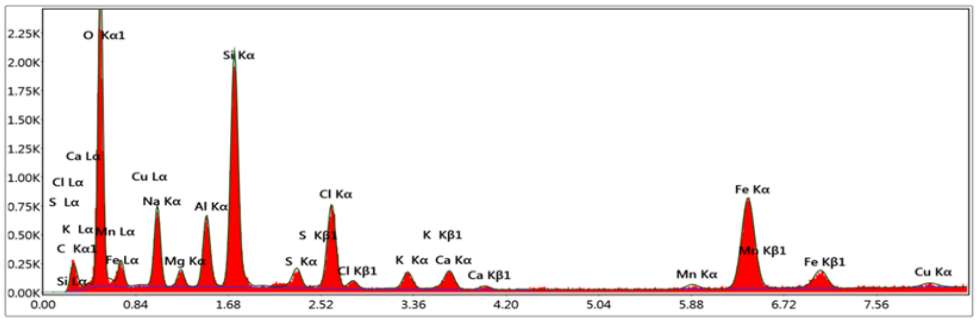
A



B



C



D

Figure (4. 27): Spectra of elements of a) BAC+FUN sample, B) BAC+FUN sample for area 1, c) BAC+FUN sample for area 2, and D) BAC+FUN sample for area 3.

Element	Weight %	Atomic %	Error %
C K	4.77	7.7	12.04
O K	50.14	60.82	8.37
NaK	3.56	3	9.79
MgK	2.24	1.79	8.16
AlK	11.26	8.1	6.17
SiK	24	16.58	5.88
S K	1	0.61	8.24
K K	1.22	0.6	5.5
CaK	1.05	0.51	5.09
TiK	0.33	0.13	8.38
FeK	0.44	0.15	6.97

A

Element	Weight %	Atomic %	Error %
O K	40.75	57.18	8.79
NaK	9.63	9.4	9.78
MgK	2.13	1.96	10.23
AlK	6.47	5.39	7.75
SiK	17.74	14.18	6.52
S K	1.63	1.14	9.56
ClK	5.59	3.54	5.08
K K	1.7	0.98	6.41
CaK	2.75	1.54	4.3
TiK	0.33	0.15	13.51
MnK	0.32	0.13	22.35
FeK	10.96	4.4	1.73

B

Element	Weight %	Atomic %	Error %
C K	9.18	15.08	10.9
O K	45.29	55.82	8.88
NaK	3.95	3.39	10.44
MgK	0.92	0.74	11.14
AlK	7.45	5.45	6.86
SiK	18.58	13.04	5.92
S K	0.7	0.43	10.87
ClK	1.48	0.82	6.73
K K	4.12	2.08	3.56
CaK	1.41	0.69	5.4
TiK	0.21	0.09	21.7
MnK	0.16	0.06	26.09
FeK	6.54	2.31	1.82

C

Element	Weight %	Atomic %	Error %
C K	10.95	17.9	13.01
O K	42.57	52.23	9.22
NaK	11.15	9.52	10.56
MgK	1.5	1.21	13.51
AlK	4.61	3.36	9.11
SiK	11.86	8.29	6.97
S K	0.95	0.58	14.17
ClK	4.38	2.43	5.32
K K	0.91	0.46	10.77
CaK	1.1	0.54	7.97
MnK	0.38	0.14	26.36
FeK	9.08	3.19	2.52
CuK	0.55	0.17	23.08

D

Figure (4. 28): Quantification of elements a) BAC+FUN sample, B) BAC+FUN sample for area 1, c) BAC+FUN sample for area 2, and D) BAC+FUN sample for area 3.

The morphological and microstructural features of contaminated sediments and biological treated sediments were characterized by SEM. From the SEM image (4.23) of contaminated sediment, it can be seen that the sample is composed by grains of 200- 500μm in size. According to the tables figure (4.25), EDS quantitative microanalysis indicated the presence of Si, Ti, Mg, Al, Ca, Fe, K,O, and C in the contaminated sediments. The EDS for the remediated sediments showed Si, Al, C, and O as main components.

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Chapter Five: Conclusions and Future Developments

In the present thesis, the contaminated marine sediments from Bagnoli harbor have been subjected to an experimental study including biological treatments and analyses, geotechnical investigations, chemical analyses and scanning electron microscopy observations. Both the untreated conditions and the effects of three different biological remediation treatments were evaluated.

In the marine biology laboratory (DISVA Department), the sample was experimentally treated using different microorganisms (i.e., bacteria only, fungi only and a mix of bacteria and fungi) and the samples were kept under monitoring for 87 days. In order to evaluate the biological activities, temperature, pH, DNA extraction and abundancy were monitored. It was observed that the performance of the sediments sample treated with the mix of bacteria and fungi showed the highest degradation comparing to the other samples during all the monitoring periods. Moreover, during the treatment time, it has been observed a significant reduction in concentration especially of hydrocarbons (LMW-PAH and HMW-PAH). On contrary, the biological treatment using (BAC+FUN) does not affect or increase the bioavailability of metals, so the mobility of heavy metals is unpredictable.

In parallel with biological investigations, geotechnical characteristics of the sediments has been evaluated in the environmental geotechnics laboratory (SIMAU department). We evaluated compressibility and hydraulic conductivity of untreated sediments and those of the sediments after 28 days of treatment in order to: (1) assess the effects of the biological treatments and the related performances in a permeable system of capping of the sediments in the seabed (2) evaluate the possible reuse of the treated sediment in different geotechnical applications such as, earthworks (e.g. road embankments) or land reclamation by coastal hydraulic fills.

In the one-dimensional consolidation test (incremental load type), the applied stresses range from 6.25 to 800 kPa. The minimum time for each load stage was 24 hours. The compressibility curves show that the biological treatments have no significant effects on the deformability of the sediments. With reference to the hydraulic conductivity, the permeability tests (rigid wall permeameters) highlight a high permeability, in the range of 10-5-10-4 cm/s for all the tested samples, confirming no significant effect of the treatment also for permeability characteristics of the sediments. The high permeability in in accordance with the sandy grain size of the sediments of concern, confirmed also by SEM observations.

Regarding the investigations of the leaching capability of the sediments, the column tests together with ICP-MS allowed to collect the leachate and to measure the concentration of inorganic compounds in the leachate itself. Zinc, Manganese and Nickel were detected in the leachate in measurable concentrations. In particular, the leached mass of Nickel was significantly reduced by the biological treatments resulting one order of magnitude lower than that leached by the untreated sample.

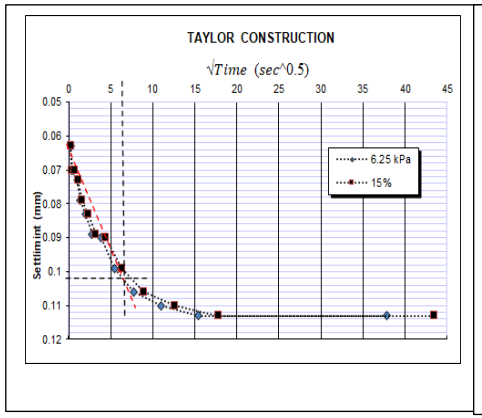
In addition, focusing on the treatment with the mix of bacteria and fungi (the most promising type among those here evaluated) it is possible to state that: (1) no changes in the amount of mobile/bioavailable fraction of metals in the sediments are registered after the treatment as demonstrated by biological analyses and that (2) the chemical composition (in terms of chemical elements) of sediments seems not to be altered by this treatment, as demonstrated by EDS micro-analyses performed by SEM.

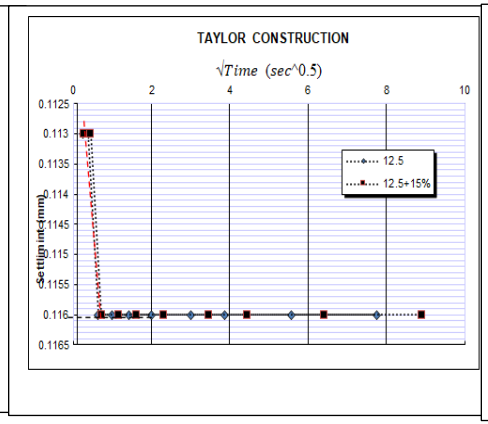
On the basis of the observations made in this work, some future developments can be proposed:

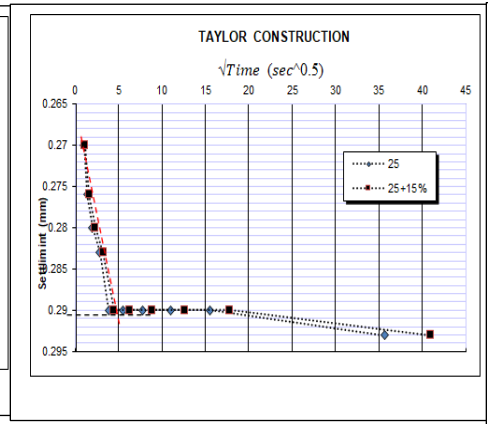
- This study examined the sediment only from one location. In order to have a complete picture abut Bagnoli harbor, additional sediments from different locations are recommended to be studied.
- Since the biological reaction is producing greenhouse gases (GHGs), their development should be considered worthwhile and investigated.
- In case of in-situ remediation, it is recommended to study the effect of dynamic change of seabed on biodegradability of contamination.

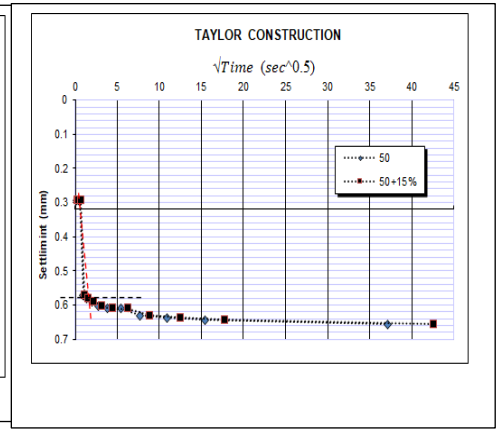
Appendix

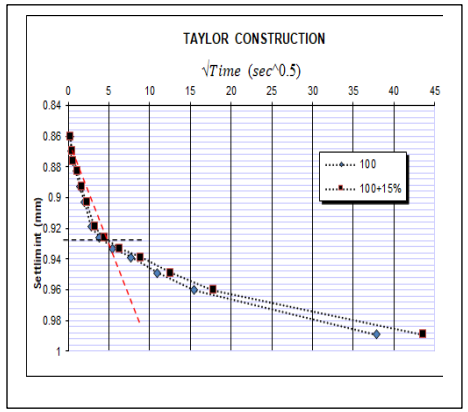
- Coefficient of Consolidation (c_v) Using Taylor Construction.
  - **CTR sample**

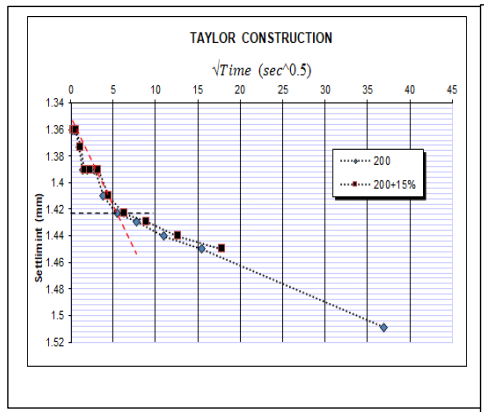


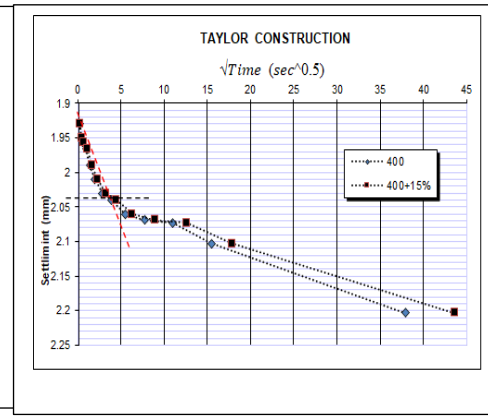




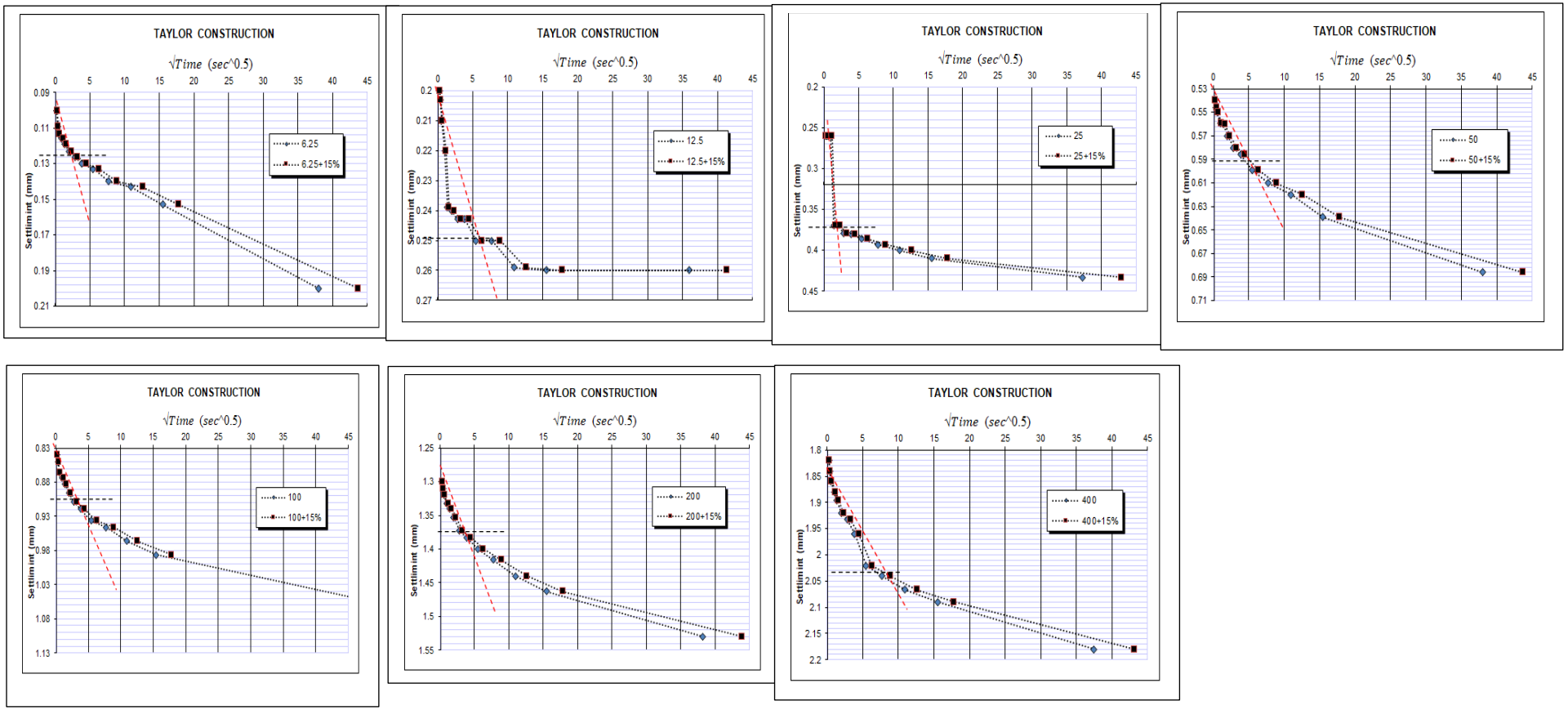




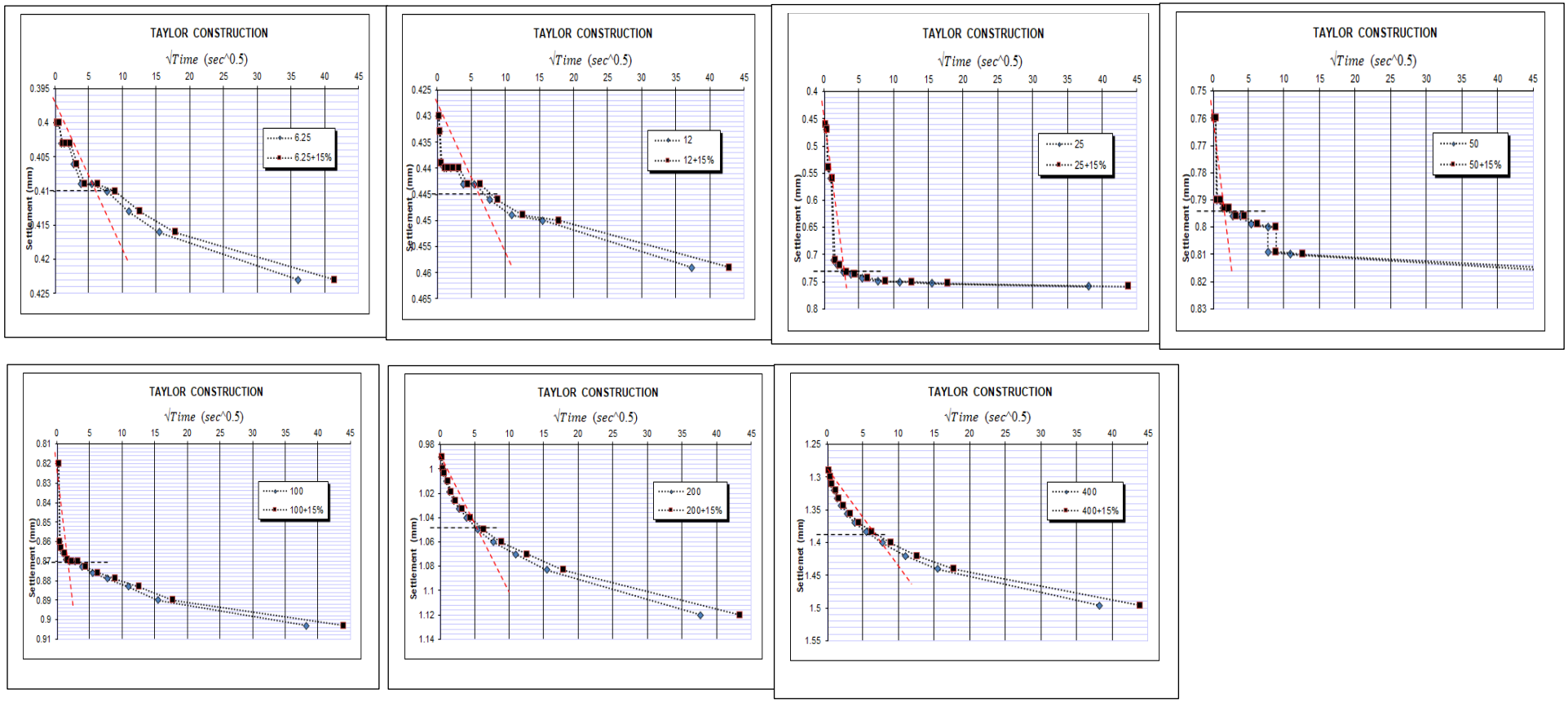




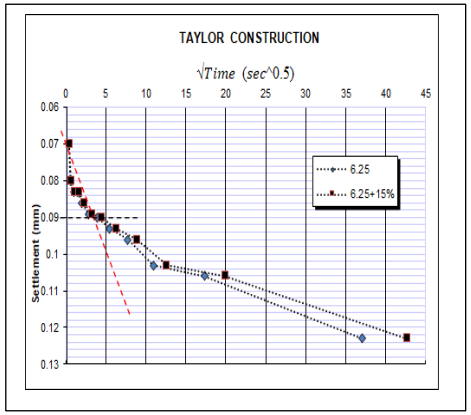
- BAC sample

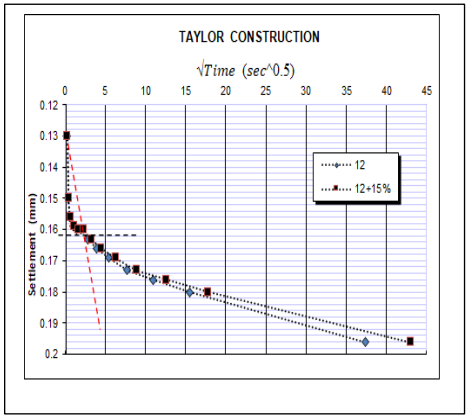

TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
6.25
6.25+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
12.5
12.5+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
25
25+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
50
50+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
100
100+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
200
200+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlimint (mm)
400
400+15%

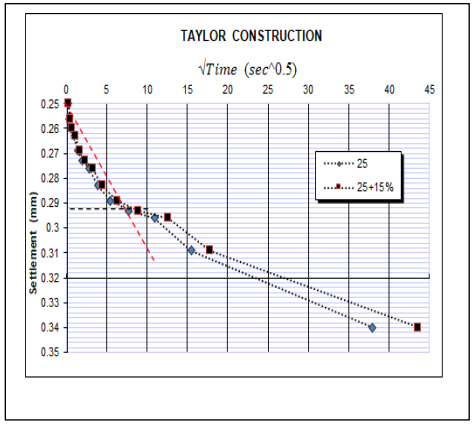
➢ FUN Sample

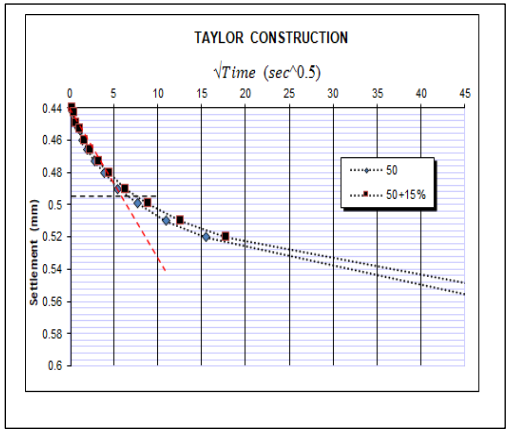

TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
6.25
6.25+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
12
12+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
25
25+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
50
50+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
100
100+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlement (mm)
200
200+15%
TAYLOR CONSTRUCTION
√Time (sec^0.5)
Settlemet (mm)
400
400+15%

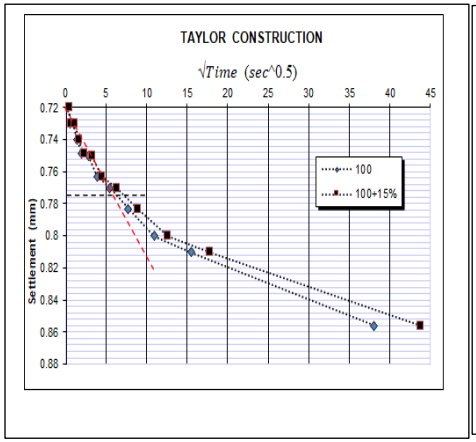
- BAC+FUN Sample

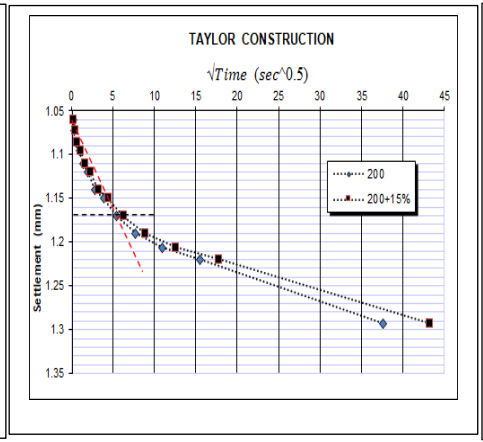


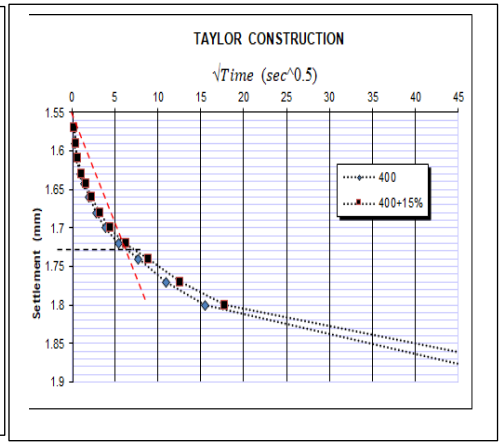












Calculations

Using Taylor's method, t_{90} can be found from graphs above. Then the value Cv can be found using the formula

$$C_v = \frac{0.848H^2}{t_{90}}$$

Where H is the height of sample.

The Secondary Consolidation calculated using the following formula:

$$C_\alpha = \frac{\Delta\varepsilon}{logt_2 - logt_1}$$

$$m_v = \frac{\Delta e}{\Delta\sigma(1 + e_0)}$$

$$k = m_v * C_v * \gamma_w$$

CTR Sample

stress ksi	t90	primary consolidation (mm)	C_v (mm2/s)	Cα	mv (kPa)	k (cm/sec)
0						
6.25	38.44	0.105556	1.990947		0.0004211	8.224E-07
12.5	0.36	0.116333	212.5889		2.526E-05	5.269E-06
25	20.25	0.293556	3.779358		0.0007453	2.763E-06
50	1.44	0.627778	53.14722	0.0175	0.0007642	3.984E-05
100	23.04	0.935556	3.321701	0.04248	0.0001358	4.425E-07
200	23.04	1.431111	3.321701	0.049498	7.842E-05	2.555E-07
400	20.25	2.053333	3.779358	0.057139	7.184E-05	2.664E-07

BAC Sample

stress ksi	t90	primary consolidation (mm)	Cv (mm2/s)	Cα	mv (kPa)	k (cm/sec)
0						
6.25	14.44	0.131222	5.3	0.031439	0.000842	4.38E-06
12.5	25	0.254444	3.06128	0.012851	0.000421	1.26E-06
25	16	0.37	4.78325	0.022495	0.000728	3.42E-06
50	27.04	0.601111	2.830325	0.049973	0.000307	8.53E-07
100	23.04	0.93	3.321701	0.061075	0.000263	8.58E-07
200	24.01	1.39	3.187505	0.06	0.000121	3.79E-07
400	27.04	2.063333	2.830325	0.099658	9.47E-05	2.63E-07

FUN Sample

stress ksi	t90	primary consolidation (mm)	Cv (mm2/s)	Cα	mv (kPa)	k (cm/sec)
0						
6.25	23.04	0.411444	3.321701	0.010052	0.000194	6.311E-07
12.5	23.04	0.446889	3.321701	0.008305	0.000135	4.391E-07
25	7.84	0.773333	9.761735	0.019238	0.001259	1.206E-05
50	10.24	0.798222	7.473828	0.006108	0.000124	9.107E-07
100	2.25	0.876556	34.01422	0.011627	8.74E-05	2.915E-06
200	27.04	1.057556	2.830325	0.032471	6.84E-05	1.9E-07
400	27.04	1.391111	2.830325	0.068567	5.42E-05	1.505E-07

BAC+FUN Sample

stress ksi	t90	primary consolidation (mm)	Cv (mm2/s)	Cα	mv (kPa)	k (cm/sec)
0						
6.25	20.25	0.092222	3.779358	0.01971	0.00176	6.53E-06
12.5	13.69	0.164444	5.590358	0.017395	0.000362	1.99E-06
25	24.01	0.294444	3.187505	0.047002	0.000379	1.18E-06
50	25	0.5	3.06128	0.04	0.000295	8.85E-07
100	27.04	0.784444	2.830325	0.060222	0.000154	4.27E-07
200	27.04	1.183333	2.830325	0.079994	0.000123	3.4E-07
400	27.04	1.748889	2.830325	0.089957	8.16E-05	2.27E-07