CHAPTER ONE

1.0 INTRODUCTION

Environmental pollution caused by the release of a wide range of compounds from various industries as a result of industrialization is now a major concern worldwide. Thousands of hazardous waste sites have been generated globally due to the accumulation of xenobiotics in soil and water over a long period of time (Boricha and Fulekar, 2009). Metal contaminants are commonly found in soils and water. Metal contaminants can be produced through industrial processes such as mining, refining, and electroplating. These metal contaminants pose adverse health effects to those who live near these polluted sites through breathing, eating, drinking, and skin contact which are all possible exposure routes to metal contaminants. Metals such as mercury, lead, and arsenic can be toxic to the kidneys, decrease mental capabilities and can cause weakness, headaches, abdominal cramps, diarrhoea, and anaemia (USEPA, 2004). Chronic exposure to these contaminants can cause permanent kidney and brain damage (USEPA, 2004). At low concentrations, some metals are important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species of organisms.

A key factor to the remediation of metals is that metals are non-destructible, but can be transformed through sorption, methylation, complexation, and changes in valence state by various microorganisms. These transformations affect the mobility and bioavailability of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination. Bacteria such as *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Citrobacter*, *Klebsiella*, and *Rhodococcus* are organisms that are commonly used in bioremediation mechanisms (Kozlowski and Walkowrak, 2002). These mechanisms include bioaugmentation, which is the addition of inoculum of microorganisms with known pollutant transformation abilities into the contaminated environment and biostimulation, which

is the addition of nutrients, enzymes and bulking agent such as wood chips and/or nutrients such as N/P/K to supplement the indigenous microbes of the contaminated site (Kozlowski and Walkowrak, 2002). These organisms are often used in the bioremediation of cadmium (Kozlowski and Walkowrak, 2002). *Alcaligenes* and *Pseudomonas* have been used in the bioremediation of chromium (Kozlowski and Walkowrak, 2002). Likewise, organisms like *Escherichia* and *Pseudomonas* have been used in the bioremediation of copper (Kozlowski and Walkowrak, 2002). According to Mueller *et al.* (1997) bioremediation which is also referred to as bioreclamation and biorestoration, can be described as the process whereby contaminants such as organic wastes are biologically degraded under controlled conditions to an innocuous state. The main purpose for carrying out bioremediation is to remove contaminants from the natural environment and/or convert the contaminants to a less harmful product using the indigenous bacterial community of the contaminated environment. Bioremediation strategies are developed to promote the bacterial metabolism of contaminants, by adjusting the water, air and nutrient supply (Bamforth and Singleton, 2005).

According to a document released by the United States Environmental Protection Agency (USEPA, 1995), it states that bioremediation is one of the most promising technological approaches to the problem of hazardous waste. This process relies on the ability of microorganisms such as bacteria or fungi to transform hazardous chemicals into less toxic or non-toxic substances. There are several reasons why such biological transformation is often more attractive than direct chemical or physical treatment. Some of these reasons include:

- ability of the microorganisms to directly degrade contaminants (in the casse of organic compounds) rather than merely transferring them from one medium to another.
- ability of the microorganisms to employ metabolic degradation pathways that can terminate with benign waste products such as carbon dioxide and water.
- ability of the microorganisms to derive the food energy necessary to degrade contaminants from the contaminants themselves.
- microrganisms can be used *in situ* to minimize disturbance of the clean-up site.

Cattle dung slurry also called gomeya is a mixture of cattle dung and urine in a ratio of approximately 3:1. It contains crude fibre (cellulose with lignin), crude protein, cellulose, hemi cellulose, and minerals like nitrogen, potassium, traces of sulphur, iron, magnesium, calcium, cobalt, manganese and so forth (Nene, 1999). Bacterial composition of cattle dung includes various species of bacteria such as *Bacillus* species, *Corynebacterium* species, Fecal *Streptococcus*, *Pseudomonas sp., Sarcina, E. coli* and *Lactobacillus* species, fungi such as *Aspergillus, Mucor spp., Rhizopus stolonifer, Rhizopus sp., Penicillium,* and *Trichoderma* various species of protozoa and yeasts such as *Saccharomyces* and *Candida* (Randhawa and Kullar, 2011). Majority of bacteria are cellulose, hemicelluloses, and pectin fermenters. Cattle dung comprises undigested fibre, sloughed off intestinal epithelium, some excreted products derived from bile (pigments), intestinal bacteria, and mucus. The bile pigment biliverdin is mainly present in cattle dung giving it its green color. Bile salts give dung its emulsifying properties by conferring hydrophilic coat to the otherwise hydrophobic droplets (Randhawa and Kullar, 2011). Some of this bacterial consortium from the animal waste has been assessed for the bioremediation of hazardous waste compounds.

1.1 STATEMENT OF THE PROBLEM

The accumulation and persistence of toxic materials in soil represents a major problem. These persistent compounds pose a major threat to the environment. For example, accumulation of heavy metals in the soil affects the flora and fauna of such an environment. The heavy metals may also be leached as a result of rainfall or accumulation of water in the environment which with time may get to the underground water. Heavy metals may also accumulate in some plants and lead to biomagnifications in human consumers of such plant product. The use of conventional technologies such as stabilization electro-kinetic systems, vitrification, incineration, excavation and landfill, soil washing, soil flushing and solidification in treating contaminated soil are costly and not environmentally friendly.

1.2 JUSTIFICATION

Recently, researchers have discovered that bacterial processes are effective in cleaning up radioactive and metallic contaminants (two of the most common and most recalcitrant components of hazardous waste sites) (NABIR, 2003). The use of

microorganisms for bioremediation of soil contaminated with effluents from rolled steel industry and soils containing heavy metal leachates will serve as an alternative to the physical and chemical treatment methods which are costly and not eco-friendly and also improve the quality of the soil.

1.3 AIMS AND OBJECTIVES

1.3.1 AIMS

The aim of this research is to bioremediate soil contaminated with heavy metals using bacterial inoculum with and without cattle dung slurry (the organic amendment).

1.3.2 OBJECTIVES

The specific objectives of this research are:

1. Assessment and determination of the physical and chemical parameters of the contaminated soil in order to have background knowledge of the seasonal variation in the heavy metal contamination of the soil.
2. Isolation, screening and identification of microorganisms from heavy metal contaminated soil using phenotypic and molecular techniques.
3. Bioremediation of the contaminated soil with and without cattle dung slurry.
4. Evaluation of the ability of the treated soil to support plant growth using *Corchorus olitorius.*
5. Determination of the proximate and heavy metals composition of harvested plants and the treated soil samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy metals

According to Etherington (1982), the term "heavy metals" refers to some elements that have their density greater than 5 g/cm^3. Raskin *et al.* (1994) defined heavy metal as any element that has metallic properties such as ductility, conductivity, density, stability as cations, ligand specificity and an atomic number greater than 20 while Jarup (2003) defined heavy metals as metals with density higher than 5 mg/mL. However, the term is now being used to refer to metals such as arsenic, cadmium, chromium, copper, lead, nickel, molybdenum, vanadium and zinc. Also of interest are metals such as aluminium, cobalt, strontium and other rare metals (Suruchi and Khanna, 2011).

Several metals are essential for biological systems and must be present in a certain concentration range. In fact, they provide essential co-factors for metalloproteins and enzymes and as such when they occur in too low concentrations can lead to a decrease in metabolic activity. However at high concentrations, metals can act in a deleterious manner by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecule (Collins and Stotzky, 1989). Heavy metals exist in colloidal, ionic, particulate and dissolved phases. The soluble forms of metal elements are generally ionized or unionized organometallic chelates. Heavy metals are normally present at low concentrations in freshwater (Le Faucheur *et al*., 2006). The discharge of wastewaters from a wide variety of industries such as electroplating, metal finishing, leather tanning, chrome preparation, production of batteries, phosphate fertilizers, pigments, stabilizers, and alloys had impacted both the aquatic and soil environments negatively (El-Nady and Atta, 1996; Stephens and Calder, 2005). All soils contain trace amounts of metals, the concentration of metals in uncontaminated soil is determined primarily by the geology of the parent material from which the soil was formed (McLean and Bledsoe, 1992).

Usually heavy metals are released into the environment as a result of both natural and anthropogenic activities as shown in Table 2.1. Natural sources responsible for the release of heavy metals into the environment include emissions from volcanoes, transport of continental dust and the weathering of metal-enriched rocks (Ernst, 1998). Anthropogenic sources which contribute to an increased input of heavy metals in soils include: the exploration of mines and smelters, the application of manures, fertilizers, metal based pesticides and metal-enriched sewage sludge in agriculture, combustion of fossil fuels, metallurgical industries and electronics (this include the manufacturing, usage and disposal) and military training (Alloway, 1995). All these activities may affect the uptake of heavy metals by modifying the physical and chemical properties of the soil such as pH, organic matter and bioavailability of heavy metals in the soil, making heavy metals to become ubiquitous in the environment (Whatmuff, 2002; McBride, 2003; Yusuf and Osibanjo, 2006) and humans are exposed to them through various ways (Khan *et al.,* 2007; Wilson and Pyatt, 2007).

The extent of soil pollution by heavy metals and base metal ions some of which are soil micronutrients is very alarming. It has been observed that the larger the urban area, the lower the quality of the environment as a result of pollution (Eddy, 2004). Mining and smelting operations are important causes of heavy metal contamination in the environment due to activities such as mineral excavation, ore transportation, smelting and refining, and disposal of the tailings and waste waters around mines (Dudka and Adriano, 1997; Navarro *et al.,* 2008). Heavy metals such as cadmium, copper, lead, chromium and mercury are important environmental contaminants most especially in areas with high anthropogenic activities (Suruchi and Khanna, 2011).

Heavy metals have two major important roles they can play which can be termed as the beneficial and harmful roles.

Some of the beneficial roles played by heavy metals are seen in the physiological functions which they have in the body systems, example of such functions include: the stabilization of DNA and RNA by zinc. Copper is involved in collagen synthesis, dopamine hydrolase and amine oxidases. However, metals that form compounds of biological constituents can be toxic, carcinogenic or mutagenic even at very low concentrations (Picardo *et al*., 2009).

On the other hand, heavy metals are very harmful due to their non destructible nature, long biological half lives and their potential to accumulate in different body parts. Most heavy metals are extremely toxic because of their solubility in water. Even at low concentrations, heavy metals have damaging effects on man and animals; this is because there is no suitable mechanism for their elimination from the body (Abii, 2012). Heavy metals pollution represents serious problem for human health and for life in general.

Heavy metals are persistent environmental contaminants which may be deposited on the surfaces and then adsorbed into the tissues of vegetables. Plants take up heavy metals by adsorbing them from deposits on the parts of the plants exposed to the air from polluted environment as well as from contaminated soil (Kkairiah *et al.*, 2004; Al-Jassir *et al.*, 2005; Kachenko and Singh, 2006; Singh and Kumar, 2006; Sharma *et al*., 2008a,b; Suruchi and Khanna, 2011)). The major concern with the uptake of these contaminants by plants is that they can accumulate in the plant resulting in their presence in plant products consumed by humans. Environment Canada (1996) reported that heavy metals might adversely affect specific tissues, reproduction and development. This may also cause anaemia, nervous system disorders and depressed immune systems, resulting in mortality and effects on population levels.

Heavy metals are important environmental contaminants threatening the health of human population and natural ecosystems. Heavy metals can impact negatively on the quality of agricultural soils, including phytotoxicity and transfer of heavy metals to the human diet from crop uptake (Nicholson *et al.*, 2003). Adverse environmental impacts from excessive heavy metals dispersed from mine and smelter sites include contamination of water and soil, phytotoxicity, soil erosion, and potential risks to human health (McLaughlin and Singh, 1999; Adriano, 2001; Pruvot *et al*., 2006). Heavy metal contamination of agricultural soils and crops in the vicinity of mining areas has been regarded as a source of great environmental concern (Wcisło *et al.*, 2002; Liu *et al.*, 2005; Kachenko and Singh, 2006).

Table 2.1: Sources of heavy metals contamination in the environment

Anthropogenic sources	Natural sources
Arsenic: Pesticides, wood preservatives, biosolids, ore mining and smelting	Weathering of minerals
Cadmium: Paints and pigments, plastic stabilizers, electroplating, phosphate fertilizers, battery	Erosion and volcanic eruptions
Chromium: Tanneries, steel industries, fly ash	Forest fires and biogenic source
Copper: Pesticides, fertilizers, biosolids, ore mining and smelting	Particles released by vegetation
Mercury: Silver-gold mining, coal combustion, medical waste	
Nickel: Effluent, kitchen appliances, surgical instruments, automobile batteries	
Lead: Aerial emission from combustion of leaded fuel,batteries waste, insecticide and herbicides	

Source: Dixit *et al.,* 2015.

2.2 Heavy metal contamination

Heavy metal contaminants are commonly found in soils, sediments, and water and they can be produced through various industrial processes such as mining, refining and electroplating. According to Ross (1994), the anthropogenic sources of metal contamination can be divided to five main groups:

i. metalliferous mining and smelting which releases metals such as arsenic, cadmium, lead and mercury into the environment
ii. effluent from industries which releases metals such as arsenic, cadmium, chromium, cobalt, copper, mercury, nickel and zinc into the environment
iii. atmospheric deposition which releases metals such as arsenic, cadmium, chromium, copper, lead, mercury and uranium into the environment
iv. agriculture which releases metals such as arsenic, cadmium, copper, lead, selenium, uranium and zinc into the environment and
v. waste disposal which releases arsenic, cadmium, chromium, copper, lead, mercury and zinc into the environment.

Haavisto (2002) discovered that in Finland, most cases of heavy metals contamination of soil were caused by waste treatment plants, saw mills, wood impregnation plants, shooting ranges, garages and scrap yards and about 38% of these metal contaminated sites are located in groundwater areas or close to residential areas. Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust and industrial activities are the major sources of heavy metals contamination of soil. Increased metal uptake by food crops grown on such contaminated soils is often observed (Singh *et al*., 2004; Chen *et al*., 2005). Heavy metal contamination of soils especially soils with low pH leads to serious problems in the food web. At low pH, trace elements become abundant and consequently the plants absorb more quantities of toxic elements (Arsova, 1998; Alvarez *et al.*, 1998; Benkova, 2005; Dinev and Bojinova, 2006). Absorption and accumulation of metals in plants are complicated processes and these are defined by the behaviour of the metal, the soil characteristics and the properties of the biological agents (Japenga *et al*., 2005; Dinev and Vassilev, 2006).

Heavy metal contamination and accumulation in soils and plants is of increasing concern because of the potential human health risks. Food chain contamination is one

of the important pathways for the entry of these toxic contaminants into the human body. Heavy metal accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to plant transfer factors of the metals (Rattan *et al*., 2005). The consumption of food contaminated with heavy metal can seriously deplete some essential nutrients in the body which can result in decreasing immunological defense, it can also result in intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Iyengar and Nair, 2000; Tu¨rkdogan *et al*., 2003). The heavy metals can accumulate within the body organs over time and constitute serious disruption to normal body function (Jarup, 2003; Sathawara *et al.*, 2004; Curtis and Smith, 2008). The damage associated with these metals is of great concern throughout the world because of their toxic and mutagenic effects even at low concentration (Das, 1990).

Water contamination by heavy metals at times is inevitable due to natural processes such as weathering of rocks and anthropogenic activities (effluents from industrial, agricultural and at times domestic sewage). Waste water from industries involved in activities such as mining, electroplating, paint production or chemical laboratories often contains high concentrations of heavy metals including cadmium, copper and lead (Suruchi and Khanna, 2011).

2.3 Mobility of heavy metal contaminant

Heavy metals in soil may exist in various chemical forms, and this have an effect on their solubility thus results in a direct impact on their mobility and biological availability (Xian, 1989). For example, the high mobility of cadmium in soil makes it very available for plants and is also responsible for its availability in the food chain (Di Toppi *et al*., 1999). Metals solubility in soils depends mainly on the soil pH, organic carbon, Cation Exchange Capacity (CEC), redox conditions and clay contents (Hough *et al*., 2003; Walker *et al.*, 2003; Kashem and Singh, 2004; Rieuwerts *et al*., 2006). For example at pH ranges between 4.0-8.5, metal cations are mobile while anions tend to transform to oxide minerals. At high pH levels, cations adsorb unto mineral surfaces and metal anions are mobilized. Hydrous metal oxides of iron, aluminum, and manganese can affect metal concentrations because these minerals are capable of

removing cations and anions. Microbes can convert metal contaminants to less harmful end products which can result in the immobilization of such metal contaminants (NRC, 1993). The immobility of metals is primarily caused by reactions that keep metals in a solid phase or cause metals to precipitate (Evanko and Dzombak, 1997).

2.4 Impacts of heavy metal contamination

Metal waste is commonly found in soil, sediments, and water. Prolonged exposure to low level of toxic elements have damaging effects on human beings and other animals (Ikeda *et al.*, 2000), since there is no efficient mechanism for their elimination. The detrimental impact becomes apparent only after several years of exposure (Bahemuka and Mubofu, 1999).

2.4.1 Impact of heavy metals on microorganisms

Metals play an integral role in the life processes of microorganisms. Some metals, such as calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickel and zinc are essential and serves as micronutrients and/or macroelements which are used for redox-processes, to stabilize molecules through electrostatic interactions, as components of various enzymes and for regulation of osmotic pressure (Bruins *et al*., 2000). Many other metals such as silver, aluminium, cadmium, gold, lead and mercury have no biological role and are non essential and can be potentially toxic to microorganisms (Bruins *et al.*, 2000).

Recently, numerous laboratory and field studies have demonstrated the adverse effect of metals on the soil ecosystem. Significant reductions in bacterial biomass have been found in metal contaminated soils compared with uncontaminated soils, this is because heavy metals contamination results in the death and elimation of susceptible bacterial biomass (Frostegård *et al*., 1993; Fliessbach *et al*., 1994; Roane and Kellogg, 1996; Konopka *et al*., 1999). Also, many studies have shown that metal contamination causes a shift within the soil bacterial community by reducing the sensitivity of microbes to the metals, that is microbes which were sensitive to the heavy metals initially become less sensitive after a period of time (Capone *et al.*, 1983; Malizewska *et al*., 1985; Bååth, 1989; Said and Lewis, 1991; Roane and Kellogg, 1996; Dahlin *et al*., 1997; Bååth *et al*., 1998a, 1998b; Khan and Scullion, 2000). Toxicity of nonessential metals

occurs through the displacement of essential metals from their native binding sites or through ligand interactions (Nies, 1999; Bruins *et al*., 2000). Metals such as Hg^{2+}, Cd^{2+} and Ag^{2+} tend to bind to sulphur hydryl (SH) groups, and thus inhibit the activity of sensitive enzymes (Nies, 1999).

At high concentrations, both essential and nonessential metals can damage cell membranes, alter enzyme specificity, disrupt cellular functions and damage the structure of DNA (Bruins *et al*., 2000). In order to have a physiological or toxic effect, most metal ions have to enter the bacterial cell. Metal ions cannot be degraded or modified like toxic organic compounds, some possible mechanisms used by microorganisms for metal resistance include:

i. exclusion by permeability barrier
ii. intra- and extra-cellular sequestration
iii. active efflux pumps
iv. enzymatic reduction
v. reduction in the sensitivity of cellular targets to metal ions

The presence of one or more of these resistance mechanisms allows microorganisms to function in metal contaminated environments (Ji and Silver, 1995; Nies and Silver, 1995; Nies, 1999; Rensing *et al*., 1999).

2.4.2 Impact of heavy metals on plants

Heavy metal contamination in vegetables cannot be underestimated as these food stuffs are important components of human diet (Marshall, 2004; Wang *et al*., 2005; Radwan and Salama, 2006; Khan *et al*., 2008). Vegetables constitute an important part of human diet since they contain carbohydrates, proteins, vitamins, minerals, fibres, antioxidants and micro nutrients which are required for human health. They also act as neutralizing agents for acidic substances formed during digestion (Thompson and Kelly, 1990; Nicoli *et al*., 1999; Oke and Hamburger, 2002).

Heavy metals uptake by plants grown on polluted soils has been studied to a considerable extent (Wong, 1996; Wong *et al*., 1996; Sukreeyapongse *et al*., 2002; Yusuf *et al*., 2003; Suruchi and Khanna, 2011). Heavy metal uptake via roots from contaminated soils and surface water, and direct deposition of heavy metal

contaminants from the atmosphere unto plant surfaces can lead to contamination of plant by heavy metals. When these metals exceed the physiological demand of plant, they may not only be toxic to the plants, but could also enter into the food chain, become biomagnified and pose serious health concern to human (Sugiyama, 1994).

Crops and vegetables grown on soils contaminated with heavy metals have the tendency of accumulating heavy metals depending on the nature of the vegetables. Some vegetables have a greater potential to accumulate higher concentrations of heavy metals than others (Odoh and Kolawole, 2011). The biotoxic effects of heavy metals on plants depend upon the concentrations and oxidation states of heavy metals, source of heavy metal and mode of deposition (Duruibe *et al.*, 2007). The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, the concentration of heavy metals in the soil, the nature of the soil, the degree of maturity of the plants at the time of the harvest and exposure period to heavy metals (Scott *et al*., 1996; Voutsa *et al*., 1996). Elevated levels of heavy metals in vegetables have been reported as a result of long term use of treated and untreated waste water (Adeniyi, 1996; Sinha *et al.*, 2005; Sharma *et al* 2006, 2007). Thus, it is extremely important to monitor heavy metal contents in waste water to be used in irrigation of soil in order to prevent the entry of heavy metals into food chain (Dudka and Miller, 1999). As a result of higher concentrations of heavy metals in soils, there have been reports of inhibition in some plant activities such as root growth, shoot development and various metabolic processes and this has also resulted into chlorosis, damage to root tips, reduced water and nutrient uptake and damage to enzyme system (Baisberg-Pahlsson, 1989; Sanita di Toppi and Gabbrielli, 1999).

Among the heavy metal elements, cadmium (Cd) and chromium (Cr) are of special concern because of their toxicity to plants even at low concentrations (Shukla *et al*., 2007). Cadmium is particularly dangerous pollutant due to its high solubility and high toxicity in water (Pinto *et al*., 2004). Cadmium is a toxic heavy metal, causing phytotoxicity; its uptake and accumulation in plants pose a potential threat to human health (Shah and Dubey, 1997). Its accumulation causes reductions in photosynthesis, diminishes water and nutrient uptake (Sanità di Toppi and Gabbrielli, 1999), and

results in visible symptoms of injury in plants, such as chlorosis, growth inhibition, browning of root tips, and finally plant death (Kahle, 1993). High contents of cadmium in soil retards plant growth, reduces biomass production (Rai *et al*., 2005), adversely affects mineral assimilation and induces changes in various physiological and biochemical characteristics of plants (Scebba *et al*., 2006). In some plant species, the interactions of cadmium and metal nutrients have resulted in changes in the plant nutrient concentration and composition (Peralta-Videa *et al*., 2002). Similarly, elevated levels of chromium in the soil results in retardation of plant growth, damage to the roots, reduces yield and hampers productivity (Sharma *et al*., 2003).

Iron is essential for the synthesis of chlorophyll and activates a number of respiratory enzymes in plants. The deficiency of iron results in severe chlorosis of leaves in plants (Shuaibu *et al*., 2013). Lead toxicity has become important because of its constant increase in the environment. High concentration of lead may lead to the reduction in root hair development and stunted growth due to reduced photosynthetic rate in plants, which is as a result of stomata closure by the deposition of lead (Sarkar and Jana, 1986). Lead exerts deleterious effects on morphology, growth and photosynthetic processes of plants and causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition (Singh *et al.*, 1997; Sharma and Dubey, 2005). Uptake of metals by plants especially vegetables is one of the major pathways through which soil-metals enter into food chain and is subsequently bio-accumulated to high concentrations causing serious risk to human health when plant based food stuffs are consumed (Cui *et al.*, 2004). The health risks will depend on the chemical composition of the heavy metal, its physical characteristics, the vegetables cultivated and the consumption rate (Xian, 1987). Consumption of vegetable have increased in recent years due to the awareness that vegetables contain certain nutritionally important compounds necessary for human survival and are often called protective food due to their functions of preventing disease of human body (Aualiita and Pickering, 1987; Badawy and El-Motaium, 2003), hence, increasing the danger associated with consumption of vegetables contaminated with heavy metals. According to Abii (2012), the desire for food safety and security in recent times has stimulated research on the danger associated with the consumption of food contaminated with heavy metals and toxins. Thus information

about heavy metal concentrations in food products and their dietary intake is very important for assessing the risk to human health (Mushtaq and Khan, 2010).

2.4.3 Impact of heavy metals on human

Possible routes of exposure to heavy metal contaminants in the environment include breathing, eating, drinking and skin contact. Metals such as mercury, lead and arsenic are potentially toxic to the kidneys, decrease mental capabilities, cause weakness, headaches, abdominal cramps, diarrhea and anemia. Chronic exposure to these contaminants can cause permanent kidney and brain damage (USEPA, 2004). Consuming food contaminated by Pb, Hg, As, Cd and other metals can seriously deplete body stores of Fe, vitamin C and other essential nutrients, leading to decreased immunological defences, intrauterine growth retardation, impaired psycho-social faculties and disabilities associated with malnutrition (Iyengar and Nair, 2000).

Cadmium is used in some industries for coating steel, glass and plastics (including polyvinyl chloride) and also for the Ni-Cd battery production and automotive tires. It has special importance due to its long half life and it can exert toxic effects on almost all systems of the human body (Tsalev, 1993). Cadmium is a non-essential element in foods and natural waters and it accumulates principally in the kidney and liver (Divrikli *et al*., 2006). Cadmium is capable of being carcinogenic and mutagenic; it is also an endocrine disruptor and can lead to lung damage and fragile bones. It also affects calcium regulation in biological systems (Degraeve, 1981; Salem *et al*., 2000). Cadmium has been reported to be very dangerous causing kidney damage, cancer, diarrhea, and vomiting (Abbas *et al.,* 2010; Tandi *et al.,* 2005). Cadmium has been found to be associated with occurrences of Itai-Itai, a disease under which patients show a wide range of symptoms such as low grade of bone mineralization, high rate of fractures, increased rate of osteoporosis and intense bone associated pain (Fridberg *et al.,* 1974).

Chromium has been reported to be capable of causing kidney and liver damage, alteration of genetic materials, lung cancer, skin rashes, stomach upset, ulcers, respiratory problems and weakening of the immune system. The toxicity of chromium can also cause hair loss (Salem *et al*., 2000; Dixit *et al.*, 2015). Lead is very harmful even at very low concentrations and can result in damaging the nervous system, bone,

liver, pancreases, teeth and gum (Fergusson, 1990; Bakidere and Yaman, 2008). Lead is also considered to be a potential carcinogen and is responsible for causing a number of diseases such as cardiovascular, kidney, blood, nervous and bone diseases (Jarup, 2003). Excess exposure of children to lead causes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, risk of cardiovascular disease (Salem *et al*., 2000; Padmavathiamma and Li, 2007; Wuana and Okieimen, 2011). Serious systemic health problems can develop as a result of excessive dietary accumulation of heavy metals such as cadmium and lead in the human body (Oliver, 1997).

Zinc and Copper are essential elements needed for the proper functioning of the body, however excessive concentration in food and feed plant products are of great concern because of their toxicity to humans and animals (Kabata-Pendias and Mukherjee, 2007). Zinc is the least toxic of all the heavy metals and it is an essential element in human diet as it is required to maintain the functioning of the immune system. Too much zinc in human diet may not be as hazardous or detrimental to health as zinc deficiency; this is because deficiency of zinc in the diet may be highly detrimental to human health. Regular consumption of vegetables rich in zinc may assist in preventing the adverse effect of zinc deficiency which results in retarded growth and delayed sexual maturation because of its role in nucleic acid metabolism and protein synthesis (Barminas *et al.*, 1998). The recommended dietary allowance for zinc is 15 mg/day for men and 12 mg/day for women according to Agency for Toxic Substances and Disease Registry (ATSDR, 1994), however high concentration of zinc in vegetables may cause vomiting, renal damage, cramps etc (Shuaibu *et al*., 2013). Copper is an essential micronutrient which functions as a biocatalyst, required for body pigmentation in addition to iron, it helps maintain a healthy central nervous system, prevents anaemia and is interrelated with the functions of zinc and iron in the body (Akinyele and Osibajo, 1982). Copper toxicity induces iron deficiency, lipid peroxidation and destruction of membranes (Zaidi *et al*., 2005). Elevated levels of copper in the body can cause brain and kidney damage, liver cirrhosis, chronic anemia as well as stomach and intestine irritation (Salem *et al*., 2000; Wuana and Okieimen, 2011).

Nickel (II) containing wastewaters are common contaminants in the environment as Ni^{2+} is used in a number of industries such as electroplating, battery manufacturing such as Nickel/iron (Ni/Fe) storage batteries, mining, metal finishing, forging and also in the production of ferrous steel cutlery (Greenwood and Earnshaw, 1993). Nickel also plays some role in body functions including enzyme functions. It occurs naturally more in plants than in animal flesh. It activates some enzyme systems in trace amount but its toxicity at higher levels is more prominent (Divrikli *et al.*, 2006). Nickel can cause allergic skin diseases such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation. It can also be immunotoxic, neurotoxic, genotoxic, can affect fertility and cause hair loss (Salem *et al*., 2000; Khan *et al.*, 2007; Duda-Chodak and Baszczyk, 2008; Das *et al.*, 2008). High level of nickel may also result in zinc or iron deficiency as well as enzymic malfunctioning (Jarup, 2003). Higher concentrations of nickel can cause cancer of the lungs, nose and bone. Dermatitis (nickel itch) is one of the most frequent effects of exposure to nickel from coins and jewellery. High concentration of Ni(II) in ingested water may cause gastrointestinal distress, nausea, vomiting, diarrhoea, pulmonary fibrosis, renal edema, skin dermatitis and severe damage to lungs and kidneys, (Erdogan *et al*., 2005; Meena *et al*., 2005).

High level of iron is injurious to humans, this is because exposure to high levels of iron dust may cause respiratory diseases such as chronic bronchitis and ventilation difficulties in human (Shuaibu *et al*., 2013). Cobalt has little direct activity on its own in the body as it is an integral component of vitamin B_{12} and as such its effects, sources and uses are very similar to that of vitamin B_{12}. It is involved in preventing and treating pernicious anaemia and also helps in red blood cell production. Cobalt also supports normal nervous system functions (Sobukola *et al*., 2008). Very little information has been reported on its concentrations in food materials.

2.5 Remediation techniques for polluted soil

The accumulation and persistence of toxic materials in soil represents a major problem today. Various toxic contaminants are generated as by-products from various industries such as petroleum and petrochemical, pulp and paper, chemical industries and which may be released into the environment or spilled accidentally. Metals do not undergo either chemically or biologically induced degradation that can alter or reduce their toxicity over time (Knox *et al.*, 2000). That is to say, heavy metals cannot be

destroyed biologically or chemically but are only transformed from one oxidation state to another. As a consequence of the alteration of its oxidation state, the metal may become either:

(i) more water soluble and can be removed by leaching

(ii) inherently less toxic

(iii) less water soluble so that it precipitates and then becomes less bioavailable or removed from the contaminated site

(iv) volatilized and removed from the polluted area (Garbisu and Alkorta, 1997).

Remediation of metals often involves five general approaches: isolation, immobilization, mobilization, physical separation, and extraction (Evanko and Dzombak, 1997). Immobilization and mobilization involve bioremediation processes. Industries use a combination of more than one approach to properly treat metal-contaminated sites. The combination of the approaches can be cost-effective.

2.5.1 Conventional techniques used in remediation of contaminants

There are various conventional remediation techniques used in cleaning heavy metals polluted environments. These techniques are classified as *in situ* and *ex situ* techniques and they include: solidification and stabilization, soil vitrification, soil incineration, excavation and landfill, soil washing, soil flushing and electro kinetic systems. Each of the conventional remediation technology has specific benefits and limitations (MADEP, 1993). The conventional methods used in *ex situ* soil remediation though effective are often too expensive due to high cost involved in the disposal of the contaminated soil, transportation and backfill of the original site with clean soil (Ryan *et al.*, 2001; Zhu *et al.*, 2004). Both *in situ* and *ex situ* techniques are further classified as either biological or non biological remediation techniques.

2.5.1.1 Biological techniques used in remediation of contaminated soil

i. Bioventing: this technique is divided into the aerobic and anaerobic types. Aerobic bioventing involves supplying oxygen to contaminated unsaturated soils with low oxygen concentrations to facilitate aerobic bacterial biodegradation. Oxygen is typically introduced by air injection wells that push air into the subsurface. The microbes present in the contaminated soil then use the supplied oxygen, oxidize the contaminants to gain energy and carbon for growth (USEPA 1995a and 1995b).

Anaerobic bioventing is an emerging technology that may be useful in treating highly chlorinated compounds, such as pentachlorophenol (PCP), some polychlorinated biphenyls (PCBs), and pesticides such as lindane and dichlorodiphenyltrichloroethane (DDT). In this technique, same type of gas delivery system used in aerobic bioventing is utilized but instead of injecting air, nitrogen and electron donors (e.g. hydrogen and carbon dioxide) are used. The nitrogen displaces the soil oxygen, and the electron donor gas facilitates bacterial dechlorination. Volatile and semivolatile organic compounds may be produced during anaerobic bioventing that are not anaerobically degradable. Volatile compounds may be aerobically degraded in the soil surrounding the treatment zone. Semivolatile compounds may be treated by following anaerobic bioventing with aerobic bioventing. Since aerobic and anaerobic bioventing share similar gas delivery systems, the switch can be made by simply changing the injected gas (USEPA 1995a and 1995b). Figure 2.1 shows an iilustration of a bioventing system according to Held and Dörr (2000). The advantages of this technique are: it utilizes readily available equipment, it is easy to install, it creates minimal disturbance to the treatment site, may not require costly off gas treatment and it is easily combinable with other technologies (such as air sparging and groundwater extraction). However its disadvantages include: high concentrations of gases used may be toxic to microorganisms, it is not applicable for certain site conditions (e..g. it is not suitable for soils with low permeability), it sometimes requires nutrients and air injection wells, it is capable of treating only unsaturated zones of soils, it needs other methods to treat saturated zones of soils and groundwater (Castelo-Grande and Barbosa, 2003).

ii. Land farming: depending on how deep into the soil the contaminant is, the contaminated soil can either be tilled without requiring any excavation in the case of very shallow contamination covering only the top soil region, however for contamination that has gone deeper into the soil matrix i.e. beyond the top soil layer, this technique requires the excavation and application of contaminated material on soil surface and periodically tilling it to mix and aerate the material (Harmsen *et al.*, 2007; Maciel *et al.*, 2009). In this technique, the reduction observed in the concentrations of the contaminant may sometimes be due to volatilization rather than biodegradation (Souza *et al.*, 2009; Sanscartier *et al.*, 2010). Figure 2.2 shows a schematic diagram of the process of land farming according to FRTR (2000). According to Castelo-Grande

and Barbosa (2003), the advantages of this technique include: relative simple design and implementation and it requires short treatment duration of about six months to two years under optimal conditions. However, the disadvantages include: the land mass required for this technique is high, also dust and vapor generation during the use of this technique may result in air pollution.

2.5.1.2 Non-biological techniques used in remediation of contaminated soil

i. Electrokinetic separation remediation: this is an emerging and innovative technique which is used to complement the limitations encountered in the use of traditional technology and to treat fine-grained soils. The method is suitable for removing contaminants effectively from soil with low permeability (for example, clay soil). *In situ* electrokinetic separation remediation can be applied to treat low permeable soils contaminated with heavy metals, radionuclides and organic pollutants. The principle behind this method is the application of a low level direct current electric potential through electrodes, which are placed into the contaminated soil. Ionic contaminants are transported to the oppositely charged electrode by electro-migration. In addition, electro-osmotic flow provides a driving force for the movement of soluble contaminants (Gomes *et al.*, 2012). According to Huang *et al.* (2012), though the technology has been known and utilised for more than a decade, its application in the removal of hydrophobic and strongly adsorbed pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) especially from low permeability soils is limited, hence solubilising agents are therefore used in these cases to enhance the removal efficiency of PAHs. Figure 2.3 shows a schematic diagram of electrokinetic separation remediation adapted from NAVFAC (2000). The advantages of this technique are: it is an *in situ* technology that has little disturbance on the environment since soil removal is not required and metals are actually removed from soil unlike some other conventional techniques e.g. stabilization, which leaves the metals in the soil. The disadvantages are: the effectiveness of the technique is reduced in alkaline soils and it requires soil moisture (Castelo-Grande and Barbosa, 2003).

ii. Solidification and Stabilization: “solidification” refers to a process in which materials are added to the waste to produce an immobile mass.

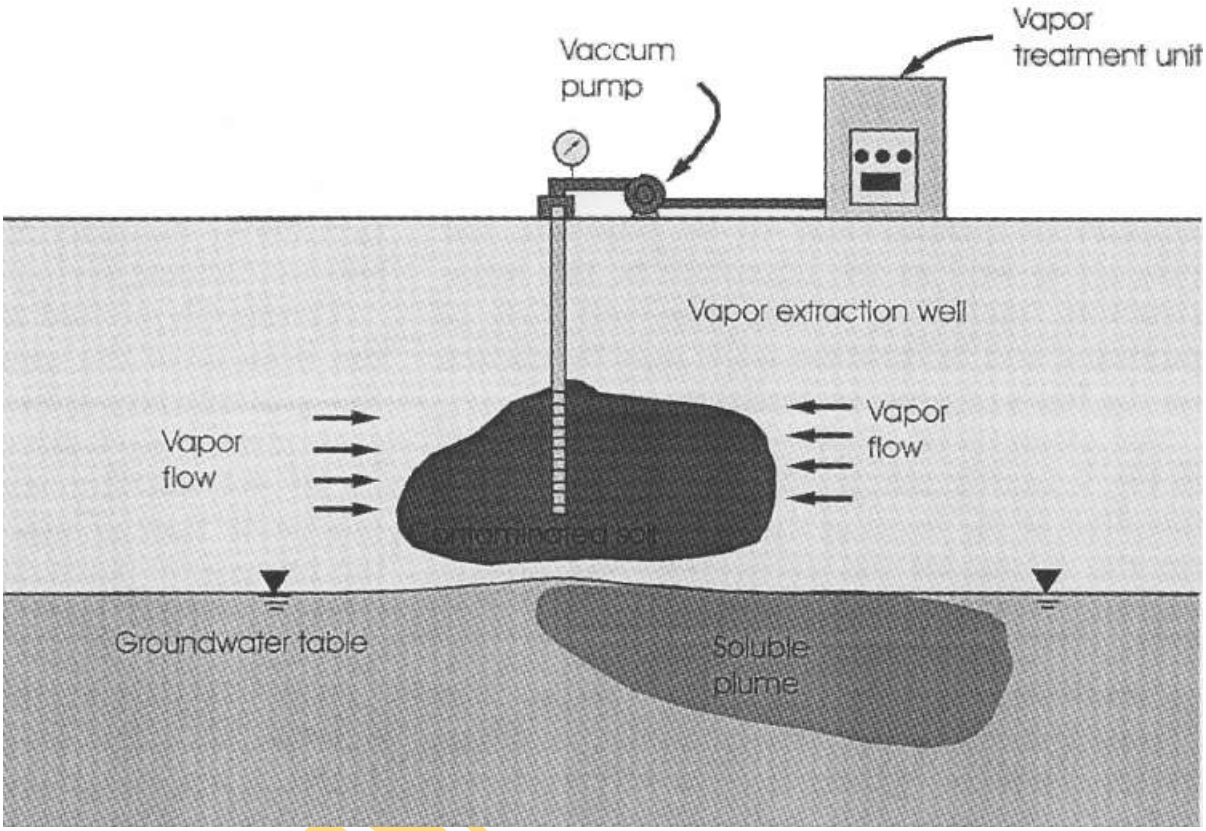


Figure 2.1: Illustration of bioventing system (Held and Dörr, 2000).

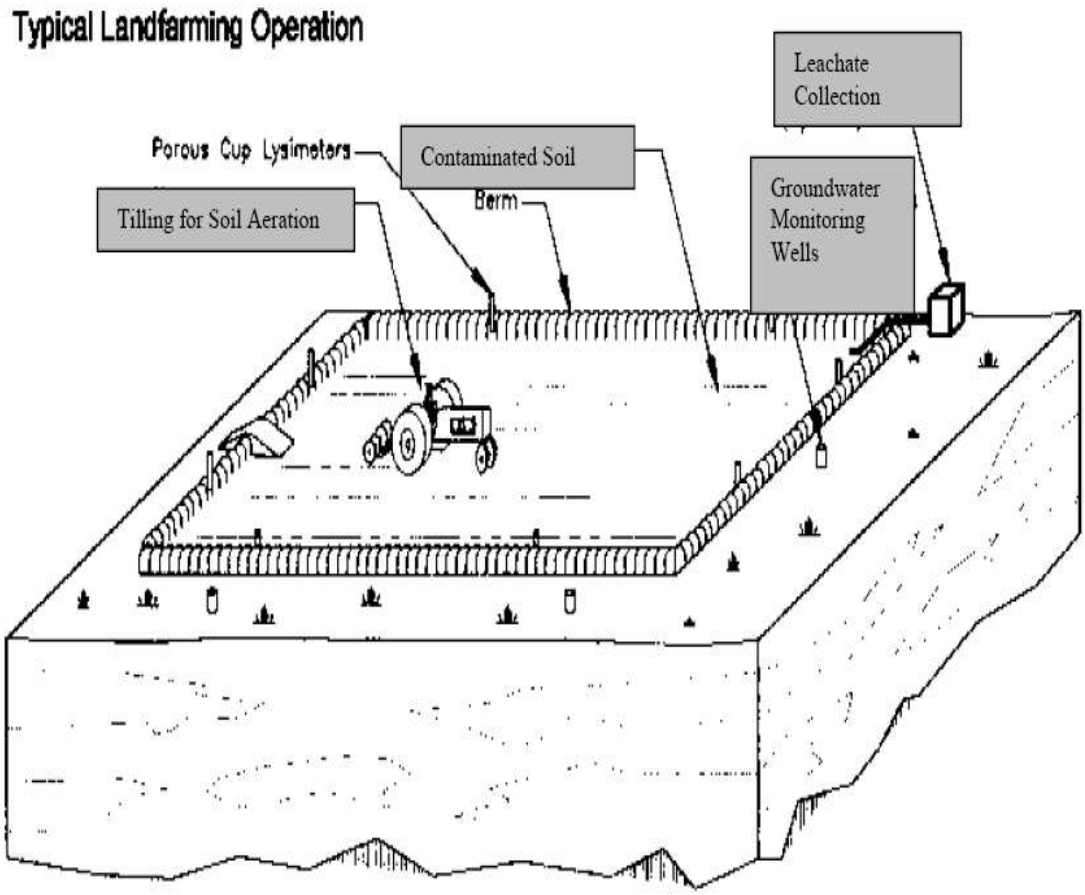


Figure 2.2: Schematic diagram of the process of land farming (FRTR, 2000)

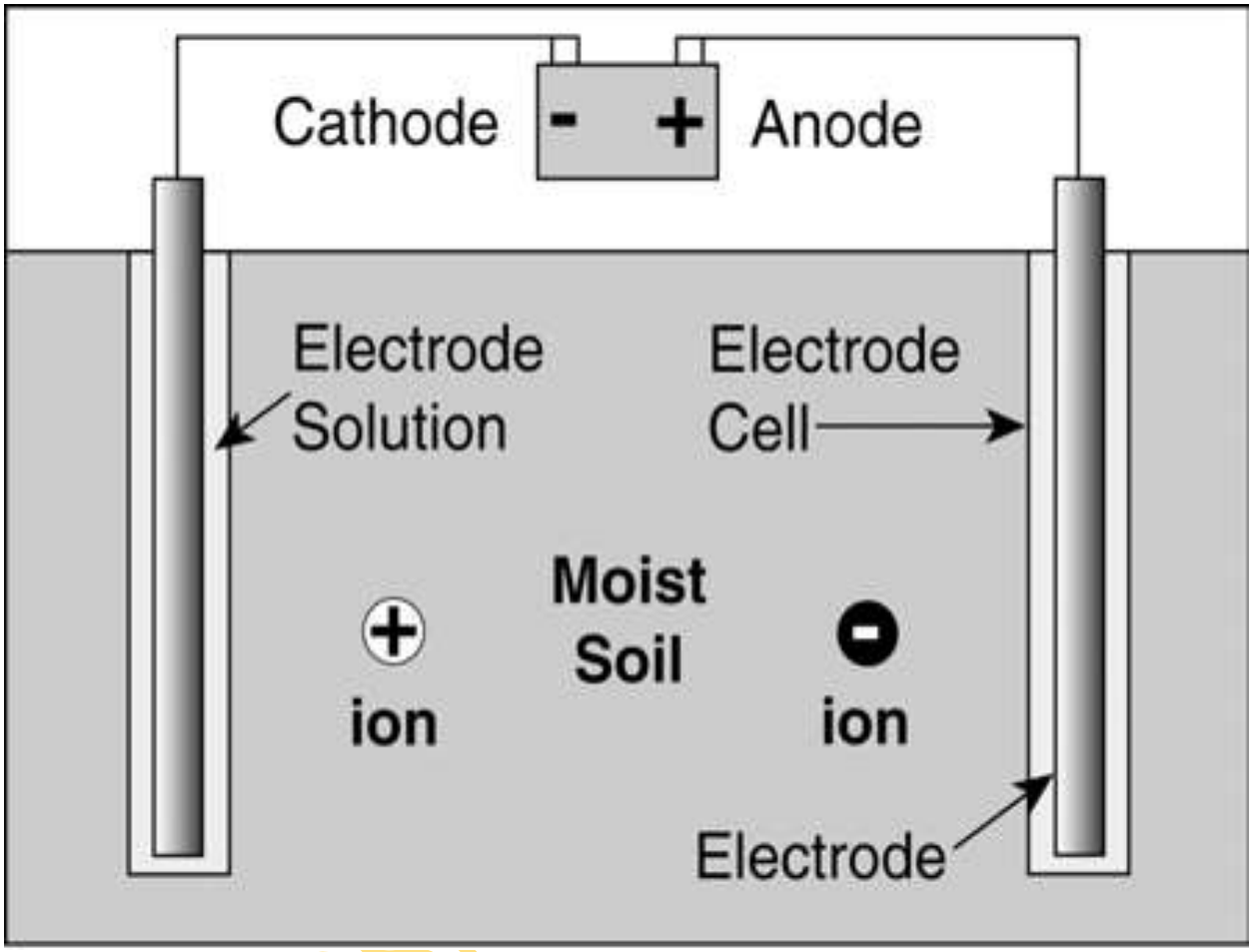


Figure 2.3: Schematic diagram of Electrokinetic separation remediation (NAVFAC, 2000).

This may or may not involve a chemical bonding between the toxic contaminant and the additive whereas "stabilization" refers to converting a waste to a more chemically stable form. This conversion may include solidification, but it often times include the use of physicochemical reaction to transform the contaminants to a less toxic form (USEPA, 1999; Pensaert, 2008). Solidification is a technique that encapsulates hazardous waste into a solid material of high structural integrity. It eliminates free liquids, reduces hazardous constituent mobility by lowering waste permeability, minimizes constituent tendency to leach and provides stability for handling, transport and disposal. The process of solidifying fine waste particles is termed microencapsulation while macroencapsulation is the process of solidifying wastes in large blocks or containers. The solubility, mobility and toxicity of hazardous wastes are reduced by stabilization technologies. Combination of solidification and stabilization techniques are effective for treating soils containing metals, asbestos, radioactive materials, organics, corrosive cyanide compounds and semi-volatile organics (USEPA, 2009). Figure 2.4 shows a diagram of solidification and stabilization according to FRTR (2000). Factors that may limit the applicability and effectiveness of the process include: environmental conditions which may affect the long-term immobilization of contaminants; some processes result in a significant increase in volume of the contaminant (up to double of the original volume); certain wastes are incompatible with combination of the two processes; treatability studies are generally required; Volatile Organic Compounds (VOCs) are generally not immobilized; long-term effectiveness has not been demonstrated for many contaminant/process combinations (Raghavan *et al.*, 1989; FRTR, 2000).

iii. Soil vapour extraction: this is a physical-chemical treatment method in which volatile contaminants are treated using a venting and *ex-situ* gas treatment system. This technique has been effective in reducing concentrations of VOCs and certain semi-volatile organic compounds. Principally, a vacuum is applied to the soil matrix to create a negative pressure gradient that causes movement of vapours toward extraction wells. Volatile contaminants are readily removed from the subsurface through the extraction wells. The collected vapours are then treated and discharged to the atmosphere or where permitted, re-injected to the subsurface (Suthersan, 1999; Soares *et al.*, 2010). Figure 2.5 shows a soil vapor extraction system according to FRTR

(2000). The advantages of this technique include: it has proven performance with readily available equipments which are easy to install; it has minimal disturbance to site operations; has very short treatment times (6-48 months). However, the disadvantages are: the effectiveness decreases when applied to sites with low permeability; can only treat the unsaturated zone; the extracted vapour if discharged to the atmosphere may require costly treatment (Castelo-Grande and Barbosa, 2003).

iv. Soil washing: this is an *ex situ* treatment technology for the remediation of contaminated soil. The technique is suitable for the cleanup of soils contaminated with organic, inorganic and radioactive compounds. The selection of soil washing for a particular contamination problem will depend on a variety of factors. One important factor to consider is whether the contamination is specific to particular groups of particles within the soil and whether these particles can be removed from the contaminant-free bulk of particles by physical or physical and chemical processes (Griffiths, 1995). The technique involves mixing the solvent (water) and contaminated soil in an extractor vessel (Balba *et al*,1998; Pavel and Gavrilescu, 2008). The mixing dissolves the organic contaminant into the solvent. The solvent and dissolved contaminants are then placed in a separator where the solute and solvent are separated and treated. The soils can be stockpiled, tested and used as inert material. Figure 2.6 shows the diagram of soil washing according to Dadrasnia *et al*. (2013). The advantages of this technique include: it is capable of reducing the volume of contaminant, therefore, further treatment or disposal is less problematic and it is commercially available. The disadvantage include: the toxicity of the contaminant remains unchanged, although volume is reduced; the technique is less effective when soil contains a high percentage of silt and clay; costs associated with the disposal of the subsequent waste streams must be considered (Castelo-Grande and Barbosa, 2003).

v. Incineration: this is a thermal treatment technique for the remediation of soils polluted with organic compounds. It is the most widely used method. It is very expensive and generates problems with air emissions and noise (Flores *et al.*, 2012). Incineration technology is intended to permanently destroy organic contaminants. It is an integrated system of components for waste preparation, feeding, combustion, and emissions control. Central to the system is the combustion chamber, or the incinerator.

There are four major types of incinerator: rotary kiln, fluidized bed, liquid injection, and infrared. Figure 2.7 shows the schematic diagram of incineration (FRTR, 2000). The advantages include the reduction in the toxicity and volume especially in the case of organic contaminants; it is widely used and available commercially. However, its disadvantages are: metals are not destroyed and end up in the flue gases or in the ashes; there is often resistance to incineration by the community and screening may be needed in certain types of soils such as clay soils or soils containing rocks (Castelo-Grande and Barbosa, 2003).

2.5.2 Plant remediation (Phytoremediation)

The use of plants to clean wastewater is quite old. Hartman (1975) reported plant species that could accumulate high levels of metals in their leaves. According to Byers (1935), the genus *Astragalus* accumulated up to 0.6 % selenium in dry shoot biomass. There have been reports of plants that accumulated nickel up to 1% in shoots, followed by high zinc accumulation in shoots of *Thlaspi caerulescens* (Rascio, 1977). The idea to use plants for cleaning heavy metal contaminated soils was reintroduced by Chaney (1983). Later, numerous researchers used plants to remediate soil and wastewater (Kisku *et al*., 2000; Kaushik *et al*., 2005; Sawalha *et al*., 2008).

Phytoremediation is now a widely acceptable technology which may provide an alternative to cleaning wastewater and contaminated soil because of its cost-effectiveness, environment-friendliness, aesthetically pleasant nature and equal applicability for the removal of both organic and inorganic contaminants present in soil, water and air (Yu *et al*., 2007).

Phytoremediation employs the use of plants to remove organic and/or inorganic contaminants from biota (phytoextraction), volatilization of contaminants by plants from the soil into the atmosphere (phytovolatilization), or stabilization of an inorganic into a less soluble form (phytostabilization). Phytoremediation is inexpensive, effective, can be implemented *in situ*, and is environmentally friendly (Chaney *et al.*, 1997; Trapp and Karlson, 2001; Zavoda *et al.*, 2001). Phytoremediation can be used to clean up contaminated sites in several ways such as phytovolatilization, phytostabilization, phytoaccumulation or extraction and phytodegradation by plants (Cunningham *et al*., 1997; Flathman and Lanza, 1998).

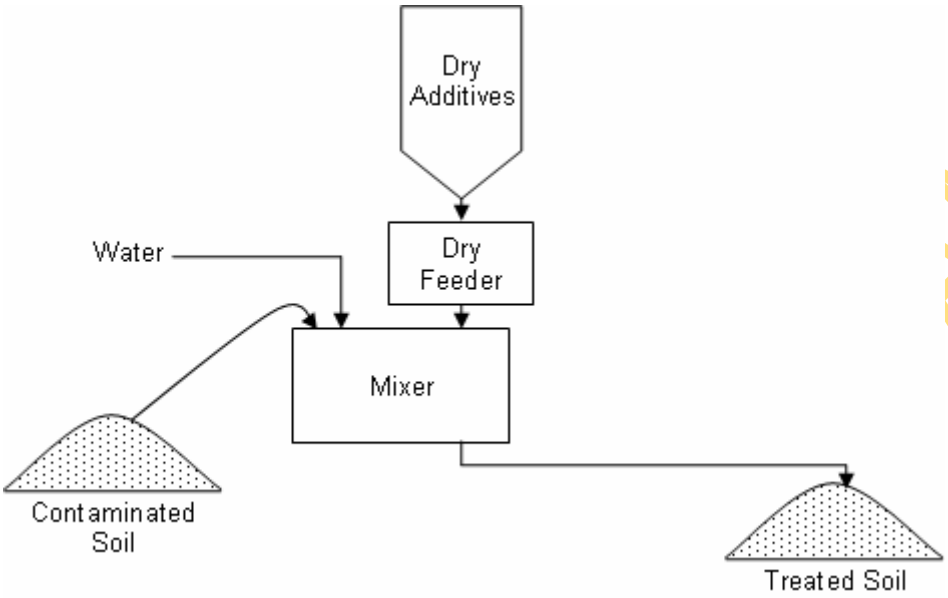


Figure 2.4: Schematic diagram of solidification and stabilization (FRTR, 2000).

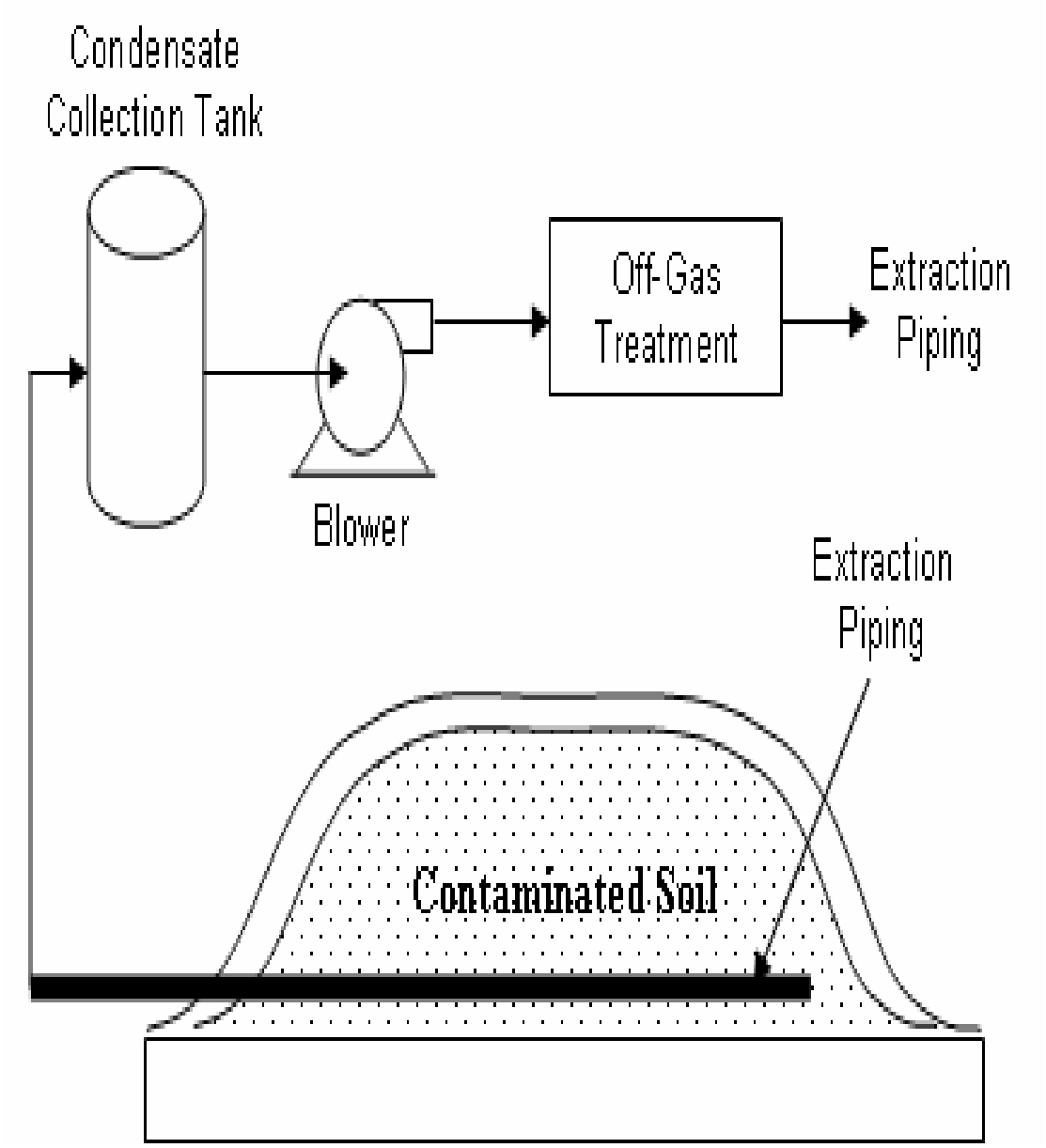


Figure 2.5: Schematic diagram of Soil Vapor Extraction System (FRTR, 2000)

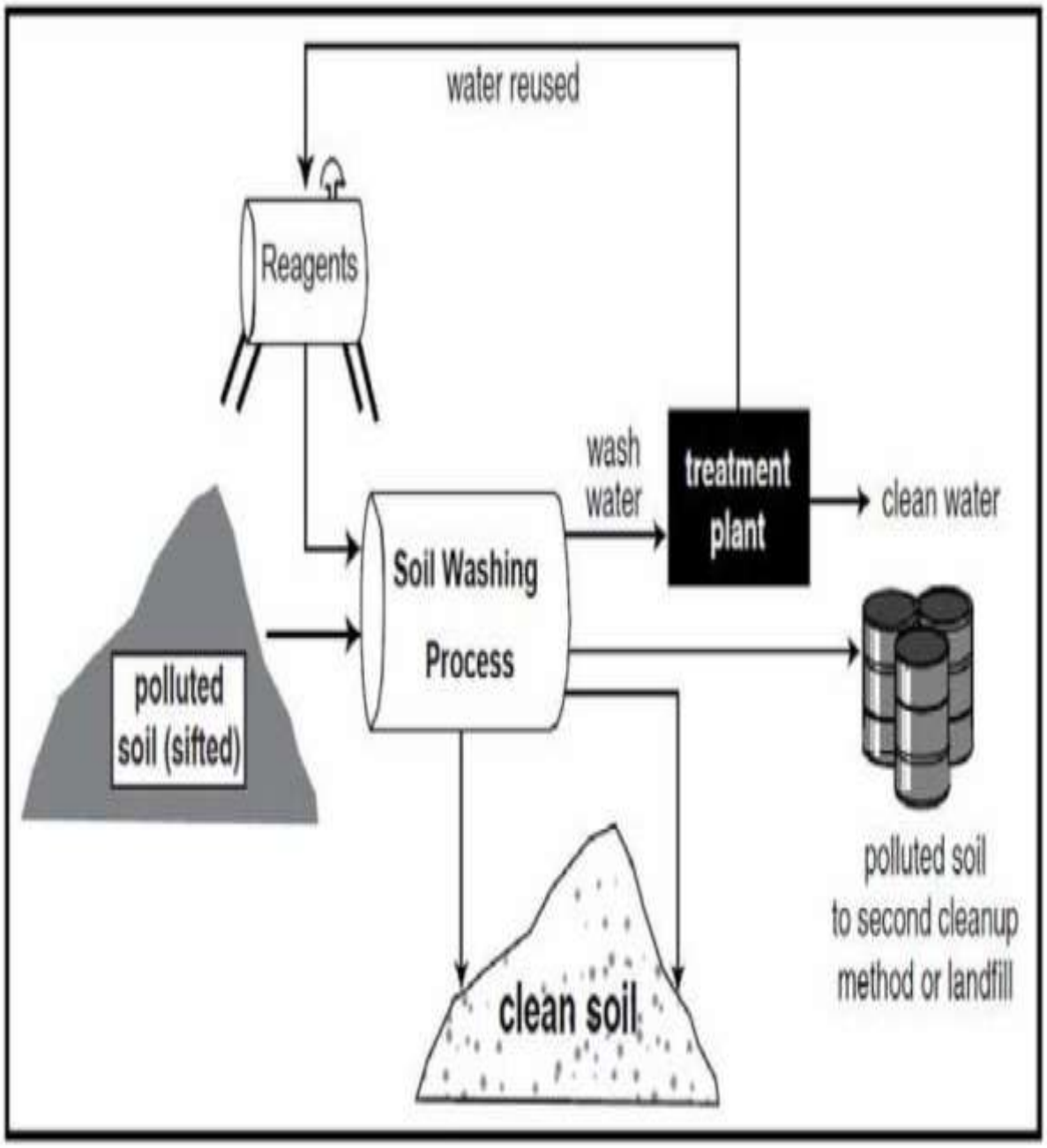


Figure 2.6: Schematic diagram of soil washing (Dadrasnia *et al*., 2013).

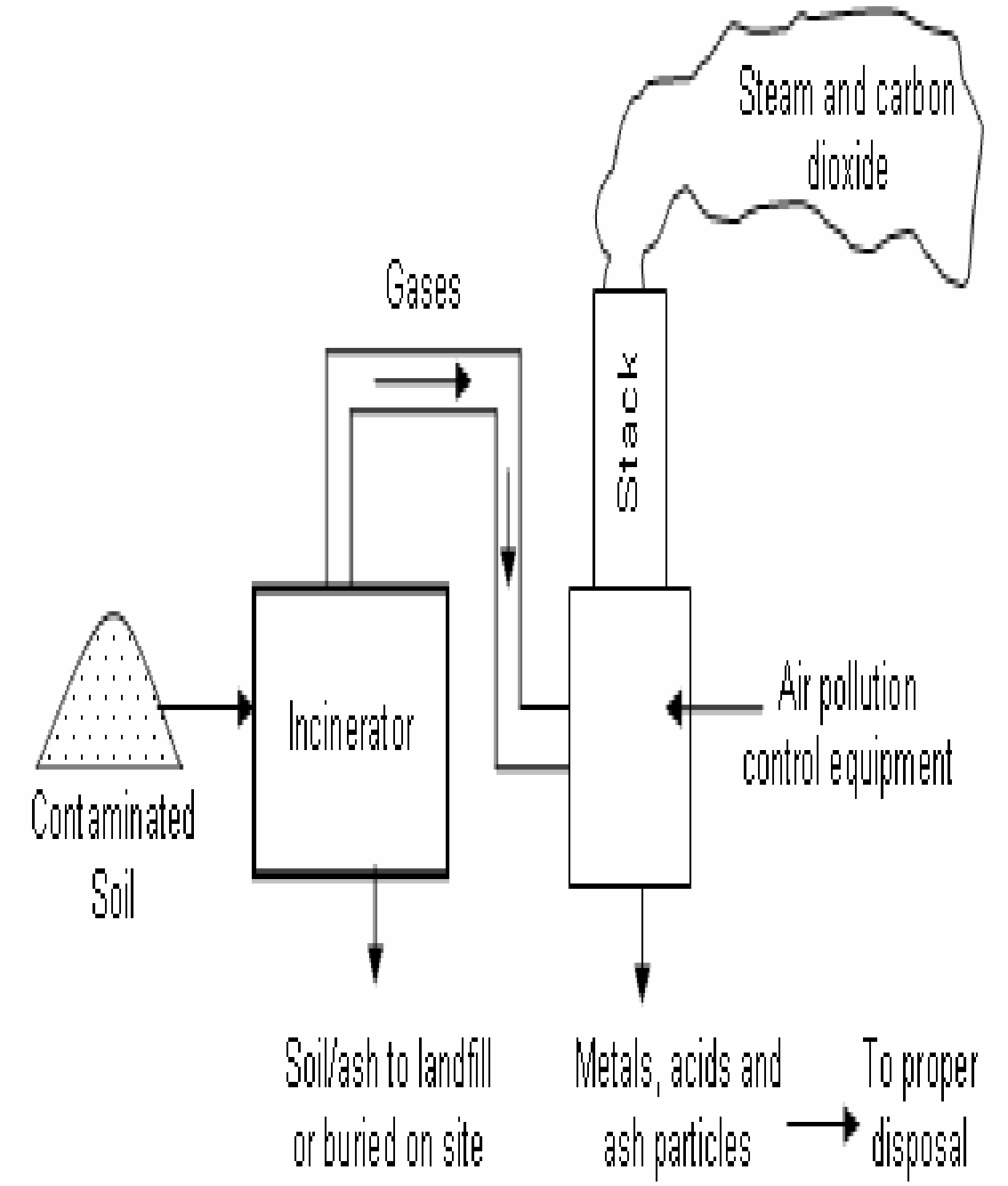


Figure 2.7: Schematic diagram of incineration (FRTR, 2000)

Phytoextraction is applicable to sites that contain low to moderate levels of metal pollution, this is because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. Plants being considered for phytoextraction should be tolerant of the targeted metal and be efficient at translocating them from roots to the harvestable above ground portions of the plant.

Phytoremediation of heavy metals from soils also known as phytoextraction is a process which uses the uptake capabilities of plants. Plants can accumulate metals that are essential for growth and development (such as Cu, Mn, Fe, Zn, Mo, and possibly Ni) and also some metals that have no known biological function (such as Cd, Cr, Pb, Co, Ag, Se, Hg) (Baker and Brooks, 1989; Raskin *et al.*, 1994; Brooks, 1998). Hence, plants have been described as solar-driven pumping stations which can remove these contaminants from the environment (Cunningham *et al.*, 1995). Readily bioavailable metals for plant uptake include Cd, Ni, Zn, As, Se, and Cu. Moderately bioavailable metals are Co, Mn, and Fe; while Pb, Cr, and U are not very bioavailable (Miller, 1996).

Presently, there are two strategies of phytoextraction: (i) continuous phytoextraction, using hyperaccumulators and (ii) chelate-assisted or induced phytoextraction (Salt *et al.*, 1998). The first strategy of metal phytoextraction depends on the natural ability of some plants to accumulate, translocate and resist high amounts of metals over the complete growth cycle. Hyperaccumulators are the most suitable plants since they can accumulate 10-500 times higher levels of heavy metals compared with other plants (Chaney *et al.*, 1997). Also there has been report by Oyedele *et al.* (2006) in which they reported that *Corchorus olitorius* has the potential to decontaminate soils contaminated with heavy metals. The possibility of contaminating the food chain is one of the main problems associated with phytoextraction techniques. However, this challenge is being tackled when hyperaccumulator plants are used. This is because many hyperaccumulator species belong to the *Brassicaceae* family which has a high content of thyocianates which makes it non-palatable to animals, thus reducing the chances of bioaccumulation of metals in the food chain during phytoextraction programs (Navari-Izzo and Quartacci, 2001).

Chelate-assisted or induced phytoextraction is based on the fact that the application of metal chelates such as nitrilotriacetate, ethylene diamine tetraacetic acid (EDTA) and ethylene diamine disuccinate to the soil significantly enhances metal accumulation by plants. Under many circumstances, depending on the metal itself, it is common to find cases of low bioavailability in the soil, thus preventing the remediation process (a large proportion of many metals remains sorbed to solid soil constituents). Fortunately, the discovery that the application of certain chelates to the soil increases the translocation of heavy metals from soil into the shoots has opened a wide range of possibilities for this field of metal phytoextraction (Blaylock *et al.*, 1997). However, the application of synthetic chelates to the soil must be done carefully because of their potential toxicity.

2.5.3 Bacterial remediation (Bioremediation)

Bioremediation technology uses microorganisms to reduce, contain, or transform contaminants present in soils, sediments, water, and air to benign products (NABIR, 2003). Bioremediation is an innovative and promising technology available for removal of heavy metals and recovery of the heavy metals in polluted water and lands. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. It uses relatively low-cost techniques, which generally have high public acceptance and can often be carried out on site. Harder (2004), estimated that bioremediation accounts for 5 to 10 percent of all pollution treatment and has been used successfully in cleaning up the illegal dumping of used engine oil which contained heavy metals as well as hydrocarbons. Since microorganisms have developed various strategies for their survival in heavy metal-polluted habitats, these organisms are known to develop and adopt different detoxifying mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization, which can be exploited for bioremediation either *ex situ* or *in situ* (Gadd, 2000; Lim *et al*., 2003; Malik, 2004; Lin and Lin, 2005). Microorganisms can assimilate heavy metals actively (bioaccumulation) and/or passively (adsorption) (Hussein *et al*., 2001). The bacterial cell walls, which consist mainly of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups (Scott and Karanjkar, 1992). Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination.

Bioremediation promotes the growth of microorganisms to degrade contaminants by utilizing those contaminants as carbon and energy sources. The bioremediation systems in operation today rely on microorganisms native to contaminated sites, encouraging them to work by supplying them with the optimum levels of nutrients and other chemicals essentials for their metabolism (Iram *et al.,* 2009; Ajaz *et al*., 2010). Bioremediation strategies are developed to promote the bacterial metabolism of contaminants, by adjusting the water, air and nutrient supply. This is accomplished by the biostimulation (the addition of a bulking agent such as wood chips and/or nutrients such as N/P/K) and bioaugmentation (often an inoculum of microorganisms with known pollutant transformation abilities) of the contaminated environment (Bamforth and Singleton, 2005).

Bioremediation techniques are typically more economical than traditional methods of waste treatment such as incineration, absorbent/adsorbent techniques, catalytic destruction, etc. Bioremediation technologies are improving as greater knowledge and experience are being gained in the field. Bioremediation application can be more effective where environmental conditions permit bacterial growth and activity; its application often involves the manipulation of environmental parameters to allow bacterial growth and degradation to proceed at a faster rate (Fulekar, 2005). Although most microorganisms have detoxification abilities (i.e. mineralization, transformation and/or immobilization of contaminants), however bacteria play a crucial role in it (Diaz, 2004). Bacteria have developed strategies for obtaining energy from virtually every compound under oxic or anoxic conditions (using alternative final electron acceptors such as nitrate, sulfate, and ferric ions) (Ferhan *et al*., 2002).

Microorganisms can detoxify metals through different methods such as valence transformation, extracellular chemical precipitation or volatilization. Also, they can enzymatically reduce some metals in metabolic processes that are not related to metal assimilation (Lovley, 1993). Many microorganisms also produce siderophores, iron complexing molecules, some of which have high affinity for heavy metals. For example in *Pseudomonas aeruginosa* and *Alcaligenes eutrophus*, siderophore synthesis can be induced by the presence of high iron concentrations (Höfte *et al.*, 1994; Gilis *et al.*, 1996). Sulphate reducing bacteria have been studied and seen to be able to carry out bioprecipitation, a process that convert sulfate to hydrogen sulfide,

which in turn reacts with heavy metals to form insoluble metal sulfides such as zinc sulfide and cadmium sulfide (White *et al.*, 1998; Iwamoto and Nasu, 2001). Microorganisms can also enzymatically reduce other metals such as technetium, vanadium, molybdenum, gold, silver, etc. but these processes have not been studied extensively (Lovley, 1993).

Some organisms which have being involved in bioremediation processes include *Pseudomonas* species such as *P. aeruginosa*, *P. ambigua*, *P.fluorescens*, other bacterial species such as *Bacillus cereus, B. subtilis, E. coli* (ATCC 33456), *Achromobacter eurydice, Micrococcus roseus, Enterobacter cloacae, Desulfovibrio desulfuricans* and *D. vulgaris, Shewanella alga* BrY-MT have been reported to be effective in bioremediation of various contaminants (Lovley, 1994; Guha *et al*., 2001; Camargo *et al*., 2003).

In situ bioremediation has the ability to transform contaminants to less toxic compounds, making this a promising environmental cleanup technique. It accelerates contaminant desorption and dissolution by treating contaminants close to their source. Methods such as pump-and-treat only remove or destroy contaminants in groundwater, but not those contaminants which are already absorbed to soil or solids in the aquifer (NRC, 1993). There have been various reports in the use of bioremediation techniques for cleaning up oil spills (Hoff, 1993; Swannell and Head, 1994; Lee, 1999; Prince *et al*., 1999) using agricultural fertilizers as the main source of nutrient supply (Swannelll *et al*., 1996).

2.6 Cattle dung slurry/ Gomeya

Cattle dung slurry/gomeya usually referred to as a waste product can enhance the degradation of contaminants in the environment (Randhawa and Kullar, 2011). Cattle dung slurry is a cheap and easily available rich source of microflora. It is a mixture of cattle dung and urine in a ratio of around 3:1 respectively (Randhawa and Kullar, 2011). It contains crude fibre (cellulose with lignin), crude protein, cellulose, hemicellulose, and minerals such as nitrogen, potassium, traces of sulphur, iron, magnesium, calcium, cobalt, manganese etc (Nene, 1999). Bacterial composition of cattle dung slurry comprises of about 60 species of bacteria such as *Bacillus* species, *Corynebacterium* species, Fecal *Streptococcus*, *Pseudomonas* sp., *Sarcina*, *E. coli* and

Lactobacillus species, fungi such as *Aspergillus, Rhizopus, Penicillium* and *Trichoderma*, about 100 species of protozoa and yeasts such as *Saccharomyces* and *Candida*. Majority of the bacteria contained in cattle dung are cellulose, hemicelluloses, and pectin fermenters. Cattle dung comprises undigested fibre, sloughed off intestinal epithelium, some excreted products derived from bile (pigments), intestinal bacteria, and mucus. The bile pigment biliverdin is mainly present in cattle dung (herbivore) giving it its green color. Presence of bile salts in cattle dung gives it its emulsifying properties by conferring hydrophilic coat to otherwise hydrophobic droplets (Randhawa and Kullar, 2011).

There have been reports of laboratory investigations on the use of organic nutrients such as cattle dung and poultry droppings in the bioremediation of oil polluted sites (Amadi and Ue-Bari, 1992; Obire and Akinde, 2006). When these organic nutrients are added to polluted sites, they act both as a source of microorganisms and nutrients for microorganisms (Obire *et al.,* 2008). According to Adedokun and Ataga (2007), soil amendments or additives are needed to increase the activities of microbes and for effective bioremediation of polluted soil. For instance, Okolo *et al*. (2005) reported increased degradation of crude oil in soil augmented with poultry manure while Mbah *et al.* (2009) reported that amendment of spent oil contaminated soil with organic wastes led to improved soil physical properties and increased agronomic parameters of such soil. Davies and Wilson (2005) reported that soil amendments improve the physical properties of such soil like water retention, water permeability, water infiltration, drainage, aeration and structure of soil. This will lead to a more efficient remediation of polluted soil. However, there is dearth of information on the use of bioremediation technique incorporated with gomeya as nutrient source for the cleanup of heavy metal contaminated sites.

2.7 Sterilization of soil

According to Williams-Linera and Ewel (1984), steam sterilization can change some of the chemical properties of the soil and thereby affect the higher plants growing in it. For instance, in a comparative study of three soil samples carried out by Skipper and Westermann (1973), autoclaving produced variable changes in pH, there was an increase in pH of one sample, decrease in another, and no change in the third soil

sample; concentration of potassium was observed to have increased in two of the samples whereas there was a reduction in the concentration of potassium of the third sample; concentrations of nitrogen and phosphorus were found to have increase in two of the soil sample while it remained the same in the third sample, there was no changes in the concentration of calcium. Other researchers reported a decrease in the concentration of organic matter and magnesium, while the effect of soil sterilization on the concentrations of phosphorus, potassium and calcium were observed to be small and variable (Kitur and Frye, 1983). Steam sterilization increased the concentration of extractable manganese in the soil approximately four- to eightfold (Fujimoto and Sherman, 1945). Most procedures used in soil sterilization have been reported to alter soil physical and chemical properties and consequently modify quantitatively and qualitatively soil – xenobiotic - microbe interactions (Shawa *et al*., 1999).

2.8 *Corchorus olitorius*

Corchorus olitorius commonly called jute or Jew mallow belongs to the Tiliaceae family (Nkomo and Kambizi, 2009; Aluko *et al.*, 2014). *Corchorus olitorius* originated from South China from where it was introduced to India and Pakistan. However, it has been found to grow as a wild plant in many parts of India as well as China and many parts of Australia and Africa especially in southwestern Nigeria. It is one of the most popular vegetables in every home; hence it is grown in nearly all home gardens, market gardens near the city and truck gardens around the world (Aluko *et al.,* 2014).

Corchorus olitorius is an erect herb that varies from 20 cm to approximately 1.5m in height depending on the cultivar. The stems are angular with simple oblong to lanceolate leaves that have serrated margins and distinct hair-like teeth at the base. The bright yellow flowers are small and the fruit is an angular capsule. *Corchorus* seeds show a high degree of dormancy which can be broken by means of hot water treatment (Schippers *et al*., 2002a). It has been recorded that *C. olitorius* can be naturally established from seeds and tolerates a wide range of soils and climates (Oladiran, 1986; Nkomo and Kambizi, 2009).

The leaves of *C. olitorius* on the average, contains 85-87g water, 5-6g protein, 0.7g oil, 5g carbohydrate, 1-5g fibre, 250 – 266mg Ca, 4-8mg iron, 3000iu vitamin A, 0.1mg

thiamine, 0.3mg riboflavin, 1.5mg nicotinamide and 53 – 100mg ascorbic acid (per 100g). The leaves of *C. olitorius* are popularly used in soup preparation and traditional medicine for the treatment of fever, chronic infection of the bladder (cystitis), cold and tumours (Oboh *et al*., 2009). The young shoot tips can be eaten raw or cooked and it contains high levels of protein and vitamin C (Shittu and Ogunmoyela, 2001). Jute is usually recommended for pregnant women and nursing mothers because it is believed to be rich in iron (Oyedele *et al*., 2006; Aluko *et al*., 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Initial study of the sampling site

The study location was a site contaminated with heavy metal as a result of the activities of a steel rolling company situated in Alomaja (latitude: 7.2675N and longitude: 3.8578E) in Oluyole Local Government Area of Ibadan Oyo State, South-western Nigeria. An initial study was carried out during the dry and rainy season on the site in order to obtain background knowledge of the seasonal variations in the physical and chemical parameters of the study site. Composite soil sample was collected from different points by using soil auger to collect the samples from the topsoil (0cm to 15cm depth). Sampling was done twice (in the peak of dry season and during the rainy season). The soil surrounding the steel rolling company was observed in order to know if there were deviations from the normal physical characteristics such as colour, texture, odour and deposition of effluents from the rolled steel industry and these were considered as indicators of pollution. The samples were thereafter transported to laboratory for microbiological and chemical analysis within 24 hours of collection.

3.2 Sample collection

Five hundred kilogram of composite soil sample to be used for bioremediation study and planting was collected using soil auger from the top soil (0-15 cm) at different points in the study site in sack bags and was transported to the Department of Microbiology, University of Ibadan, Ibadan.

3.3 Collection of cattle dung slurry (gomeya)

Cattle dung slurry (gomeya) used as organic amendment in this study was collected in large nylon bags from a commercial pen in Bodija Market (latitude: 7.4351^{o}N, longitude: 3.9143^{o}E), in Ibadan North Local Government Area of Oyo State, South

western Nigeria and was transported to the Department of Microbiology, University of Ibadan, Ibadan.

3.4 Collection of *Corchorus olitorius* seeds

Seeds of *Corchorus olitorius* already treated with scarification method (in order to break the dormancy) used in this study were obtained from Agronomy Department, Faculty of Agriculture, University of Ibadan, Ibadan, Nigeria.

3.5 Analysis of heavy metals contaminated soil sample

The soil samples collected were thoroughly mixed using hand trowel to obtain a composite sample. The following analyses were carried out on the composite samples for both the dry and rainy season samples.

3.5.1 Determination of pH

Hydrogen ion concentration (pH 1:1 H_2O) was determined using a glass electrode pH meter (Hanna instruments HI2210). A paste was formed from the composite soil sample by weighing 20 g of soil into a 50 mL beaker, 20 mL of water was added in small increments and the soil sample was stirred with a spatula until it was saturated. The paste was stirred at regular intervals of 30 minutes after which the glass electrode was inserted into the paste and the pH was read (Bates, 1954).

3.5.2 Determination of Exchangeable Acidity

Exchangeable acidity (EA) is defined as total titratable acidity of the soil and it represents the acidity due to exchangeable hydronium and aluminium ions. The exchangeable acidity was determined using the KCl extraction method following the method of Mclean (1965). Five grams of the dried composite soil sample was weighed into a 50 mL centrifuge tube and 30 mL of 1 M KCl was added. The centrifuge tube was tightly closed with a rubber stopper and was put on a reciprocating shaker to shake for 1 hour. The content was centrifuged at 2,000 rpm for 15 minutes and the clear supernatant was carefully decanted into a 100 mL volumetric flask. Thirty milliliters of 1 M KCl was added to the same soil sample and allowed to shake on the shaker for 30 minutes, after which it was centrifuged at 2,000 rpm for 15 minutes and the clear supernatant was carefully decanted into the same 100 mL volumetric flask containing

the first supernatant (this step was repeated) and the clear supernatant was decanted into the same volumetric flask and the volume was made up to mark by adding 1 M KCl. Fifty milliliters from the KCl extract was pipetted into a 250 mL Erlenmeyer flask, 5 drops of phenolphthalein indicator was added and this was titrated against 0.01 M NaOH to a permanent pink endpoint with alternate stirring and standing (the amount of base used is equivalent to the total amount of acidity (H+Al) in the aliquot taken). One drop of 0.01 M HCl was added to the same flask to bring the solution back to colorless after which 10 mL of NaF solution was added. The solution was stirred constantly, 2 drops of the indicator was added and was titrated with 0.01 M HCl until the colour of the solution disappears and does not return within two minutes. The milliequivalent of acid used was equal to the amount of exchangeable Al in the solution. The milliequivalent of total aciditity obtained in the above step was subtracted from that obtained when titrated against KCl and this gives the milliequicvalent of the exchangeable hydrogen (acidity) which was expressed in meq/100 g of soil.

3.5.3 Determination of mineral contents of the soil

These analyses were conducted to determine the concentration of minerals such as nitrogen, carbon, calcium, magnesium, available phosphorus, sodium, potassium present in the composite soil sample.

3.5.3.1 Determination of organic Carbon

The organic matter in the soil sample in the form of carbon was determined using the Walkey-Black wet oxidation method as described by Page (1982). The composite soil sample was ground so that it can pass through a 0.5 mm mesh and 1.0 g of the soil was weighed into a 250 mL Erlenmeyer flask. Ten millilitres of 1 N $K_2Cr_2O_7$ solution was pipetted into the flask and swirled gently so as to disperse the soil, 20 mL of concentrated H_2SO_4 was rapidly added using a glass pipette and the flask was gently swirled immediately in order to ensure proper mixture of the reagent. The flask was swirled more vigorously for a minute after which it was rotated and allowed to stand on an asbestos sheet for 30 minutes. Distilled water was added to the solution after 30 minutes, this was immediately followed by the addition of 3-4 drops of 0.025 M O-phenanthroline-ferrous complex (ferroin) indicator after which it was titrated against

0.5 N ferrous sulphate solution. As the reaction approached the endpoint, the solution took on a greenish cast and then turned dark green, at this point, the ferrous solution was added drop by drop until the colour of the solution changed sharply from blue to red.

3.5.3.2 Determination of Nitrogen

The total nitrogen in the composite soil sample was determined using the macro-Kjeldahl method as described by Page (1982). The composite soil sample was air dried, ground and sieved using a 0.5 mm mesh. Ten grams of the sieved soil sample was weighed into a dry 500 mL macro-Kjeldahl flask and 20 mL of distilled water was added. The flask was swirled for a few minutes and was allowed to stand for 30 minutes. A tablet of mercury catalyst was added together with 10 g of K_2SO_4 followed by the addition of 30 mL of concentrated H_2SO_4 using a glass pipette. The flask was cautiously heated using low heat on the digestion stand until all water in the sample was removed and frothing ceased, after this the heat was increased until the digest cleared. The mixture was boiled for 5 hours using regulated heat so that the H_2SO_4 condenses about midway up the neck of the flask. The flask was allowed to cool and 100 mL of distilled water was slowly added to it. The digest was carefully transferred into another clean macro-Kjeldahl flask (750 mL), while the entire soil particle was retained in the original digestion flask, this is because soil particle can cause severe bumping during Kjeldahl distillation.

The soil residue was washed with 50 mL of distilled water four times and the aliquots were transferred into the new flask. Fifty millilitres of H_3BO_3 indicator solution was put into a 500 mL Erlenmeyer flask and this was placed under the condenser of the distillation apparatus with the end of the condenser about ten centimetres above the surface of the H_3BO_3 solution. The 750 mL macro-Kjeldahl flask was attached to the distillation apparatus, 150 mL of 10 M NaOH was added through the distillation flask by opening the funnel stopcock and distillation was commenced immediately. The condenser was kept cool by allowing sufficient cold water to flow through and the heat was regulated so as to minimize frothing and suck back. The distillation was stopped upon collection of 150 mL distillate. The NH_4-N in the distillate was determined by titrating it against 0.01 M standard HCl using a 25 mL burette graduated at 0.1 mL interval. The change in colour of the distillate from green to pink indicated the end

point of the titration. The nitrogen content of the soil in percentage was calculated using the following formula:

$$\%N = \frac{T \times M \times 14 \times 100}{\text{weight of soil used}}$$

where T= titre value and M= molarity of HCl, 14= molecular weight of Nitrogen

3.5.3.3 Determination of Phosphorus

The phosphorus in the composite soil sample was analysed using the vanado-molybdate method (AOAC, 2012). Ten millilitres of the sample solution from wet digestion was pipetted into a 100 mL volumetric flask and 60 mL of distilled water was added. Within 5 minutes, 20 mL of vanado-molybdate reagent was added and the mixture was diluted. This was mixed and allowed to stand for 10 minutes. The percent transmittance was determined at 400 nm and phosphorus was extrapolated from a curve drawn using KH_2PO_4 as standards.

3.5.3.4 Determination of calcium and magnesium

Extraction was carried out on the soil samples using methods described by Mehlich (1953) and Watanabe and Olsen (1965). The composite soil sample was air dried, ground and sieved using a 0.5 mm mesh. Five grams of the sieved soil sample was weighed into a 50 mL glass beaker, 25 mL of Mehlich-1 extracting solution was added. The extraction flask was placed on a reciprocating mechanical shaker for five minutes after which it was filtered into 20 mL plastic scintillation vials. This was kept for use as the soil extract.

The calcium and magnesium in the composite soil sample was determined by versenate titration method following the method described by AOAC (2012).

For calcium determination, 50 mL of distilled water together with 20 mL of 20% KOH and 20 mL of the extract obtained from the soil sample were put into a 250 mL Erlenmeyer flask, 0.05 g of calcein indicator powder was added and this was titrated against 0.02 N versenate until the fluorescent green colour disappears leaving a yellowish pink colour which indicated the endpoint of the titration. Calcium in milliequivalent/litre was calculated using the following formula:

$$Ca^{2+}\ (meq/L) = \frac{N \times V_1 \times 1000}{20}$$

Where N= Normality of versenate, V_1= volume of versenate

For the determination of calcium and magnesium in the composite soil sample, 50ml of distilled water was measured into an Erlenmeyer flask; 25 mL of concentrated ammonia solution was added to it together with 20 mL of the extract from the soil sample. Five drops of the eriochrome black T indicator was added to the solution followed by the addition of 1 mL of 2% NaCN. This was titrated against 0.02 N versenate to a bright blue end point. The total calcium plus magnesium in milliequivalent/litre in the sample aliquot used was given by:

$$Ca^{2+} + Mg^{2+} \text{ (meq/L)} = \frac{N \times V_2 \times 1000}{20}$$

Where N= Normality of versenate, V_2= volume of versenate

The amount of Mg^{2+} (in meq/100 g of soil sample) was obtained by subtracting the value obtained for Ca^{2+} (meq/L) from the value obtained for $Ca^{2+} + Mg^{2+}$ (meq/L).

3.5.4 Determination of Concentration of Heavy metals

The determination of concentration of heavy metals such as cadmium, iron, copper, lead, chromium, zinc, nickel, cobalt and manganese present in the soil sample was determined using the wet digestion procedure (SSSA, 1971). This was carried out by weighing 0.5 g of the 0.5 mm sieved soil into a 100 mL Berzellius beaker, 5 mL HNO_3 and 2 mL $HClO_4$ was added and covered with a watch glass. This was digested in a fume cupboard by heating it to a final volume of 3 to 5 mL. Ten to fifteen millilitres of water was added and the digest solution was filtered through an acid washed filter paper into a 50 mL volumetric flask. It was diluted to volume with deionized water and the filter paper was washed with water. The filtrate was used to determine the concentration of heavy metals present in the sample using Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer (AAS).

3.5.5 Microbiological analysis

3.5.5.1 Total Viable Bacteria Count (TVBC) and Isolation of Pure Bacteria

The determination of the total viable bacteria count (TVBC) was carried out in triplicates. The agar medium and the diluents used were sterilized at 121^{o}C for 15 minutes. One gram of the thoroughly mixed composite soil samples was suspended in 9 mL of sterile distilled water and serially diluted (Olutiola *et al*., 2000). One millilitre dilutions 10^{-1} to 10^{-4} were inoculated into sterile Petri dishes and already prepared and cooled nutrient agar (Lab M, United Kingdom) was added to it using the pour plate

technique as described by Olutiola *et al*. (2000). Inoculated plates were incubated at 37^{o}C for 24 hours in an inverted position after which distinct bacteria colonies were counted. Morphologically distinct bacteria colonies were subcultured by streaking on fresh nutrient agar plates until pure bacteria colonies were obtained. Pure cultures of each bacteria strain were stored on nutrient agar slants at 4^{o}C for further studies.

3.5.5.2 Identification of microorganisms

Pure culture of the bacterial isolates were characterized and identified using Cowan and Steel (1993) and Holt *et al.* (2000) after the following tests were carried out:

Gram Staining

This test was carried out to differentiate the bacterial isolates into Gram positive and Gram negative isolates based on their Gram reaction, and to detect their morphology and cell arrangements. The test was carried out according to the method of Olutiola *et al.* (2000). Smear from 18-24 hours old culture was prepared on microscope slides for all the organisms, this was heat fixed. Thereafter they were flooded with crystal violet solution for 60 seconds and was poured off. The slides were rinsed with Gram's iodine solution and the iodine was allowed to react for 60 seconds before it was rinsed off with distilled water, this was followed by rinsing the slides with 95% ethanol until no violet colour of the crystal violet was observed. The slides were then rinsed with water after which they were counterstained with safranin for 30 seconds and rinsed off with water and allowed to air dry. The slides were viewed under the oil immersion power of the light microscope. Gram positive organisms stained purple colour while Gram negative organisms stained pink. The organisms were also observed for their cell morphology and arrangement.

Catalase test

This test was carried out to detect the presence of catalase (an enzyme) in an organism. This enzyme converts hydrogen peroxide to water and oxygen, oxygen is liberated as gas which is observed as effervescence.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_20 + O_2$$

Three percent hydrogen peroxide was prepared; a drop of this was placed on a clean glass slide and an 18-24 hour old culture of the test organism was placed on the slide

and observed. Effervescence caused by liberation of oxygen gas indicated presence of catalase, hence a positive result while absence of effervescence indicated negative result (Olutiola *et al*., 2000).

Oxidase test

This was carried out to detect the presence of cytochrome C in the test organisms. Whatman no. 1 filter paper (CAT No. 1001 110) was moistened with tetramethyl-p-phenylene diamine hydrochloride (Sigma-Aldrich). A streak of 18-24 hours old culture of the test organism was made on the moistened filter paper and this was observed for colour change. A colour change to purple on the filter paper within 10-15 seconds indicated a positive result while negative result was indicated by no colour change or delayed colour change (Olutiola *et al*., 2000).

Potassium Hydroxide test

Like the Gram's stain reaction, the potassium hydroxide (KOH) test is based on the differences in the bacterial cell wall composition. The cell wall of Gram-negative bacteria is easily disrupted when exposed to dilute alkali solutions while the tough thick peptidoglycan wall of Gram-positives do not lyse. When the cell walls are disrupted, the suspension in KOH becomes viscous due to the release of relatively unfragmented threads of deoxyribonucleic acid. The KOH test is a test that can be used to confirm the Gram's staining reaction. Following the method described by Halebian *et al.* (1981) and Carlone *et al.* (1983), a drop of 3% KOH (Sigma-Aldrich) was placed on a clean glass slide, with the aid of an inoculating loop, a large colony of the test organism was picked from a 18-24 hour old culture, this was mixed with the solution, homogenized and allowed to wait for a minute. The loop was then used to lift the mixture up to about 1-2cm. For Gram negative bacteria, the KOH solution characteristically became very viscous and mucoid with a string of the solution following the loop while Gram positive bacteria display no reaction and no string formation.

Citrate Utilization test

This test was carried out to differentiate the isolates based on their ability to utilize citrate as sole carbon source, the test was carried out as described by Olutiola *et al.*

*(*2000). Citrate agar slants were prepared according to manufacturer's instruction (see appendix 1), this was inoculated with a peptone water culture of the test organism using an inoculating loop. The slants were incubated at 37^{o}C for 2-5 days. A change in colour of the slant from green to blue indicates the utilization of citrate as sole carbon source.

Indole test

This test is important in the differentiation of coliforms and it depends on the production of indole from tryptophan by the organism. Tubes of tryptone water (Oxoid M0087) were inoculated with a loopful of 18-24 hours old broth culture of test organism and the tubes were incubated for 5-7 days at 37^{o}C. At the end of the incubation period, 0.5 mL of Kovac's reagent (produced by Kermel) was added, this was observed for the formation of a red ring at the top of the broth, indicating a positive reaction while no colour change or ring formation indicated a negative indole reaction (Olutiola *et al*., 2000).

Hydrogen sulphide production test

This test was carried out to determine the ability of isolates to decompose organic sulphur compounds such as sulphite to produce hydrogen sulphide. The test was carried out as described by Olutiola *et al*. (2000). Whatman no. 1 filter paper (CAT No. 1001 110) to be used as the indicator paper was soaked till it was saturated with solution of lead acetate and was allowed to dry. Thiosulphate broth containing peptone water and 0.01% of thiosulphate was prepared, dispensed into test tubes. The dried filter paper and the thiosulphate broth were sterilized at 121^{o}C for 15 minutes. The tubes containing the thiosulphate broth were inoculated with a loopful of a broth culture of the test organism and the filter paper already soaked with lead acetate was inserted three-quarter down into the test tube so that it did not make contact with the content of the test tube. Blackening of the filter paper indicated the production of hydrogen sulphide after 48 hours of incubation.

Urease test

This test was carried out to determine the ability of the isolates to produce urease which is an enzyme that breaks down urea to release ammonia. The test was carried

out according to the method of Olutiola *et al.* (2000). Basal medium for the detection of urease production was prepared following the composition of urease agar (Hi media M1828) and distributed into bottles; this was sterilized at 121^{o}C for 15 minutes. Urea solution was sterilized using membrane filter and added to the basal medium to give a concentration of 2% urea, the bottles were slanted after which they were streaked with the test organisms and incubated for up to 7 days. It was examined for the production of red colouration which was an indication that the organisms were urease producers, non urease producers shows no colour change.

Motility Test

This test was carried out to determine if the organism was motile, the medium used was formulated using the composition of Sulphate production, Indole production and Motility test agar (SIM medium (see appendix I)), which is a medium used to detect hydrogen sulphide production, indole formation and motility test. The medium was prepared by dissolving 13 g of nutrient broth and 3 g of agar-agar into one litre of distilled water and dispensed as 10 mL volumes into test tubes. This was autoclaved at 121^{o}C for 15 minutes after which it was allowed to cool. The semi-solid medium was stab inoculated with the test organism and incubated at 35^{o}C for 24 hours after which it was observed for motility of the organisms. Organisms that grew along the line of stab only were classified as non motile, whereas those that grew and ramified away from the line of stab were classified as motile organisms.

Sugar fermentation test:

This test was carried out to determine the ability of the isolate to ferment various sugars which were all products of BDH Chemicals Ltd., Poole, England. The medium used consist of 1% of appropriate sugar and 1% peptone water and phenol red was used as an indicator, all dissolved in 100 mL of distilled water (Olutiola *et al*., 2000). The sugars used include sucrose, glucose, sorbitol, lactose, inositol, mannose and arabinose. The medium prepared for each sugar test was dispensed as 10 mL volume into test tubes and Durham's tube was inserted into the test tube in an inverted position and this was sterilized at 121^{o}C for 10 minutes. Each of the test tubes was inoculated with the test organism and incubated for 5-7 days during which it was observed for colour change and gas production. Acid production was indicated by a change in the

colour of the medium from red to yellow and gas production was indicated by displacement of the solution in the Durham's tube by air.

3.6 Molecular characterization of the isolates

3.6.1 Extraction of Total Genomic DNA

Total genomic DNA was extracted from the bacterial isolates for molecular characterization at the Bioscience Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, using QIAamp DNA Mini Kit (250) cat no 51306 as directed in the handbook (Qiagen, 2003). Each bacterial strain was inoculated into 5 mL Nutrient broth (234000 BD Difco) for 72 hours at 37^{o}C. In order to obtain compact pellet, 1.5 mL of the culture was put into an Eppendorf tube, and was spun in a microcentrifuge for 5 minutes at 7500 rpm. The supernatant was discarded and the pellet was resuspended in buffer ATL (which was supplied with the QIAamp DNA Mini Kit used) to make total volume of 180 µL. Exactly 20µL of Proteinase K was added to this mixture and was vortexed, and incubated at 56°C until the cells were completely lysed. The mixture was placed in a shaking water bath during incubation to disperse the sample. Lysis was completed within three hours. The Eppendorf tube was briefly centrifuged to remove droplets from the inside of the lid. Thereafter, 4µL RNAase (100 mg/mL) was added to the mixture, this was mixed by pulse-vortexing for 15 seconds and incubated for 2 minutes at room temperature. The Eppendorf tube containing the mixture was briefly centrifuged to remove droplets from inside the lid before adding 200 µL buffer AL to the sample. This was mixed again by pulse-vortexing for 15 seconds to ensure that the sample and buffer AL were thoroughly mixed to yield a homogeneous solution and incubated at 70°C for 10 minutes. This was followed by addition of 230 µL ethanol (96–100%) to the sample and mixed by pulse-vortexing for 30 seconds. The mixture together with the precipitate formed upon the addition of the alcohol was carefully applied to the QIAamp Spin Column without wetting the rim. The cap was closed and was centrifuged at 6000 rpm for 1 minute. The QIAamp Spin Column was placed in a clean 2 mL collection tube provided with the kit and the filtrate was discarded. The QIAamp Spin Column was carefully opened and 500 µL of buffer AW1 was added without wetting the rim. The cap was closed and was centrifuged at 6, 000 rpm for 1 minute after which it was placed in a collection tube and the filtrate was discarded. The

QIAamp Spin Column was carefully opened and 500 µL of buffer AW2 was added without wetting the rim. The cap was closed and was centrifuged at 6, 000 rpm for 1 minute after which it was placed in a collection tube and the filtrate was discarded. Centrifugation was again carried out at full speed of 14,000 rpm for 3 minutes. The QIAamp Spin Column was placed in a clean Eppendorf tube and the collection tube containing the filtrate was discarded. In order to obtain the purified DNA, the QIAamp Spin Column was carefully opened and 200 µL of preheated (70^{o}C) buffer AE was added. This was incubated at 70^{o}C for 1 minute and then centrifuged at 6,000 rpm for 1 minute. The filtrate solution was placed into the spin column and 200 µL of preheated (70^{o}C) buffer AE was added. This was incubated at 70^{o}C for 1 minute and then centrifuged at 6,000 rpm for 1 minute (in order to increase the DNA yield), after which the spin column was discarded and the obtained DNA was loaded unto agarose gel and nanodrop spectrophotometry was performed.

3.6.2 Polymerase Chain Reaction (PCR) Amplification

The PCR amplification was performed using Applied Biosystems Thermocycler, model 9800 with 1.5 µL of DNA extract in a total volume of 25 µL The PCR master mix contained 1.0 µL of 10X PCR reaction buffer, 1.0 µL of 25 mM $MgCl_2$, 0.5µL of 5pMol forward primer (8F (5´ 3´)), 0.5 µL of 5pMol reverse primer (1392R (5´ 3´)), 1.0 µL of DMSO, 0.8 µL of 2.5 mM DNTPs, 0.1 µL of Taq DNA polymerase, 2.0 µL of 10 ng/µL and 3.1 µL of ultra pure PCR water to make a total volume of 10 µL. Initial denaturation was carried out at 94°C for 3 minutes, which was followed by thirty-six cycles of amplification at 94°C for 30 seconds, annealing was done at 56°C for 30 seconds and extension at 72°C for 45 seconds. A final extension phase was performed at 72°C for 10 minutes.

3.6.3 Purification of amplified product

The PCR product was purified by PEG-NaCl method. The sample was mixed with X 0.6 volume of PEG-NaCl which was made up of 20% (PEG(MW6000) and 2.5 M NaCl) and incubated for 20 minutes at 37°C. The precipitate was collected by centrifugation at 3,800 rpm for 20 minutes. The pellet was washed with 70% ethanol, air dried and dissolved in 12 µL sterile distilled water.

3.6.4 DNA Sequencing

The sample was sequenced using a 16-well Applied Biosystems sequencing plate following the manufacturer's instructions. The thermocycling for the sequencing reactions began with an initial denaturation at 94^{o}C for 2 minutes, followed by 35 cycles of PCR consisting of denaturation at 94^{o}C for 10 seconds, annealing at 50 ^{o}C for 10 seconds and extension at 60^{o}C for 4 minutes using primers 704F (5´ 3´) and 907R (5´ 3´). The samples were purified using standard protocols described by Applied Biosystems (Foster City, USA). To this, 10 µL of Hi-Di formamide was added and vortexed briefly. The DNA was denatured by incubating at 95°C for 3 min, kept on ice for 5-10 min. Sequencing reaction was done with the attached file and loaded on the 3130xl Genetic analyzer (Applied Biosystems) following the manufacturer's instructions. The obtained sequences of bacterial 16S rRNA were analysed using Sequence Scanner (Applied Biosystems) software and the 16S rRNA sequence contigs were generated using Chromas Pro.

3.6.5 Identification of the Bacterial Isolates

The obtained 16S rRNA sequence contigs were used for a Basic Local Alignment Search Tool (BLAST) at the GenBank database hosted at the National Centre for Biotechnology Information (NCBI) to confirm the identity of the isolates. This was done by entering the obtained 16S rRNA sequence contigs into the query dialogue box of the National Centre for Biotechnology Information (NCBI) website with the instruction to limit the search to referenced organisms in the GenBank database. The gene sequences of related organisms were dispayed and the gene sequence of organisms showing high relatedness were copied to be used for the construction of the phylogenetic tree.

3.6.6 Phylogenetic Analyses

The 16S rRNA gene sequences obtained from the GenBank database of the National Centre for Biotechnology Information (NCBI) were aligned using the Molecular Evolutionary Genetics Analysis (MEGA) software version 6 following the method described by Hall (2013). The evolutionary history of the bacterial isolates was inferred using the Neighbor-Joining method as described by Saitou and Nei (1987). The optimal tree with the sum of branch length = 14.28219804 was shown (in the

result section). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches as described by Felsenstein (1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.,* 2004) and in the units of the number of base substitutions per site. The analysis involved 36 nucleotide sequences. All positions with less than 95% site coverage were eliminated i.e. fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 564 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 using the method described by Tamura *et al.* (2013).

3.7 Determination of the isolates ability to tolerate heavy metals

The ability of bacterial isolates to tolerate increasing concentration of heavy metals such as chromium, cadmium, lead, copper, cobalt, nickel and zinc was determined quantitatively using the agar diffusion method following the method described by Narasimhulu *et al.* (2010). Concentration of heavy metals in nutrient agar medium was gradually increased from 100-500 µg/mL. The screening was done by streaking a 24 hours old culture of the test organism on nutrient agar plate supplemented with 100 µg/ mL of the salt of the heavy metals of interest and was incubated for five days. Isolates that grew at this concentration were sub-cultured on nutrient agar plates supplemented with higher concentration until 500 µg/mL concentration of heavy metal at increasing level of 50 µg/mL. The concentration at which the bacterial isolates failed to grow after five days of incubation was considered as the minimum inhibitory concentration (MIC) of the heavy metal.

3.8 Bioremediation of contaminated soil sample

3.8.1 Preparation of soil

Composite soil sample collected from the heavy metals contaminated steel rolling site was initially treated as recommended by Saeed and Rafique (1980) and Iqbal *et al.* (2011). According to this method, samples were air dried in the sunlight for a day and then sieved using a 0.5 mm nylon mesh sieve. The soil sample was then sequentially sterilized using hot air oven at 105^{o}C for one hour, after which it was aseptically

packaged by weighing 5 kg into sterile polythene bags that will be used for the planting exercise.

3.8.2 Preparation of bacterial inocula

Three bacterial isolates that showed very high MIC were selected for the bioremediation exercise. These were *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) as revealed by molecular characterization. Working solution for the bioremediation exercise was prepared by inoculating each of these isolates into peptone water broth and incubating till a cell density of 7.6×10^{11} cfu/mL was obtained. However, for bioremediation exercise using mixed culture, the working solution was allowed to reach a cell density of 1.5×10^{12} cfu/ml (Okparanma *et al.*, 2009).

3.8.3.1 Preparation of cattle dung slurry (gomeya)

Cattle dung slurry (gomeya) is a mixture of cattle dung and urine in a ratio of approximately 3:1 weight/volume (w/v) respectively (Randhawa and Kullar, 2011). The cattle dung slurry was sterilized at 105^{o}C for 1 hour, and 100g of this was added to the experimental set up (5 kg of soil) following the method described by Ayotamuno *et al.* (2006) and Njoku *et al.* (2012).

3.8.3.2 Analysis of the sterilized cattle dung

The sterilized cattle dung slurry was analyzed for its proximate matter such as percentage crude fibre, crude protein, ash and ether extract and heavy metal content such as iron, copper, zinc, cadmium, cobalt, lead, chromium and nickel using the same methods as described in section 3.5.3 and 3.5.4.

3.8.4 Bioremediation experimental setup

Twenty milliliters of the working solution of the bacterial isolate was introduced into each experimental setup. There were sixteen experimental groups in all and each group had five replicates. The experimental groups are as shown below:

Groups

A Sterilized soil treated with *Alcaligenes aquatilis* (TS11)

B Sterilized soil treated with *Pseudomonas mucidolens* (E63)

C	Sterilized soil treated with *Bacillus cereus* (E12Ciia)
AB	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63)
AC	Sterilized soil treated with consortia of *Alcaligenes aquatilis* TS11) and *Bacillus cereus* (E12Ciia)
BC	Sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
ABC	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
AM	Sterilized soil treated with *Alcaligenes aquatilis* (TS11) and sterilized cattle dung slurry (gomeya)
BM	Sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)
CM	Sterilized soil treated with *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)
ABM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)
ACM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)
BCM	Sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)
ABCM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)
Control	Sterilized soil alone without bacterial or organic nutrient amendment
ControlM	Unsterilized soil containing sterilized cattle dung slurry (gomeya)

When the working solution of the bacterial isolates was added to the soil, it was aseptically mixed with the soil using sterile glass rod, so as to ensure proper mixing of the isolates with the soil sample. Also, after the addition of the Cattle dung slurry, the

soil was turned using a sterile hand trowel to ensure that there is proper mixture of the soil and organic nutrient.

3.9 Planting using potted plant experiment

3.9.1 Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) having 16 treatments with 5 replicates. The total number of pots used were 80, treated seeds of *C. olitorius* were spread (at least six) in each pot.

3.9.2 Planting Experiment

After planting, the plants were watered twice daily (100 ml/time) for the first two weeks of planting and later reduced to once till the experiment was terminated eight weeks after planting (WAP), so as to prevent the leaching of nutrient from the treatments. Data were collected starting from 2 WAP on a weekly basis on the following parameters: plant height, stem diameter and number of leaves.

3.9.2.1 Plant height

Plant height was measured using a measuring tape. The plant height was measured from the rhizoplane to the apical tip of the plant in centimeters starting from 2WAP till the eighth week when the experiment was terminated (Elings, 2000).

3.9.2.2 Stem diameter

This was measured using a vernier caliper below the first nodes of the plant (Elings, 2000).

3.9.2.3 Number of leaves

This was obtained by counting the number of leaves per plant manually (Elings, 2000).

3.10 Post Bioremediation and Post Harvest analysis

3.10.1 Analysis of the harvested plants

At the end of the experiment, the plants were harvested per group and the following parameters were determined: wet and dry weight, moisture content, dry matter, %

crude protein, % ash, % ether extract (fat), % crude fibre and heavy metal content using standard analytical methods as described by AOAC (2012).

3.10.1.1 Determination of fresh, moisture content and dry weight of harvested plants

The fresh weight of the plant was obtained by putting the freshly harvested plants on a measuring scale and taking note of the readings. Moisture content of the plant samples was determined by weighing two grams of the freshly harvested plant samples into a silica dish which had been previously ignited and weighed. The plant was dried in the steam oven at 100^{o}C to a constant weight and was cooled for 10 minutes in a dessicator each time before weighing till a constant weight was observed. The moisture content of the plant sample was calculated using the following formula:

$$\%\text{Moisture content} = \frac{\text{weight of plant taken - weight of plant after dying}}{\text{weight of plant taken}} \times 100$$

The dry weight of the plants was obtained by measuring the final weight of the plant samples after the moisture content had being removed (AOAC, 2012).

3.10.1.2 Determination of dry matter of harvested plants

The dry matter content of plant is the ratio of of the plant dry mass to its fresh mass. It is the amount of material remaining after removing the moisture content. Hence, the dry matter of the plant sample was determined by subtracting the moisture content obtained for each plant sample from 100 (Cozzolino and Labandera, 2002). The dry matter was calculated using:

Dry matter = 100 - %moisture content

3.10.1.3 Determination of ash content of harvested plants

The percentage ash content of the plant samples was determined by charring the residue obtained from the moisture content determination using Vecstar muffle furnace (Model EF3, Chesterfield, UK) for 3 hours at 450^{o}C. The remaining inorganic material after charring was cooled in a desiccator, weighed and the ash content was determined (AOAC, 2012).

$$\%\ \text{Ash} = \frac{\text{weight of sample remaining after charring}}{\text{weight of original sample}} \times 100$$

3.10.1.4 Determination of percentage crude protein of harvested plants

The percentage crude protein present in the plant sample was determined using the microKjeldahl distillation method. The procedure used was as follow:

Two grams of the dried ground plant sample was weighed into a Kjeldahl flask, 5 g of anhydrous sodium sulphate, a speck of selenium followed by the addition of 25 mL of concentrated H_2SO_4. This was placed in the fume cupboard and heated gently for 5-10 minutes until frothing nearly stopped, the flame was turned on fully and the contents of the flask was digested until it assumed a green colour, after which it was digested for 15 minutes more. The flask was allowed to cool and was diluted with 50 mL of distil water before the flask became cold. The content of the flask was then turned into a 250 mL flask and all the contents of the first flask were rinsed and added to the new one. The 250 mL flask was made up to mark in order to determine the percentage nitrogen. The apparatus to be used was steamed out for about 10 minutes and then the steam generator removed from the heat source while the developing vacuum removed the condensed water. The steam generator was placed on a heat source and 5 mL of the nitrogen containing digest was pipette into the body of the apparatus via the small funnel aperture. Mercury catalyst was added together with 1 mL of 1% sodium thiosulphate to prevent the formation of mercury-ammonia complexes. Also 5 mL of 60% NaOH solution was added. A 100 mL Erlenmeyer flask containing 10 mL of boric acid plus indicator (2 parts of 0.2%methyl red+3 parts of 0.2% of bromocresol green) was placed at receiving tip of the condenser. When all was ready, the funnel plug was carefully lifted and most of the caustic soda was allowed to run into the apparatus. The receiving flask was held off the condenser tip while the caustic soda was running into the apparatus so as to prevent any violent suck back, after which the flask was returned to its normal position as soon as possible. The distillation was continued for 2 minutes (AOAC, 2012). The content of the receiving flask was titrated against 0.01 N HCl and the percentage crude protein was calculated using:

$$\% \text{ Crude protein} = \frac{0.00014 \times \text{volume of acid} \times 250 \times 100 \times 6.25}{5 \times W}$$

where W= weight in grams of the sample digested.

3.10.1.5 Determination of percentage ether extract of harvested plants

The percentage ether extract was obtained by following this procedure:

A soxhlet extractor with a reflux condenser and a small flask which had been dried previously and weighed was set up. One gram of the sample was weighed and transferred to a fat free extraction thimble, this was plugged lightly with cotton wool and then placed in the extractor after which petroleum ether was added at distillation point 40-60^{o}C until it siphons over once. More ether was added until the barrel of the extractor was half full, the condenser was replaced and the joints were checked to ensure they were tight after which it was placed on water bath. The source of heat was adjusted so that the ether boils gently and was allowed to siphon over 10 to 12 times. When the ether was short of siphoning over, the flask was detached and the content of the extractor barrel was siphoned into the ether stock bottle and drained well. The thimble was removed and dried on a clock glass on the bench top away from a flame, after which the condenser and the flask was replaced and the distillation of the ether was continued until the ether flask was practically dry. The flask which now contains all the oil was detached, the exterior was cleaned and it was dried in the oven until a constant weight was obtained, the difference between the initial weight and the final weight gave the weight of the oil (AOAC, 2012). The extracted residue was kept to be used for analysis of the crude fibre while the percentage ether content was calculated using the following formula:

$$\%\text{Ether extract} = \frac{\text{weight of oil}}{\text{weight of plant sample}} \times 100$$

3.10.1.6 Determination of percentage crude fibre of harvested plants

The percentage crude fibre present in the plant sample was determined as follows:

Twenty three millilitres of 10% sulphuric acid was measured with a pipette into a beaker, 175 mL of water was added and was allowed to boil. The residue from the ether extraction was transferred into a 1000 mL Erlenmeyer flask, fitted with air condenser.The acid from the first step was added and allowed to boil while taking note of the time the boiling started. The flame was adjusted so that the liquid began to boil gently and this was continued for 30 minutes. Particles from the side of the flask were constantly rinsed back by importing a circular motion to the flask. A piece of close textured linen was cut to fit over a Büchner funnel and was secured with an elastic band. Boiling water was poured into the funnel and allowed to remain there until the funnel was hot. The water was removed by suction. The liquid was boiled for 30 minutes after which it was poured into the funnel and filtered by suction. The time of

filtration was not allowed to exceed 10 minutes. The residue was washed with boiling water until it was free of acid and was returned into a digesting flask using a thin spatula. Two hundred millilitres of 1.25% NaOH solution (which was prepared by adding 25 mL of 10% NaOH to 175mL of distil water and was allowed to boil) was added, this was allowed to boil within a minute and was then allowed to boil gently and steadily for 30 minutes. It was then filtered through a Whatman No. 4 (15 cm) filter paper, washed with boiling water until it was free of acid. The residue was washed twice with 95% alcohol and then three times with petroleum ether using small quantities. The residue was allowed to drain, then transferred to a clean silica dish and dried in the oven to a constant weight. It was ignited to burn off all organic matter, after which it was cooled and weighed. The percentage crude fibre was given as loss on ignition (AOAC, 2012).

3.10.1.7 Determination of heavy metal content of harvested plants

Heavy metal contents of the plant sample were determined using the wet digestion procedure (SSSA, 1971). This was carried out by weighing 1 g of the dried ground plant tissue into a 100 mL Berzellius beaker, 5 mL HNO_3 and 2 mL $HClO_4$ was added and covered with a watch glass. This was digested in a fume cupboard by heating it to a final volume of 3 to 5 mL. Ten to fifteen millilitres of water was added and the digest solution was filtered through an acid washed filter paper into a 50 mL volumetric flask. It was diluted to volume with deionized water and the filter paper was washed with water. The filtrate was used to determine the concentration of heavy metals present in the sample using Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer (AAS).

3.10.2 Analysis of the bioremediated soil samples

The five replicates in each treatment group were pooled together and mixed well in order to obtain a composite sample. This was then analysed for the following parameters: pH, organic carbon, nitrogen, available phosphorus, exchangeable acidity, calcium, potassium, sodium, magnesium, manganese, iron, copper, zinc, lead, cadmium, chromium, cobalt and nickel. The methodology used in this was the same as that used for the initial soil analysis described in sections 3.5.1 to 3.5.4.

3.11 Data Analysis

All data obtained were analysed and reported as mean ± standard deviation of five measurements and analysed using univariate analysis of variance and Duncan Post Hoc test to determine significant differences ($p \geq 0.05$) between treatments using Statistical Package for Social Science Research version 17 (SPSS).

CHAPTER FOUR

4.0 RESULTS

4.1 Sampling site

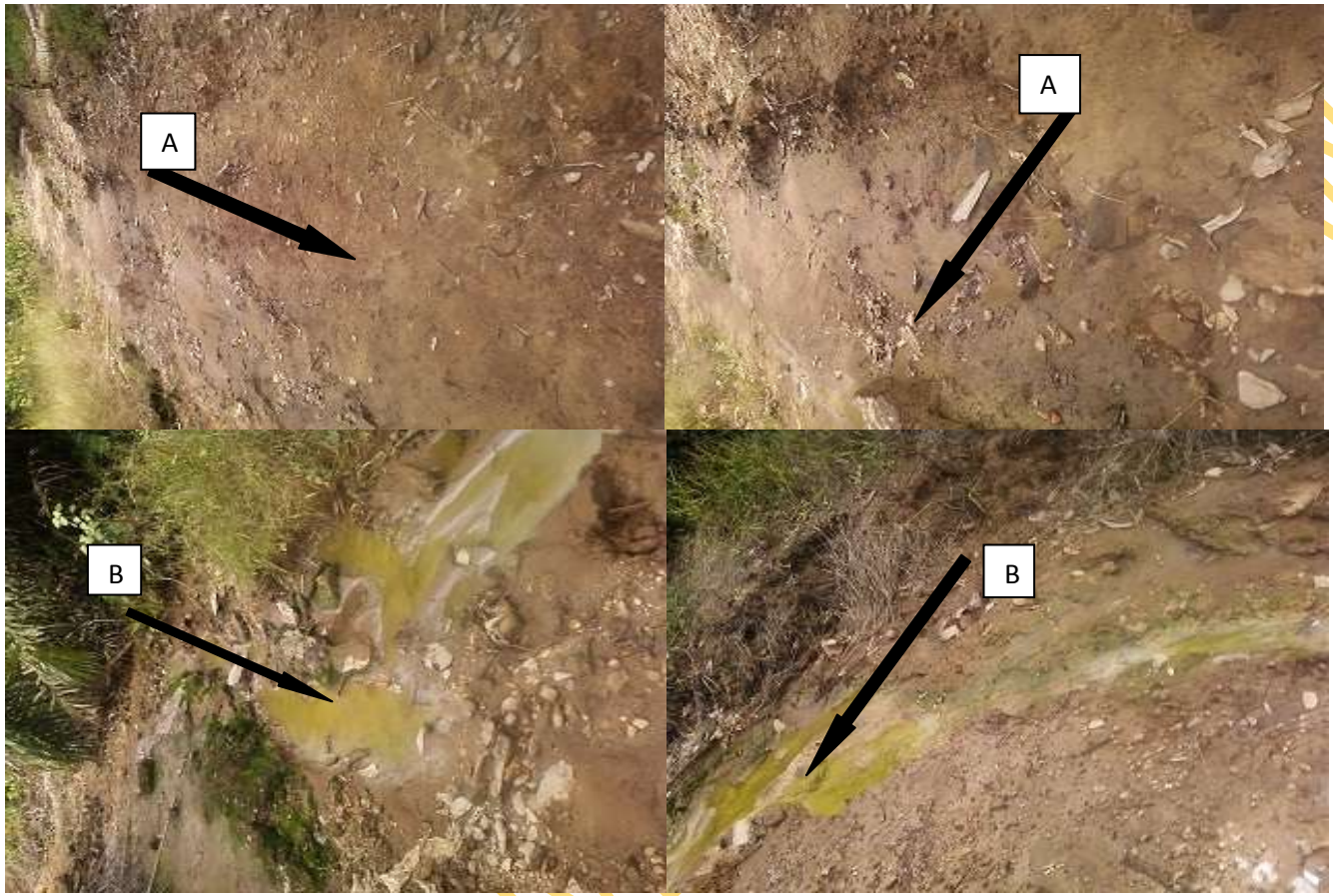
Physical observation of the site where the soil sample used in theis study was collected revealed that was the soil could not support plant growth as a result of contamination caused by the released of effluents by the steel rolling company in that area as shown by plate 4.1.

4.2.1 Physical and chemical properties of the contaminated soil sample

The soil samples collected from the sampling site was dark brown in colour with a characteristic choking odour which is peculiar to heavy metal contaminated sites. Tables 4.1 shows the result of the initial analysis carried out to determine the physical and chemical and heavy metals of the composite soil samples collected during the rainy and dry season. It was observed that the soil from the study site had higher physical and chemical and heavy metal content during the dry season than the rainy season. For instance, heavy metals such as cadmium, lead, cobalt, nickel and chromium had concentrations of 3.0, 2333.6, 13.7, 40.6 and 1678.7 mg/kg, respectively during the dry season and concentrations of 0.5, 1505.5, 10.5, 31.5 and 1526.0 mg/kg, respectively during the rainy season.

4.2.2 Physical and chemical properties of the cattle dung slurry (gomeya)

Table 4.2 shows the proximate and heavy metal content of the cattle dung slurry (gomeya). The values obtained for proximate mineral such as crude protein, ash and ether extract (fat) were 4.9, 19.8 and 1.1 mg/100g, respectively, while for heavy metals such as cadmium, lead, cobalt, chromium and nickel were 53.5,77.0, 50.2, 88.1 and 74.2 mg/kg, respectively.



Keys: A- bare land which could not support plant growth

B- effluents from rolling steel company passing through the land

Plate 4.1: Sampling site showing effect of effluent on the environment

Table 4.1: Physical and chemical properties of the heavy metals contaminated soil sample during dry and rainy seasons

Parameters	Dry season	Rainy season
pH	6.5	7.0
Total organic carbon (TOC) (g/kg)	42.4	40.8
Total Nitrogen (g/kg)	4.0	3.9
Exchangeable Acidity (meq/100g)	0.4	0.4
Available Phosphorus (mg/kg)	14.0	13.3
Ca (cmol/kg)	2.6	2.7
K (cmol/kg)	0.3	0.4
Na (cmol/kg)	0.5	0.3
Mg (cmol/kg)	0.5	0.4
Mn (mg/kg)	99.4	98.5
Fe (mg/kg)	24.8	23.8
Cu (mg/kg)	2.8	1.0
Zn (mg/kg)	2.6	1.4
Cd (mg/kg)	3.0	0.5
Pb (mg/kg)	2333.6	1505.5
Co (mg/kg)	13.7	10.5
Ni (mg/kg)	40.6	31.5
Cr (mg/kg)	1678.7	1526.0

Table 4.2: Physical and chemical properties of the cattle dung slurry (gomeya)

	Parameter (%)	Amount present (mg/100g)
Proximate minerals	crude protein	4.9
	ash	19.8
	ether extract (fat)	1.1
	crude fibre	47.0
Heavy metals	Fe	4675.0
	Cu	24.9
	Zn	16.8
	Cd	53.5
	Pb	77.0
	Co	50.2
	Cr	88.1
	Ni	74.2

4.3 Total Viable Bacterial Counts (TVBC)

It was observed that not many bacterial isolate could grow in the contaminated soil, the mean TVBC ranged from 7-28 cfu/ml, hence the low bacterial load of the soil sample from the contaminated site.

4.4 Biochemical characteristics of the bacterial isolates

Table 4.3 shows the biochemical characteristics of bacterial isolates obtained from the composite soil sample. The bacterial isolates were *Proteus mirabilis*, *Pseudomonas* sp, *P. fluorescens, P. azotoformans, P. putida, Alcaligenes faecalis*, *Providencia* sp, *Bacillus mycoides, B. subtilis* and *Enterobacter* sp. Figure 4.1 shows the frequency of occurrence of the isolated bacteria based on the biochemical identification. The thirty-six isolates were distributed as follows: *Pseudomonas* sp (52.77%), Proteus *mirabilis* (13.89%), *Alcaligenes faecalis* (13.89%), *Enterobacter* sp (8.33%), *Providencia* sp (5.56%) and *Bacillus* sp (5.56%).

4.5 Molecular and Phylogenetic Characteristics of the Bacterial isolates

The result of the 16S rRNA sequences of thirty-five out of the thirty six bacterial isolates obtained in this study is shown in Table 4.5 (one isolate had short nucleotide sequence and could not be identified). Based on the data base information available on National Centre for Biotechnology Information (NCBI) site using the Basic Local Alignment Search Tool (BLAST), the isolates were classified and identified using the highest percentage similarity with organism of the nearest homology. Twenty-seven (77.14%) of the isolates belong to the group Gamma (ɤ) proteobacteria and in the genera *Proteus, Azotobacter, Pseudomonas, Providencia, Shewanella, Citrobacter and Pantoea* while five (14.29%) of the isolates belong to the Beta (β) proteobacteria and in the genera *Alcaligenes, Paenalcaligenes, Castellaniella.* Two (5.71%) of the bacterial isolates belong to the group of Firmicutes in the genera *Bacillus,* only one (2.86%) isolate was found to belong to the group Alpha (α) proteobacteria in the genera *Brucella* as shown in Figure 4.2.

Figure 4.3 shows the evolutionary relationship of taxa (phylogenetic tree) of the isolates identified using biochemical tests constructed with Molecular Evolution Genetics Analysis (MEGA) version 6.

Table 4.3: Biochemical characteristics of bacterial isolates from composite soil sample

S/N	Isolate Code	Gram Reaction	Shape	Catalase	Oxidase	KOH	Methyl red	Vogfes Proskauer	Citrate utilization	Indole	Endospore	Hydrogen Sulphhide	Urease	Motility	TSI	Glucose	Mannose	Sorbitol	Sucrose	Arabinose	Inositol	Lactose	*Probable Organism*
1	E36ai	-	Rod	+	+	+	+	+	+	-	-	-	+	+	rr	-	-	-	-	-	-	-	*Alcaligenes faecalis*
2	61	-	Rod	+	-	+	±	-	-	+	-	+	+	+	ry	±	-	-	-	-	-	-	*Proteus mirabilis*
3	E12Ciib	-	Rod	+	-	+	±	-	-	+	-	+	+	+	ry	±	-	-	-	-	-	-	*Proteus mirabilis*
4	SA4iv	-	Rod	+	-	+	±	-	-	+	-	+	+	+	ry	±	-	-	-	-	-	-	*Proteus mirabilis*
5	E13bii	-	Rod	+	-	+	±	-	-	+	-	+	+	+	ry	±	-	-	-	-	-	-	*Proteus mirabilis*
6	EC61b	-	Rod	+	-	+	±	-	-	+	-	+	+	+	ry	±	-	-	-	-	-	-	*Proteus mirabilis*
7	EC1aii	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
8	615	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
9	TS11	-	Rod	+	+	+	+	+	+	-	-	-	+	+	rr	-	-	-	-	-	-	-	*Alcaligenes faecalis*
10	E69	-	Rod	+	+	+	+	+	+	-	-	-	+	+	rr	-	-	-	-	-	-	-	*Alcaligenes faecalis*
11	E13aiib	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas putida*
12	EC61a	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
13	E12C	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
14	SA42bi	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
15	E63	-	Rod	+	+	+	+	-	+	-	-	-	-		rr	-	-	-	-	-	-	-	*Pseudomonas azotoformans*
16	EC2iii	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas fluorescens*
17	E11iib	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
18	E13ciiia	-	Small rods	+	-	+	+	-	+	+	-	-	-	+	ry	+	-	-	-	-	-	-	*Providencia sp*
19	E11dii	-	Small rods	+	-	+	+	-	+	+	-	-	-	+	ry	+	-	-	-	-	-	-	*Providencia sp*

S/N	Isolate Code	Gram Reaction	Shape	Catalase	Oxidase	KOH	Methyl red	Vogfes Proskauer	Citrate utilization	Indole	Endospore	Hydrogen Sulphhide	Urease	Motility	TSI	Glucose	Mannose	Sorbitol	Sucrose	Arabinose	Inositol	Lactose	*Probable Organism*
20	6	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
21	TS2b	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
22	SA43i	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
23	E12Ciia	+	Rod	+	+	-	-	+	+	-	+	+	+	-	rr	+	±	-	±	-	-	-	*Bacillus mycoides*
24	E13bi	-	Rod	+	+	+	+	+	+	-	-	-	+	+	rr	-	-	-	-	-	-	-	*Alcaligenes faecalis*
25	TS1	-	Rod	+	-	+	-	+	±	-	-	-	-	+	yy	+	+	+	+	+	+	+	*Enterobacter sp*
26	TS2a	-	Rod	+	-	+	-	+	±	-	-	-	-	+	yy	+	+	+	+	+	+	+	*Enterobacter sp*
27	714	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas putida*
28	65iia	-	Rod	+	-	+	-	+	±	-	-	-	-	+	yy	+	+	+	+	+	+	+	*Enterobacter sp*
29	652b	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
30	EC61c	-	Rod	+	+	+	+	+	+	-	-	-	+	+	rr	-	-	-	-	-	-	-	*Alcaligenes faecalis*
31	TS14	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas fluorescens*
32	661b	+	Rod	+	-	-	-	+	+	-	+	+	-	+	yy	+	-	-	±	-	-	-	*Bacillus subtilis*
33	661bi	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas fluorescens*
34	SA46	-	Rod	+	+	+	+	-	+	-	-	-	-	+	ry	-	-	-	-	-	-	-	*Pseudomonas sp*
35	TS9	-	Rod	+	+	+	+	-	+	-	-	-	-		rr	-	-	-	-	-	-	-	*Pseudomonas azotoformans*
36	SA43i	-	Rod	+	+	+	+	-	+	-	-	-	-	+	rr	-	-	-	-	-	-	-	*Pseudomonas putida*

Keys: + characteristics present (positive), - characteristics absent (negative), rr red slant, red butt (no fermentation in both slant and butt), ry red slant yellow butt (fermentation occurred in butt but not the slant), yy yellow slant yellow butt (fermentation in both slant and butt).

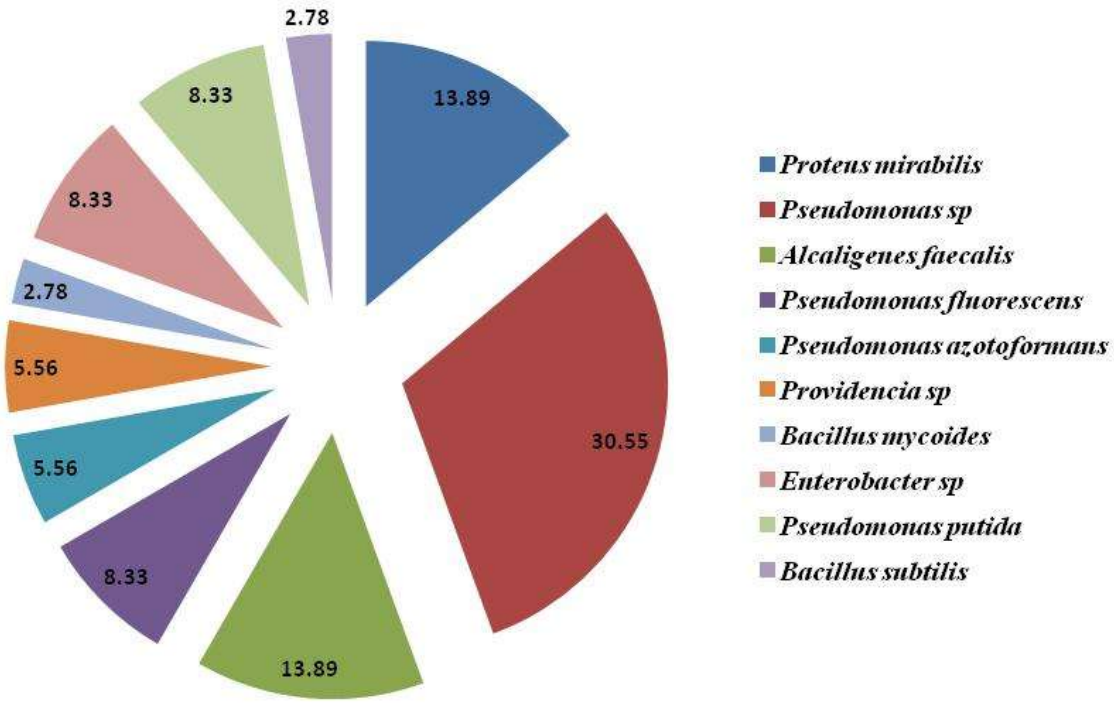


Figure 4.1: Frequency of occurrence of bacterial isolates

Table 4.4: Phylogenetic Identities of the bacterial isolates using BLAST

S/N	Isolate Code	Probable organism identified with biochemical test	Length of the nucleotide sequences	% similarity	Accession number of nearest Homology	Name of the organism from NCBI	Phylogenetic group
1	E36ai	*Alcaligenes faecalis*	802	99	NR 025357.1	*Alcaligenes faecalis subsp. parafaecalis*	β- proteobacteria
2	61	*Proteus mirabilis*	973	91	NR 074898.1	*Proteus mirabilis*	ɤ- proteobacteria
3	E12Ciib	*Proteus mirabilis*	963	100	NR 074898.1	*Proteus mirabilis*	ɤ- proteobacteria
4	SA4iv	*Proteus mirabilis*	973	70	NR 074898.1	*Proteus mirabilis*	ɤ- proteobacteria
5	E13bii	*Proteus mirabilis*	878	99	NR 113344.1	*Proteus mirabilis*	ɤ- proteobacteria
6	EC61b	*Proteus mirabilis*	1008	57	NR O74898.1	*Proteus mirabilis*	ɤ- proteobacteria
7	EC1aii	*Pseudomonas sp*	826	70	NR 029063.1	*Pseudomonas rhizosphaerae*	ɤ- proteobacteria
8	615	*Pseudomonas sp*	1037	99	NR 074597.1	*Pseudomonas syringae*	ɤ- proteobacteria
9	TS11	*Alcaligenes faecalis*	973	77	NR 114959.1	*Alcaligenes aquatilis*	β- proteobacteria
10	E69	*Alcaligenes faecalis*	956	70	NR 116967.1	*Paenalcaligenes hominis*	β- proteobacteria
11	E13aiib	*Pseudomonas putida*	927	99	NR 074596.1	*Pseudomonas putida*	ɤ- proteobacteria
12	EC61a	*Pseudomonas sp*	966	100	NR 025588.1	*Pseudomonas proteolytica*	ɤ- proteobacteria
13	E12C	*Pseudomonas sp*	923	98	NR 074798.1	*Shewanella oneidensis*	ɤ- proteobacteria
14	SA42bi	*Pseudomonas sp*	964	100	NR 026395.1	*Pseudomonas graminis*	ɤ- proteobacteria
15	E63	*Pseudomonas azotoformans*	902	76	NR 043422.1	*Pseudomonas mucidolens*	ɤ- proteobacteria
16	E11iib	*Pseudomonas sp*	936	95	NR 112075.1	*Pseudomonas veronii*	ɤ- proteobacteria

S/N	Isolate Code	Probable organism identified with biochemical test	Length of the nucleotide sequences	% similarity	Accession number of nearest Homology	Name of the organism from NCBI	Phylogenetic group
17	E13ciiia	*Providencia sp*	981	95	NR 102978.1	*Providencia stuartii*	ɤ- proteobacteria
18	E11dii	*Providencia sp*	943	90	NR 042412.1	*Providencia heimbachae*	ɤ- proteobacteria
19	6	*Pseudomonas sp*	977	53	NR 041296.1	*Shewanella hafniensis*	ɤ- proteobacteria
20	TS2b	*Pseudomonas sp*	994	35	NR 119141.1	*Shewanella putrefaciens*	ɤ- proteobacteria
21	SA43i	*Pseudomonas sp*	769	100	NR 114233.1	*Shewanella decolorationis*	ɤ- proteobacteria
22	E12Ciia	*Bacillus mycoides*	946	97	NR 114582.1	*Bacillus cereus*	Firmicutes
23	E13bi	*Alcaligenes faecalis*	803	97	NR 044802.1	*Castellaniella denitrificans*	β- proteobacteria
24	TS1	*Enterobacter sp*	971	96	NR 102823.1	*Citrobacter koseri*	ɤ- proteobacteria
25	TS2a	*Enterobacter sp*	1004	95	NR 126319.1	*Cedecea lapagei*	ɤ- proteobacteria
26	714	*Pseudomonas putida*	972	99	NR 040992.1	*Pseudomonas japonica*	ɤ- proteobacteria
27	65iia	*Enterobacter sp*	936	97	NR 111998.1	*Pantoea agglomerans*	ɤ- proteobacteria
28	652b	*Pseudomonas sp*	975	93	NR 116732.1	*Shewanella xiamenensis*	ɤ- proteobacteria
29	EC61c	*Alcaligenes faecalis*	988	98	NR 025357.1	*Alcaligenes faecalis subsp. parafaecalis*	β- proteobacteria
30	TS14	*Pseudomonas fluorescens*	718	99	NR 028706.1	*Pseudomonas veronii*	ɤ- proteobacteria
31	661b	*Bacillus subtilis*	915	77	NR 113945.1	*Bacillus safensis*	Firmicutes
32	661bi	*Pseudomonas fluorescens*	669	100	NR 028986.1	*Pseudomonas poae*	ɤ- proteobacteria
33	SA46	*Pseudomonas sp*	952	56	NR 103935.1	*Brucella suis*	α- proteobacteria
34	TS9	*Pseudomonas azotoformans*	973	99	NR 102514.1	*Pseudomonas poae*	ɤ- proteobacteria
35	SA43i	*Pseudomonas putida*	978	86	NR 074739.1	*Pseudomonas putida*	ɤ- proteobacteria

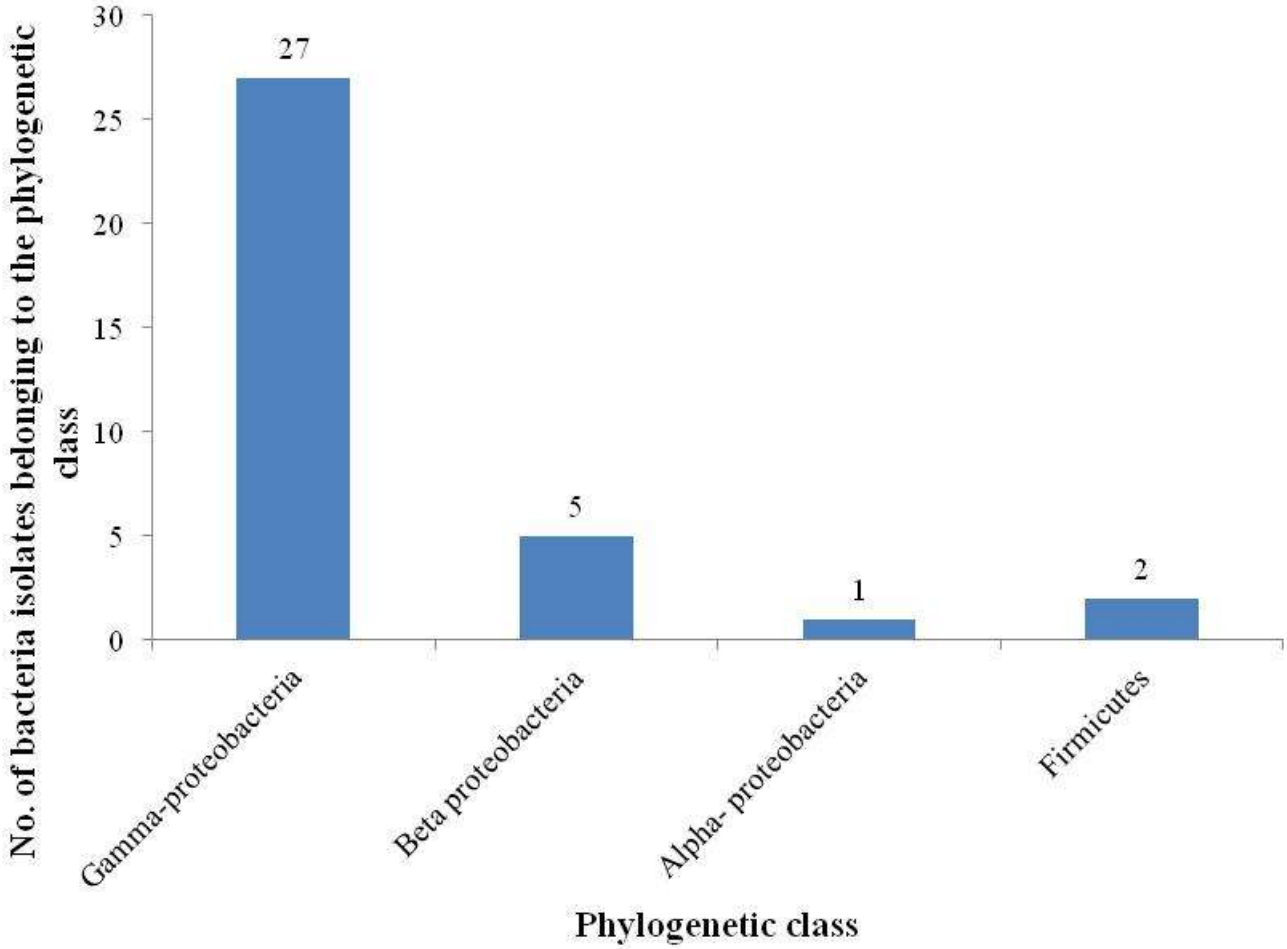


Figure 4.2: Distribution of the isolated bacteria into phylogenetic class

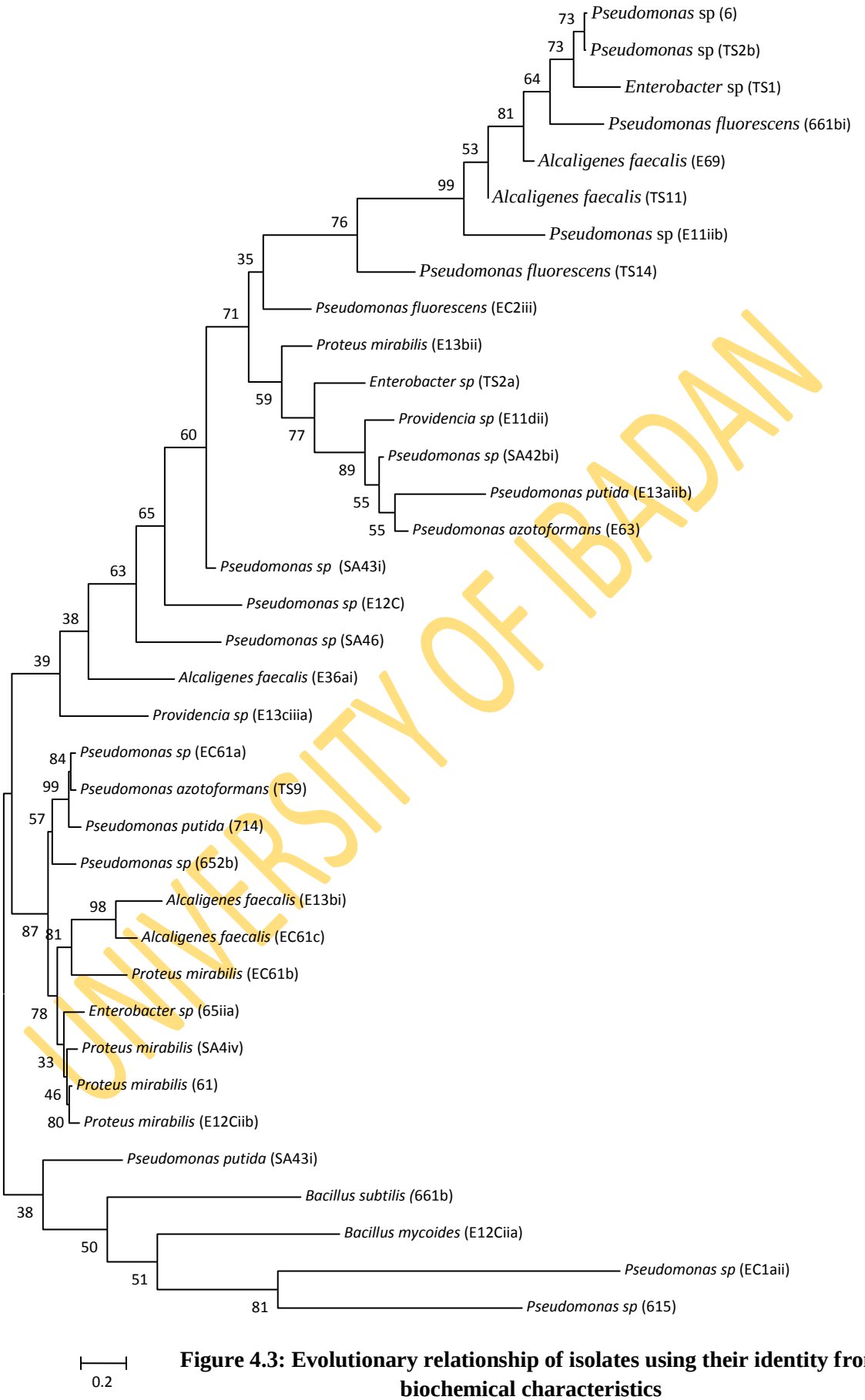


Figure 4.3: Evolutionary relationship of isolates using their identity from biochemical characteristics

Proteus mirabilis (61, E12Ciib, EC61b and SA4iv) showed 91,100, 57 and 70% 16S rRNA homology with *Proteus mirabilis* (NR 074898.1) in NCBI Genbank database (Table 4.4). On the phylogenetic tree, *P. mirabilis* (61 and E12Ciib) clustered together with 80% sequence alignment and they both clustered with *P. mirabilis* (SA4iv) with 46% sequence alignment. *P. mirabilis* (EC61b) did not form a cluster with them but rather it clustered with *Alcaligenes faecalis* (E13bi and EC61C) with 81% sequence alignment (Figure 4.3).

Alcaligenes faecalis (E36ai and EC61C) showed 99 and 98% 16S rRNA homology with *A. faecalis* subsp. *parafaecalis* (NR 025357.1) in NCBI Genbank database (Table 4.4), however, the two isolates did not cluster together on the phylogenetic tree, rather *A. faecalis* (EC61C) clustered with *A. faecalis* (E13bi) (which had 97% 16S rRNA homology with *Castellaniella denitrificans* (NR 044802.1) in NCBI Genbank database) (Table 4.4) with 98% sequence alignment.

Pseudomonas sp (EC61a) clustered with *P. azotoformans* (TS9) with 84% sequence alignment while they both clustered with *P. putida* (714) with 99% sequence alignment on the phylogenetic tree (Figure 4.3). *Pseudomonas* sp (EC61a), *P. putida* (714), *P. azotoformans* (TS9) showed 100, 99 and 99% 16S rRNA homology with *P. proteolytica* (NR 025588.1), *P. japonica* (NR 040992.1) and *P. poae* (NR 102514.1) respectively in NCBI Genbank database. *Pseudomonas* sp (16 and TS2b) clustered together with 73% sequence alignment on the phylogenetic tree but they showed 53 and 35% 16S rRNA homology to *Shewanella hafniensis* (NR 041296.1) and *S. putrefaciens* (NR 119141.1), respectively in NCBI Genbank database (Table 4.4).

Bacillus mycoides (E12Ciia) clustered with *B. subtilis* (661b) with 50% sequence alignment on the phylogenetic tree (Figure 4.3) and they both showed 97 and 77% 16S rRNA homology to *B. cereus* (NR 114582.1) and *B. safensis* (NR 113945.1) respectively in NCBI Genbank database (Table 4.4).

It was observed that some isolates identified by the biochemical tests as *Pseudomonas* sp shared 16s rRNA similarities with *Shewanella* sp in the NCBI Genbank database. For example, *Pseudomonas* sp (E12C, 6 and TS2b) showed 98, 53 and 35% 16S rRNA homology

to *Shewanella oneidensis (*NR 074798.1), *S. hafniensis* (NR 041296.1) and *S. putrefaciens* (NR 119141.1) respectively in NCBI Genbank database (Table 4.4).

4.6 Tolerance of the bacterial isolates to heavy metals

Table 4.5 shows the tolerance of bacterial isolates to heavy metals. The isolates were observed to show different tolerance level to the heavy metal salts used. Some of the isolates also showed colorations when growing on agar plates incorporated with the heavy metal salts. Four of the isolates identified molecularly as *Proteus mirabilis* (61), *Pseudomonas veronii* (E11iib), *Cedecea lapagei* (TS2a) and *Pseudomonas poae* (TS9) could not tolerate the heavy metals at all and therefore did not grow at all the concentrations tested for the various heavy metals. It was observed that zinc was tolerated by most of the bacterial isolates, as only nine isolates failed to grow from 100-500 µg/ml compared to twenty one isolates for cadmium, sixteen isolates for copper, fifteen isolates for chromium, thirteen for nickel, fourteen for lead and seventeen for cobalt which could not tolerate the heavy metal salts at any concentration and hence their inability to grow.

Most of the bacterial isolates were observed to tolerate between 150 and 350 µg/mL of the different heavy metal salts used with the exception of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) which still tolerated some of the heavy metal salts up to 450 µg/mL. However, none of the bacterial isolates grew at 500 µg/mL.

4.7 Monitored agronomic parameters of planted *C. olitorius*

Based on the treatment groups, the results obtained are divided into two major categories which are treatments containing sterilized contaminated soil with bacterial inoculum as the only additive and treatments containing sterilized contaminated soil with bacterial inoculum together with organic amendment (gomeya).

4.7.1 Plant height

Significant difference was observed in the plant height of the treatments starting from 2WAP to 7 WAP when the experiment was terminated (at $p \geq 0.05$). It was observed that the various treatment groups varied in their performance.

Table 4.5: Tolerance of bacterial isolates to salts of heavy metals

S/N	Isolate Code	Name of organism from NCBI database	MIC of isolate (µg/ml)						
			Cadmium	Copper	Chromium	Nickel	Lead	Cobalt	Zinc
1	E36ai	*Alcaligenes faecalis subsp. parafaecalis*	200	300	-	-	-	200	300
2	61	*Proteus mirabilis*	-	-	-	-	-	-	-
3	E12Ciib	*Proteus mirabilis*	-	300	350	300	350	200	350
4	SA4iv	*Proteus mirabilis*	250	-	-	-	-	-	-
5	E13bii	*Proteus mirabilis*	-	300	350	300	350	250	350
6	EC61b	*Proteus mirabilis*	-	-	350	300	300	150	300
7	EC1aii	*Pseudomonas rhizosphaerae*	250	-	350	300	300	150	300
8	615	*Pseudomonas syringae*	350	300	350	300	350	-	-
9	TS11	*Alcaligenes aquatilis*	400	400	400	400	400	-	-
10	E69	*Paenalcaligenes hominis*	-	250	-	-	-	-	300
11	E13aiib	*Pseudomonas putida*	-	300	300	300	350	300	350
12	EC61a	*Pseudomonas proteolytica*	-	-	200	200	300	-	300
13	E12C	*Shewanella oneidensis*	-	300	300	300	300	250	300
14	SA42bi	*Pseudomonas graminis*	-	300	300	-	150	250	300

S/N	Isolate Code	Name of organism from NCBI database	MIC of isolate (µg/ml)						
			Cadmium	Copper	Chromium	Nickel	Lead	Cobalt	Zinc
15	E63	*Pseudomonas mucidolens*	200	150	400	350	400	350	400
16	EC2iii	*Pseudomonas fluorescens*	200	-	-	-	300	-	300
17	E11iib	*Pseudomonas veronii*	-	-	-	-	-	-	-
18	E13ciiia	*Providencia stuartii*	-	300	200	300	300	300	300
19	E11dii	*Providencia heimbachae*	200	-	200	300	-	300	-
20	6	*Shewanella hafniensis*	-	300	200	300	300	300	300
21	TS2b	*Shewanella putrefaciens*	-	-	200	300	300	250	300
22	SA43i	*Shewanella decolorationis*	250	300	200	300	300	200	300
23	E12Ciia	*Bacillus cereus*	450	450	250	400	450	150	300
24	E13bi	*Castellaniella denitrificans*	300	-	-	300	-	-	300
25	TS1	*Citrobacter koseri*	-	300	300	300	300	150	300
26	TS2a	*Cedecea lapagei*	-	-	-	-	-	-	-
27	714	*Pseudomonas japonica*	250	300	-	300	300	300	300
28	65iia	*Pantoea agglomerans*	300	-	-	300	150	150	300
29	652b	*Shewanella xiamenensis*	-	-	-	300	150	-	-

S/N	Isolate Code	Name of organism from NCBI database	MIC of isolate (µg/ml)						
			Cadmium	Copper	Chromium	Nickel	Lead	Cobalt	Zinc
30	EC61c	*Alcaligenes faecalis subsp. parafaecalis*	300	-	-	300	-	-	150
31	TS14	*Pseudomonas veronii*	-	300	-	-	-	-	300
32	661b	*Bacillus safensis*	-	300	-	-	-	-	300
33	661bi	*Pseudomonas poae*	-	300	-	-	-	-	300
34	SA46	*Brucella suis*	-	300	300	200	300	200	300
35	TS9	*Pseudomonas poae*	-	-	-	-	-	-	-
36	SA43i	*Pseudomonas putida*	300	-	-	-	-	-	300

Key: - organisms which failed to grow at a particular soncentration of the heavy metal salts

For instance, treatment BC had the least height of 2.76±0.15 cm at 2 WAP, however at 6 WAP, the highest height was observed in treatment ABC with 7.84±0.69 cm, while the least height was observed in treatment B with 4.68±0.41 cm, and this trend was maintained till the experiment was terminated. The control had the highest height of 4.10±0.55 cm as at 2WAP and at 7WAP it had a height of 5.86±0.57 cm. Table 4.6 shows the mean weekly result of the of plant height of treatments containing only bacterial inoculum.

Table 4.7 shows the mean weekly result of the of plant height of treatments which received bacterial inoculum and gomeya. It was observed that from 2WAP till 7WAP when the experiment was terminated, ControlM had the highest height with 6.40±0.89 cm and 22.94±4.30 cm at 2WAP and 7WAP, respectively. However among the treatments that received bacterial inoculum together with gomeya, the highest height at 7WAP was observed in the treatment BCM with 9.24±1.78 cm, while the least was observed in the treatment AM with 4.42±0.49 cm.

4.7.2 Number of leaves per plant

The results obtained for number of leaves/plant revealed that there was significant difference in the number of leaves per plant among the treatments starting from 2WAP (at $P \leq 0.05$). Table 4.8 shows the mean weekly result of the number of leaves/plant of treatments containing only bacterial inoculum.. Treatment A had the highest number of leaves/plant from 2WAP till 4 WAP having an average of 4.00±0.00 and 5.20±0.45, respectively but by 5WAP till the 7WAP when the experiment was terminated, treatment ABC had the highest number of leaves/plant with an average of 5.80±0.45.

Table 4.9 shows the mean weekly result of the number of leaves/plant of treatments which received bacterial inoculum and gomeya. It was observed ControlM had the highest number of leaves/plant from 2WAP till 7WAP with an average of 4.00±0.00 and 9.40±1.82 leaves, respectively. By 7WAP, it was observed that among the treatments which received bacterial inoculum and gomeya, the highest number of leaves/plant was obtained in treatment BCM with an average of 5.60±0.55 number of leaves while the least number of leaves/plant was observed in treatments AM and ACM with each having an average of 5.00±0.00 leaves.

Table 4.6: Height of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum only

Treatment	Weeks after planting					
	2	3	4	5	6	7
A	3.60±0.55^{b}	4.74 ±0.63^{ef}	4.76±0.61^{hi}	4.90±0.65^{jk}	4.92±0.62^{nop}	4.94±0.63st
B	3.20±0.45^{bcd}	4.24 ±0.43^{fg}	4.26±0.42^{i}	4.66±0.42^{k}	4.68±0.41^{p}	4.70±0.40^{t}
C	3.00±0.00^{cd}	4.16 ±0.05^{g}	4.26±0.05^{i}	4.72±0.13^{k}	4.72±0.13^{op}	4.74±0.11^{t}
AB	3.30±0.45^{bc}	4.73 ±0.27^{ef}	4.86±0.27^{h}	5.30±0.31^{j,k}	5.30±0.31^{mno}	5.30±0.31^{rst}
AC	3.00±0.00^{cd}	5.06 ±0.13^{e}	5.18±0.08^{h}	5.40±0.10^{j,k}	5.36±0.09^{mn}	5.38±0.08^{rs}
BC	2.76±0.15^{d}	4.84 ±0.42^{e}	4.98±0.38^{h}	5.32±0.27^{j,k}	5.32±0.27^{mno}	5.32±0.27^{rst}
ABC	3.14±0.22^{bcd}	4.12 ±0.08^{g}	4.34±0.11^{i}	5.66±1.37^{j}	7.64±0.69^{l}	7.84±0.69^{q}
Control	4.10±0.55^{a}	4.92 ±0.70^{e}	5.28±0.53^{h}	5.74±0.57^{j}	5.86±0.57^{m}	5.86±0.57^{r}

***values with the same letters on each column are not significantly different from each other at p≤0.05

Key:

A	Plants grown on sterilized soil treated with *Alcaligenes aquatilis* (TS11)
B	Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63)
C	Plants grown on sterilized soil treated with *Bacillus cereus (*E12Ciia)
AB	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63)
AC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia)
BC	Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
ABC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis*(TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
Control	Plants grown on sterilized soil alone without bacterial or organic nutrient amendment

Table 4.7: Height of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum and gomeya

Treatment	Weeks after planting					
	2	3	4	5	6	7
AM	2.86±0.11^{c}	3.66 ±0.38^{g}	3.92±0.29^{k}	4.40±0.45^{o}	4.40±0.45^{s}	4.42±0.49^{w}
BM	3.04±0.11^{bc}	5.04 ±0.11^{f}	5.18±0.08^{j}	6.02±0.28^{mn}	6.68±0.36^{qrs}	6.74±0.41 ^{vw}
CM	3.58±0.40^{b}	6.06 ±0.68^{e}	6.30±0.51^{i}	6.72±0.50^{m}	6.84±0.43^{qrs}	7.32±0.30^{uv}
ABM	3.30±0.23^{bc}	4.08 ±0.16^{g}	4.22±0.15^{k}	4.90±0.10^{no}	6.36±0.40^{qrs}	6.64±0.34^{vw}
ACM	3.14±0.22^{bc}	5.08 ±0.13^{f}	5.20±0.10^{j}	5.76±0.11^{mno}	5.82±0.11^{rs}	6.00±0.10^{vw}
BCM	3.18±0.20^{bc}	5.04 ±0.15^{f}	5.56±0.55^{j}	6.66±1.32^{m}	8.86±2.05^{q}	9.24±1.78^{u}
ABCM	3.50±0.21^{b}	4.68 ±0.28^{f}	5.74±1.02^{ij}	6.20±1.10^{mn}	7.98±0.92^{qr}	8.31±0.85^{uv}
ControlM	6.40±0.89^{a}	7.70 ±0.64^{d}	8.50±0.73^{h}	11.18±2.28^{l}	17.72±4.47^{p}	22.94±4.30^{t}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

AM Plants grown on sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized cattle dung slurry (gomeya)

BM Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

CM Plants grown on sterilized soil treated with *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

ACM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

BCM Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABCM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ControlM Plants grown on sterilized soil containing sterilized cattle dung slurry (gomeya)

Table 4.8: Number of leaves/plant of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum only

Treatment	Weeks after planting					
	2	3	4	5	6	7
A	4.00±0.00^{a}	5.20 ±0.45^{e}	5.20±0.45^{h}	5.20±0.45^{l}	5.20±0.45^{n}	5.20±0.45^{p}
B	3.40±0.55^{abc}	4.80 ±0.45^{ef}	4.80±0.45^{hij}	5.00±0.00^{l}	5.00±0.00^{n}	5.00±0.00^{p}
C	3.60±0.55^{ab}	5.00 ±0.00^{ef}	5.00±0.00^{hi}	5.00±0.00^{l}	5.00±0.00^{n}	5.00±0.00^{p}
AB	3.20±0.45^{bcd}	4.60 ±0.55^{ef}	4.60±0.55^{ij}	4.80±0.45^{l}	4.80±0.45^{n}	4.80±0.45^{p}
AC	2.60±0.0.55^{d}	4.40 ±0.55^{fg}	5.00±0.00^{hi}	5.00±0.00^{l}	5.00±0.00^{n}	5.00±0.00^{p}
BC	2.80±0.45^{cd}	4.20 ±0.45^{g}	4.40±0.55^{j}	4.80±0.45^{l}	4.80±0.45^{n}	4.80±0.45^{p}
ABC	3.60±0.55^{ab}	4.80 ±0.45^{ef}	5.00±0.00^{hi}	5.80±0.45^{k}	5.80±0.45^{m}	5.80±0.45^{o}
Control	3.00±0.00^{bcd}	5.00 ±0.00^{e,f}	5.00±0.00^{hi}	5.00±0.00^{l}	5.00±0.00^{n}	5.00±0.00^{p}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

A	Plants grown on sterilized soil treated with *Alcaligenes aquatilis* (TS11)
B	Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63)
C	Plants grown on sterilized soil treated with *Bacillus cereus (*E12Ciia)
AB	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63)
AC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia)
BC	Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
ABC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis*(TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
Control	Plants grown on sterilized soil alone without bacterial or organic nutrient amendment

Table 4.9: Number of leaves/plant of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum and gomeya

Treatment	Weeks after planting					
	2	3	4	5	6	7
AM	3.00±0.00^{c}	5.00 ±0.00^{e}	5.00±0.00^{h}	5.00±0.00^{k}	5.00±0.00^{m}	5.00±0.00^{o}
BM	3.20±0.45^{bc}	4.60 ±0.89^{ef}	5.40±0.55^{h}	5.40±0.55^{jk}	5.40±0.55^{m}	5.40±0.55^{o}
CM	3.20±0.45^{bc}	5.00 ±0.00^{e}	5.00±0.00^{h}	5.00±0.00^{k}	5.20±0.45^{m}	5.40±0.55^{o}
ABM	3.20±0.45^{bc}	5.00 ±0.00^{e}	5.00±0.00^{h}	5.00±0.00^{k}	5.40±0.55^{m}	5.40±0.55^{o}
ACM	3.00±0.00^{c}	5.00 ±0.00^{e}	5.00±0.00^{h}	5.00±0.00^{k}	5.00±0.00^{m}	5.00±0.00^{o}
BCM	3.20±0.45^{bc}	4.40 ±0.55^{f}	5.40±0.55^{h}	5.60±0.55^{j}	5.60±0.55^{m}	5.60±0.55^{o}
ABCM	3.60±0.55^{ab}	5.00 ±0.00^{e}	5.00±0.00^{h}	5.40±0.55^{jk}	5.40±0.55^{m}	5.40±0.55^{o}
ControlM	4.00±0.00^{a}	6.32 ±0.45^{d}	6.40±0.55^{g}	6.40±0.55^{i}	7.80±1.30^{l}	9.40±1.82^{n}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

AM Plants grown on sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized cattle dung slurry (gomeya)

BM Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

CM Plants grown on sterilized soil treated with *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

ACM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

BCM Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABCM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ControlM Plants grown on sterilized soil containing sterilized cattle dung slurry (gomeya)

4.7.3 Plant stem diameter

Plant stem diameter obtained in this study revealed significant difference in the results obtained from the treatments starting from 2WAP till 7WAP except at 3 WAP (at $p \leq 0.05$). Table 4.10 shows the mean weekly result of the plant stem diameter of treatments which received only bacterial inoculum. By 7WAP, the highest stem diameter was observed in treatment ABC which had an average of 1.35±0.00 mm while the smallest stem diameter was observed in treatments B, AB, AC and BC with each having an average of 1.20±0.00 mm each.

Table 4.11 shows the mean weekly result of the plant stem diameter of treatments which received bacterial inoculum and gomeya. It was observed that there was significant difference in the plant stem diameter of the treatments from 2WAp till 7WAP. ControlM had the highest stem diameter with an average of 2.20±0.20 cm at 7WAP when the experiment was terminated. Among other treatments which received bacterial inoculum and gomeya, at 7WAP when the experiment was terminated, treatment ABCM had the biggest diameter with an average of 1.36±0.05 mm, while the smallest stem diameter was observed in treatment AM with an average of 1.22±0.04 mm.

4.8 Post Harvest observations

4.8.1 Proximate matter of the harvested plant

Tables 4.12 and 4.13 show the proximate matter of the harvested *Corchorus olitorius* from treatments which received only bacterial inoculum and those that received bacterial inoculum and gomeya, respectively.

4.8.1.1 Fresh weight of the harvested plant

Significant difference ($p \leq 0.05$) was observed in the values obtained for fresh weight of plants harvested from both the treatments which received only bacterial inoculum and those that received bacterial inoculum and gomeya. For those which received only bacterial inoculum, plants grown on treatment ABC had the highest fresh weight with an average of 2.54±0.57 g, this was followed by those harvested from the Control which had an average weight of 1.10±0.13 g.

Table 4.10: Stem diameter of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum only

Treatment	Weeks after planting					
	2	3	4	5	6	7
A	1.06±0.05^{bc}	1.20±0.07^{d}	1.20±0.07^{f}	1.22±0.04^{i,j}	1.22±0.04^{l}	1.22±0.04^{p}
B	1.04±0.05^{bc}	1.16±0.05^{d}	1.20±0.00^{f}	1.20±0.00^{j}	1.20±0.00^{l}	1.20±0.00^{p}
C	1.04±0.05^{bc}	1.18±0.08^{d}	1.22±0.04^{f}	1.24±0.05^{i}	1.24±0.05^{l}	1.24±0.05^{o}
AB	1.00±0.00^{c}	1.16±0.05^{d}	1.18±0.05^{f}	1.20±0.00^{j}	1.20±0.00^{l}	1.20±0.00^{p}
AC	1.10±0.00^{ab}	1.20±0.00^{d}	1.20±0.00^{f}	1.20±0.00^{j}	1.20±0.00^{l}	1.20±0.00^{p}
BC	1.10±0.00^{ab}	1.20±0.00^{d}	1.20±0.00^{f}	1.20±0.00^{j}	1.20±0.00^{l}	1.20±0.00^{p}
ABC	1.14±0.05^{a}	1.24±0.05^{d}	1.30±0.00^{e}	1.34±0.02^{g}	1.35±0.00^{k}	1.35±0.00^{m}
Control	1.14±0.05^{a}	1.24±0.05^{d}	1.30±0.00^{e}	1.30±0.00^{h}	1.33±0.04^{k}	1.33±0.04^{n}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

A	Plants grown on sterilized soil treated with *Alcaligenes aquatilis* (TS11)
B	Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63)
C	Plants grown on sterilized soil treated with *Bacillus cereus (*E12Ciia)
AB	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63)
AC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia)
BC	Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
ABC	Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis*(TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia)
Control	Plants grown on sterilized soil alone without bacterial or organic nutrient amendment

Table 4.11: Stem diameter of *Corchorus olitorius* at different weeks after planting in treatments which received bacterial inoculum and gomeya

Treatment	Weeks after planting					
	2	3	4	5	6	7
AM	1.10±0.00^{bc}	1.20±0.00^{e}	1.20±0.00^{h}	1.22±0.04^{k}	1.22±0.04^{n}	1.22±0.04^{r}
BM	1.12±0.45^{bc}	1.22±0.45^{e}	1.30±0.00^{g}	1.31±0.02^{j}	1.31±0.02^{mn}	1.31±0.02^{mqr}
CM	1.12±0.45^{bc}	1.24±0.55^{e}	1.30±0.00^{g}	1.30±0.00^{j}	1.30±0.00^{mn}	1.30±0.00^{qr}
ABM	1.12±0.45^{bc}	1.24±0.55^{e}	1.30±0.00^{g}	1.30±0.00^{j}	1.30±0.00^{mn}	1.31±0.02^{qr}
ACM	1.06±0.55^{c}	1.20±0.00^{e}	1.28±0.04^{g}	1.30±0.00^{j}	1.30±0.00^{mn}	1.30±0.00^{qr}
BCM	1.10±0.07^{bc}	1.22±0.04^{e}	1.30±0.00^{g}	1.32±0.02^{j}	1.35±0.00^{mn}	1.35±0.00^{qr}
ABCM	1.16±0.05^{ab}	1.24±0.05^{e}	1.30±0.00^{g}	1.31±0.01^{j}	1.36±0.05^{m}	1.36±0.05^{q}
ControlM	1.22±0.04^{a}	1.42±0.04^{d}	1.54±0.05^{f}	1.61±0.11^{i}	2.00±0.24^{l}	2.20±0.20^{p}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

AM Plants grown on sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized cattle dung slurry (gomeya)

BM Plants grown on sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

CM Plants grown on sterilized soil treated with *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized cattle dung slurry (gomeya)

ACM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

BCM Plants grown on sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ABCM Plants grown on sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) and sterilized cattle dung slurry (gomeya)

ControlM Plants grown on sterilized soil containing sterilized cattle dung slurry (gomeya)

The least fresh weight was observed in plants harvested from treatment A which had an average of 0.22±0.05 g as shown in Table 4.12. It was observed that among plants harvested from the treatments which received bacterial inoculum and gomeya, those from treatment BM had an average weight 9.65±9.12 g showing the highest fresh weight; this was followed by plants harvested from ControlM which had an average weight of 8.60±0.03 g. The least fresh weight was observed in plants harvested from treatment AM which had an average weight of 0.06±0.00 g as shown in Table 4.13.

4.8.1.2 Dry weight of the harvested plant

Significant difference ($p \leq 0.05$) was observed in the values obtained for dry weight of plants harvested from both the treatments which received only bacterial inoculum and those which received bacterial inoculum and gomeya. In the treatments which received only bacterial inoculum, plants harvested from treatment ABC had the highest dry weight with an average of 0.99±0.17 g; this was followed by those harvested from Control which had an average dry weight of 0.63±0.24 g. The least dry weight was found in plants harvested from treatment A with an average weight of 0.10±0.01 g as shown in Table 4.12. In the case of plants harvested from treatments which received both bacterial inoculum and gomeya, the highest dry weight was recorded in plants harvested from ControlM with an average of 4.22±0.16 g; this was followed by those from treatment BM and BCM which had an average weight of 1.08±0.81 and 1.08±0.99 g. The least dry weight was observed in treatment AM which had an average weight of 0.29±0.04 g as shown in Table 4.13.

4.8.1.3 Moisture content

There was no significant difference ($p \leq 0.05$)in the values obtained for moisture (%) of plants harvested from both the treatments which received only bacterial inoculum and those which received bacterial inoculum and gomeya. However, for the groups which received only bacterial inoculum, plants harvested from treatment C had the highest moisture (%) with an average of 62.00±0.14; this was followed by plants harvested from treatment B with an average of 61.90±6.79. The least moisture (%) was however observed in plants harvested from Control which had an average of 43.65±15.34 as shown in Table 4.12. In the case of plants harvested from the groups which received bacterial inoculum and gomeya, treatment BM had the highest moisture (%) and this

Table 4.12: Proximate matter content of harvested *C. olitorius* from treatment groups which received only bacterial inoculum

Treatment	Proximate matter							
	Fresh weight (g)	Dry weight (g)	% Moisture	Dry matter	% Crude protein	% Ash	% Ether extract (fat)	% Crude fibre
A	0.22±0.05^{c}	0.10±0.01^{f}	55.00±7.07^{g}	45.00±7.07^{h}	12.24±1.90^{i,j}	19.06±1.47^{o}	1.40±0.15^{s}	22.62±0.55^{t}
B	0.26±0.03^{c}	0.10±0.03^{f}	61.90±6.79^{g}	38.10±6.79^{h}	7.92±1.17^{jkl}	20.08±0.11^{no}	1.66±00.22^{rs}	19.78±0.53^{vw}
C	0.36±0.09^{c}	0.14±0.04^{f}	62.00±0.14^{g}	38.00±0.14^{h}	15.82±1.90^{i}	14.97±0.69^{p}	1.93±0.25^{qr}	18.72±0.25^{w}
AB	0.33±0.13^{c}	0.15±0.08^{f}	56.25±8.84^{g}	43.75±8.84^{h}	10.39±3.18^{j,k}	17.88±1.62^{o}	1.91±0.15^{qr}	19.62±0.54^{vw}
AC	0.38±0.05^{c}	0.14±0.01^{f}	61.10±3.25^{g}	38.90±3.25^{h}	11.03±2.08^{j}	18.16±1.48^{o}	1.35±0.22^{s}	18.95±0.96^{vw}
BC	0.29±0.07^{c}	0.13±0.04^{f}	51.95±26.36^{g}	48.05±26.38^{h}	6.58±0.03^{k,l}	21.65±0.78^{n}	2.00±0.05^{qr}	19.88±0.47^{vw}
ABC	2.54±0.57^{a}	0.99±0.17^{d}	60.75±2.05^{g}	39.25±2.05^{h}	4.44±1.33^{l}	24.57±0.62^{m}	2.00±0.20^{q,r}	21.72±0.99^{tu}
Control	1.10±0.13^{b}	0.63±0.24^{e}	43.65±15.34^{g}	56.35±15.34^{h}	5.57±0.80^{l}	22.56±0.63^{mn}	2.11±0.06^{q}	20.61±0.78^{uv}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:
A - Plants harvested from sterilized soil treated with *Alcaligenes aquatilis* (TS11), B - Plants harvested from sterilized soil treated with *Pseudomonas mucidolens* (E63), C - Plants harvested from sterilized soil treated with *Bacillus mycoides (*E12Ciia), AB - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63), AC - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia), BC - Plants harvested from sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia), ABC - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia), Control - Plants harvested from sterilized soil alone without bacterial or organic nutrient amendment.

Table 4.13: Proximate matter content of harvested *C. olitorius* from treatment groups which received bacterial inoculum and gomeya

Treatment	Proximate matter							
	Fresh weight	Dry weight	% Moisture	Dry matter	% Crude protein	% Ash	% Ether extract (fat)	% Crude fibre
AM	0.60±0.00^{b}	0.29±0.04^{d}	51.70±7.07^{e}	48.30±7.07^{f}	7.18±0.45^{hi}	23.61±0.57^{jk}	1.99±0.18^{o}	18.73±0.43^{t}
BM	9.65±9.12^{a}	1.08±0.81^{d}	72.65±34.29^{e}	27.35±34.29^{f}	14.55±5.72^{g}	17.85±0.33^{m,}	1.55±00.08^{pq}	18.60±2.10^{t}
CM	1.34±0.79^{b}	0.44±0.34^{d}	69.30±7.21^{e}	30.39±6.77^{f}	13.14±4.11^{gh}	19.66±0.93^{lm}	1.12±0.18^{,r}	18.33±1.36^{t}
ABM	1.12±0.18^{b}	0.66±0.13^{d}	41.20±1.70^{e}	58.80±1.70^{f}	8.93±1.05^{ghi}	16.10±1.28^{n}	1.26±0.15^{qr}	19.61±0.79^{t}
ACM	1.90±0.31^{ab}	0.67±0.37^{d}	62.85±25.81^{e}	37.15±25.81^{f}	8.68±0.91^{ghi}	17.90±0.42^{mn}	1.90±0.14^{op}	20.80±0.41^{t}
BCM	2.60±1.08^{ab}	1.08±0.99^{d}	63.10±22.77^{e}	36.90±22.77^{f}	9.81±1.49^{ghi}	18.07±0.93^{mn}	1.93±0.27^{op}	19.65±0.50^{,t}
ABCM	2.28±0.34^{ab}	1.07±0.19^{d}	52.00±0.00^{e}	48.00±0.00^{f}	8.74±1.96^{ghi}	21.63±2.06^{kl}	2.05±0.08^{o}	20.61±0.59st
ControlM	8.60±0.03^{ab}	4.22±0.16^{c}	51.00±2.12^{e}	49.00±2.12^{f}	5.79±1.22^{i}	25.41±0.85^{j}	1.69±0.09^{op}	22.58±0.63^{s}

***values with the same letters on each column are not significantly different from each other at $p \leq 0.05$

Key:

AM - Plants harvested from sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized Cattle dung slurry (gomeya), BM - Plants harvested from sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya), CM - Plants harvested from sterilized soil treated with *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ABM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya), ACM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), BCM - Plants harvested from sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ABCM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ControlM - Plants harvested from unsterilized soil containing sterilized Cattle dung slurry (gomeya)

was followed by treatment CM which had an average of 72.65±34.29 and 69.30±7.21, respectively. The least moisture (%) was observed in treatment ABM which had an average of 41.20±1.70 as shown in Table 4.13.

4.8.1.4 Dry matter content

There was no significant difference ($p \leq 0.05$) in the values obtained for dry matter of plants harvested from both the treatments which received only bacterial inoculum and those which received bacterial inoculum and gomeya. The treatments which received only bacterial inoculum, the highest dry matter content was obtained in the plants harvested from Control and was followed by those harvested from treatment BC with an average of 56.35±15.34 and 48.05±26.38 g, respectively. The least dry matter content was obtained in plants harvested from treatment C which had an average of 38.00±0.14 g (Table 4.12). While in plants harvested from the treatments which received bacterial inoculum and gomeya, the highest dry matter content was observed in the plants harvested from treatment ABM and was followed by those harvested from ControlM with an average of 58.80±1.70 and 49.00±2.12 g, respectively. The least dry matter content was observed in treatment CM with an average of 30.39±6.77 g (Table 4.13).

4.8.1.5 Crude protein content of the harvested plant

Significant difference ($p \leq 0.05$) was observed in the values obtained for the crude protein of plants harvested from both the treatment groups which received only bacterial inoculum and those that received bacterial inoculum and gomeya. The highest crude protein was obtained in plants harvested from treatment C and this was followed by those harvested from treatment A with an average of 15.82±1.90 and 12.24±1.90 respectively, the least crude protein was however obtained in plants harvested from treatment ABC which had an average of 4.44±1.33 (Table 4.12). The result obtained for the plants harvested from the treatments which received both bacterial inoculum and gomeya showed that treatment BM had the highest crude protein followed by treatment CM with an average of 14.55±5.72 and 13.14±4.11, respectively while the least crude protein was obtained in the plants harvested from ControlM having an average of 5.79±1.22 (Table 4.13).

4.8.1.6 Ash content of the harvested plant

There was significant difference ($p \leq 0.05$) in the values obtained for the ash content of plants harvested from both the treatments which received only bacterial inoculum and those that received bacterial inoculum and gomeya. In the treatments that received only bacterial inoculum, it was observed that plants harvested from treatment ABC had the highest percentage ash content; this was followed by the plants harvested from Control with average values of 24.57±0.62 22.56±0.63, respectively. The least percentage ash content content was obtained in plants harvested from treatment C which had an average of 14.97±0.69 (Table 4.12). The treatments that received bacterial inoculum and gomeya, the highest ash content was obtained in the plants harvested from ControlM followed by those harvested from treatment AM with average values of 25.41±0.85 and 23.61±0.57, respectively. The least ash content was observed in plants harvested from treatment ABM which had a value of 16.10±1.28 (Table 4.13).

4.8.1.7 Ether extract of the harvested plant

There was significant difference ($p \leq 0.05$) in the values obtained for the ether extract of plants harvested from both the treatments which received only bacterial inoculum and those that received bacterial inoculum and gomeya. In the treatments that received only bacterial inoculum, it was observed that plants harvested from Control had the highest ether extract with an average of 2.11±0.06, this was followed by those harvested from treated BC and ABC which had an average values of 2.00±0.05 and 2.00±0.20, respectively. The least ether extract was observed in the plants harvested from treatment AC with an average of 1.35±0.22 (Table 4.12). In the treatments which received bacterial inoculum and gomeya, the highest ether extract was observed in plants harvested from treatment ABCM followed by those harvested from treatment AM with an average values of 2.05±0.08 and 1.99±0.18, respectively. The least ether extract was observed in plants harvested from treatment CM which had an average of 1.12±0.18 (Table 4.13).

4.8.1.8 Crude fibre of the harvested plant

There was significant difference ($p \leq 0.05$) in the values obtained for the crude fibre (%) of plants harvested from both the treatments which received only bacterial inoculum and those which received bacterial inoculum and gomeya. In the treatments that received only bacterial inoculum, it was observed that plants harvested from treatment A had the highest crude fibre followed by those harvested from treatment ABC with average values of 22.62±0.5 and 21.72±0.99, respectively. The least crude fibre was observed in plants harvested from treatment C which had an average value of 18.72±0.25 (Table 4.12). In the treatments which received bacterial inoculum and gomeya, the highest crude fibre was observed in plants harvested from ControlM followed by the ones harvested from treatment ACM with average values of 22.58±0.63 and 20.80±0.41, respectively. The least crude fibre was however observed in plants harvested from treatment CM which had an average of 18.33±1.36 (Table 4.13).

4.8.2 Heavy metal of the harvested plant

Tables 4.14 and 4.15 shows the heavy metal content of the harvested *Corchorus olitorius* harvested from treatment groups which received only bacterial inoculum and those which received bacterial inoculum and gomeya respectively.

4.8.2.1 Iron

There was significant difference ($p \leq 0.05$) in the values obtained for the iron content of plants harvested from the two treatments. It was observed that in treatments which received only bacterial inoculum, plants harvested from treatment ABC had the highest iron content with average value of 37.95±1.20 mg/kg, while the lowest iron content was observed in plants harvested from treatment AB which had an average of 24.95±1.77 mg/kg. However, it was observed that plants harvested from Control had a high iron content of 38.10±0.85 mg/kg as shown in Table 4.14. In the treatments which received bacterial inoculum and gomeya, plants harvested from ControlM had the highest iron content followed by those harvested from treatment ABM with average values of 42.35±1.06 and 38.40±1.13 mg/kg respectively. The lowest iron content was observed in plants harvested from treatment CM which had a value of 25.75±3.61 mg/kg as shown in Table 4.15.

Table 4.14: Heavy metal content of harvested *C. olitorius* from treatment groups which received only bacterial inoculum

Treatment	Heavy metals							
	Iron	Copper	Zinc	Cadmium	Lead	Cobalt	Chromium	Nickel
A	25.35±0.49^{c}	36.05±2.05^{d}	17.45±1.34^{g}	35.55±8.56^{i}	37.70±0.85^{l}	30.50±1.56^{n}	27.00±0.71^{p}	29.00±13.29^{s}
B	26.95±0.64^{bc}	31.70±2.26^{de}	16.50±0.85^{g}	36.45±4.60^{i}	37.65±5.02^{l}	27.70±1.41^{n}	20.75±1.48^{q}	29.00±14.57^{s}
C	28.40±1.70^{b}	29.15±1.34^{de}	17.00±0.28^{g}	32.95±6.43^{i}	34.55±2.47^{l}	28..35±6.86^{n}	24.80±3.82^{pq}	26.70±8.63^{s,t}
AB	24.95±1.77^{c}	30.05±3.60^{de}	16.10±0.85^{g}	34.85±8.13^{i}	36.30±4.81^{l}	29.35±1.06^{n}	24.40±2.97^{pq}	29.35±11.52^{s}
AC	28.35±1.77^{b}	30.55±6.01^{de}	15.90±0.71^{g}	27.85±11.67^{i,j}	32.50±2.55^{l}	28.55±1.48^{n}	26.40±2.40^{p}	27.70±9.76^{s,t}
BC	28.45±049^{b}	31.00±3.68^{de}	16.10±0.85^{g}	12.00±12.02^{jk}	31.85±1.77^{l}	26.80±0.99^{n}	26.50±2.55^{p}	31.25±11.10^{s}
ABC	37.95±1.20^{a}	20.20±4.95^{f}	4.25±0.21^{h}	4.40±0.28^{k}	10.90±0.85^{m}	6.30±0.71^{o}	4.15±0.64^{r}	3.20±0.85^{t}
Control	38.10±0.85^{a}	25.80±0.85^{ef}	5.95±0.78^{h}	4.80±0.71^{k}	9.15±0.64^{m}	5.90±0.71^{o}	4.00±0.14^{r}	3.25±0.63^{t}

***values with the same letters on each column are not significantly different from each other at p≤0.05

Key:
A - Plants harvested from sterilized soil treated with *Alcaligenes aquatilis* (TS11), B - Plants harvested from sterilized soil treated with *Pseudomonas mucidolens* (E63), C - Plants harvested from sterilized soil treated with *Bacillus mycoides (*E12Ciia), AB - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63), AC - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia), BC - Plants harvested from sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia), ABC - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia), Control - Plants harvested from sterilized soil alone without bacterial or organic nutrient amendment.

Table 4.15: Heavy metal content of harvested *C. olitorius* from treatment groups which received bacterial inoculum and gomeya

Treatment	Heavy metals							
	Iron	**Copper**	**Zinc**	**Cadmium**	**Lead**	**Cobalt**	**Chromium**	**Nickel**
AM	27.90±0.57^{de}	28.25±1.63^{f}	11.85±4.60^{g}	22.95±11.38^{h}	26.35±13.93^{j}	16.80±12.02^{k}	13.75±11.81^{l}	19.35±18.88^{m}
BM	38.35±2.90^{ab}	27.50±1.70^{f}	5.50±1.41^{g}	7.30±1.56^{hi}	13.05±3.89^{j}	6.45±0.07^{k}	5.30±0.57^{l}	3.90±0.28^{m}
CM	25.75±3.61^{e}	24.50±6.93^{f}	9.40±6.08^{g}	19.95±15.06^{hi}	21.20±14.28^{j}	17.60±13.44^{k}	12.90±9.48^{l}	13.60±12.30^{m}
ABM	38.40±1.13^{ab}	22.40±1.41^{f}	4.90±0.99^{g}	8.70±2.83^{hi}	12.55±5.44^{j}	7.25±3.32^{k}	6.85±0.07^{l}	5.20±0.14^{m}
ACM	35.30±1.13^{bc}	22.00±1.70^{f}	4.95±0.49^{g}	5.75±0.64^{i}	10.40±0.99^{j}	7.95±0.64^{k}	5.60±0.99^{l}	4.50±1.56^{m}
BCM	32.00±1.98^{cd}	19.95±1.77^{f}	4.85±1.91^{g}	5.00±0.14^{i}	9.10±1.27^{j}	6.60±0.57^{k}	4.55±1.91^{l}	3.50±0.85^{m}
ABCM	37.80±1.98^{ab}	22.85±2.05^{f}	4.90±1.00^{g}	4.20±0.57^{i}	10.30±0.71^{j}	6.70±1.98^{k}	5.00±0.28^{l}	3.65±0.92^{m}
ControlM	42.35±1.06^{a}	24.90±4.67^{f}	7.85±4.45^{g}	5.50±0.57^{i}	10.00±1.56^{j}	6.05±0.35^{k}	4.70±0.85^{l}	3.15±1.06^{m}

***values with the same letters on each column are not significantly different from each other at p≤0.05

Key:
AM - Plants harvested from sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized Cattle dung slurry (gomeya), BM - Plants harvested from sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya), CM - Plants harvested from sterilized soil treated with *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ABM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya), ACM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), BCM - Plants harvested from sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ABCM - Plants harvested from sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya), ControlM - Plants harvested from unsterilized soil containing sterilized Cattle dung slurry (gomeya)

4.8.2.2 Copper

There was significant difference ($p \leq 0.05$) in the values obtained for the copper content of plants harvested from the treatments which received only bacterial inoculum, but there was no significant difference in the values obtained from those which received both bacterial inoculum and gomeya. Among treatments which received only bacterial inoculum, plants harvested from treatment A had the highest copper content followed by plants harvested from treatment B with average values of 36.05±2.05 and 31.70±2.26 mg/kg, respectively, while the lowest copper content was observed in plants harvested from treatment ABC which had an average of 20.20±4.95 mg/kg (Table 4.14). In the treatments which received bacterial inoculum and gomeya, plants harvested from treatment AM had the highest copper content followed by those harvested from treatment BM with average values of 28.25±1.63 and 27.50±1.70 mg/kg, respectively. The lowest copper content was observed in plants harvested from treatment BCM which had a value of 19.95±1.77 mg/kg (Table 4.15).

4.8.2.3 Zinc

There was significant difference ($p \leq 0.05$) in the values obtained for the zinc content of plants harvested from the treatments which received only bacterial inoculum, but there was no significant difference ($p \leq 0.05$) in the values obtained from those which received bacterial inoculum and gomeya. Among treatments which received only bacterial inoculum, plants harvested from treatment A had the highest zinc content followed by plants harvested from treatment C with average values of 17.45±1.34 and 17.00±0.28 mg/kg, respectively, while the lowest zinc content was observed in plants harvested from treatment ABC which had an average of 4.25±0.21 mg/kg (Table 4.14). It was observed that among treatments that received bacterial inoculum and gomeya, plants harvested from treatment AM had the highest zinc content followed by those harvested from treatment CM with average values of 11.85±4.60 and 9.40±6.08 mg/kg, respectively. The lowest zinc content was observed in plants harvested from treatment BCM which had a value of 4.85±1.91 mg/kg (Table 4.15).

4.8.2.4 Cadmium

There was significant difference ($p \leq 0.05$) in the values obtained for the cadmium content of plants harvested from both the treatments. Among treatments which received only bacterial inoculum, it was observed that plants harvested from treatment B had the highest cadmium content followed by plants harvested from treatment A with average values of 36.45±4.60 and 35.55±8.56 mg/kg, respectively, while the lowest cadmium content was observed in plants harvested from treatment ABC which had an average of 4.40±0.28 mg/kg (Table 4.14). Among the treatments which received both bacterial inoculum and gomeya, plants harvested from treatment AM had the highest cadmium content followed by those harvested from treatment CM with average values of 22.95±11.38 and 19.95±15.06 mg/kg, respectively. The lowest cadmium content was observed in plants harvested from treatment ABCM which had a value of 4.20±0.57 mg/kg (Table 4.15).

4.8.2.5 Lead

Significant difference ($p \leq 0.05$) was observed in the values obtained for the lead content of plants harvested from the treatments which received only bacterial inoculum, but there was no significant difference in the values obtained from those which received bacterial inoculum and gomeya. Among treatments which received only bacterial inoculum, plants harvested from treatment A had the highest lead content followed by plants harvested from treatment B with average values of 37.70±0.85 and 37.65±5.02 mg/kg, respectively, while the lowest lead content was observed in plants harvested from Control which had an average of 9.15±0.64 mg/kg (Table 4.14). In the treatments which received both bacterial inoculum and gomeya, plants harvested from treatment AM had the highest lead content followed by those harvested from treatment CM with average values of 26.35±13.93 and 21.20±14.28 mg/kg respectively. The lowest lead content was observed in plants harvested from treatment BCM which had a value of 9.10±1.27 mg/kg (Table 4.15).

4.8.2.6 Cobalt

There was significant difference ($p \leq 0.05$) in the values obtained for the cobalt content of plants harvested from the treatments which received only bacterial inoculum, but no significant differencewas observed in the values obtained from those which received

both bacterial inoculum and gomeya. In treatments which received only bacterial inoculum, plants harvested from treatment A had the highest cobalt content followed by plants harvested from treatment AB with average values of 30.50±1.56 and 29.35±1.06 mg/kg, respectively (Table 4.14). However, in treatments which received bacterial inoculum and gomeya, plants harvested from treatment CM had the highest cobalt content with an average value of 17.60±13.44. The lowest cobalt content was observed in plants harvested from ControlM which had a value of 6.05±0.35 mg/kg (Table 4.15).

4.8.2.7 Chromium

There was significant difference ($p \leq 0.05$) in the values obtained for the chromium content of plants harvested from the treatments which received only bacterial inoculum, but there was no significant difference in the values obtained from those which received bacterial inoculum and gomeya. Plants harvested from treatment A had the highest chromium content mong palnts harvested from treatments which received only bacterial inoculum with an average value of 27.00±0.71 (Table 4.14). In the treatments which received bacterial inoculum and gomeya, plants harvested from treatment AM had the highest chromium content with an average value of 13.75±11.81. The lowest chromium content was observed in plants harvested from treatment BCM which had a value of 4.55±1.91 mg/kg (Table 4.15).

4.8.2.8 Nickel

There was significant difference ($p \leq 0.05$) in the values obtained for the nickel content of plants harvested from the treatments which received only bacterial inoculum, but there was no significant difference in the values obtained from those which received both bacterial inoculum and gomeya. Among treatments which received only bacterial inoculum, plants harvested from treatment BC had the highest nickel content followed by plants harvested from treatment AB with average values of 31.25±11.10 and 29.35±11.52 mg/kg, respectively, while the lowest nickel content was observed in plants harvested from treatment ABC which had an average of 3.20±0.85 mg/kg. In the treatment groups which received bacterial inoculum and gomeya, plants harvested from treatment AM had the highest nickel content followed by those harvested from treatment CM with average values of 19.35±18.88 and 13.60±12.30 mg/kg

respectively (Table 4.14). The lowest nickel content was observed in plants harvested from ControlM which had a value of 3.15±1.06 mg/kg (Table 4.15).

4.8.3 Post bioremediation and post harvest physical and chemical properties of the treated soil samples

The result of the post bioremediation and post harvest physical and chemical analysis of the treated soil samples treated with both bacterial inoculum as well as inoculum and gomeya are shown in Tables 4.16 and 4.17.

4.8.3.1 pH of the treated soil sample

It was observed that there was increase in the pH of the treated soil in all the groups with a range of 8.41 in soil sample B to 8.52 observed in soil sample C for soils treated using only bacterial inoculum as shown in Table 4.16 and a range of 8.48 in soil sample BCM to 8.84 in ControlM for soils treated using bacterial inoculum and gomeya as shown in Table 4.16 compared with a value of 6.45 and 7.00 observed in the contaminated soils during the initial analysis carried out during the dry and rainy season respectively (Table 4.1).

4.8.3.2 Exchangeable acidity (EA) of the treated soil sample

Among the samples treated using only bacterial inoculum, there was an increase in the EA in treatment A, C, AB, AC, AB and Control with values ranging from 0.40 to 0.50 meq/100g compared with 0.37 and 0.35 meq/100g obtainable in the in the contaminated soils during the initial analysis (Table 4.1) during the dry and rainy season respectively.

However, reduction was observed in the EA of soil samples B and BC with each having a value of 0.30 meq/100g respectively. In the samples treated using bacterial inoculum and gomeya, it was observed that in treatment AM, BM, CM, ABM and ABCM there was an increase in the EA with values ranging from 0.40 to 0.50 meq/100g compared with 0.37 and 0.35 meq/100g obtainable in the contaminated soils during the initial analysis conducted during the dry and rainy season respectively (Table 4.1), whereas for soil samples BCM, ACM and ControlM, reduction in EA was observed with each having a final EA of 0.30 meq/100g.

Table 4.16: Physical and chemical properties of soil bioremediated with only bacterial inoculum after harvesting the *C. olitorius*

PARAMETERS	A	B	C	AB	AC	BC	ABC	CONTROL
pH	8.50	8.41	8.52	8.49	8.50	8.41	8.51	8.44
T.O.C (g/kg)	63.52	59.98	65.11	60.74	61.93	67.89	67.09	63.92
T/N (g/kg)	6.57	6.20	6.74	6.28	6.41	7.02	6.94	6.61
Exchangeable Acidity (meq/100g)	0.40	0.30	0.50	0.40	0.50	0.30	0.40	0.40
Available Phosphorus (mg/kg)	42.87	33.20	66.48	39.30	39.78	31.46	25.70	49.67
Ca (Cmol/kg)	86.20	105.66	128.11	107.41	99.80	125.62	94.84	101.42
K (Cmol/kg)	2.75	2.78	14.32	2.06	2.42	1.99	0.97	6.04
Na (Cmol/kg)	8.04	8.70	12.39	10.22	9.13	8.91	9.35	8.91
Mg (Cmol/kg)	0.53	1.17	1.08	1.17	0.86	1.23	0.90	1.19
Mn (mg/kg)	450.0	515.0	738.0	867.0	581.0	756.0	558.0	910.0
Fe (mg/kg)	17.4	13.5	17.2	15.2	10.2	12.1	14.1	23.5
Cu (mg/kg)	4.12	1.61	2.04	0.97	1.07	2.01	1.51	5.84
Zn (mg/kg)	3.84	1.04	1.68	1.12	0.93	1.68	0.99	4.92
Cd (mg/kg)	2.2	2.6	2.6	2.2	1.8	2.1	2.0	4.8
Pb (mg/kg)	21.3	20.8	40.5	24.3	21.1	20.8	22.6	1360.56
Co (mg/kg)	1.04	1.51	1.68	1.86	1.04	1.33	1.75	8.72
Ni (mg/kg)	2.23	1.06	0.93	1.22	0.86	1.04	1.43	1.21
Cr (mg/kg)	1.96	1.81	3.36	3.04	2.17	2.23	3.06	1240.87

Key:

A	Sterilized soil treated with *Alcaligenes aquatilis* (TS11)
B	Sterilized soil treated with *Pseudomonas mucidolens* (E63)
C	Sterilized soil treated with *Bacillus mycoides* (E12Ciia)
AB	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63)
AC	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia)
BC	Sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia)
ABC	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia)
Control	Sterilized soil alone without bacterial or organic nutrient amendment

Table 4.17: Physical and chemical properties of soil bioremediated with bacterial inoculum and gomeya after harvesting the *C. olitorius*

PARAMETERS	AM	BM	CM	ABM	ACM	BCM	ABCM	CONTROLM
pH	8.76	8.73	8.74	8.62	8.51	8.48	8.74	8.84
T.O.C (g/kg)	69.48	65.11	63.97	59.95	63.52	69.08	67.09	70.27
T/N (g/kg)	7.19	6.34	6.61	6.20	6.57	7.15	6.94	7.27
Exchangeable Acidity (meq/100g)	0.40	0.40	0.40	0.50	0.30	0.30	0.50	0.30
Available Phosphorus (mg/kg)	38.26	85.15	63.38	53.01	53.35	22.95	59.46	66.49
Ca (Cmol/kg)	117.39	140.96	130.99	74.10	97.93	102.05	133.98	94.69
K (Cmol/kg)	1.15	18.74	8.49	10.81	4.62	0.95	9.51	5.82
Na (Cmol/kg)	9.13	11.30	10.00	9.13	9.13	7.61	9.57	8.26
Mg (Cmol/kg)	1.31	1.22	1.41	0.90	0.98	1.42	1.38	0.88
Mn (mg/kg)	505.0	590.0	814.0	344.0	438.0	306.0	594.0	561.0
Fe (mg/kg)	14.3	16.4	14.2	12.7	21.3	17.4	10.4	12.3
Cu (mg/kg)	1.23	5.94	1.61	2.60	3.08	3.84	3.04	3.31
Zn (mg/kg)	0.87	3.34	1.16	1.41	1.91	1.87	1.93	1.74
Cd (mg/kg)	2.3	7.2	5.3	3.1	2.4	2.9	3.3	5.1
Pb (mg/kg)	23.4	46.5	42.6	37.4	23.2	26.1	39.8	45.7
Co (mg/kg)	1.11	1.09	0.98	1.03	1.16	1.23	0.92	1.46
Ni (mg/kg)	0.90	0.88	0.92	1.00	1.03	1.11	0.86	1.21
Cr (mg/kg)	2.31	2.26	3.10	2.63	2.68	2.41	2.30	2.91

Key:

AM	Sterilized soil treated with *Alcaligenes aquatilis (*TS11) and sterilized Cattle dung slurry (gomeya)
BM	Sterilized soil treated with *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya)
CM	Sterilized soil treated with *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya)
ABM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Pseudomonas mucidolens* (E63) and sterilized Cattle dung slurry (gomeya)
ACM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya)
BCM	Sterilized soil treated with consortia of *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya)
ABCM	Sterilized soil treated with consortia of *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus mycoides* (E12Ciia) and sterilized Cattle dung slurry (gomeya)
ControlM	Sterilized soil containing sterilized Cattle dung slurry (gomeya)

4.8.3.3 Mineral content

4.8.3.3.1 Total organic carbon (TOC) of the treated soil sample

There was an increase in the TOC of the treated soil in all the groups with a range of 59.98 g/kg in sample B to 67.89 g/kg in sample BC for soils treated using only bacterial inoculum and a range of 59.95 g/kg in sample ABM to 69.48 g/kg in sample AM for soils treated using bacterial inoculum and gomeya compared with a value of 40.80 and 42.44 g/kg observed in the contaminated soils during the initial analysis (Table 4.1) during the dry and rainy season respectively.

4.8.3.3.2 Total nitrogen of the soil after bioremediation

There was an increase in the total nitrogen of the treated soil in all the groups with a range of 6.20 g/kg in sample B to 7.02 g/kg in sample BC for soils treated using only bacterial inoculum, while in soils treated using bacterial inoculum and gomeya, a range of 6.20 in sample ABM to 7.27 g/kg in ControlM was observed compared with a value of 3.90 and 3.98 g/kg observed in the contaminated soils during the initial analysis (Table 4.1) during the dry and rainy season respectively.

4.8.3.3.3 Available phosphorus of the soil after bioremediation

There was an increase in the available phosphorus of the treated soil in all the groups with a range of 25.70 mg/kg in sample ABC to 66.48 mg/kg in sample C for soils treated using only bacterial inoculum as shown in Table 4.16 and a range of 22.95 mg/kg in sample BCM to 85.15 mg/kg in sample BM for soils treated using bacterial inoculum and gomeya (Table 4.17) compared with a value of 13.26 and 14.00 mg/kg observed in the contaminated soils during the initial analysis (Table 4.1) during the dry and rainy season respectively.

4.8.3.3.4 Calcium content of the soil after treatment

There was an increase in the calcium content of the treated soil in all the groups with a range of 86.20 cmol/kg in sample A to 128.11 cmol/kg in sample C for soils treated using only bacterial inoculum (Table 4.16) and a range of 74.10 cmol/kg in sample ABM to 140.96 cmol/kg in sample BM for soils treated using bacterial inoculum and gomeya (Table 4.17) compared with a value of 2.56 and 2.68 cmol/kg observed in the

contaminated soils during the initial analysis (Table 4.1) during the dry and rainy season respectively.

4.8.3.3.5 Potassium content of the bioremediated soil samples

There was an increase in the potassium content of the treated soil when compared with the result obtained in the initial analysis of the contaminated soil (Table 4.1). Sample A had concentration of 0.97 cmol/kg while sample C had a concentration of 14.32 cmol/kg in soils treated using only bacterial inoculum. The same trend was observed in soil treated with both bacterial inoculum and gomeya. Treatment BCM had a concentration of 0.95 cmol/kg and BM had a concentration of 18.74 cmol/kg compared with concentrations of 0.34 and 0.36 cmol/kg observed in the initial analysis of the contaminated soil before treatment (Table 4.1).

4.8.3.3.6 Sodium content of the bioremediated soil samples

There was an increase in the sodium content of the treated soil when compared with the result obtained in the initial analysis of the contaminated soil (Table 4.1). Sample A had concentration of 8.04 cmol/kg while sample C had a concentration of 12.39 cmol/kg in soils treated using only bacterial inoculum. The same trend was observed in soil treated with both bacterial inoculum and gomeya. Treatment BCM had a concentration of 7.61 cmol/kg and BM had a concentration of 11.30 cmol/kg compared with concentrations of 0.34 and 0.36 cmol/kg observed in the initial analysis of the contaminated soil before treatment.

4.8.3.3.7 Magnesium content of the treated soil samples

There was an increase in the magnesium content of the treated soil in all the groups with a range of 0.53 cmol/kg in sample A to 1.23 cmol/kg in sample BC for soils treated using only bacterial inoculum and a range of 0.88 cmol/kg in ControlM to 1.42 cmol/kg in sample BCM for soils treated using bacterial inoculum and gomeya compared with a value of 0.41 and 0.50 cmol/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1).

4.8.3.4 Concentration of heavy metals in the treated soil samples

It was observed that the performance of the treatments varied in respect to the concentration of the heavy metal concentration of the soil samples after the bioremediation exercise. Some heavy metals such as iron, lead, cobalt, nickel and chromium were greatly reduced in all the treatment groups, while the concentration of some like manganese increased in all the treatment groups whereas the concentration of some heavy metals such as copper, zinc and cadmium varied from one treatment group to another. This is shown in Tables 4.16 and 4.17.

4.8.3.4.1 Concentration of manganese in the treated soil samples

There was an increase in the final concentration of manganese in all the treatments. In the soils treated with only bacterial inoculum, the highest concentration of manganese was observed in sample AB which had a concentration of 867.0 mg/kg while in the treatments groups treated with bacterial inoculum and gomeya, the highest concentration was observed in treatment CM with a concentration of 814.00 mg/kg compared with an initial concentration of 98.50 and 99.43 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1).

4.8.3.4.2 Concentration of iron in the treated soil samples

There was a decrease in the final concentration of iron in all the treatment groups for soils treated with only bacterial inoculum and those treated with both bacterial inoculum and gomeya respectively. In the soils treated with only bacterial inoculum, the lowest concentration was observed in treatment AC which had a concentration of 10.20 mg/kg (Table 4.16) while in the groups treated with bacterial inoculum and gomeya, the lowest concentration was observed in treatment ABCM with a concentration of 10.40 mg/kg (Table 4.17) compared with an initial concentration of 23.80 and 24.76 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1).

4.8.3.4.3 Concentration of copper in the treated soil samples

It was observed that there was an increase in the concentration of copper in soils treated with only bacterial inoculum and those treated with both bacterial inoculum

and gomeya, respectively, compared with the concentration of 1.04 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy season (Table 4.1) except in treatment group AB which had a concentration of 0.97 mg/kg.

Reduction in the concentration of copper was observed in samples B, C, BC, AC and ABC for soils treated with only bacterial inoculum with a range of 1.07-2.04 mg/kg compared with the concentration of 2.67 mg/kg observed in the contaminated soil samples used for the initial analysis during the dry season (Table 4.1).

However, an increase in the concentration of copper was observed in samples A, BM and Control with concentrations of 4.12, 5.94 and 5.84 mg/kg respectively compared with the concentration of 2.67 mg/kg observed in the contaminated soil samples used for the initial analysis during the dry season (Table 4.1).

In samples AM, CM and ABM, the concentration of copper was reduced to a range of 1.23-2.60 mg/kg compared with the concentration of 2.67 mg/kg observed in the contaminated soil samples used for the initial analysis during the dry season (Table 4.1).

4.8.3.4.4 Concentration of zinc in the bioremediated soils

Reduction was observed in the concentration of zinc in samples B, AB, AC, ABC, AM among soils treated with only bacterial inoculum and those treated with both bacterial inoculum and gomeya respectively, compared with the concentration of 1.36 and 2.55 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1). The highest reduction was observed in treatment AM with a concentration of 0.87 mg/kg. In treatment A and BM, the concentration of zinc was increased with values of 3.84 and 3.34 mg/kg, respectively.

4.8.3.4.5 Concentration of cadmium in the bioremediated soils

There was an increase in the concentration of cadmium in all the treatments compared with the concentration of 0.50 mg/kg observed observed in the contaminated soil samples used for the initial analysis during the rainy season (Table 4.1).

However, reduction in the concentration of cadmium was observed in samples A, B, C, AB, AC, BC, ABC, AM, ACM and BCM with a range of 1.8 to 2.9 mg/kg compared with the concentration of 2.98 observed in the contaminated soil samples

used for the initial analysis during the dry season (Table 4.1). The concentration of cadmium was increased above its concentration during the dry season in samples BM, CM, ABM, ABCM, Control and ControlM with a range of 3.1to 7.2 mg/kg.

4.8.3.4.6 Concentration of lead in the treated soil samples

Reduction was observed in the final concentration of lead in all the treatments. In the soils treated with only bacterial inoculum, the lowest concentration was observed in treatment B and BC which had a concentration of 20.8 mg/kg respectively while in the groups treated with bacterial inoculum and gomeya, the lowest concentration was observed in treatment AM with a concentration of 23.40 mg/kg compared with an initial concentration of 1505.50 and 2333.55 mg/kg observed in the contaminated soil samples during the rainy and dry season respectively (Table 4.1).

There was slight reduction in the concentration of lead in the Control group which had a final concentration of 1306.56 mg/kg compared with an initial concentration of 1505.50 and 2333.55 mg/kg observed in observed in the contaminated soil samples.

4.8.3.4.7 Concentration of cobalt in the bioremediated soil samples

There was a decrease in the final concentration of cobalt in all the treatments. In the soils treated with only bacterial inoculum, the lowest concentration was observed in treatment A and AC which had a concentration of 1.04 mg/kg respectively while in the groups treated with bacterial inoculum and gomeya, the lowest concentration was observed in treatment ABCM with a concentration of 0.92 mg/kg compared with an initial concentration of 10.50 and 13.65 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1).

4.8.3.4.8 Concentration of nickel in the bioremediated soil samples

There was a decrease in the final concentration of nickel in all the treatments. In the soils treated with only bacterial inoculum, the lowest concentration was observed in treatment AC which had a concentration of 0.86 mg/kg respectively while in the groups treated with bacterial inoculum and gomeya, the lowest concentration was observed in treatment ABCM with a concentration of 0.86 mg/kg compared with an

initial concentration of 31.50 and 40.56 mg/kg which was observed during the rainy and dry season respectively (Table 4.1).

4.8.3.4.9 Concentration of chromium in the bioremediated soil samples

There was a decrease in the final concentration of chromium in all the treatments. In the soils treated with only bacterial inoculum, the lowest concentration was observed in treatment B which had a concentration of 1.81 mg/kg respectively while in the groups treated with bacterial inoculum and gomeya, the lowest concentration was observed in treatment BM with a concentration of 2.26 mg/kg compared with an initial concentration of 1526.00 and 1678.67 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1). There was slight reduction in the concentration of chromium in the Control group which had a final concentration of 1240.87 to an initial concentration of 1526.00 and 1678.67 mg/kg observed in the contaminated soil samples used for the initial analysis during the rainy and dry season respectively (Table 4.1).

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Description of the study site

The area surrounding the study site was heavily contaminated with heavy metals as a result of the release of effluent by the steel rolling industry in the area into the surrounding soil. It was observed that the soil could not support plant growth and some plants which could adapt to the heavy metal in the environment were either drying up or had a yellow colour; all these deviations from normal physical characteristics of a healthy soil and plant indicated a high degree of pollution of the study area by heavy metals. This is in accordance with the report of Revathi *et al.* (2011), who reported that plant growth is not sustained in soils heavily polluted with heavy metals. The observed deviations in the physical characteristics of the soil samples were in agreement with changes observed in a soil contaminated by acid mine drainage water as reported by Bitala *et al.* (2009).

Analysis of the contaminated soil sample done before treatment revealed that the concentration of the heavy metals were higher during the dry season than during the wet or rainy season. This can be attributed to the effect of leaching which is more evident during the wet or rainy season compared to the dry season and also the rates of deposition of suspended particles are generally higher during the dry season compared to the wet or rainy season. This is in agreement with the findings of Kilicel (1999).

Concentrations of some heavy metals present in the contaminated site in this study was found to exceed the concentrations recommended by WHO and USEPA. For instance, heavy metals such as cadmium, lead, chromium and cobalt had concentrations of 0.50-2.98 mg/kg, 1505.50-2333.55 mg/kg, 1526.00-1678.67 mg/kg and 10.50-13.65 mg/kg, respectively which exceeded the recommended concentrations of 0.003, 10, 2 and 8 mg/kg by WHO and USEPA for each of the respective heavy metals (WHO, 1984; USEPA, 2004; Parizanganeh *et al.*, 2012 and Ezejiofor *et al.*, 2013). However,

minerals such as calcium, potassium, magnesium and sodium which gave a concentration of 2.56-2.68, 0.34-0.36, 0.41-0.50 and 0.30-0.50 cmol/kg respectively were below the recommended values of 10-20, 0.6-1.2, 3-8 and 0.7-1.2 cmol/kg (WHO, 1984; USEPA, 2004; Parizanganeh *et al.*, 2012 and Ezejiofor *et al.*, 2013). These minerals are essential to plant growth and development hence reduction in their bioavailability will lead to reduced plant growth. A major reason for the reduction in bioavailability of these important minerals could be the high contamination of the soil with heavy metals. According to Chibuike and Obiora (2014), the presence of heavy metals in a soil may affect the availability of other element especially the minerals in the soil.

Though earlier studies has shown that cattle dung slurry contains high numbers of saprophytic fungi and heterotrophic bacteria such as *Acinetobacter* sp, *Bacillus* sp, *Alcaligenes* spp, *Serratia* spp. and *Pseudomonas* spp. which can be involved in the bioremediation of heavy metals (Obire *et al*,. 2008; Boricha and Fulekar, 2010; Randhawa and Kullar, 2011). Majority of these organisms are not spore formers and therefore are destroyed by the application of heat during the sterilization of the Cattle dung slurry. Analysis of the sterilized cattle dung slurry (gomeya) revealed a concentration of 4675, 24.85, 16.83, 53.5, 77.0, 50.2 and 88.1 mg/kg of iron, copper, zinc, cadmium, lead cobalt and chromium respectively. This was contrary to concentrations of 3040, 44.1, <0.002 and<0.002 mg/kg of iron, copper, zinc, cadmium and lead, respectively in cattle dung observed in an earlier study by Tripathi *et al.* (2004), they also reported the absence of cobalt and chromium in the cattle dung they analysed. Though, there was dearth of information on the use of gomeya on heavy metals contaminated soil, previous studies have shown that the use of cattle dung as organic nutrient source has shown good promises in remediation of crude oil contaminated soil (Orji *et al*., 2012).

5.2 Bacterial population of heavy metal contaminated site

Thirty six bacterial isolates were obtained from the contaminated sites namely *Proteus mirabilis* (5), *Pseudomonas* sp (11), *P. fluorescens* (3), *P. azotoformans* (2), *P. Putida* (3) *Alcaligenes faecalis* (5), *Providencia* sp (2), *Bacillus mycoides* (1), *B. subtilis* (1) and *Enterobacter* sp (3). All the bacteria isolated in this study had been indicated in

previous studies as having potential of being used in bioremediation of heavy metal (Höfte *et al.*, 1994; Gilis *et al.*, 1996; Lovley, 1994; Guha *et al*., 2001; Camargo *et al*., 2003).

5.3 Molecular and Phylogenetic Characteristics of the Bacterial isolates

Analysis of the 16S rRNA nucleotide sequence is often used to identify and classify organisms while phylogenetic relationships between closely related species are usually defined based on the highly conserve regions of their aligned sequences. The diversity of the microorganisms isolated in this study was observed to belong to four divisions. Twenty-seven (77.14%) of the isolates belong to the group Gamma (ɤ) proteobacteria which are in the genera *Proteus, Azotobacter, Pseudomonas, Providencia, Shewanella, Citrobacter* and *Pantoea*, while five (14.29%) of the isolates belong to the Beta (β) proteobacteria which are in the genera *Alcaligenes, Paenalcaligenes* and *Castellaniella.* Two (5.71%) of the bacterial isolates belong to the group of Firmicutes in the genera *Bacillus,* only one (2.86%) isolate was found to belong to the group Alpha (α) proteobacteria in the genera *Brucella.*

Majority of these isolates especially *Pseudomonas, Providencia, Shewanella, Alcaligenes* and *Bacillus* species have been cited in earlier studies as having ability to tolerate heavy metals in the environment and also remediate heavy metals (Chang *et al*., 1997; Hassen *et al*., 1998; Roane *et al*., 2001; Magyarosy *et al*., 2002; Rajendran *et al*., 2003; De *et al*, 2008; Pandey and Fulekar, 2012).

5.4 Tolerance of the bacterial isolates to heavy metals

The bacterial isolates were observed to show different tolerance level to the heavy metal salts. Some of the isolates showed colorations when growing on agar plates incorporated with the heavy metal salts as a result of absorbing the colours of the heavy metal salts. Majority of the isolates were able to grow between 150 and 300µg/ml of the various metal salts concentration, however, some of the bacterial isolates were able to grow at 350 µg/ml to 450 µg/ml of the heavy metals, whereas about four isolates failed to grow at any of the concentrations of the heavy metals. This was in agreement with an earlier study carried out by Narasimhulu *et al.* (2010), in which the authors observed that bacterial isolates grow at different Minimum Inhibitory Concentration (MIC) when cultured on agar plates supplemented with heavy

metal salts. *Alcaligenes aquaatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia) showed very high MIC and were therefore used for the bioremediation exercise in this study.

5.5 Observed agronomic parameters

The pH of the treated soil obtained in this study were 8.41-8.52 in soils treated using only bacterial inoculum, while a range of 8.18-8.76 was obtained in soils treated using bacterial inoculum and gomeya. This pH is suitable for the growth and yield of *C. olitorius*, according to Facciola (1990), *C. olitorius* does well in acid, neutral and basic (alkaline) soils, it tolerates soil pH of 4.5 to 8.0. However extreme pH conditions will reduce the availability of iron in the soil and therefore cause yellowing between leaf veins (Palada and Chang, 2003).

A plant height of 7.84 cm in ABC and 9.24 cm in BCM at 7 WAP was obtained in treatments which received only bacterial inoculum and bacterial inoculum and gomeya respectively. ControlM which contained unsterilized contaminated soil and gomeya showed a final height of 22.94 cm at 7 WAP. The values obtained for plant height in this study are low compared to that obtained by Ogunrinde and Fasimirin (2011) in which they obtained a mean height of 105.03 cm at 7WAP for *C. olitorius* planted in an uncontaminated soil. The stunted growth of plants observed in this study may be as a result of the effect of heavy metals contamination on the soil. Adenipekun et *al.* (2013) reported similar observation in a study in which they observed a height range of 4.083-11.183 cm in *C. olitorius* grown on a soil contaminated with oil and remediated using *Pleurotus pulmonarius* at 5WAP.

The data obtained for number of leaves at 7 WAP in this study, are 5.80, 5.60 and 9.40 for treatment ABC, BCM and ControlM, respectively. This is similar to that obtained by Adenipekun *et al.* (2013), in which they observed a range of 4.500-10.666 number of leaves in *C. olitorius* grown on a *Pleurotus pulmonarius* remediated soil contaminated with oil after 5WAP.

The biggest stem diameter observed among the different treatment groups in this study were found in treatment ABC (1.35 mm), ABCM (1.36 mm) and ControlM (2.20 mm).

This is comparable to that obtained by Adenipekun *et al.* (2013), in which they observed a range of 0.128-1.05 mm for stem diameter of *C. olitorius* grown on a *Pleurotus pulmonarius* remediated soil contaminated with oil after 5WAP.

Comparing data obtained for the different agronomic parameters in groups treated with only bacterial inoculum and those treated with both bacterial inoculum and gomeya, it was observed that the treatments which received gomeya perfomed better than the others. This is because the addition of organic matter in the form of gomeya improves the soil properties and hence the fertility of the soils for the cultivation of crops, this is in agreement with Revathi *et al.* (2011), who reported that addition of organic amendment to soil helps in improving the soil properties and fertility. A possible explanation for the performance of ControlM which contained unsterilized contaminated soil with organic amendment (gomeya) is that the indigenous microflora of the soil are well adapted to the environment and therefore their lag phase during the treatment was short or absent and their bioremediation abilities were improved as a result of the addition of gomeya (Adedokun and Ataga, 2007; Medjor *et al.*, 2012).

The reduction in plant height in most of the treatment groups (especially those containing sterilized soil) could be attributed to the adverse effect of heavy metals contamination of the soil on the cell elongation and cell expansion. The effects of toxic substances on plants are dependent on the amount of toxic substance taken up from a given environment. The toxicity of some metal may be so high that plant growth is retarded before a large quantity of an element can be translocated (Haghiri, 1973). Reduced shoot and seedling length in metal contaminated soil could be as a result of the reduction in meristematic cells present in this region and some enzyme contained in cotyledon and endosperm. In uncontaminated soils, plant cells become active and begin to digest and store food which is converted into the soluble form and transported to the radicle and plumule tips e.g. enzyme amylase convert starch into sugar while protease act on protein, however when the activities of different enzymes become affected as a result of heavy metals contamination, food will not reach the radicle and plumule and as such affects the shoot and seedling length (Kabir *et al.*, 2009). Also in a study by Dalal and Bairgi (1985), they observed reduction in seed germination, root; shoot and seedling length of jute varieties, *Corchorus olitorius* cv.

JRO 524 and C*apsular corchorus* JRC 321 at different levels of Pb concentration in the soil particularly at 20 mg/L

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5.6 Post bioremediation and post harvest observations

5.6.1 Analysis of the harvested plants

In order to derive the desired benefits from the consumption of vegetables and plant materials, care must be taken to ensure that non contaminated vegetables especially those which are free from accumulation of heavy metals are consumed because of the health hazard of these metals on man even at trace concentrations. Vegetables and other plants have the ability to absorb physiologically toxic trace metals (Khan and Frankland, 1983) and there are various health hazards that can arise from consumption of vegetables with high metal contents (Byrne, 1998). The bioaccumulation of metals in vegetables has also been shown to distort the composition of beneficial nutrients in them. For example, the accumulation of chromium has led to decrease in protein and chlorophyll contents of *Najas indica* and other plants (Sarita *et al.,* 2002).

Analysis of the harvested *C. olitorius* plants revealed that for proximate matter such has % crude fibre, dry weight, ether extract (fat) and fresh weight most of the treatment groups performed well having a range of 18.34-22.62; 0.10-4.22; 1.11-2.06 and 0.22-9.65 respectively compared to values of 20.30; 0.34; 0.12 and 1.11 respectively obtained by some authors (Ndlovu and Afolayan, 2008; Adenipekun *et al.* 2013 and Yekeen *et al.*, 2013).

Although crude fibre has little nutritional value, however, adequate intake of dietary fibre can lower the serum cholesterol level and aids absorption of trace elements in the gut as well as reduced the risk of coronary heart disease, diabetes, constipation, hypertension, colon and breast cancer (Asuk *et al*., 2015; Hayat *et al*., 2014; Gqaza *et al*., 2013; Ishida *et al*., 2000). *C. olitorius* was found to be rich in fibre and this is beneficial nutritionally. The ether extract (Fat) content reported in this study is moderate when compared to those from other plants. Dietary fats function in the increase of palatability of food by absorbing and retaining flavours (Antia *et al*., 2006).

Other proximate matters such as % ash, % crude protein, dry matter and % moisture had a range of 14.97-25.41; 5.57-15.82; 27.35-56.35; and 43.65-72.65 respectively and they were low compared to values of 21-21.40; 21.12; 16.8 and 84.28 respectively obtained by Onwordi *et al.* (2009) and Acho *et al.* (2014).

High ash content in food is a measure of high deposit of mineral contents (Akpabio *et al*., 2012). The value obtained in this study suggests that the *C. olitorius* harvested was moderately rich in mineral elements. One major reason why the harvested *C. olitorius* is not high in fibre content is because the harvested plants were observed to absorb heavy metals and this could have affected its mineral content.

Dietary proteins are important for natural synthesis and maintenance of body tissues, enzymes and hormones as well as other substances required for healthy functioning of the body system (Hayat *et al*., 2014). The protein value obtained from this study 4.44±1.33 to 15.82±1.90 suggests that *C. olitorius* can effectively contribute to the daily protein needed. Even though the value is low compared to protein values from other reports, Gqaza *et al*. (2013) however said that any plant food that provides more than 12 % of its caloric value from protein is considered a good source of protein.

George (2003) reported that though high moisture content is important in maintaining the protoplasmic content of the plant cells, it however makes the vegetables perishable and susceptible to spoilage by micro-organisms during storage. The moisture content obtained for *C. olitorius* in this study though low compared to that of some authors, shows that the harvested *C.olitorius* can be easily susceptible to spoilage by micro-organisms during storage.

The analysis of the harvested *C. olitorius* plant revealed the presence of heavy metals in the leaves; this is an indication that there was an uptake of these heavy metals by the roots of *C. olitorius* and this was followed by its translocation which is in agreement with Peralta-Videa *et al.* (2002). According to earlier studies by Hough *et al*. (2003); Walker *et al.* (2003), Kashem and Singh, (2004) and Rieuwerts *et al*. (2006), it was reported that at pH ranges between 4.0-8.5, metal cations are mobile while anions tend to transform to oxide minerals, thus increasing their concentration in the environment, the pH of the treated soil samples were found to be between 8.41 and 8.84, this could

be one of the reasons the heavy metals were easily absorbed and translocated in the plant. According to Muhammad *et al*. (2008), leafy vegetables grown in heavy metal contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils. This is because they are capable of absorbing these metals through their roots. As earlier reported by Akan *et al*. (2009), vegetables accumulate heavy metals in their edible and non-edible parts as can be seen in the accumulation of heavy metals in the leaves and stems of *C. olitorius* in this study.

The analysis of the harvested *C. olitorius* plant revealed that the content of iron (Fe) and zinc (Zn) in the harvested plants was low having a range of 24.95-42.35 and 4.25-17.45 mg/kg respectively compared to the recommended standard of 60 mg/kg (FAO/WHO, 2001; Sanyaolu *et al.,* 2011 and Ayejuyo *et al.,* 2014). The values obtained for Zn in this study, 24.95-42.35 mg/kg was higher when compared to those reported by Sobukola *et al.* (2010), who reported zinc levels of 0.011, 0.070 and 0.050 mg/kg in the leaves of bitter leaf, water leaf and cabbage, respectively.

The nickel content of the harvested *C. olitorius* plants varies; some were higher than the recommended standard while some plants harvested form treatment AM, CM, ABM, ACM, BCM, ABCM, Control and ControlM had lower values of 3.90; 5.20; 4.50; 3.50; 3.20; 3.65; 3.25 and 3.15 mg/kg respectively when compared to the standard range of 10 mg/kg (FAO/WHO, 2001).

The concentration of Cu in this study, 19.95-36.05 was higher than that reported by Elbagermi *et al*. (2012), who reported values of 5.00, 5.75 and 5.32 mg/kg for the concentration of Cu in carrot, cucumber and spinach respectively.

The concentration of other heavy metals such as cadmium, chromium, copper and lead of the harvested plant ranged from 4.20-36.45; 4.00-27.00; 19.95-36.05 and 9.10-37.70 mg/kg respectively which exceeded the recommended range when compared to standards of 0.2, 2.3, 0.3 and 0.30 mg/kg respectively (FAO/WHO, 2001; Sanyaolu *et al.,* 2011; Yebpella *et al.,* 2011 and Ayejuyo *et al.,* 2014).

5.6.2 Physical and chemical properties of treated soil

The post bioremediation and post harvest analysis of the treated soils revealed an increase in the pH, total organic carbon, total nitrogen and available phosphorus of the treated soils. The pH and available phosphorus observed in the treated soil in this study were higher than the pH of 5.1-6.5 and available phosphorus of 20 mg/kg observed in studies by Brady and Weil (2008) and Holland *et al*. (1989), respectively. The pH observed in this study indicated that all the treatment groups were within a pH range that was conducive for bioremediation to take place; this is because according to Nkereuwem *et al.* (2010), the best pH for optimum bioremediation to take place ranges between 5.5 to 8.8.

In the case of the exchangeable acidity some treatments like B, BC, ACM, BCM and ControlM had concentrations of 0.30 meq/100 g compared to the concentration of 0.35-0.37 meq/ 100g observed in the initial analysis of the contaminated soil. Other treatments in both the groups treated with only bacterial inoculum and those treated with bacterial inoculum and gomeya had a concentration of 0.40- 0.50 meq/100 g each. It was observed that all the treatments had higher exchangeable acidity when compared to the EA of 0.2 meq/100 g obtained in a study by Masarirambi *et al.* (2012).

An increase was observed in the concentration of minerals such as calcium, potassium and sodium of the treated soils. The data obtained during the initial analysis of the contaminated soil for calcium, potassium and sodium were 2.56-2.68, 0.34-0.36 and 0.30-0.50 Cmol/kg respectively. In the treatments treated with only bacterial inoculum, it was observed that the concentrations of calcium, potassium and sodium ranged 86.20 in treatment A to 128.11 Cmol/kg in treatment C; 0.97 in treatment ABC to 14.32 Cmol/kg in treatment in C and 8.04 in treatment A and 12.39 Cmol/kg in treatment C respectively. While in the groups treated with bacterial inoculum and gomeya, the concentrations of calcium, potassium and sodium ranged 74.10 in treatment ABM to 140.96 Cmol/kg in treatment BM; 0.95 in treatment BCM to 18.74 Cmol/kg in treatment BM and 7.61 in treatment BCM to 11.30 Cmol/kg in treatment BM respectively. These concentrations were above the concentrations of 10-20; 0.6-1.2 and 0.7-1.2 Cmol/kg observed by Holland *et al.* (1989) for calcium, potassium and sodium, respectively. One major factor responsible for the increase in the mineral content of the

treated soil samples is the decrease in the concentration of heavy metals present in the soil as supported by Chibuike and Obiora (2014).

The concentration of magnesium in the groups treated with only bacterial inoculum and both bacterial inoculum and gomeya ranged from 0.53 in treatment A to 1.23 Cmol/kg in treatment BC and 0.88 in ControlM to 1.41 Cmol/kg in CM, these concentrations were higher than the concentration of 0.41-0.50 Cmol/kg observed in the initial analysis of the contaminated soil. However, the ranges were lower compared to a range of 3-8 Cmol/kg observed by Brady and Weil (2008).

The isolates used in this study were able to achieve high reduction in heavy metal concentration in the soil. For instance, in the groups which received only bacterial inoculum, treatment AC achieved a 57.99% reduction on the concentration of iron in the soil; treatments B and BC had a 98.92% reduction on the concentration of lead in the soil; treatments A and AC gave a 91.39% reduction on the concentration of cobalt, treatment C had a 97.42% reduction on the concentration of nickel while treatment B had a reduction of 99.89% on the concentration of chromium, while in the groups treated with bacterial inoculum and gomeya, treatment ABCM achieved a 57.17% reduction on the concentration of iron in the soil; treatment AM had a 98.78% reduction on the concentration of lead in the soil; treatments ABCM gave a 92.38% reduction on the concentration of cobalt, treatment ABCM had a 97.61% reduction on the concentration of nickel while treatment BM had a reduction of 99.86% on the concentration of chromium after 7 weeks of bioremediation. This is comparable to results from earlier studies. For instance, Chang *et al*. (1997) reported a *Pseudomonas aeruginosa* PU21 (Rip64) strain with a metal uptake efficiency of 80% within 2 days while Roane *et al*. (2001) reported a *Bacillus* strain H9 with a metal uptake efficiency of 36% within 48 hours. Magyarosy *et al*. (2002) also reported a *Pseudomonas* spp with a metal uptake efficiency of 98% within 4 days. Though this isolates did not work as rapid as those earlier cited, this can be attributed to the fact that soils contaminated with heavy metals are poor in nutrients and bacterial diversity which results in impeded rates of remediation (White *et al*., 2006).

Though the isolates were able to reduce the concentrations of these heavy metals in the soil, it was however observed that the concentrations of these heavy metals were still above the recommendation of 0.300, 10, 8, 40 and 2.0 mg/kg respectively of WHO and USEPA (WHO, 1984; USEPA, 2004; Parizanganeh *et al.*, 2012 and Ezejiofor *et al.*, 2013).

The concentration of manganese increased in all the treatment groups having a range of 306.0 mg/kg in group BCM to 867.0 in group AB mg/kg compared to the initial concentration of 99.43 and 98.50 mg/kg observed during the dry and rainy season respectively. This is in agreement with Tanaka *et al.* (2001), in which it was reported that some soil sterilization methods have an impact on the concentration of heavy metals especially manganese in the soil.

The reduction in the concentration of heavy metals such as lead, cobalt, nickel and chromium in the Control group which had a concentration of 1360.56, 8.72, 23.54 and 1240.87 mg/kg respectively compared to the initial concentrations of 1505.50-2333.55; 10.50-13.65; 31.50-40.56 and 1526.00-1678.67 mg/kg observed during the rainy and dry season respectively could be attributed to the effect of phytoremediation. This is in agreement with Oyedele *et al.* (2006) in which they reported that *C. olitorius* has the potential to decontaminate soils contaminated with heavy metals.

The results obtained in this study, has shown that the use of bacterial consortia rather than individual microorganism works more effectively in bioremediation of heavy metals. This is in agreement with observations made by Nwadinigwe and Onyeidu (2012). It has also been observed that bioaugmentation (i.e. the use of bacterial inoculum) worked better than biostimulation (addition of gomeya to stimulate indigenous microflora as seen in ControlM) in reducing heavy metals in this study. This is similar to the findings of Bento *et al.* (2003), in which they observed that bioaugmentation was more effective than biostimulation in the bioremediation of soil contaminated by diesel oil. Factors which could favour bioaugmentation over biostimulation include the fact that bacterial cells used for bioaugmentation are still young (since they are often harvested freshly before use and are often in the

exponential phase of growth) and hence are viable requiring a very short or no lag phase when introduced into the environment. Also, the indigenous microbes used in biostimulation might be stressed as a result of their previous exposure to the heavy metals contamination which reduces their ability to survive and degrade the toxic pollutants (Leahy and Colwell, 1990; Adams *et al.* 2015).

However, it was observed that the combination of both bioaugmentation and biostimulation gives a better result as seen in treatments which received bacterial inoculum and gomeya. This agrees with earlier studies on bioremediation of soil contaminated with diesel and total petroleum hydrocarbon removal by microorganisms (Bento *et al.* 2004; Abdusalam and Omale, 2009).

Although microorganisms and plants can be used independently for the clean-up of polluted sites, combining these two treatments increase the efficiency of this method of remediation. The combination of bacterial activities and planting experiment reduced the concentration of heavy metals in the soil. This is because the microorganisms acted in synergism with the plants for effective remediation. This is in agreement with Chibuike and Obiora (2013), who stated that synergism between microbes and plants can boost effective remediation of pollutants . This synergistic relationship promotes the exchange of water and nutrients established between plant roots and the microorganisms, enhancing plant growth in the process (Nanda and Abraham, 2013). Plant processes promote the removal of contaminants from the soil and water either directly or indirectly. Direct processes include plant uptake into roots or shoots and transformation, storage, or transpiration of the contaminants by bacterial, soil, and root interactions within the rhizosphere (Hutchinson *et al*., 2003). Plants transform certain contaminants through oxidation and reduction reactions, conjugation phase and deposition of conjugates into vacuoles and cell walls (Subramanian and Shanks, 2003).

CHAPTER SIX

6.0 SUMMARY AND CONCLUSIONS

This study investigated the possibility of using bacterial inoculum (either singly or as a consortium) with and/or without nutrient amendment in the form of cattle dung (gomeya) in remediating soil contaminated with heavy metals using bioaugmentation and biostimulation techniques.

Heavy metals contaminated soil sample was purposively collected from a contaminated site and thirty six bacteria isolates were obtained from the soil sample. The isolates were screened for their tolerance to heavy metals using heavy metals salts, three isolates showing the highest tolerance to heavy metals were selected and used for bioremediation of the contaminated soil. The three isolates were identified as *Alcaligenes aquatilis* (TS11), *Pseudomonas mucidolens* (E63) and *Bacillus cereus* (E12Ciia).

Molecular characterization based on the 16S rRNA nucleotide sequence of the isolates obtained in this study identified the bacteria isoltates. They were member of the genera *Proteus, Azotobacter, Pseudomonas, Providencia, Shewanella, Citrobacter, Pantoea, Alcaligenes, Paenalcaligenes, Castellaniella, Bacillus* and *Brucella.*

From the results obtained in this study, the following observations were arrived at:

i. Soils surrounding the steel rolling industries were heavily contaminated with high concentration of heavy metals.
ii. The ability of bacteria isolates especially *Pseudomonas* sp. to remediate heavy metals particularly lead and chromium.
iii. Biostimulation of heavy metals contaminated soil with organic amendment supported plant growth more than bioaugmentation technique alone.
iv. Bioaugmentation achieved better heavy metals removal from the contaminated soil compared with biostimulation.

v. The combination of both bioaugmentation and biostimulation gives a better result in reducing the concentration of heavy metals in the treated soil as seen in treatments which received bacterial inoculum and gomeya.

vi. The use of bacterial consortia rather than individual microorganism works more effectively in bioremediation of heavy metals.

vii. The selected bacteria can be employed in remediation exercise involving heavy metals contamination of soil.

viii. The combination of bacterial remediation and phytoremediation reduced the concentration of heavy metals in the soil. This is because the microorganisms acted in synergism with the plants for effective remediation.

ix. Soil sterilization had effect on the planting exercise, hence the group containing unsterilized contaminated soil with cattle dung slurry as organic amendment (biostimulation) performed better in terms of agronomy parameters such as plant height, number of leaves, stem diameter and in the plant yield.

x. It can however be concluded that increasing the number of days used for the experimental set-up and increasing the bacterial inoculum may also increase the percentage of heavy metals remediated by the bacterial isolate.

REFERENCES

Abbas, M.; Pawoem, Z.; Igba, S.; Riazuddin, L.M.; Ahmed, M. and Bhutto, R. 2010. Monitoring of toxic metals (Cd, Pb, As, Hg) in vegetable of Sindh, Pakistan. *Kuthmandu University of Science Engineering and Technology,* **16**:60-65.

Abdusalam, S. and Omale, A.B 2009. Comparison of Biostimulation and Bioaugmentation Techniques for the Remediation of Used Motor oil contaminated soil. *Brazillian Archives of Biology and Technology*, **52(3)**: 747-754.

Abii, T.A. 2012. Levels of Heavy Metals (Cr, Pb, Cd) Available for Plants within Abandoned Mechanic Workshops in Umuahia Metropolis *Research Journal of Chemical Sciences* **2(2)**:79-82.

Acho, C.F.; Zoue, L.T.; Akpa, E.E.; Yapo, V.G. and Niamke, S.L. 2014. Leafy vegetables consumed in Southern Côte d'Ivoire: a source of high value nutrients. *Journal of Animal* and *Plant Sciences*. **20(3)**:3159-3170.

Adams, G.O.; Fufeyin, P.T.; Okoro, S.E. and Ehinomen, I. 2015. Bioremediation, biostimulation and bioaugmentation: A review. *International Journal of Environmental Bioremediation and Biodegradation*, **3(1):**28-39.

Adedokun, O.M. and Ataga, A.E. 2007. Effects of amendments and bioaugumentation of soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (*Vigna unguiculata* L. Walp). *Scientific Research Essay*, **2(5)**: 147-149.

Adenipekun, C.O.; Ayanleye, O.O. and Oyetunji, O.J. 2013. Bioremediation of soil contaminated by spent diesel oil using *Pleurotus pulmonarius* Fries (Quelet) and its effects on the growth of *Corchorus olitorius* (L). *Journal of Applied Biosciences*, **68**:5366 – 5373.

Adeniyi, A.A. 1996. Determination of cadmium, copper, iron, lead manganese and zinc in water leaf (*Talinum triangulae*) in dumpsites. *Environmental International*, **22**:259-262.

Adriano, D.C. 2001. *Trace elements in terrestrial environments*: Biogeochemistry, bioavailability and risks of metals (2nd ed.). New York: Springer-Verlag.

Agency for Toxic Substances and Disease Registry (ATSDR) 1994. *Toxicological profile for Zinc and Cobalt.* US Department of Health and Human Services, Public Health Serv. 205-88-0608.

Ajaz haja mohideena, R.; Thirumalai Arasuc, V.; Narayananb, K.R. and Zahir Hussaind, M.I. 2010. Bioremediation of Heavy metal contaminated soil by the Exigobacterium and accumulation of Cd, Ni, Zn and Cu from Soil environment. *International Journal of Biological Technology*, **1(2):** 94-101.

Akan, J.C.; Abdulrahman, F.I., Ogugbuaja, V.O. and Ayodele, J.T. 2009. Heavy metals and anion levels in some samples of vegetables grown within the vicinity of Challawa indust. Area, Kano State, Nigeria. *American Journal of Applied Sciences*, **6(3):**534-542.

Akinyele, I.O. and Osibajo, O. 1982. Levels of trace elements in hospital diet. *Food Chemistry*, **8**:247-251.

Akpabio, U.D.; Udo, U.E. and Akpakpan, A.E. 2012. Proximate composition and nutrient analysis of *Aneilema aequinoctiale* leaves. *Asian Journal of Plant Science and Research*, **2(5):**607-612.

Al-Jassir, M.S.; Shaker, A and Khalid, M.A. 2005. Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh city, Saudi Arabia. *Bulletin of Environmental Contamination and Toxicology*, **75**:1020-1027.

Alloway, B.J. 1995. *Heavy metals in soils*. Blackie, Glasgow, Scotland.

Aluko, O.A.; Olanipekun, T.O.; Olasoji, J.O.; Abiola, I.O.; Adeniyan, O.N.; Olanipekun, S.O.; Omenna, E.C.; Kareem, K.O. and Douglas, A.I. 2014. Effect of organic and inorganic fertilizer on the yield and nutrient composition of jute mallow. *Global Journal of Agriculture Research*, **2(3):** 1-9.

Alvarez, G.; Jimenez, B.; Carral, G. and Gutierez, M. 1998. Aluminium distribution and soil acidity in a Mediterranean mountain area. *Proceedings of 16th World Congress of Soil Science*, **1**:457-460.

Amadi, A. and Ue Bari, Y. 1992. Use of poultry manure for amendment of oil polluted soils in relation to growth of maize (*Zea mays* L.). *Environmental International* **18**: 521-527.

Antia, B.S.; Akpan, E.J.; Okon, P.A. and Umoren I.U. 2006. Nutritive and antinutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pakistan Journal of Nutrition*, **5**:166-168.

Arsova, A. 1998. Bioecological testing of carbonate deposit designed for liming copper polluted acid soil. *Journal of Balkan Ecology*, **3**:85-89.

Association of Analytical Chemists (AOAC) 2012. Official Methods of Analysis 19th Ed. Association of Analytical Chemists, Washington D.C

Asuk, A.A.; Agiang, M.A.; Dasofunjo, K. and Willie, A.J. 2015. The biomedical significance of the phytochemical, proximate and mineral compositions of the leaf, stem bark and root of Jatropha curcas. *Asian Pacific Journal of Tropical Biomedicine*, **5(8):**650-657.

Aualiita, T.U. and Pickering, W.F. 1987. The specific sorption of trace amounts of Cu, Pb and Cd by inorganic particulates. *Water Air and Soil Pollution*, **35**:171–185.

Ayejuyo, O.O.; Osundiya, M.O.; Olowu, R.A.; Bamgboye, O.A. and Ogunlola, A.O. 2014. Bioaccumulation of heavy metals in frequently consumed leafy vegetable grown along Nigeria-Benin Seme Border, West Africa *Advances in Applied Science Research*, **5(1):**1-7.

Ayotamuno, J.M.; Kogbara, R.B. and Taleat, M.O. 2006. Bioremediation of a petroleum-hydrocarbon polluted agricultural soil at different levels of water application in Port Harcourt, Nigeria. *Journal Food, Agriculture Environment.* **4(3&4)**: 214-217.

Bååth, E. 1989. Effects of heavy metals in soil on bacterial processes and populations (a review). *Water Air and Soil Pollution*, **47**:335-379.

Bååth, E.; Frostegård, Å.; Montserrat, D.R. and Tunlid, A. 1998a. Bacterial community-based measurements to estimate heavy metal effects in soil. The use of phospholipid fatty acid patterns and bacterial community tolerance. *Ambio*, **27**:58-61.

Bååth, E.; Diaz-Ravina, M.; Frostegård, A. and Campbell, C.D. 1998b. Effect of metal-rich sludge amendements on the soil bacterial community. *Applied Environmental Microbiology*, **64:**238-245.

Badawy, S.H. and EL-Motaium, R.A. 2003. Fate of some heavy metals in sandy soil amended with sewage sludge and their accumulation in plants. *Egypt Journal of Environmental Science*, **43 (1):**1-17.

Bahemuka, T.E. and Mubofu, E.B. 1999. Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania. *Food Chemistry*, **66**:63–66.

Baisberg-Pahlsson, A.M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water Air and Soil Pollution*, **47(3-4**):287-319.

Baker, A.J.M. and Brooks, R.R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery*, **1**:81-126.

Bakidere, S. and Yaman, M. 2008. Determination of Lead, Cadmium and Copper in Road-Side Soil and Plants in Elazig Turkey. *Environmental Monitoring and Assessment*, **136:**401-410.

Balba, M.T.; Al-Awadhi, N. and Al-Daher, R. 1998. Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. *Journal of Microbiological Methods*, **32**:155-164.

Bamforth, S.M. and Singleton, I. 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology*, **80**:723–736.

Barminas, J.T.; Charles, M. and Emmanuel, D. 1998. Mineral composition of non-conventional leafy vegetables. *Plant foods for Human Nutrition*, **53**:29-36.

Bates, R.G. 1954. *Electrometric pH determinations*. John Wiley and Sons, Inc. New York.

Benkova, M., 2005. Colloid-chemical interactions at organo-mineral amelioration of soils, contaminated with heavy metals. PhD thesis, NPISS, Supervisors Prof DSc T.Raychev and Assoc. Prof. Dr.N.Dinev.

Bento, F.M.; Camargo, F.A.; Okeke, B. and Frankenberger-Jr., W.T. 2003. Bioremediation of soil contaminated by diesel oil. *Brazilian Journal of Microbiology*, **34**:65-68.

Bento, F.M.; Camagro, F.A.; Okeke, B.C. and Frankenberger Jr, W.T. 2004. Diversity of biosurfactanct producing microorganisms isolated from soils contaminated with diesel oil. *Microbiological Research*, **160(3)**: 249-255

Bitala, M.F.; Kweyunga, C. and Manoko, M.L.K. 2009. Levels of heavy metals and cyanide in soil, sediment and water from the vicinity of North Mara Gold Mine in Tarime District , Tanzania. A report presented to CCT.

Blaylock, M.J.; Salt, D.E.; Dushenkov, S.; Zakharova, O.; Gussman, C.; Kapulnik, Y.; Ensley, B.D. and Raskin, I. 1997. Enhanced accumulation of lead in indian mustard by soil-applied chelating agents. *Environmental Science and Technology*, **31:**860-865.

Boricha, H. and Fulekar, M.H. 2009. *Pseudomonas plecoglossicida* as a novel organism for the bioremediation of cypermethrin. *Biology and Medicine*, **1(4):** 1-10.

Boricha, H. and Fulekar, M.H. 2010. Identification of *Owenweeksia honkongenesis* as a novel organism for the remediation of pesticide-Fenvalerate *Romanian Biotechnological Letters,* **15(2),** 5104-5110.

Brady, N.C. and Weil, R.R. 2008. *The Nature and Properties of Soils.* 14 ed. Pearson-Prentice Hall, Upper Saddle River, NJ. 990 pp. ISBN: 13-978-0-13-227938-3.

Brooks, R.R., ed. 1998. *Plants That Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration, and Phytomining.* Wallingford, UK: 380 pp.

Bruins, M.R.; Kapil, S. and Oehme, F.W. 2000. Bacterial resistance to metals in the environment. *Ecotoxicology and Environmental Safety*, **45:**198-207.

Byers, H.G. 1935. Selenium occurrence in certain soils in the United States, with a discussion of related topics. *US Department of Agriculture Technical Bulletin*, **482**:1-47.

Byrne, 1998. Aerosols exposed. *Chemistry in Britain*, **34(8):** 23 - 26.

Camargo, F.A.; Okeke, B.C.; Bento, F.M. and Frankenberger, W.T. 2003. In vitro reduction of hexavalent chromium by a cell-free extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}. *Applied Microbiology and Biotechnology*, **62(5-6):**569-573.

Capone, D.G.; Reese, D. and Kiene, R.P. 1983. Effects of metals on methanogenesis, sulfate reduction, carbon dioxide evolution and bacterial biomass in anoxic salt marsh sediments. *Applied Environmental Microbiology*, **45**:1586-1591.

Carlone, G.M.; Valadez, M.J. and Pickett, M.J. 1983. Methods for distinguishing Gram-Positive from Gram-Negative Bacteria. *Journal of Clinical Microbiology*, **16:**1157-1159.

Castelo-Grande T. and Barbosa D. 2003. Soil Decontamination By Supercritical Extraction. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **2(2):**331-336.

Chaney, R.L. 1983. Plant uptake of inorganic waste constitutes. *Land treatment of hazardous wastes*. Park Ridge, NJ, Noyes Data Corp, pp 5076.

Chaney, R.L.; Malik, M.; Li, Y.M.; Brown, S.L.; Brewer, E.P.; Angle, J.S. and Baker, A.J.M. 1997. Phytoremediation of soil metals. *Current Opinion in Biotechnology*, **8**:279-284.

Chang, J.O.; Law, R. and Chang, C.C. 1997. Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Water Research*, **31**:1651–1658.

Chen, Y.X.; Wang, Y.P.; Lin, Q. and Luo, Y.M. 2005. Effect of copper-tolerant rhizospherebacteria on mobility of copper in soil and copper accumulation by *Elsholtzia splendens. Environment International*, **31**:861-866.

Chibuike, G.U and Obiora, S.C. 2013. Bioremediation of hydrocarbon-polluted soils for improved crop performance *International Journal of Environmental Sciences*, **4(3):**223-239.

Chibuike, G. U. and Obiora, S. C. 2014. Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. *Applied and Environmental Soil Science*. http://dx.doi.org/10.1155/2014/752708.

Collins, Y.E. and Stotzky, G. 1989. *Factors affecting the toxicity of heavy metals to microbes.* In: Beveridge, T.J. and Doyle, R.J. (ed). Metal ions and bacteria, Wiley, Toronto, Canada, pp. 31-90.

Cozzolino, D. and Labandera, M. 2002. Determination of dry matter and crude protein contents of undried forages by near-infrared reflectance spectroscopy. *The Journal of the Science of Food and Agriculture*, **82**:380-384.

Cowan, S.T. and Steel, K.J. 1993. *Cowan and Steel's manual for identification of medical bacteria*. Cambridge University press, Canbridge, New York.

Cui, Y.L.; Zhu, Y.G.; Zhai, R.H.; Chen, D.Y.; Huang, Y.Z.; Qiu, Y. and Liang, J.Z. 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environmental International*, **30**: 785-791.

Cunningham, S.D.; Berti, W.R. and Huang, J.W. 1995. Phytoremediation of contaminated soils. *Trends in Biotechnology*, **13(9):**393-397.

Cunningham, S.D.; Shann, J.R.; Crowley, D.E. and Anderson, T.A. 1997. *Phytoremediation of contaminated water and soil*. In : Kruger, E.L., Anderson, T.A. and Coats, J.R. eds. Phytoremediation of soil and water contaminants. ACS symposium series , Washington, DC, American Chemical Society, 664, pp 2 – 19.

Curtis, L.R. and Smith B.W. 2008**.** *Heavy metals in fertilizers: Considerations for setting regulations in Oregon*. Department of Environmental and molecular Toxicology: Oregon State university Corvallis Oregon, http: www Oregon.gov/ODA/TEST/docs/pdf/ferthes yy met.pdf

Dadrasnia, A.; Shahsavari, N. and Emenike, C. U. 2013.Remediation of contaminated sites in: *Hydrocarbon*, a textbook by INTECH. Chapter 4, pp 65-82. http://dx.doi.org/10.5772/51591

Dahlin, S.; Witter, E.; Mårtensson, A.; Turner, A. and Bååth, E. 1997. Where´s the limit? Changes in the microbiological properties of agricultural soils at low levels of metal contamination. *Soil Biology and Biochemistry*, **29:**1405-1415.

Dalal, T. and Bairgi, P.1985. Effect of Hg, As and Pb on germination and seedling growth of two Jute varieties. *Environmental Ecology*, **3**:403-407.

Das A.K. 1990. *Metal ion induced toxicity anddetoxification by chelation therapy*: In a textbook medical aspect of bio-inorganic chemistry 1st ed. CBS Dellus, 17-58.

Das, N.; Vimala, R. and Karthika, P. 2008. Biosorption of heavy metals—An overview. *Indian Journal of Biotechnology*, **7**:159–169.

Davies, J.G. and Wilson, C.R. 2005. Choosing a Soil Amendment. Colorado State University Cooperative Extension Horticulture, **7**: 235.

De, J.; Ramaiah, N. and Vardanyan, L. 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. *Marine Biotechnology*, **10(4):**471-477.

Degraeve, N. 1981. Carcinogenic, teratogenic and mutagenic effects of cadmium. *Mutation Research*, **86**:115–135.

Diaz, E. 2004. Bacterial degradation of aromatic contaminants: a paradigm of metabolic versatility. *International Microbiology*, **7**:173–180.

Dinev, N. and Bojinova, P. 2006. Ecological investigations and agricultural status in the region of lead-zinc smelter near the town of Plovdiv. Proceedings of I-st International Symposium "Ecological approaches towards the production of safety food", 19-20 Oct. 2006, Assoc "Regional science and Technology Unions"-Plovdiv, Bulgaria, 121-126.

Dinev, N. and Vassilev, A. 2006. *Phytoremediation and sustainable use of metal contaminated soil in Bulgaria.* In: Processing of IMIDRA- Soil remediation technologies, November, Madrid (CD- copies).

Di Toppi, L.S.; Lambardi, M.; Pazzagli, L.; Cappugi, G.; Durante, M. and Gabbrielli, R.1999. Response to cadmium in carrot *in vitro* plants and cell suspension cultures. *Plant Science*, **137**:119–129.

Divrikli, U.; Horzum, N.; Soylak, M. and Elci, L. 2006. Trace heavy metal contents of some spices and herbal plants from western Anatolia, Turkey. *International Journal of Food Science and Technology*, **41**:712-716.

Dixit, R.; Wasiullah, Malaviya, D.; Pandiyan, K.; Singh, U.B.; Sahu,A.; Shukla, R., Singh, B.P.; Rai, J.P.; Sharma, P.K.; Lade, H. and Paul, D. 2015. Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. *Sustainability*, **7**:2189-2212.

Duda-Chodak, A. and Baszczyk, U. 2008. The impact of nickel on human health. *Journal of Elementolology*, **13**:685–696.

Dudka, S. and Adriano, D.C. 1997. Environmental impacts of metal ore mining and processing: A review. *Journal of Environmental Quality*, **26**:590–602.

Dudka, S. and Miller, W.P. 1999. Accumulation of potentially toxic elements in plants and their transfer to human food chain. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*, **34**:681–708.

Duruibe, J.O.; Ogwuegbu, M.O.C. and Egwurugwu, J.N. 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, **2(5):**112-118.

Eddy, N.O. 2004. Physicochemical parameter of water and heavy metal content of water,sediment and fishes from Qua Iboe River Estuary. M.Sc Thesis. Michael. Okpara University of Agriculture, Umudike. Nigeria

Elbagermi, M.A.; Edwards, H.G.M. and Alajtal, A.I. 2012. Monitoring of heavy metal content in fruits and vegetables collected from population and market sites in the Misurata area of Libya. *International Scholarly Research Network*, **10**:1-5.

Elings, A. 2000. Estimation of leaf area in tropical maize. *Agronomy Journal*, **92**:436 – 444.

El-Nady, F.E. and Atta, M.M. 1996. Toxicity and bioaccumulation of heavy metals to some marine biota from the Egyptian coastal waters. *Journal of Environmental Science and Health, Part A*, **31(7):**1529–1545.

Environment Canada, 1996. The state of Canada's environment. Ottawa: Public Works and Government Services Canada.

Erdogan, S.; Onal, Y.; Akmil-Basar. C.; Bilmez-Erdemoglu, S.C.; Sarici-ozdemir, C.; Koseoglu, E. and Leduygu, G. 2005. Optimization of nickel adsorption from aqueous solution by using activated carbon prepared from waste apricot by chemical activation. *Applied Surface Science*, **252**:1324-1331.

Ernst, W.H.O**.** 1998. *The origin and ecology of contaminated, stabilized and non-ristine soils.* In: Metal-contaminated soil. Vangronsveld, J. and Cunningham, S.D. (Eds). (Chapter 2). Springer, New York, pp. 17-29.

Etherington, J.R. 1982. Environment and Plant Ecology, 2nd ed. Chichester: John Wiley & Sons: 487pp.

Evanko, C.R. and Dzombak, D.A. 1997. *Remediation of Metals-Contaminated Soil and Groundwater.* GWRTAC. www.gwrtac.org.

Ezejiofor, T.I.N; Ezejiofor, A.N.; Udebuani, A.C.; Ezeji, E.U.; Ayalogbu, E.A.; Azuwuike, C.O.; Adjero, L.A.; Ihejirika, C.E.; Ujowundu, C.O.; Nwaogu, L.A. and Ngwogu, K.O. 2013. Environmental metals contaminants load of a densely populated and heavily industrialized commercial city of Aba, Nigeria. *Journal of Toxicology and Environmental Health Sciences,* **5(1)**:1-11.

Facciola, S.C. 1990. *A source book of edible plants*. Kampony Publications. pp. 10-15.

FAO/WHO, (2001). Food additives and contaminants. Joint Codex Alimentarius Commission. FAO/WHO Food Standards Programme, ALINORM 01/12A. 1-289.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**:783-791.

Federal Remediation Technologies Roundtable (FRTR) 2000, *In-Situ BiologicalTreatment.* Remediation technologies screening matrix and reference guide, Version 4.0. www.frtr.gov/matrix2/section4/4_1.html.2004/04/07

Fergusson, J.E. 1990. *The Heavy Elements*; Chemistry of Environmental Impact and Health Effects. Pergamon Press, England, pp. 20-35.

Ferhan, M.; Ahmed, Z.; Riazuddin, S.; Rajoka, M.I. and Khalid, A.M. 2002.

Estimation andremoval of phenol in pharmaceutical industrial effluents from paracetamol and aspirin manufacturing units. *OnLine Journal of Biological Sciences,* **2**:587–590.

Flathman, P.E. and Lanza, G.R.. 1998. Phytoremediation: current views on an emerging green technology. *Journal of Soil Contamination*, **7(4):**415-432.

Flores, R.; García, M.G.; Peralta-Hernández, J.M.; Hernández-Ramírez, A.; Méndez, E. and Bustos, E. 2012. Electro-remediation in the presence of ferrous sulfate as an *ex-situ* alternative treatment for hydrocarbon polluted soil, *International Journal of Electrochemical Science*, **7**:2230 - 2239.

Fliessbach, A.; Martens, R. and Reber, H.H. 1994. Soil bacterial biomass and bacterial activity in soil treated with heavy metal contaminated sewage sludge. *Soil Biology and Biochemistry*, **26**, 1201- 1205.

Fridberg, L.; Piscator, M.; Nordberg, G.F. and Kjellstroem, T. 1974. *Cadmium in the environment.* 2nd ed. Chemical Rubber Co. Press, Cleveland, Ohio. p:24.

Frostegård, Å.; Tunlid, A. and Bååth, E. 1993. Phospholipid fatty acid compostion, biomass, and activity of bacterial communities from two soil types experimentally exposed to different heavy metals. *Applied Environmental Microbiology*, **11**:3605-3617.

Fujimoto, C.K. and Sherman, G.D. 1945. The effect of drying, heating and wetting on the level of exchangeable manganese in Hawaiian soils. *Soil Science Society of America, Proceedings,* **10**:107–112.

Fulekar, M.H. 2005. Bioremediation techniques for environment. *Indian Journal of Environmental Protection,* **25** 358–364.

Gadd, G.M. 2000. Bioremedial potential of bacterial mechanisms of metal mobilization andimmobilization. *Current Opinion in Biotechnology*, **11**:271–279.

Garbisu, C. and Alkorta, I. 1997. Bioremediation: principles and future. *Journal of Clean Technology, Environmental Toxicology and Occupational Medicine,* **6**: 1-16.

George, P.M. 2003. *Encyclopedia of foods*. Humane Press, Washington D.C, 1: 526.

Gilis, A.; Khan, M.A.; Cornelis, P.; Meyer, J.M.; Mergeay, M. and van der Lelie, D.

1996. Siderophore-mediated iron uptake in *Alcaligenes eutrophus* CH34 and identification of *ale*B encoding ferric-alcaligin E receptor. *Journal Bacteriology*, **178 (18):**5499-5507.

Gomes, H.I.; Dias-Ferreira, C. and Ribeiro, A.B. 2012. Electrokinetic remediation of organochlorines in soil: Enhancement techniques and integration with other remediation technologies, *Chemosphere*, **87:**1077-1090.

Gqaza, M. B.; Njume, C.; Goduka, I. N. and Grace, G. 2013. The proximate composition of *S. nigrum* plant-leaves consumed in the Eastern Cape Province of South Africa. *International Conference on Nutrition and Food Sciences.* DOI:10.7763/IPCBEE

Greenwood, N.N. and Earnshaw, A. 1993. *Chemistry of the Elements*. Pergamon Press, New York. pp. 404-405.

Griffiths, R.A. 1995. Soil-washing technology and practice. *Journal of Hazardous Materials*, **40**:175–189.

Guha, H.; Jayachandran, K; and Maurrasse, F. 2001. Kinetics of chromium (VI) reduction by a type strain *Shewanella alga* under different growth conditions. *Environmental Pollution*, **115**:209–218.

Haavisto, T. 2002. Contaminated sites in Finland – Overview 2001. The Finnish environment series, Helsinki, Finland (in press).

Haghiri, F. 1973. Cadmium uptake by plants. *Journal of Environmental Quality*, **2**: 93-96.

Halebian, S.; Harris, B.; Finegold, S.M. and Rolfe, R.D. 1981. Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. *Journal of Clinical Microbiology*, **13(3):**444–448.

Hall, B.G. 2013. Building Phylogenetic Trees from Molecular Data with MEGA. *Molecular Biology and Evolution (mbe.oxfordjournals.org),* **30 (5):** 1229-1235.

Harder, E. 2004. *Bioremediation of Engine oil*. Little Flower Academy. Dallas, Texas.

Harmsen, J.; Rulkens, W.H.; Sims, R.C.; Rijtema, P.E. and Zweers, A. 2007. Theory and application of landfarming to remediate polycyclic aromatic hydrocarbons and mineral oil contaminated sediments: beneficial reuse. *Journal of Environmental Quality*, **36***:*1112–1122.

Hartman W.J. (Jr). 1975. An evaluation of land treatment of municipal wastewater and

physical siting of facility installations. Washington DC; US Department of Army.

Hassen, A.; Saidi, N.; Cherif, M. and Boudabous, A. 1998. Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresource Technology*, **65**:73– 82.

Hayat, I.; Ahmad, A.; Ahmed, A.; Khalil, S. and Gulfraz, M. 2014. Exploring the potential of red kidney beans (*Phaseolus vulgaris* L.) to develop protein based product for food applications. *Journal of Animal and Plant Sciences*, **24(3)**:860-868.

Held, T. and Dörr, H. 2000 *In Situ* Remediation, *Biotechnology*, **11**, 350-370.

Hoff, R.Z. 1993. Bioremediation: an overview of its development and use for oil spill cleanup. *Marine Pollution Bulletin*, **26(9)**:476-481.

Höfte, M.; Dong, Q.; Kourambos, S.; Krishnapillai, V.; Sherratt, D.and Mergeay, M. 1994. The *sss* gene product, which affects pyoverdin production in *Pseudomonas aeruginosa* 7NSK2, is a site-specific recombinase. *Molecular Microbiology*, **14**:1011-1020.

Holland, M.D.; Allen, R.K.G.; Barten, D. and Murphy, S.T. 1989. Land Evaluation and Agricultural for Cross River National Park, Oban Division. Prepared by the Overseas Development National Resources Institute in Collaboration with WWF for the Federal Republic of Nigeria and the Cross River State government, 1189.

Holt, G.J., Krieg, N.R., Sneath, P.H., Staley, J.T. and William, S.T. 2000. *Bergey's manual of determinative bacteriology* (9th Edition). Lippincott Williams & Wilkins, 530 Walnut St, Philadelphia, PA 19106 USA.

Hough, R.L.; Young, S.D. and Crout, N.M.G. 2003. Modeling of Cd, Cu, Ni, Pb and Zn uptake by winter wheat and forage maize, from a sewage disposal form. *Soil Use and Management*, **19**:19-27.

Huang, D.; Xu, Q.; Cheng, J.; Lu, X. and Zhang, H. 2012. Electrokinetic remediation and its combined technologies for removal of organic pollutants from contaminated soils. *International Journal of Electrochemical Science*, **7**:4528 - 4544.

Hussein, H.; Krull, R.; Abou El-Ela, S.I. and Hempel, D.C. 2001. *Interaction of the*

different heavy metal ions with immobilized bacterial culture degrading xenobiotic wastewater compounds. In Proceedings of the Second International Water Association World Water Conference, Berlin, Germany, 15–19 October; pp. 15–19.

Hutchinson, S.L.; Schwab, A.P. and Banks, M.K. 2003. *Biodegradation of petroleum hydrocarbons in the rhizosphere.* In: McCutcheon, S.C. and Schnoor, J.L. (Eds.) Phytoremediation: transformation and control of contaminants. Wiley-Interscience Inc., New Jersey. pp. 355-386.

Ikeda, M.; Zhang, Z.W.; Shimbo, S.; Watanabe, T.; Nakatsuka, H.; Moon, C.S.; Matsuda-Inoguchi, N. and Higashikawa, K., 2000. Urban population exposure to lead and cadmium in east and south-east Asia. *Science of the Total Environment*, **249**: 373-384.

Iqbal, M.A.; Chaudhary, M.N.; Zaib, Z.; Imran, M.; Ali, K. and Iqbal A. 2011. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb) in agricultural soils and spring seasonal plants, irrigated by industrial waste water. *Journal of Environmental Technology and Management*, **2(1)**: 1554-2010.

Iram, S; Ahmad, I. and Stuben, D. 2009. Analysis of mines and contaminated agricultural soil samples for fungal diversity and tolerance to heavy metals. *Pakistan Journal of Botany*, **41(2):** 885-895.

Ishida, H.; Suzuno, H.; Sugiyama, N.; Innami, S.; Todokoro, T. and Maekawa, A. 2000. Nutritional evaluation of chemical component of leaves stalks and stems of sweet potatoes (*Ipomea batatas* poir). *Food Chemistry*, *68:359*-367.

Iwamoto, T. and Nasu, M. 2001. Current bioremediation practice and perspective. *Journal of Bioscience and Bioengineering,* 92**(1):**1-8.

Iyengar, V. and Nair, P. 2000. Global outlook on nutrition and the environment: meeting the challenges of the next millennium. *Science of the Total Environment*, **249**:331-346.

Japenga, J.; Bonten, L.; Kucharski, R.; Novosielska, O. and Dinev, N. 2005. Revegetation as a tool for risk containment of heavy metal polluted sites. Third International Phytotechnologies Conference. April 20-22, 2005 Atlanta, GA.

Jarup, L. 2003. Hazards of heavy metals contamination. *British Medical Bulletin*, **68**:167-182.

Ji, G. and Silver, S. 1995. Bacterial resistance mechanisms for heavy metals of

environmental concern. *Journal of Industrial Microbiology,* **14**:61-75.

Kabata-Pendias, A. and Mukherjee, A.B. 2007. Trace elements from soil to human. New York: Springer-Verlag.

Kabir, M.; Iqbal, M.Z. and Shafiq, M. 2009. Effects of Lead on Seedling Growth of *Thespesia Populnea* l. *Advances in Environmental Biology,* **3(2):** 184-190.

Kachenko, A.G. and Singh, B. 2006. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. *Water, Air and Soil Pollution,* **169**:101-123.

Kahle, H. 1993. Response of roots of trees to heavy metals. *Environmental and Experimental Botany,* **33(1):**99-119.

Kashem, M.A. and Singh, B.R. 2004. Transformation in solid phase species of metals as affected by flooding and organic matter additions in contaminated soils. *Communications in Soil Science and Plant Analysis*, **35**:1435-1456.

Kaushik, P., Garg, V.K. and Singh, B. 2005. Effect of textile wastewaters on growth performance of wheat cultivars. *Bioresource Technology*, **96:**1189–1193.

Khan, D.H. and Frankland, B. 1983. Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil profile and plant. *Plant and Soil,* **70**:335-345.

Khan, M. and Scullion, J. 2000. Effect of soil on bacterial responses to metal contamination. *Environmental Pollution*, **110**:115-125.

Khan, M.A.; Ahmad, I. and Rahman, I. 2007. Effect of environmental pollution on heavy metals content of *Withania somnifera. Journal of the Chinese Chemical Society*, **54**:339–343.

Khan, M.S.; Shil, N.C. and Noor, S. 2008. Integrated nutrient management for sustainable yield of major vegetable crops in Bangladesh. *Bangladesh Journal of Agriculture and Environment*, **4**:81-94.

Kilicel, F. 1999. Investigation of toxic heavy metals pollution in road dust at the center of Van Turkey. *Bulletin of Pure and Applied Science,* **18**:1-4.

Kisku, G.C., Barman, S.C. and Bhargava, S.K. 2000. Contamination of Soil and Plants with Potentially Toxic Elements Irrigated with Mixed Industrial Wastewater and its Impact on the Environment. *Water, Air and Soil Pollution*, **120**:121–137.

Kitur, B.K. and Frye, W.W. 1983. Effects of heating on soil chemical properties and

growth and nutrient composition of corn and millet. *Soil Science Society of America Journal*, **47**:91-94.

Kkairiah, J.; Zalifah, M.K.; in, Y.H. and Aminah, A. 2004. The uptake of heavy metals by fruit type vegetables grown in selected agricultural areas. *Pakistan Journal of Biological Sciences*, **7**:1438-1442.

Knox, A.S.; Seamans, J.C.; Mench, M.J. and Vangronseveld, J. 2000. *Remediation of metals and Radionuclides*. Contaminated soil using in situ stabilization Techniques. Macmillan Publishers, New York. pp. 21-26.

Konopka, A.; Zakharova, T.; Bischoff, M.; Oliver, L.; Nakatsu, C. and Turco, R.F. 1999. Bacterial biomass and activity in lead-contaminated soil. *Applied Environmental Microbiology*, **65**:2256-2259.

Kozlowski, C.A. and Walkowrak, W. 2002. Removal of Cr^{6+} from aqueous solutions by polymer inclusion membranes. *Water Research*, **36**: 4870-4876.

Leahy, J.G. and Colwell, R.R. 1990. Bacterial Degradation of hydrocarbons in the environment. *Bacterial reviews*, **53(3):** 305-351.

Le Faucheur, S.; Schildknecht, F.; Behra, R. and Sigg, L. 2006. Thiols in Scenedesmus vacuolatus upon exposure to metals and metalloids. *Aquatic Toxicology*, **80**:355–61.

Lee, K. 1999. *Bioremediation of Oil Impacted Shorelines.* In: Murphy, T.and Munawar, M. (Eds.), Aquatic Restoration in Canada. Ecovision World Monograph Series, Backhuys Publishers, Leiden, Netherlands. pp. 69-85.

Lim, P.E.; Mak, K.Y.; Mohamed, N. and Noor, A.M. 2003. Removal and speciation of heavy metals along the treatment path of wastewater in subsurface-flow constructed wetlands. *Water Science and Technology*, **48**:307–313.

Lin, C.C. and Lin, H.L. 2005. Remediation of soil contaminated with the heavy metal (Cd^{2+}). *Journal of Hazardous Materials*, **122**:7–15.

Liu, W.H.; Zhao, J.Z.; Ouyang, Z.Y.; Soderlund, L. and Liu, G.H. 2005. Impacts of sewage irrigation on heavy metals distribution and contamination in Beijing, China. *Environment International*, **31**: 805-812.

Lovley, D.R. 1993. Dissimilatory metal reduction. *Annual Review of Microbiology*, **47**: 263-290.

Lovley, D.R. 1994. Bacterial reduction of iron, manganese, and other metals. *Advances in Agronomy*, **54**:175–231.

Maciel, B.M.; Santos, A.C.F.; Dias, J.C.T.; Vidal, R.O.; Dias, R.J.C., Gross, E.; Cascardo, J.C.M. and Rezende, R.P. 2009. Simple DNA extraction protocol for a 16S rRNA study of bacterial diversity in tropical landfarm soil used for bioremediation of oil waste, *Genetics and Molecular Research,*, **8**:375–388.

Magyarosy, A.; Laidlaw, R.D.; Kilaas, R.; Echer, C.; Clark, D.S. and Keasling, J.D. 2002. Nickel accumulation and nickel oxalate precipitation by *Aspergillus niger*. *Applied Microbiology and Biotechnology*, **59**:382-388.

Malik, A. 2004. Metal bioremediation through growing cells. *Environment International*, **30**:261–278.

Maliszewska, W.; Dec, S.; Wierzbicka, H. and Wozniakowska, A. 1985. The influence of various heavy metal compounds on the development and activity of soil microorganisms. *Environmental Pollution (Series A)*, **37**:195-215.

Marshall, 2004. Enhancing food chain integrity: Quality assurance mechanism for air pollution impacts on fruits and vegetables systems. Crop Post Harvest Program, Final Technical Report (R7530).

Masarirambi, M.T.; Sibandze, N.; Wahome, P.K. and Oseni, T.O. 2012. Effects of Kraal manure application rates on growth and yield of wild okra (*Corchorus olitorius* L.) in a sub-tropical environment. *Asian Journal of Agricultural Science*, **4(1)**: 89-95.

Massachusetts Department of Environmental Protection Publication (MADEP) 1993. 310 CMR 40.0000: Massachusetts Contingency Plan (MCP). Boston.

Mbah, C.N.; Nwite, J.N. and Nweke, I.A. 2009. Amelioration of Spent Oil Contaminated Ultisol with Organic Wastes and its Effect on Soil Properties and Maize (Zea mays L) Yield. *World Journal of Agricultural Sciences*, **5(2)**: 163-168.

McBride, M.B. 2003. Toxic metals in sewage sludge-amended soils: Has proportion of beneficial use discounted the risks. *Advances in Environmental Research*, **8**:5-19.

McLaughlin, M.J. and Singh, B.R. 1999. *Cadmium in soil and plants: a global perspective.* In: 'Cadmium in soils and plants'. (Eds McLaughlin, M.J. and Singh, B.R.). (The Netherlands: Kluwer Academic Publishing), pp. 13-21.

Mclean, E.O. 1965. *Aluminium*. In C.A. Black (ed), Methods of Soil Analysis. Part 2, pp 986-994.

McLean, J.E. and Bledsoe, B.E. 1992. *Behavior of Metals in Soils*. EPA Ground Water Issue, EPA 540-S-92-018: 25.

Medjor, O.W.; Egharevba, F.; Akpoveta, O.V.; Ize-lyamu, O.K. and Jatto, E.O. 2012. Kinetic Studies of Bioremediation of Hydrocarbon Contaminated Groundwater. *Research Journal of Chemical Sciences,* **2(1):** 38-44.

Meena, A.K.; Mishra, G.K.; Raj, P.K.; Rajgoapal, C. and Nagar, P.N. 2005. Removal of heavy metal ions from aqueous solutions using carbon aerogel as an adsorbent. *Journal of Harzadous Materials,* **122**:161-170.

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH4. North Carolina Soil Test Division.

Miller, R.R. 1996. *Phytoremediation*. Ground-Water Remediation Technologies Analysis Center (GWRTAC)-Technology Overview Report TO-96-03, GWRTAC-O-Series, p. 11.

Mueller, J.G.; Devereux, R.; Santavy, D.L.; Lantz, S.E.; Willis, S.G. and Pritchard, P.H. 1997. Phylogenetic and physiological comparisons of PAH-degrading bacteria from physiologically diverse soils. *Antonie Leeuwenhoek* **71**:329–343.

Muhammad, F.; Farooq, A. and Umar, R. 2008. Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrialarea. *Pakistan Journal of Botany*, **40(5)**:2099-2106.

Mushtaq, N. and Khan, K.S. 2010. Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi Region. *Pakistan Journal of Agricultural Sciences*, **47(3):**215-224.

Nanda, S. and Abraham, J. 2013. Remediation of heavy metal contaminated soil. *African Journal of Biotechnology*, **12(21)**:3099-3109.

Natural and Accelerated Bioremediation Research (NABIR) Program, Office of Biological and Environmental Research, Office of Science, U.S. Department of Energy. 2003. *What is bioremediation*. pp. 9.

Narasimhulu, K.; Rao, P.S. and Vinod, A.V. 2010. Isolation and identification of bacterials strains and study of their resistance to heavy metals and antibiotics. *Journal of Bacterial and Biochemical Technology*, **2**:074-076.

National Research Council (NRC) 1993. *In situ Bioremediation. When does it work?* National Academy Press. Washington DC, pg. 13.

Navari-Izzo, F. and Quartacci, M.F. 2001. Phytoremediation of metals. *Minerva*

Biotechnologica, **13**:73-83.

Navarro, M.C.; Pérez-Sirvent, C.; Martínez-Sánchez, M.J.; Vidal, J.; Tovar, P.J. and Bech, J. 2008. Abandoned mine sites as a source of contamination by heavy metals: A case study in a semi-arid zone. *Journal of Geochemical Exploration*, **96(2–3):**183–193.

NAVFAC 2000. TechData Sheet: A Demonstration of Electrokinetic Remediation, TDS-2084-ENV, 2 pp.

Ndlovu, J. and Afolayan, A.J. 2008. Nutritional analysis of South African wild vegetable *Corchorus olitorius* L. *Asian Journal of Plant Sciences*, **7(6)**:615-618.

Nene, Y. L. 1999. Utilizing traditional knowledge in agriculture. In *Traditional Knowledge system of India and Sri Lanka*, pp. 32– 38.

Nicholson, F.A.; Smith, S.R.; Alloway, B.J.; Carlton-Smith, C. and Chambers. B.J. 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. *The Science of the Total Environment*, **311**:205–219.

Nicoli, M.C.; Anese, M. and Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science and Technology*, **10**:94-100.

Nies, D.H. and Silver, S. 1995. Ion efflux systems involved in bacterial metal resistances. *Journal of Industrial Microbiology*, **14:**186-199.

Nies, D.H. 1999. Bacterial heavy-metal resistance. *Applied Microbiology and Biotechnology*, **51**:730-750.

Njoku, K.L., Oboh, B.O.; Akinola, M.O. and Ajasa, A.O. 2012. Comparative Effects of *Abelmoschus esculentus* (L) *Moench* (Okro) and *Corchorus olitorius* L (Jew Mallow) on Soil Contaminated with Mixture of Petroleum Products. *Research Journal of Environmental and Earth Sciences*, **4(4):**413-418.

Nkereuwem, M. E.; Edem, I. D. and O. Fagbola. 2010. Bioremediation of oil-polluted soils with organomineral fertilizer (OMF) and Mexican sunflower (*Tithonia diversifolia*). *Nigerian Journal of Agriculture, Food and Environment*, **6(1&2):**13-20.

Nkomo, M. and Kambizi, L. 2009. Effects of pre-chilling and temperature on seed germination of *Corchorus olitorius* L. (Tiliaceae) (Jew's Mallow), a wild leafy vegetable. *African Journal of Biotechnology*, **8(6):**1078-1081.

Nwadinigwe, A.O. and Onyeidu, E.G. 2012. Bioremediation of crude oil-polluted soil using bacteria and poultry manure monitored through soybean productivity. *Polish Journal of Environmental Studies,* **21(1):** 171-176.

Obire, O. and Akinde, S.B. 2006. Comparative study of the efficiency of Cattle dung and poultry manure as nutrient alternative sources in bioremediation of oil polluted soil. *Niger Delta Biologia,* **5(2)**:82-91.

Obire, O.; Anyanwu, E.C and Okigbo, R.N. 2008. Saprophytic and crude oil degrading fungi from Cattle dung and poultry droppings as bioremediating agents. *Journal of Agricultural Technology,* **4(2)**: 81-89.

Oboh, G.; Radatz, H. and Henle, T. 2009. Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of Jute (*Corchorus olitorius*) leaf. *International Journal of Food Sciences and Nutrition,* **60**:124-134.

Odoh, R. and Kolawole, S.A. 2011. Assessment of trace heavy metal contaminations of some selected vegetables irrigated with water from River Benue within Makurdi Metropolis, Benue State Nigeria. *Advances in Applied Science Research,* **2(5):**590-601.

Ogunrinde, A.T. and Fasinmirin, J.T. 2011. Soil moisture distribution pattern and yield of jute mallow (*Corchorus olitorius*) under three different soil fertility management. *Proceedings of the Environmental Management Conference*, Federal University of Agriculture, Abeokuta, Nigeria. pp373-381.

Oke, J.M. and Hamburger, M.O. 2002. Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2- diphenyl- picryl- hydrazyl radical, *African Journal of Biomedical Research,* **5**:77-79.

Okolo, J.C.; Amadi, E.N. and Odu, C.T.I. 2005. Effects of soil treatments containing poultry manure on crude oil degradation in sandy loam soil. *Applied Ecology and Environmental Research,* **3(1)**: 47-53.

Okparanma, R.N.; Ayotamuno, J.M. and Araka, P.P. 2009. Bioremediation of hydrocarbon contaminated-oil field drill-cuttings with bacterial isolates. *African Journal of Environmental Science and Technology,* **3(5):**131-140.

Oladiran, J.A. 1986. Effect of stage of harvesting and seed treatment on germination, seedling emergence and growth in *Corchorus olitorius* 'Oniyaya'. *Scientia Horticulturae,* **28**:227-233.

Oliver, M.A. 1997. Soil and human health: A review. *European Journal of Soil*

Science, **48**:573–592.

Olutiola, P.O.; Famurewa, O. and Sonntag, H.G. 2000. *An introduction to general microbiology*: A practical approach, Hygiene Institute Der Universitat Heldelberg. pp 157-180.

Onwordi, C.T.; Ogungbade; A.M. and Wusu, A.D. 2009. The proximate and mineral composition of three leafy vegetables commonly consumed in Lagos, *Nigeria African Journal of Pure and Applied Chemistry,* **3(6):**102-107.

Orji, F.A.; Abiye, A.I. and Dike, E.N. 2012. Laboratory scale bioremediation of petroleum hydrocarbon polluted mangrove swamps in the Niger delta using Cattle dung. *Malaysian Journal of Microbiology***, 8(4):** 219-228.

Oyedele, D.J.; Asonugho, C.and Awotoye, O.O. 2006. Heavy metals in soil and accumulation by edible vegetables after phosphate fertilizer application. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **5(4):**1446-1453.

Padmavathiamma, P.K. and Li, L.Y. 2007. Phytoremediation technology: Hyperaccumulation metals inplants. *Water, Air and Soil Pollution*, **184**:105–126.

Page, A.L. (eds) 1982. *Methods of soil Analysis Agronomy*. American Society of Agronomy and Soil Science, Madison, Wisconsin. No. 9. Part 2.

Palada, M.C. and Chang, L.C. 2003. Suggested Cultural Practices for Jute Mallow. *Asian Vegetable Research & Development Council Centre (AVRDC).*

Pandey, B and Fulekar, M.H. 2012. Bioremediation technology: A new horizon for environmental clean-up *Biology and Medicine,* **4(1)**: 51-59.

Parizanganeh, A.H.; Bijnavand, V., Zamani, A.A. and Hajabolfath, A. 2012. Concentration, Distribution and Comparison of Total and Bioavailable Heavy Metals in Top Soils of Bonab District in Zanjan Province. *Open Journal of Soil Science*, **2**:123-132.

Pavel, L.V and Gavrilescu, M. 2008. Overview of ex situ decontamination techniques forsoil cleanup. *Environmental Engineering and Management*, **7**:815-834.

Pensaert, S. 2008. Immobilisation, stabilisation, solidification a new approach for the treatment of contaminated soils. Case studies: London Olympics & Total Ertvelde, 15th Innovatieforum Geotechniek, 1-14.

Perarlta-Videa, J.R.; Gardea-Torresdey, J.I.; Gomez, E.; Tiemann, K.J.; Parsons, J.G.

and Carrillo, G. 2002. Effect of mixed cadmium, copper, nickel and zine at different PH upon alfalla growth and heavy metal uptake. *Environmental Pollution*, **119**:291-302.

Picardo, M.C.; Ferreira, A.C. and Da Costa, A.C. 2009. Continuous thorium biosorption-dynamic study for critical bed depth determination in a fixed-bed reactor. *Bioresource Technology*, **100**:208-210.

Pinto, A.P.; Mota, A.M.; de Varennes, A. and Pinto, F.C. 2004. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Science of the Total Environment*, **326**:239-247.

Prince, R.C.; Varadaraj, R.; Fiocco, R.J. and Lessard. R.R. 1999. Bioremediation as Oil Spill Response Tool. *Environmental Technology*, **20(8)**:891-897.

Pruvot, C.; Douay, F.; Herve, F. and Waterlot, C. 2006. Heavy metals in soil, crops and grass as a source of human exposure in the former mining areas. *Journal of Soils and Sediments*, **6**:215–220.

Qiagen, 2003. QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook.

Radwan, M.A. and Salama, A.K. 2006. Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chemical Toxicology*, **44**:1273-1278.

Raghavan R.; Dietz D.H. and Coles E. 1989. *Cleaning Excavated Soil Using Extraction Agents: A State-of the-Art Review*, EPA Report EPA 600/2-89/034. (Environmental Lab), Technical Report EL-88-24.

Rai, V.; Khatoon, S.; Bisht, S.S. and Mehrotra, S. 2005. Effect of cadmium on growth, ultramorphology of leaf and secondary metabolites of *Phyllanthus amarus* Schum and Thonn. *Chemosphere*, **61**:1644–1650.

Rajendran, P.; Muthukrishman, J. and Gunasekaran, P. 2003. Microbes in heavy metal remediation. *Indian Journal of Experimental Biology*, **41**:935-944.

Randhawa, G.R. and Kullar, J.S. 2011. Bioremediation of pharmaceuticals, pesticides, and petrochemicals with gomeya/Cattle dung. *International Scholarly Research Network. ISRN Pharmacology d*oi:10.5402/2011/362459

Rascio, W. 1977. Metal accumulation by some plants growing on Zn mine deposits. *Oikos*, **29**:250-253.

Raskin, I.; Kumar, P.B.A.N.; Dushenkov, S. and Salt, D.E. 1994. Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, **5:**285-290.

Rattan, R.K.; Datta, S.P.; Chhonkar, P.K.; Suribabu, K. and Singh, A.K., 2005. Long-

term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. *Agriculture. Ecosystem and Environment*, **109**:310-322.

Rensing, C.; Ghosh, M. and Rosen, B. 1999. Families of soft-metal-ion-transporting ATPases. *Journal of Bacteriology*, **181**:5891-5897.

Revathi, K.; Haribabu, T.E. and Sudha, P.N. 2011. Phytoremediation of Chromium contaminated soil using Sorghum plant. *International Journal of Environmental Sciences*, **2(2)**: 417-428

Rieuwerts, J.S.; Ashnore, M.R.; Farago, M.E. and Thornton, I. 2006. The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils. *Science of the Total Environment*, **366**:864-875.

Roane, T.M. and Kellogg, S.T. 1996. Characterization of bacterial communities in heavy metal contaminated soils. *Canadian Journal of Microbiology*, **42**:593-603.

Roane, T.M.; Josephson, K.L. and Pepper, I.L. 2001. Dual-bioaugmentation strategy to enhance remediation of cocontaminated soil. *Applied Environment Microbiology*, **67**:3208-3215.

Ross, S. 1994. Toxic metals in Soil-Plant Systems. John Wiley & Sons, Chichester, UK.

Ryan, J.A.; Zhang, P.; Chou, J. and Sayers, D.E. 2001. Formation of chloropyromorphite in a lead-contaminated soil amended with hydroxyapatite. *Environmental Pollution*, **50**:493–500.

Saeed, G. and Rafique, M. 1980. Government of Pakistan, Ministry of Food and Agriculture, Soil survey of Pakistan, Lahore. *Technical guide for the chemical analysis of soil and water*, Bulletin No. 14.

Said, W.A. and Lewis, D.L. 1991. Quantative assessment of the effects of metals on bacterial degradation of organic chemicals. *Applied Environmental Microbiology*, **57:**1498-1503.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**:406-425.

Salem, H.M.; Eweida, E.A.; Farag, A. 2000. Heavy metals in drinking water and their

environmental impact on human health. In *ICEHM 2000*; Cairo University: Gaza, Egypt, pp. 542–556.

Salt, D.E.; Smith, R.D. and Raskin, I. 1998. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**:643-668.

Sanità di Toppi, L. and Gabbrielli, R. 1999. Response to cadmium in higher plants. *Environmental and Experimental Botany*, **41(2):**105-130.

Sanscartier, D.; Reimer, K.; Zeeb, B. and George, K. 2010. Management of hydrocarbon-soil through bioremediation and landfill disposal at a remote location in Northern Canada. *Canadian Journal of Civil Engineering*, **37**:147–155.

Sanyaolu, V.T.; Sanyaolu, A.A.A. and Fadele, E. 2011. Spatial variation in Heavy Metal residue in *Corchorus olitorious* cultivated along a Major highway in Ikorodu- Lagos, Nigeria. *Journal of Applied Sciences and Environmental Management*, **15(2):**283-287.

Sarita, S.; Rohit, S. and Shraddha, S. 2002. Comparative studies of accumulation of Cr from metal solution and tannery effluent under repeated metal exposure by aquatic plants: its toxic effects. *Environment Monitoring and Assessment*, **80(1):**17-31.

Sarkar, A. and Jana, S. 1986. Heavy metal pollution tolerance. *Water, Air and Soil Pollution*, **27**:15-18.

Sathawara, N.G.; Parikish, D.J. and Agrwal, Y.K. 2004. Essentials heavy metals in environmental samples from western Indian. *Bulletin of Environmental Contamination and Toxicology*, **73**: 756-761.

Sawalha, M.F.; Peralta-Videa, J.R.; Duarte-Gardea, M and Gardea-Torresdey, J.L. 2008. Removal of copper, lead, and zinc from contaminated water by saltbush biomass: Analysis of the optimum binding, stripping, and binding mechanism. *Bioresource Technology*, **99**:4438–4444.

Scebba, F.; Arduini, I.; Ercoli, L. and Sebastiani, L. 2006. Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. *Biologia Plantarum*, **50**:688-692.

Schippers, R.; Maundu, P.; Imbuni, M. and Obiero, H. 2002a. *How to grow and use Jew's Mallow*. Horticultural Development Services, Baarn, The Netherlands. pp. 10.

Scott, J.A. and Karanjkar, A.M. 1992. Repeated cadmium biosorption by regenerated *Enterobacteraerogenes* biofilm attached to activated carbon. *Biotechnology Letters*, **14**:737–740.

Scott, D.; Keoghan, J.M. and Allen, B.E. 1996. Nature and low input grasses –a New Zealand high country perspective. New Zealand. *Journal of Agriculture Research,* **3**:499-512.

Shah, K. and Dubey, R.S. 1997. Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings: role of proline as a possible enzyme protectant. *Biologia Plantarum*, **40(1):**121-130.

Sharma, P. and Dubey, R.S. 2005. Lead toxicity in plants. *Brazilian Journal of Plant Physiology*, **17**:35-52.

Sharma, D.C.; Sharma, C.P. and Tripathi, R.D. 2003. Phytotoxic lesions of chromium in maize. *Chemosphere*, **51**:63–68.

Sharma, R.K.; Agrawal, M. and Marshall, F. 2006. Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. *Bulletin of Environmental Contamination and Toxicology*, **77**:312-318.

Sharma, R. K.; Agrawal, M.; Marshal, F. 2007. Heavy metal contamination in soil and vegetables in surburban arears of Varanasi, India. *Ecotoxicology and Environmental Safety*, **66**:258-266.

Sharma, R.K.; Agrawal, M. and Marshall, F.M. 2008a. Atmospheric deposition of heavy metals (Cu, Zn, Cd and Pb) in Varanasi City, India. *Environmental Monitoring and Assessment*, **142**:269-278.

Sharma, R.K.; Agrawal, M. and Marshall, F.M. 2008b. Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. *Environmental Pollution*, **154**:254-263.

Shawa, L.J.; Beatonb, Y.; Glover, L.A.; Killham, K. and Meharga, A.A. 1999. Re-inoculation of autoclaved soil as a non-sterile treatment for xenobiotic sorption and biodegradation studies. *Applied Soil Ecology*, **11:**217-226.

Shittu, T.A. and Ogunmoyela, O.A. 2001. Water blanching treatment and nutrient retention in some Nigerian green leafy vegetables. *Proceedings*, **25**:20.

Shuaibu, K.; Yahaya, M. and Abdullahi, U.K. 2013. Heavy metal levels in selected

green leafy vegetables obtained from Katsina central market, Katsina, Northwestern Nigeria. *African Journal of Pure and Applied Chemistry*, **7(5)**:179-183.

Shukla, O.P.; Dubey, S. and Rai, U.N. 2007. Preferential Accumulation of Cadmium and Chromium: Toxicity in *Bacopa monnieri* L. under Mixed Metal Treatments. *Bulletin of Environmental Contamination and Toxicology*, **78**:252–257.

Singh, S. and Kumar, M. 2006. Heavy metal load of soil, water and vegetables in peri-urban Delhi. *Environmental Monitoring and Assessment*, **120**:79-91.

Singh, R.P.; Tripathi, R.D.; Sinha, S.K.; Maheshwari, R.and Srivastava, H.S. 1997. Response of higher plants to lead contaminated environment, *Chemosphere*, **34**: 2467-2493.

Singh, K.P.; Mohon, D.; Sinha, S. And Dalwani, R. 2004. Impact assessment of treated /untreated waste water toxicants discharge by sewage treatment plants on health, agricultural and environmental quality in waste water disposal area. *Chemosphere*, **55**:227-255.

Sinha, S.; Pandey, K.; Gupta, A.K. and Bhatt, K. 2005. Accumulation of metals in vegetables and crops grown in the area irrigated with river water. *Bulletin of Environmental Contamination and Toxicology*, **74**:210-218.

Skipper, H.D. and Westermann, D.T. 1973. Comparative effects of propylene oxide, sodium azide and autoclaving on selected soil properties. *Soil Biology and Biochemistry*, **5***:*409-414.

Soares, A.; Albergaria, J.; Domingues, V.; Alvim-Ferraz Mda, C. and Delerue-Matos, C. 2010. Remediation of soils combining soil vapour extraction and bioremediation: benzene. *Chemosphere*, **80**:823-828.

Sobukola, O.P.; Awonorin, S.O.; Idowu, M.A. and Bamiro, F.O. 2008. Chemical and physical hazard profile of 'robo' processing – a street vended melon snack. *International Journal of Food Science and Technology*, **43(2):**237-242.

Sobukola, O.P.; Adeniran, O.M.; Odedairo, A.A. and Kajihausa, O.E. 2010. Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of Food Science*, **4(2):**389-393.

Soil Science Society of America (SSSA) 1971. *Instrumental methods for analysis of*

soils and plant tissue. Soil Science Society of America Inc. Madison. Wis. USA. pp. 27-32.

Souza, T.S.; Hencklein, F.A.; Angelis, D.F.; Gonc¸alves, R.A. and Fontanetti, C.S. 2009. The *Allium cepa* bioassay to evaluate landfarming soil, before and after the addition of rice hulls to accelerate organic pollutants biodegradation. *Ecotoxicology and Environmental Safety*, **72:**1363–1368.

Stephens, W.E. and Calder, A. 2005. Source and health implications of high toxic metal concentrations in illicit tobacco products. *Environmental Science and Technology*, **39**:479–488.

Subramanian, M. and Shanks, J.V. 2003. *Role of plants in the transformation of explosives*. In: McCutcheon, S.C. and Schnoor, J.L. (Eds.), Phytoremediation: Transformation and control of contaminants. Wiley-Interscience Inc., New Jersy. pp. 389-408.

Sugiyama, M. 1994. Role of cellular antioxidants in metal-induced damage. *Cell Biology and Toxicology*, **10**:1-22.

Sukreeyapongse, O.; Panichsakspatana, S. And Hansen, H. 2002. Transfer of heavy metals from sludge amended soil to vegetables and leachates. Paper presented at the 17th World Congress of Soil Science (WCSS), 14th-21st August, 2002. Thailand. Symposium No. 29, Paper No. 1969.

Suruchi and Khanna, P. 2011. Assessment of Heavy Metal Contamination in Different Vegetables Grown in and Around Urban Areas. *Research Journal of Environmental Toxicology*, **5**:162-179.

Suthersan, S.S. 1999. Soil vapour extraction, CRC Press LLC, 1-65.

Swannelll, R.P. and Head, I.M. 1994. Bioremediation comes of age. *Nature*, **368**:396-397.

Swannelll, R.P.; Lee, K. and McDonagh. M. 1996. Field Evaluations of marine oil spill bioremediation. *Microbiology Reviews*, **60**:342-365.

Tamura, K.; Nei, M. and Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*), **101**:11030-11035.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, **30**: 2725-2729.

Tanaka, S.; Wada, K.; Iwasaki, K.; Sakurai, K.; Horie, M. and Iio, K. 2001. Effect of soil disinfestation treatments on soil physicochemical properties and microbes. *Japanese Journal of Soil Science and Plant Nutrition*, **72**:554–557.

Tandi, N.K.; Nyumangara, J. and Bangara, C. 2005. Environmental and potential health effects of growing leafy vegetables on soil irrigated using sewage sludge and effluent: A case of Zn and Cu, *Journal of Environmental Science and Health*, **39:**461-471.

Thompson, H.C. and Kelly, W.C. 1990. Vegetable Crops. 5th Edition. McGraw Hill Publishing Company Ltd., New Delhi.

Trapp, S. and Karlson, U. 2001. Aspects of phytoremediation of organic contaminants. *Journal of Soils and Sediments*, **1**:37–43.

Tripathi, R.D.; Vajpayee, P.; Singh, N.; Rai, U.N.; Kumar, A.; Ali, M.B.; Kumar, B. and Yunus, M. 2004. Efficacy of various amendments for amelioration of fly-ash toxicity: growth performance and metal composition of Cassia siamea Lamk. *Chemosphere*, **54**:1581–1588.

Tsalev, D.L. 1993. *Cadmium*. In: Atomic absorption spectrometry in occupational and environmental health practice. Tsalev DL (ed), Florida, CRC press. 1:105–112.

Tu¨rkdogan, M.K.; Fevzi, K.; Kazim, K.; Ilyas, T. and Ismail, U. 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environmental Toxicology and Pharmacology*, **13**:175-179.

United State Environmental Protection Agency (USEPA) 1995. Bioremediation of hazardous wastes: Research, Development, and Field Evaluations EPA/540/R-95/532

United State Environmental Protection Agency (USEPA) 1995a. Manual: Bioventing Principles and Practices, Volume I: Bioventing Principles, EPA 540/ R-95/534a. Office of Solid Waste and Emergency Response and Office of Research and Development, pp. 94
http://nepis.epa.gov/pubtitleOSWER.htm

United State Environmental Protection Agency (USEPA) 1995b. Manual: Bioventing Principles and Practices, Volume II: Bioventing Design, EPA 540/R-95/534b. Office of Solid Waste and Emergency Response and Office of Research and Development, 94 pp.
http://nepis.epa.gov/pubtitle OSWER.htm

United State Environmental Protection Agency (USEPA) 1999. Solidification/Stabilization Resource Guide, 1-91.

USEPA. 2004. *Cleaning Up the Nation's Waste Sites: Markets and Technology Trends*. EPA 542-R-04-015.

United State Environmental Protection Agency (USEPA) 2009. Technology performance review: selecting and using solidification/stabilization treatment for site. National Service Center for Environmental Publications (NSCEP), 1-28.

Voutsa, D.; Grimanis, A. And Samara, C. 1996. Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. *Environmental Pollution,* **94(3)**:325-335.

Walker, D.J.; Clemente, R.; Roig A. and Bernal, M.P. 2003. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environmental Pollution*, **122:**303-312.

Wang, X.; Sato, T.; Xing, B. and Tao, S. 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Science of the Total Environment*, **350**:28-37.

Watanabe, F.S.; and Olsen, S.R.1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soils. *Soil Science Society of America Proceeding*, **29**:677-678.

Wcisło, E.; Ioven, D.; Kucharski, R. and Szdzuj, J. 2002. Human health risk assessment case study an abandoned metal smelter site in Poland. *Chemosphere*, **47**:507–515.

Whatmuff, M.S. 2002. Applying biosolids to acid soil in New South Wales: Are guidelines soil metal limits from other countries appropriate. *Australian Journal of Soil Research,* **40**:1041-1056.

White, P.M.; Wolf, D.C., Thomas, G.J. and Reynolds, C.M. 2006. Phytoremediation of alkylated polycyclic aromatic hydrocarbons in an oil-contaminated soil. *Water Air and Soil Pollution,* 169:207-220.

White, D.C.; Flemming, C.A.; Leung, K.T. and Macnaughton, S.J. 1998. In situ bacterial ecology for quantitative appraisal, monitoring and risk assessment of pollution remediation in soils, the subsurface, the rhizosphere and in biofilms. *Journal of Microbiological Methods,* **32:**93-105.

Williams-Linera, G. and Ewel, J.J. 1984. Effect of autoclave sterilization of a tropical an dept on seed germination and seedling growth. *Plant and Soil*, **82**:263-268.

Wilson, B. and Pyatt, F.B. 2007. Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey. UK. *Ecotoxicology and Environmental Safety*, **66**:224-231.

Wong, J.W.; Li, G.X. and Wong, M.H. 1996. The growth of *Brassica chinensis* in heavy metal contaminated sludge compost from Hong Kong. *Bioresource Technology*, **58**:209-313.

Wong, J.W.C. 1996. Heavy metal contents in vegetables and market garden soils in Hong Kong. *Environmental Technology*, **17**:407-414.

World Health Organization (WHO) 1984. Guidelines for drinking water quality: Health criteria and other supporting information. WHO, Geneva, Vol 2, pp 84-90.

Wuana, R.A. and Okieimen, F.E. 2011. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices (Ecology)*, http://dx.doi.org/10.5402/2011/402647.

Xian, X. 1987. Chemical partitioning of cadmium, zinc, lead, and copper in soils near smelters. *Journal of Environmental Science and Health, Part A*, **6**:527-541.

Xian, X. 1989. Effect of chemical forms of cadmium, zinc and lead in polluted soils on their uptake by cabbage plants. *Plant and Soil.*, **113**:257-264.

Yebpella, G.G.; Magomya, A.M.; Udiba, U.U.; Gandu, I.; Amana, S.M.; Ugboaja, V.C. and Usman, N.L. 2011. Assessment of Cd, Cu, Mn and Zn Levels in Soil, Water and vegetable Grown in Irrigated Farm along River Kubani, Zaria, Nigeria. *Journal of Applied Environmental and Biological Sciences.* **1(5):**84-89.

Yekeen, T.A.; Akintaro, O.I.; Akinboro, A. and Azeez, M.A. 2013. Evaluation of cytogenotoxic and nutrient composition of three commonly consumed vegetables in south-western Nigeria. *African Journal of Food, Agriculture, Nutirtion and Development*, **13(2)**: 7452-7466.

Yu, X.Z.; Gu, J.D. and Huang, S.Z. 2007. Hexavalent chromium induced stress and metabolic responses in hybrid willows. *Ecotoxicology*, **16**:299–309.

Yusuf, A.A.; Arowolo, T.A and Bamgbose, O. 2003. Cadmium, copper and nickel

levels in vegetables from industrial and residential areas in Lagos City, Nigeria. *Food and Chemical Toxicology*, **41**:375-378.

Yusuf, K.A. and Osibanjo, O. 2006. Trace metals in water and sediments from Ologe Lagoon, Southwestern Nigeria. *Pakistan Journal of Scientific and Industrial Research*, **49**:88-96.

Zaidi, M.I.; Asrar, A.; Mansoor, A. and Farooqui, M.A. 2005. The heavy metal concentrations along roadsides trees of Quetta and its effects on public health. *Journal of Applied Sciences*, **5(4):**708-711.

Zavoda, J.; Cutright, T.; Szpak, J. and Fallon, E. 2001. Uptake, selectivity, and inhibition ofhydroponic treatment of contaminants. *Journal of Environmental Engineering*, **127**:502-508.

Zhu, Y.G.; Chen, S.B. and Yang, J.C. 2004. Effects of soil amendments on lead uptake by two vegetable crops from a lead-contaminated soil from Anhui, China. *Environment International*, **30:**351–356.

APPENDICES

APPENDIX 1

LIST OF MEDIA AND THEIR COMPOSITION

NUTRIENT AGAR

Code: LAB 008

This is a general purpose medium for the cultivation of organisms that are not demanding in their nutritional requirements e.g. organisms that can be isolated from air, water, dust etc. Nutrient Agar is suitable for teaching and demonstration purposes, it is isotonic and can be enriched with biological fluids such as sterile blood and egg yolk.

Composition

Ingredients	**Gram/Litre**
Peptone	5.0
Beef Extract	3.0
Sodium chloride	8.0
Agar No. 2	12.0
pH	7.3 ± 0.2

Directions

Twenty eight grams of powder was weighed and dispersed in 1 litre of deionised water. It was allowed to soak for 10 minutes, swirled to mix then sterilised by autoclaving for 15 minutes at 121°C. It was cooled to 47°C, mixed well and then poured into Petri plates.

NUTRIENT BROTH

Code:LAB 014

This is a general purpose broth which can be used for the sterility testing for aerobic organisms. This broth can also be used as the suspending medium for cooked meat granules for the cultivation of anaerobic organisms.

Composition

Ingredients	**Gram/Litre**
Beef Extract	10.0
Peptone	10.0
Sodium chloride	5.0
pH	7.3 ± 0.2

Directions

Twenty five grams of powder was weighed and dispersed in 1 litre of deionised water. It was allowed to soak for 10 minutes, swirled to mix then dispensed into tubes or bottles, and sterilised for 15 minutes at 121°C.

SIMMONS CITRATE AGAR

Code: LAB 069

This is a medium devised by Simmons in 1926 to help in the differentiation of enteric bacteria. Certain Enterobacteriacae have the ability to utilize citrate as the sole source of carbon and utilize inorganic ammonium salts as the sole source of nitrogen resulting in an increase in alkalinity. Bromothymol Blue is used as a pH indicator.

Composition

Ingredients	**Gram/Litre**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar No. 2	15.0
pH	6.9 ± 0.2

Directions

Twenty four grams of powder was weighed and dispersed in 1 litre of deionised water. It was allowed to soak for 10 minutes and swirled to mix then heated to dissolve the agar and solids. It was dispensed into tubes or bottles then sterilised by autoclaving at 121°C for 15 minutes and was allowed to set as slopes.

SIM MEDIUM (Oxoid)

Code: CM0435

A medium for the differentiation of enteric bacteria on the basis of sulphide production, indole production and motility.

Composition

Ingredients	**Gram/Litre**
Tryptone	20.0
Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Agar	3.5
pH	7.3 ± 0.2

Directions

Thirty grams of the powder was suspended in 1 litre of distilled water and boiled to dissolve the medium completely. It was dispensed into final containers and sterilised by autoclaving for 15 minutes at 121°C.

PEPTONE WATER

Code: LAB104

This is a general purpose growth medium that can be used as a base for carbohydrate fermentation studies. The medium has a high level of tryptone making it suitable for use in the indole test.

Composition

Ingredients	**Gram/Litre**
Bacteriological peptone	10.00
Sodium Chloride	5.00
pH	7.2 ± 0.2

Directions

Fifteen grams of powder was weighed and dispersed in 1 litre of deionised water. It was allowed to dissolve then distributed into final containers. It was sterilised by autoclaving at 121°C for 15 minutes.

TRYPTONE WATER

Code: Oxoid CM0087

This is a liquid medium for the production of indole by microorganisms.

Composition

Ingredients	**Gram/Litre**
Tryptone	10.0
NaCl	5.0
pH	7.5 ± 0.2

Fifteen grams of powder was dissolved in 1 L of distilled water, distributed into test tubes and sterilized at by autoclaving at 121°C for 15 minutes.

BASAL MEDIUM FOR UREASE PRODUCTION

(following the composition of Urease agar (Himedia M1828)

Composition

Ingredients	**Gram/Litre**
Yeast extract	0.100
Monopotassium phosphate	0.091
Disodium phosphate	0.095
Phenol red	0.010
pH	6.8± 0.2

The basal medium was prepared by weighimg 20.30 grams of its composition in 1 L of distilled water, it was mixed well and sterilized by autoclaving at 121 °C for 15 minutes and then allowed to cool to 45 °C, urea solution was sterilized using membrane filter and added to the basal medium to give a concentration of 2% urea, the agar was allowed to set as slope.

APPENDIX 2

Figures A 1-35 shows the evolutionary relationships of taxa (phylogenetic tree). The evolutionary history was inferred using the Neighbour-Joining method following the method described by Saitou and Nei (1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches following the method described by Felsenstein (1985). The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method as described by Tamura *et al*. (2004) and are in the units of the number of base substitutions per site. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. All evolutionary analyses involved 11 nucleotide sequences and were conducted in MEGA6 following the method described by Tamura *et al.* (2013).

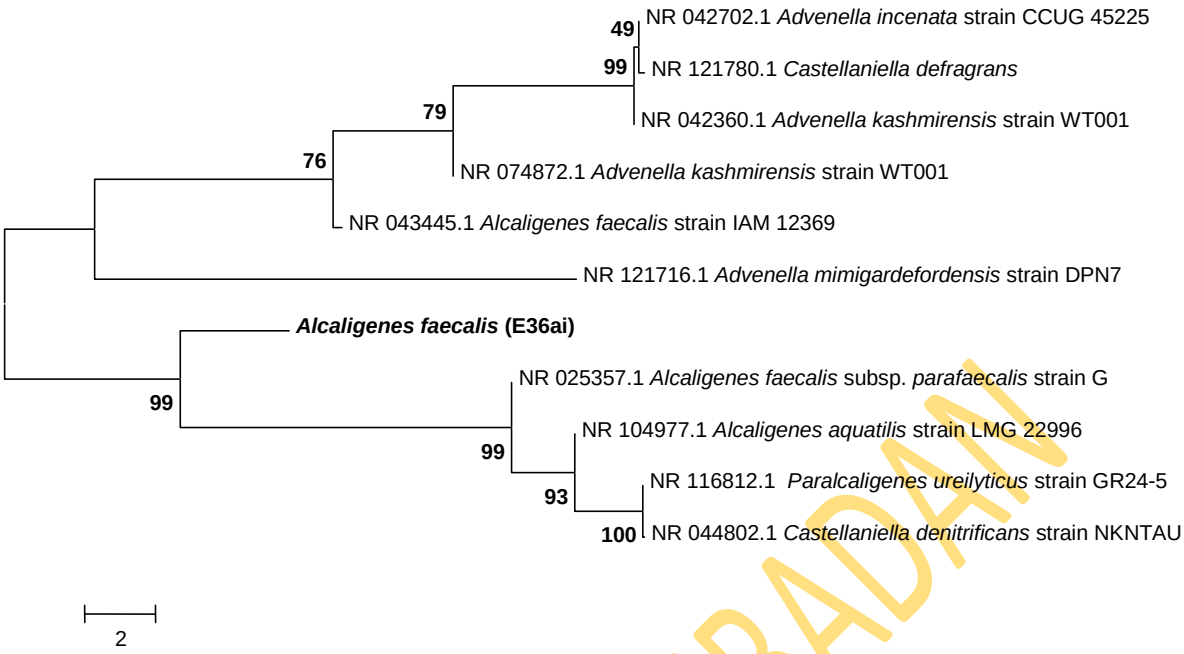

Figure A 1a: Evolutionary relationship of *Alcaligenes faecalis* (E36ai)

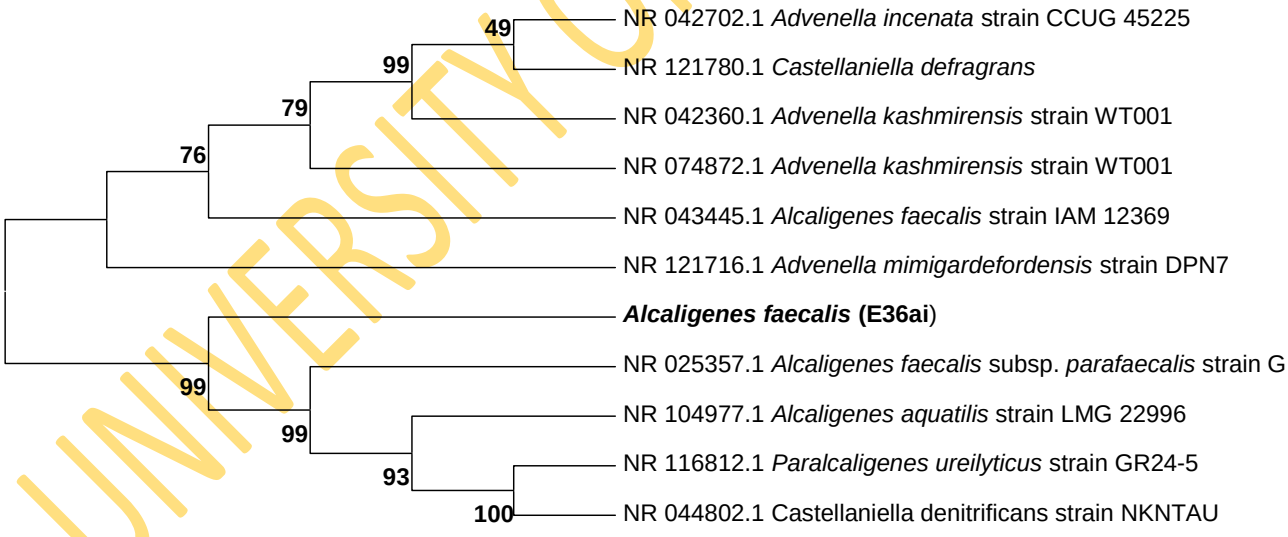

Figure A 1b: Evolutionary relationship of *Alcaligenes faecalis* (E36ai) showing topology

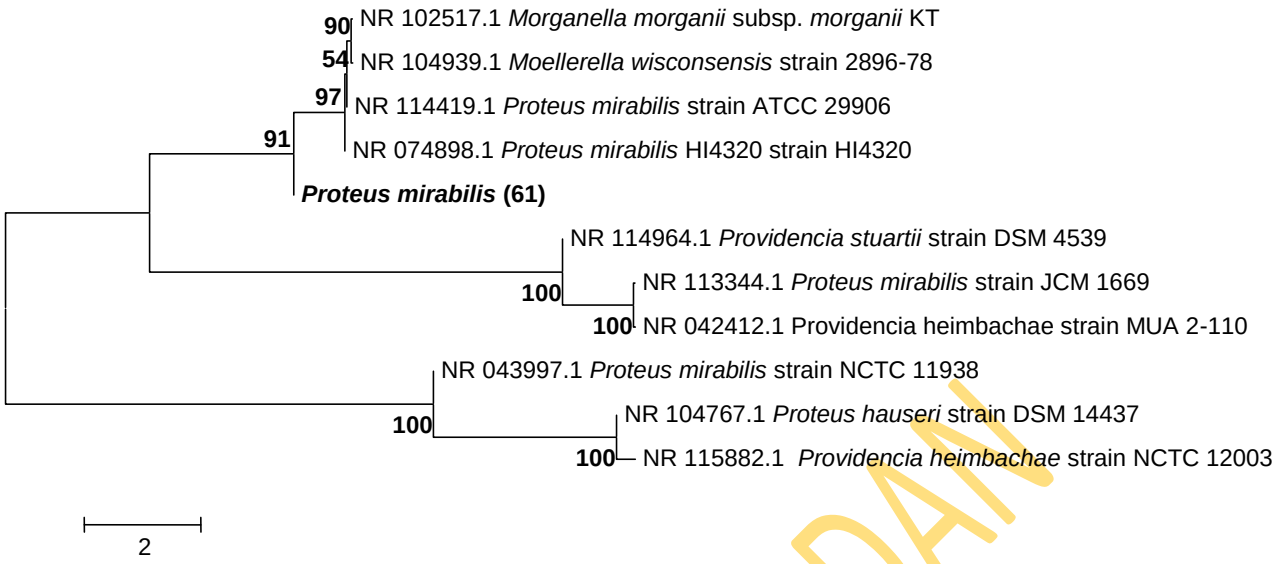

Figure A 2a: Evolutionary relationship of *Proteus mirabilis* (61)

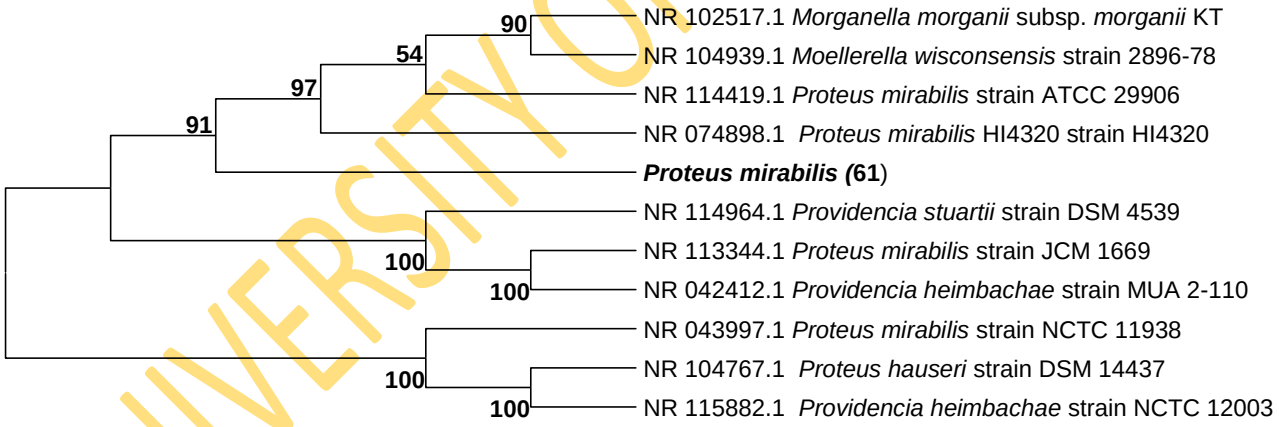

Figure A 2b: Evolutionary relationship of *Proteus mirabilis* (61) showing topology

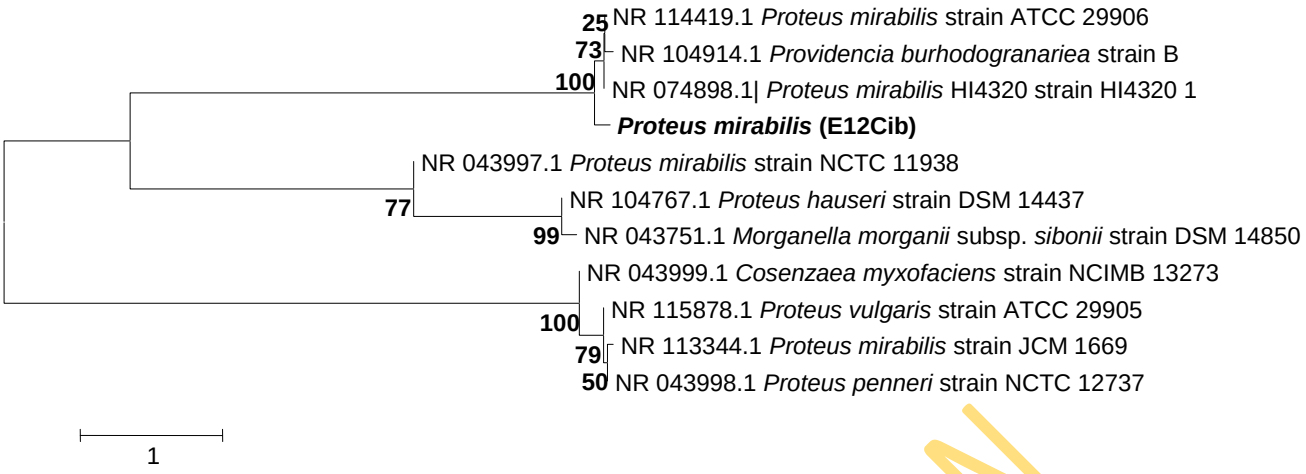

Figure A 3a: Evolutionary relationship of *Proteus mirabilis* (E12Cib)

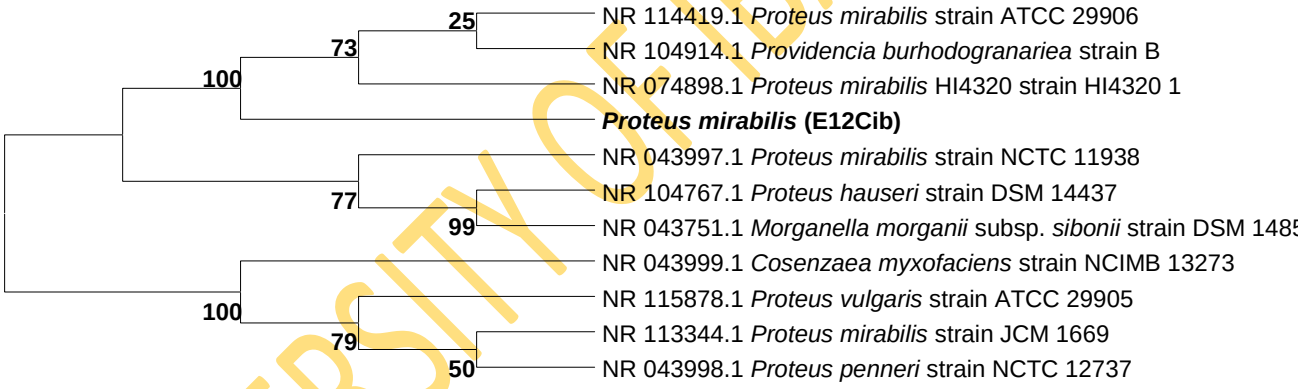

Figure A 3b: Evolutionary relationship of *Proteus mirabilis* (E12Cib) showing topology

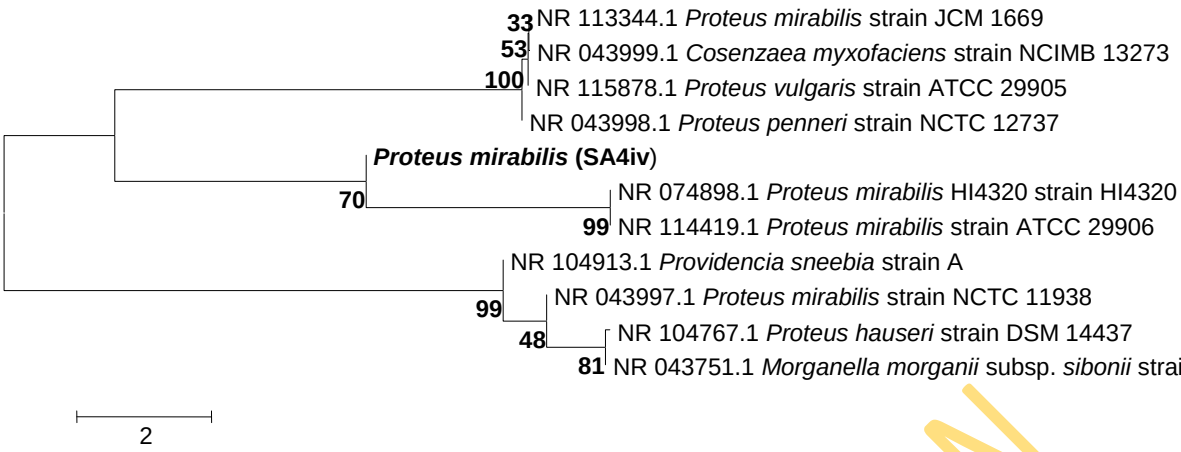

Figure A 4a: Evolutionary relationship of *Proteus mirabilis* (SA4iv)

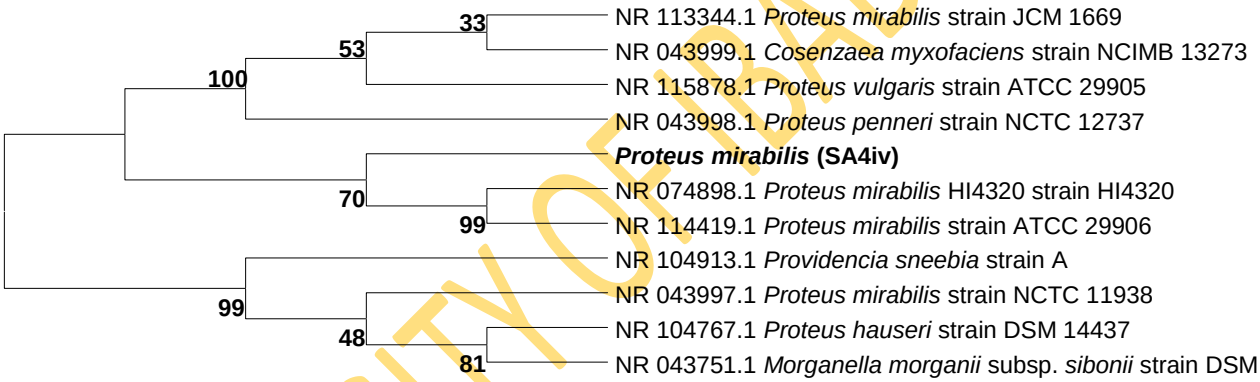

Figure A 4b: Evolutionary relationship of *Proteus mirabilis* (SA4iv) showing topology

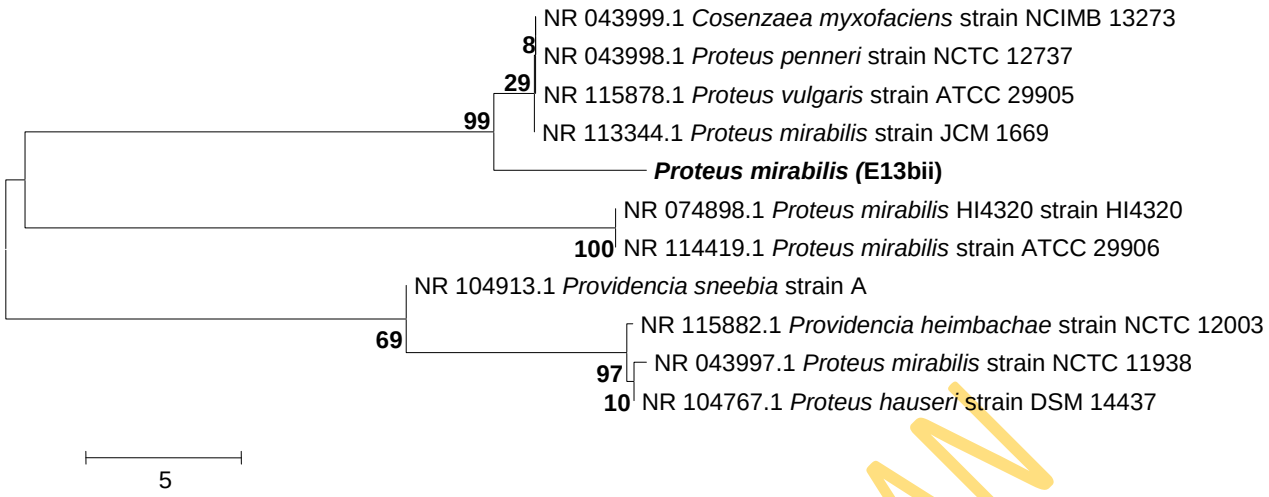

Figure A 5a: Evolutionary relationship of *Proteus mirabilis* (E13bii)

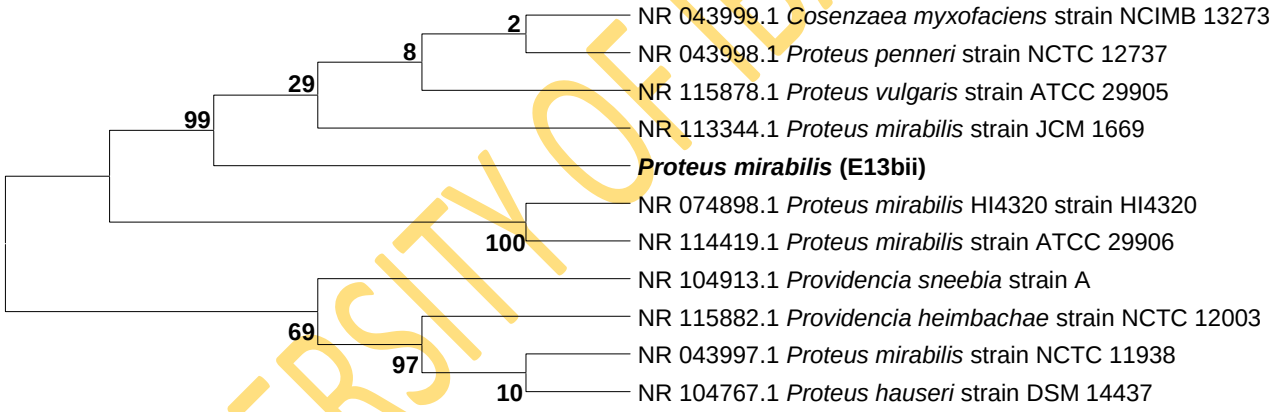

Figure A 5b: Evolutionary relationship of *Proteus mirabilis* (E13bii) showing topology

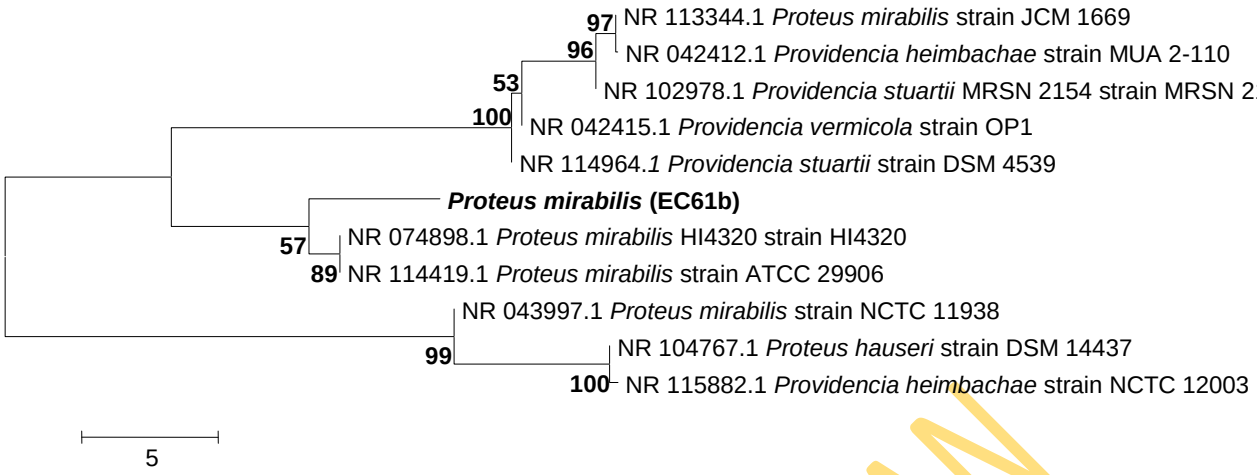

Figure A 6a: Evolutionary relationship of *Proteus mirabilis* (EC61b)

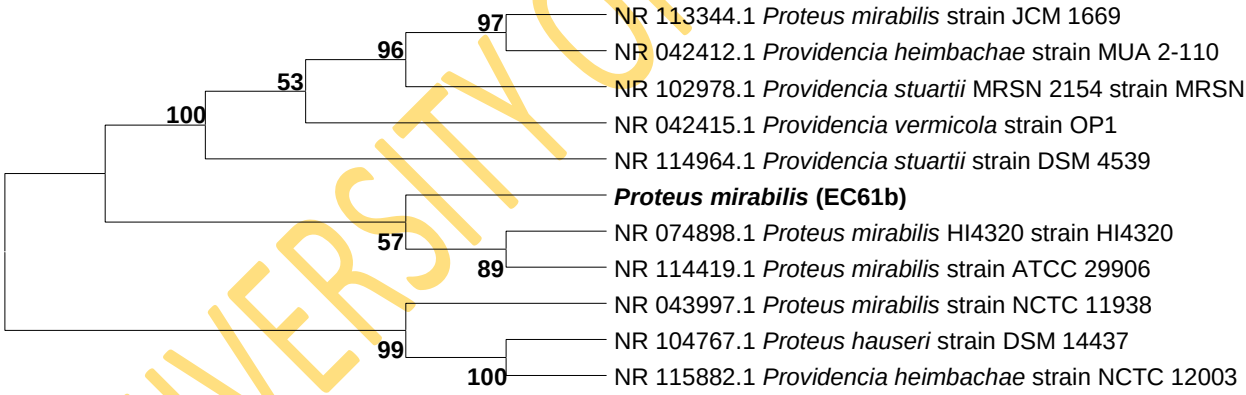

Figure A 6b: Evolutionary relationship of *Proteus mirabilis* (EC61b) showing topology

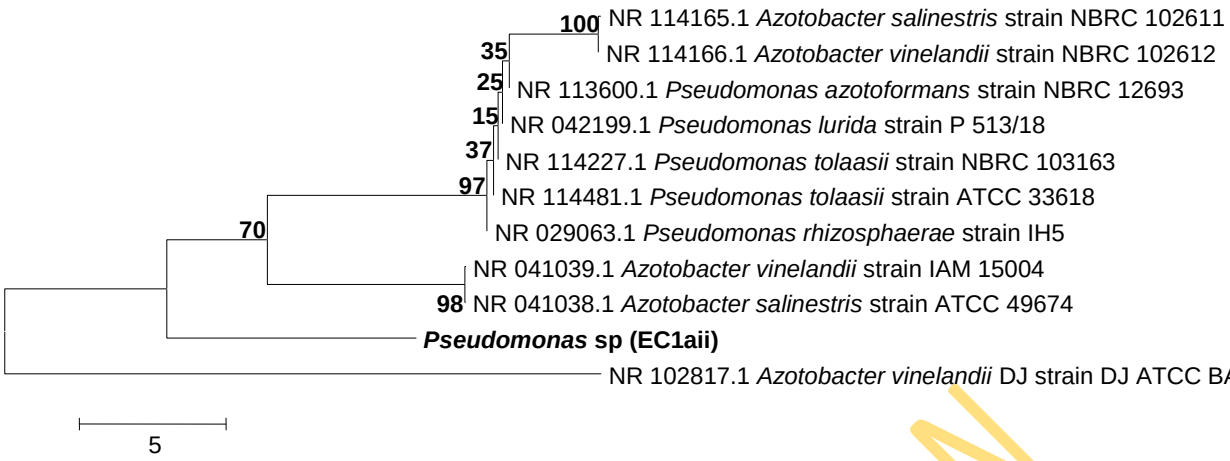

Figure A 7a: Evolutionary relationship of *Pseudomonas* sp (ECIaii)

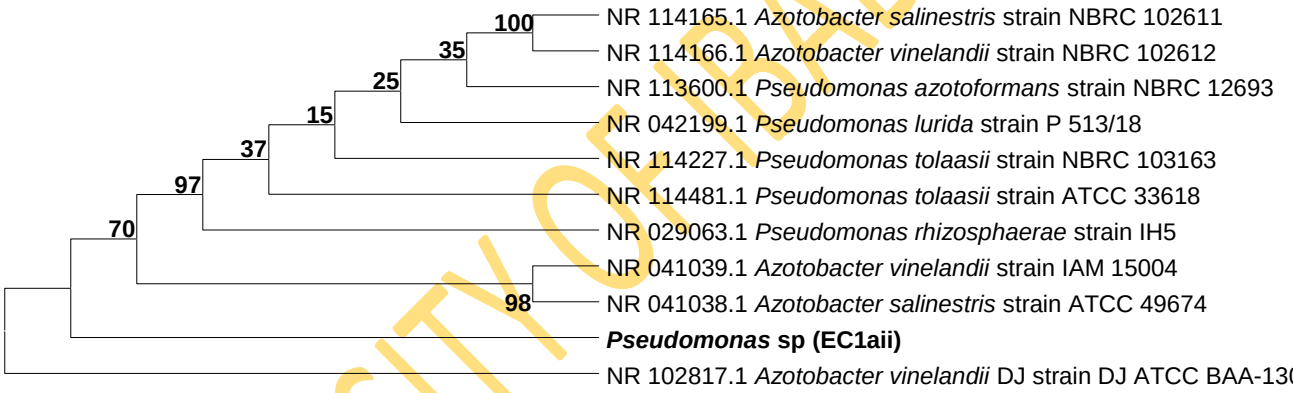

Figure A 7b: Evolutionary relationship of *Pseudomonas* sp (EC1aii) showing topology

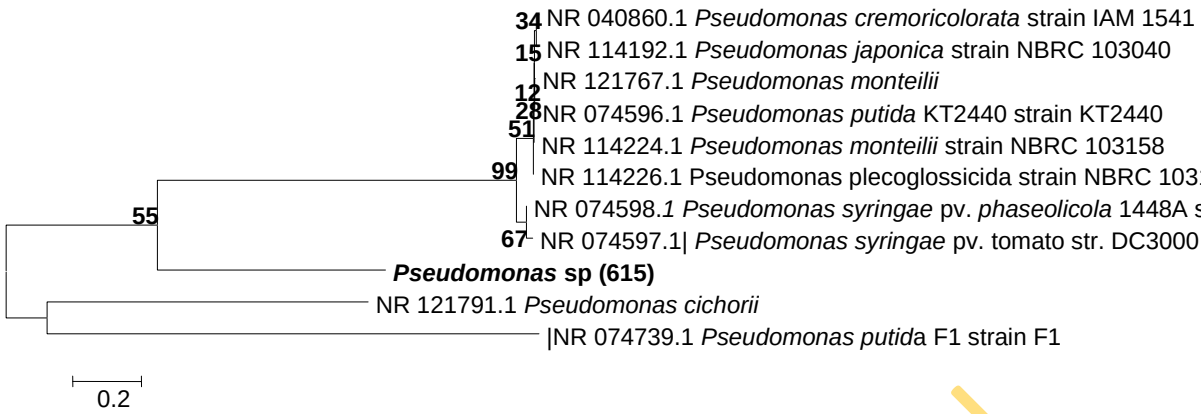

Figure A 8a: Evolutionary relationship of *Pseudomonas* sp (615)

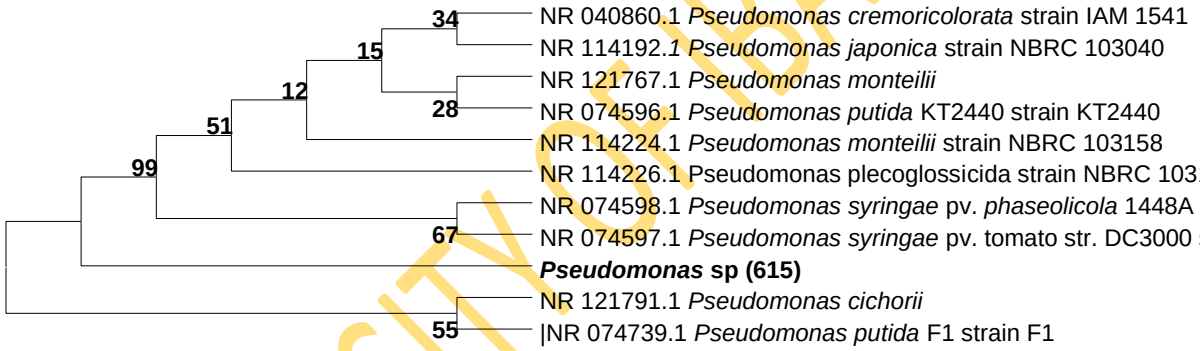

Figure A 8b: Evolutionary relationship of *Pseudomons* sp (615) showing topology

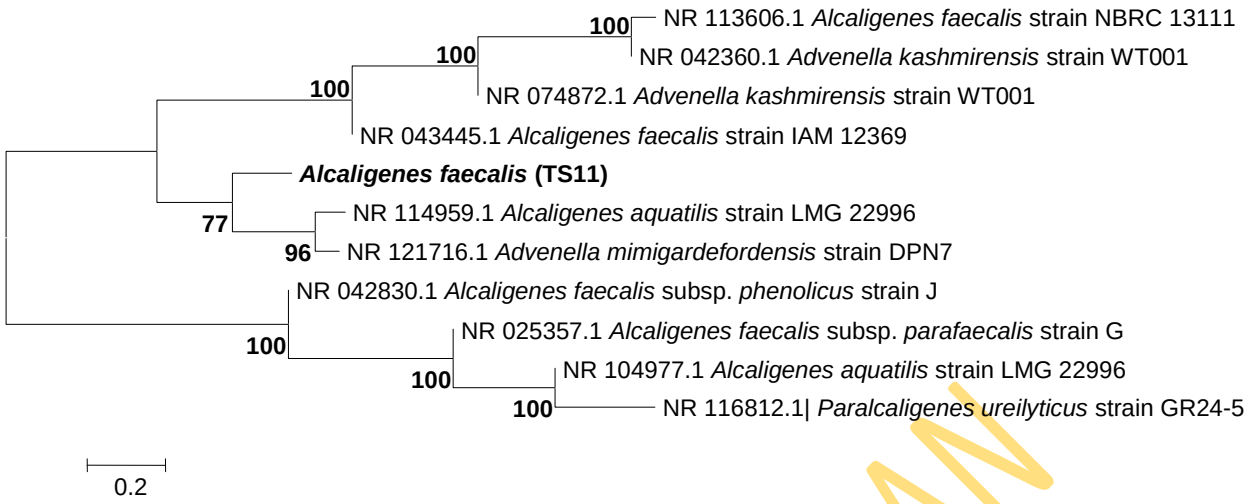

Figure A 9a: Evolutionary relationship of *Alcaligenes faecalis* (TS11)

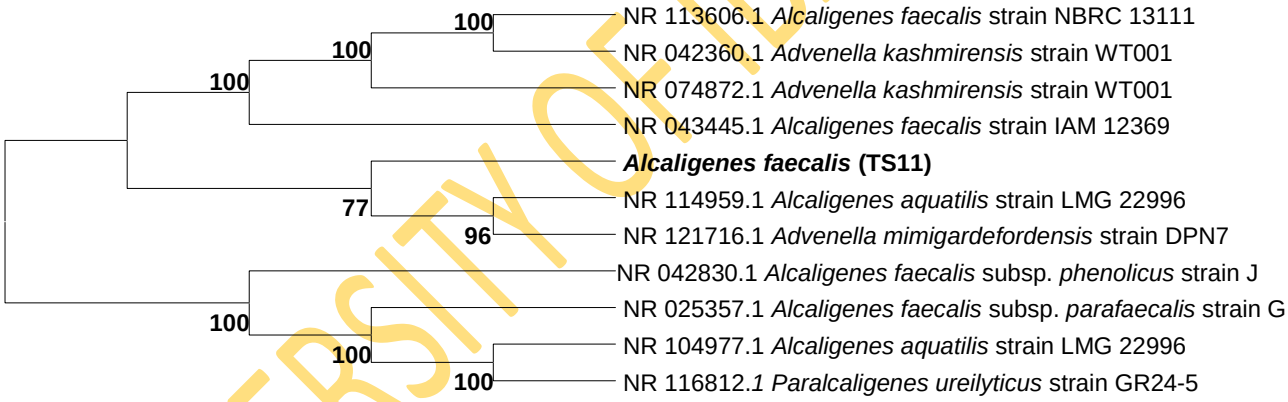

Figure A 9b: Evolutionary relationship of *Alcaligenes faecalis* (TS11) showing topology

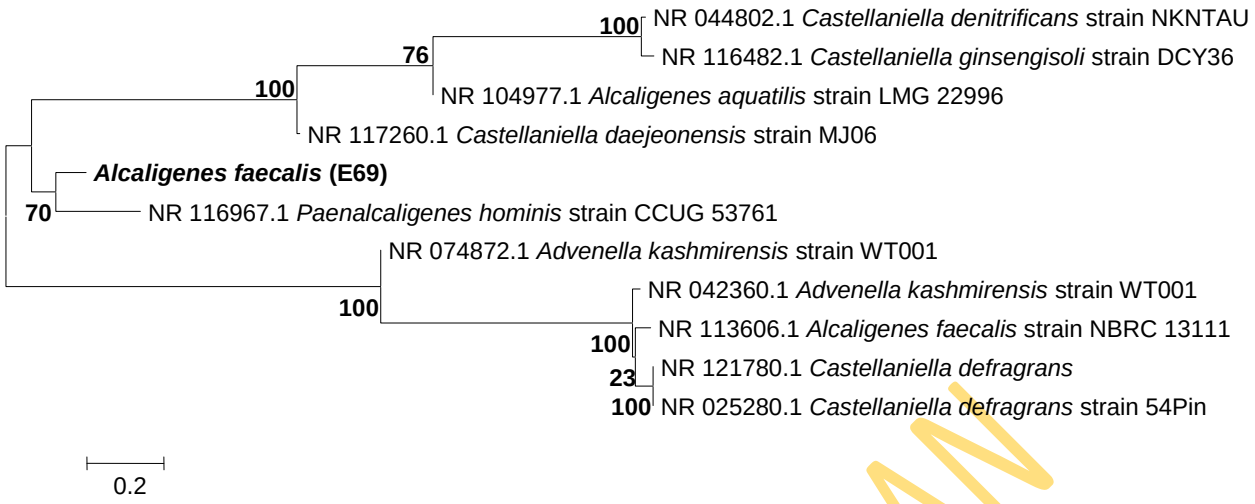

Figure A 10a: Evolutionary relationship of *Alcaligenes faecalis* (E69)

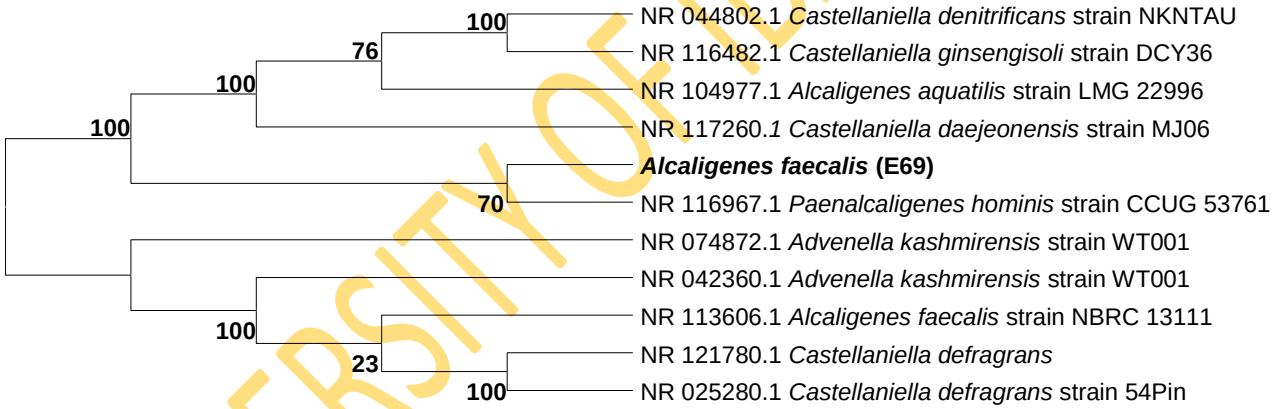

Figure A 10b: Evolutionary relationship of *Alcaligenes faecalis* (E69) showing topology

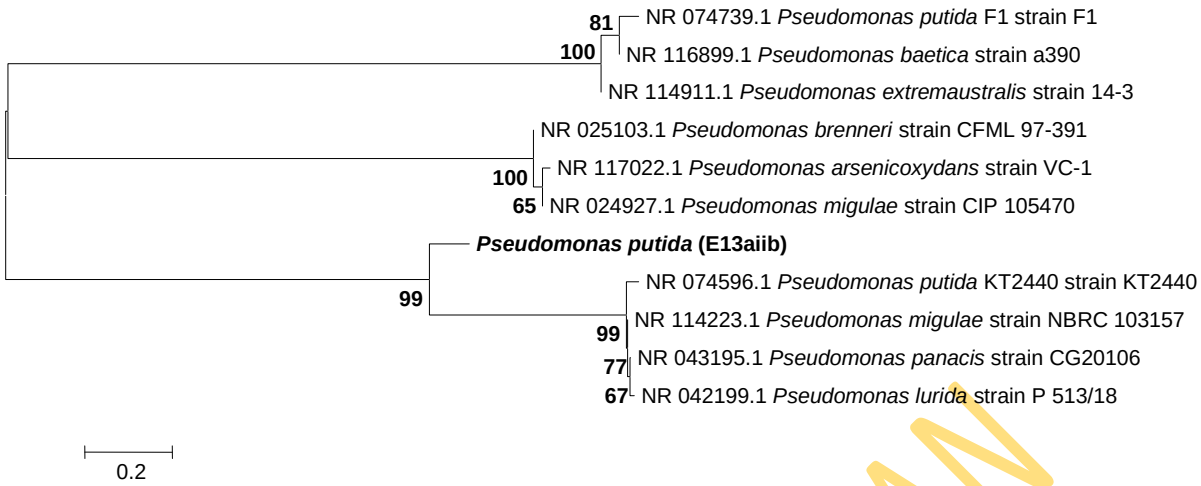

Figure A 11a: Evolutionary relationship of *Pseudomonas putida* (E13aiib)

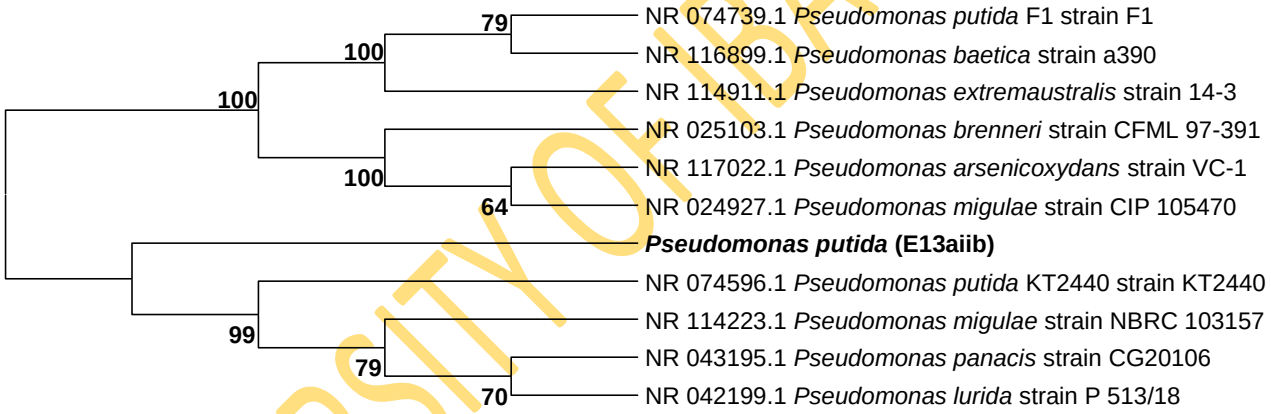

Figure A 11b: Evolutionary relationship of *Alcaligenes faecalis* (E69) showing topology

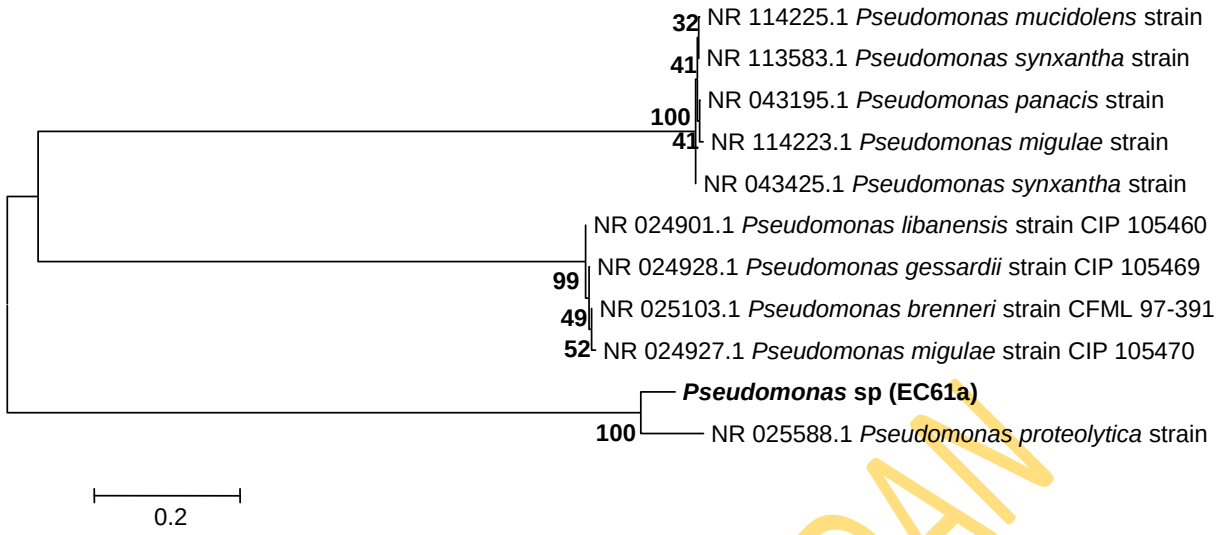

Figure A 12a: Evolutionary relationship of *Pseudomonas* sp (EC61a)

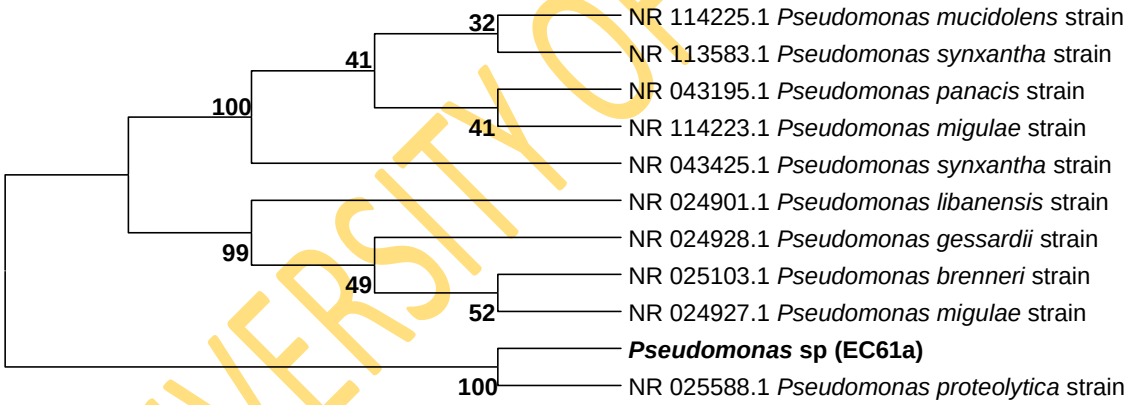

Figure A 12b: Evolutionary relationship of *Pseudomonas* sp (EC61a)showing topology

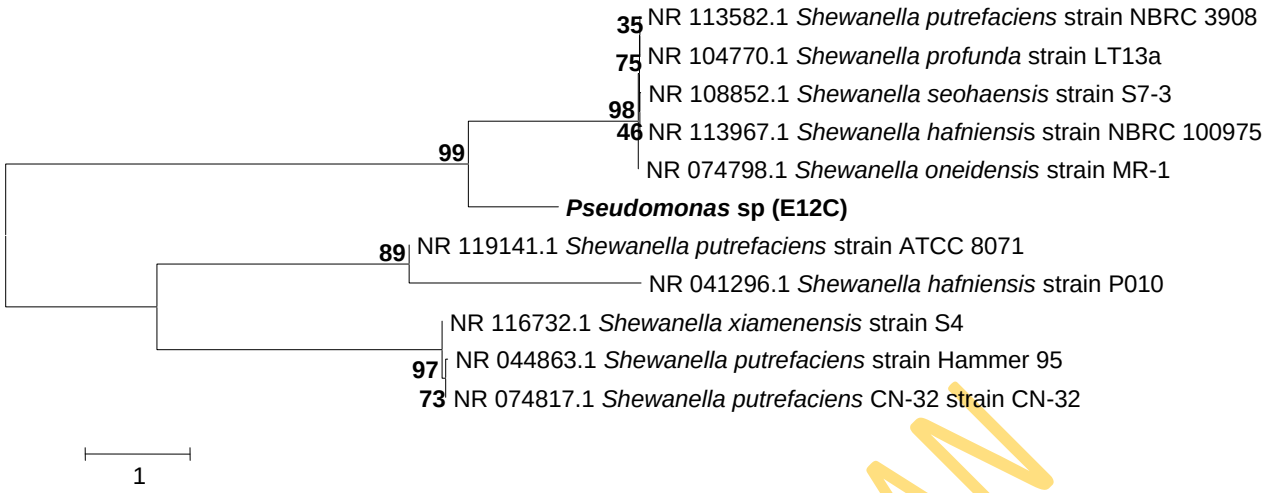

Figure A 13a: Evolutionary relationship of *Pseudomonas* sp (E12c)

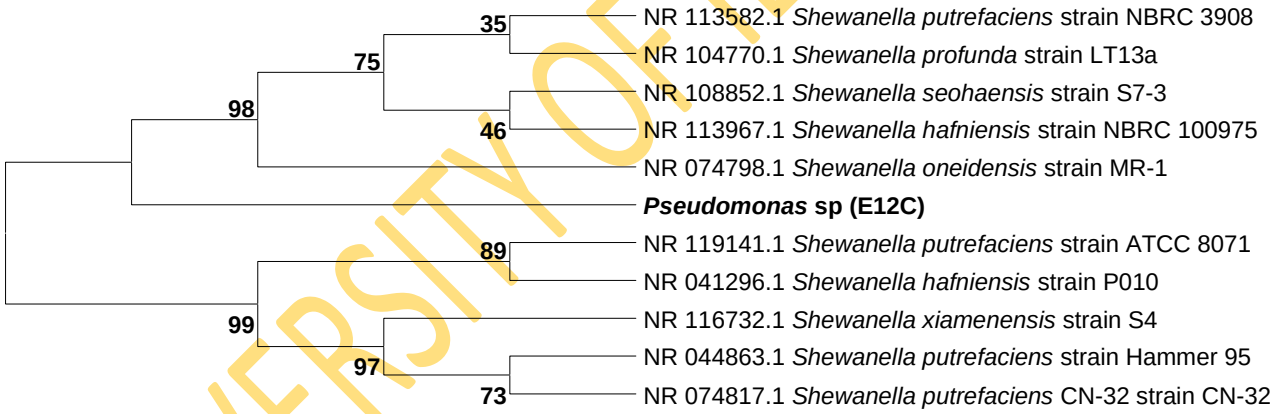

Figure A 13b: Evolutionary relationship of *Pseudomonas* sp (E12C)showing topology

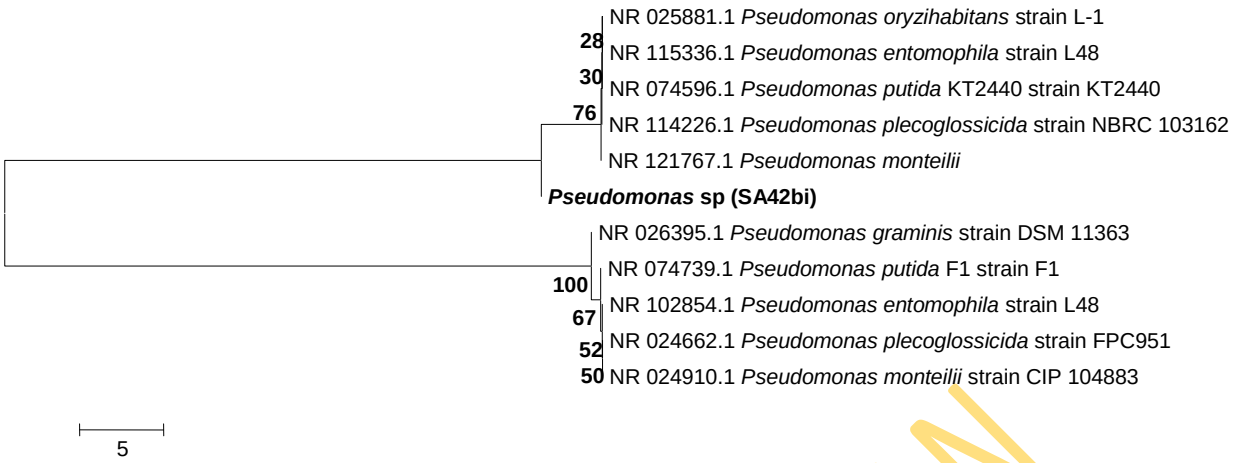

Figure A 14a: Evolutionary relationship of *Pseudomonas* sp (SA42bi)

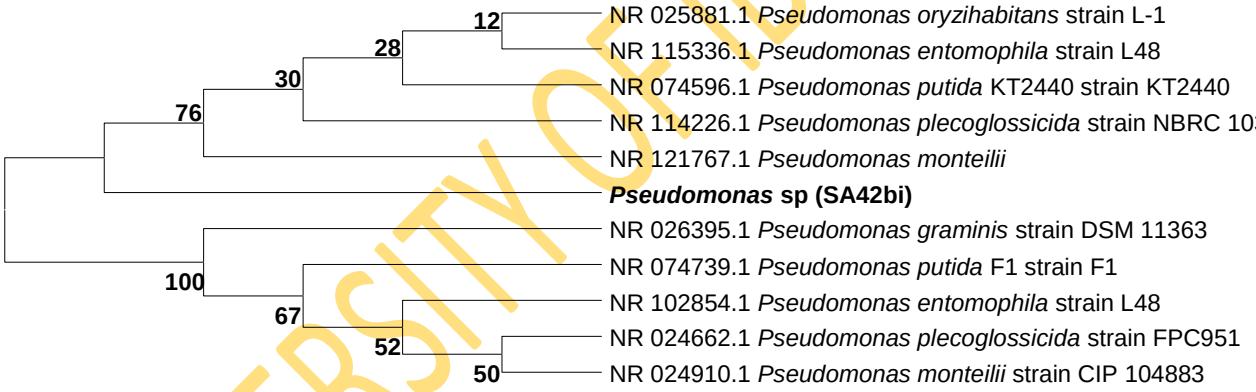

Figure A 14b: Evolutionary relationship of *Pseudomonas* sp (SA42bi) showing topology

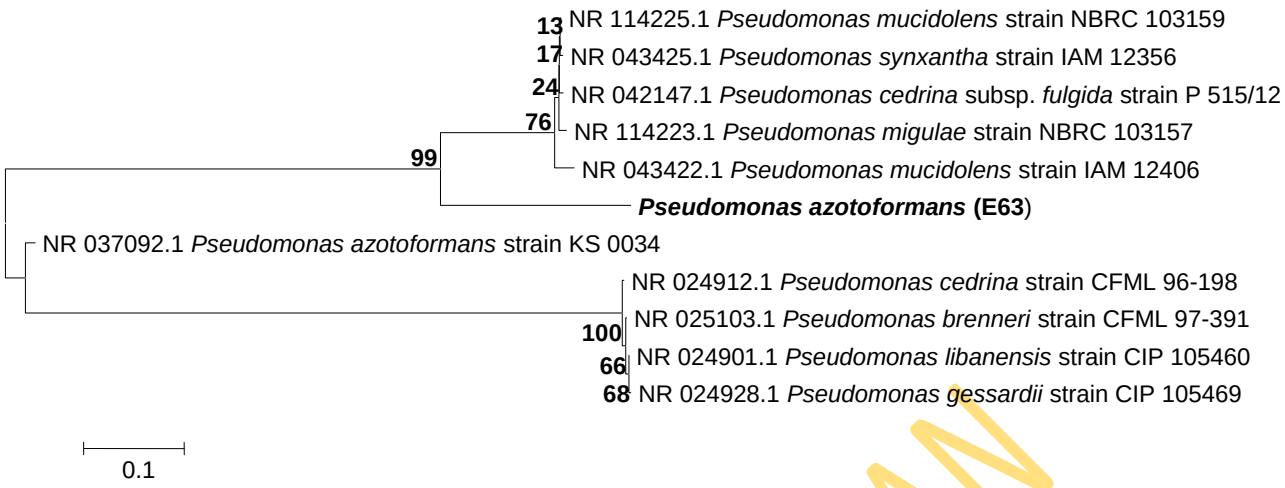

Figure A 15a: Evolutionary relationship of *Pseudomonas azotoformans* (E63)

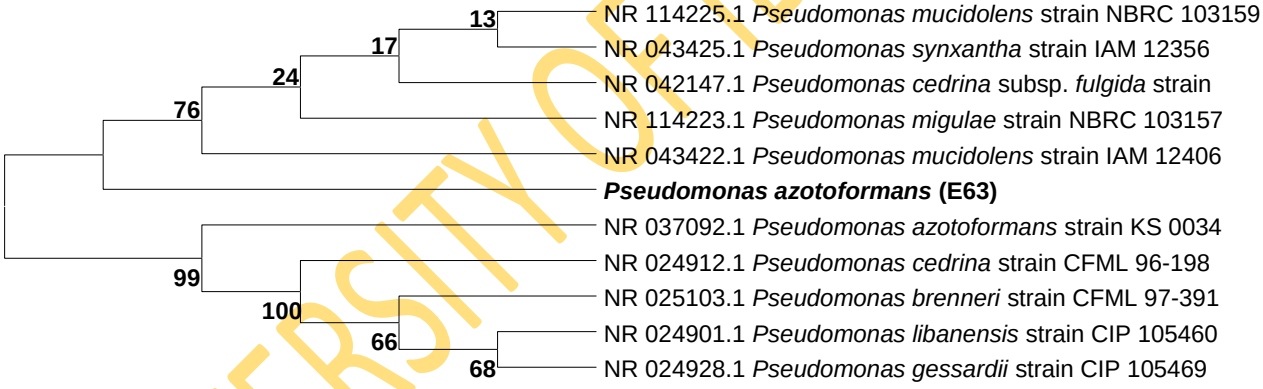

Figure A 15b: Evolutionary relationship of *Pseudomonas azotoformans* (E63) showing topology

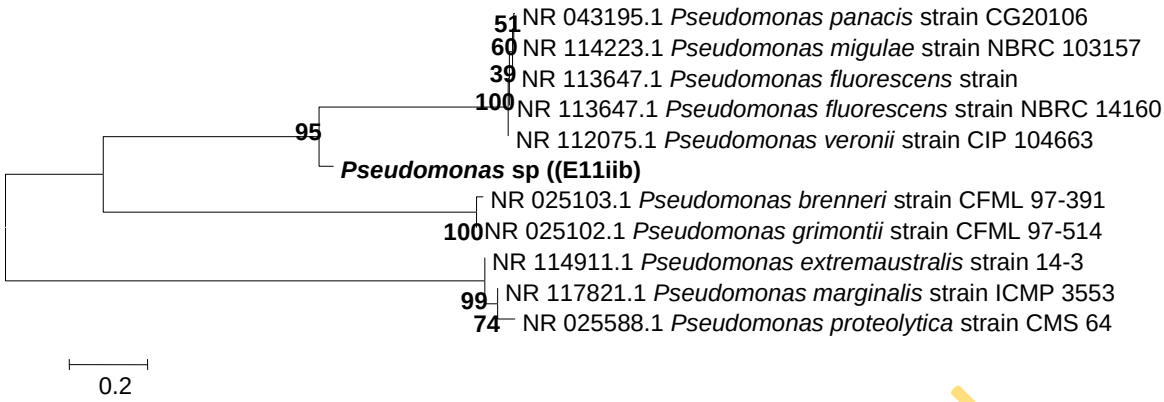

Figure A 16a: Evolutionary relationship of *Pseudomonas* sp (E11iib)

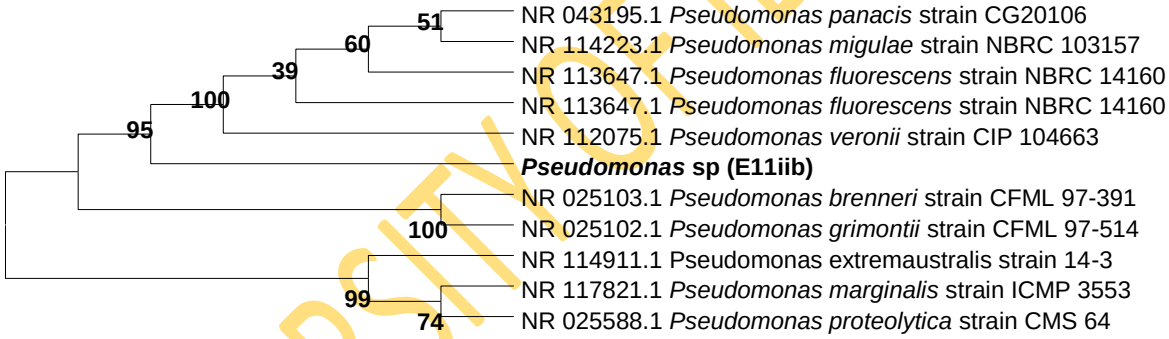

Figure A 16b: Evolutionary relationship of *Pseudomonas* sp (E11iib) showing topology

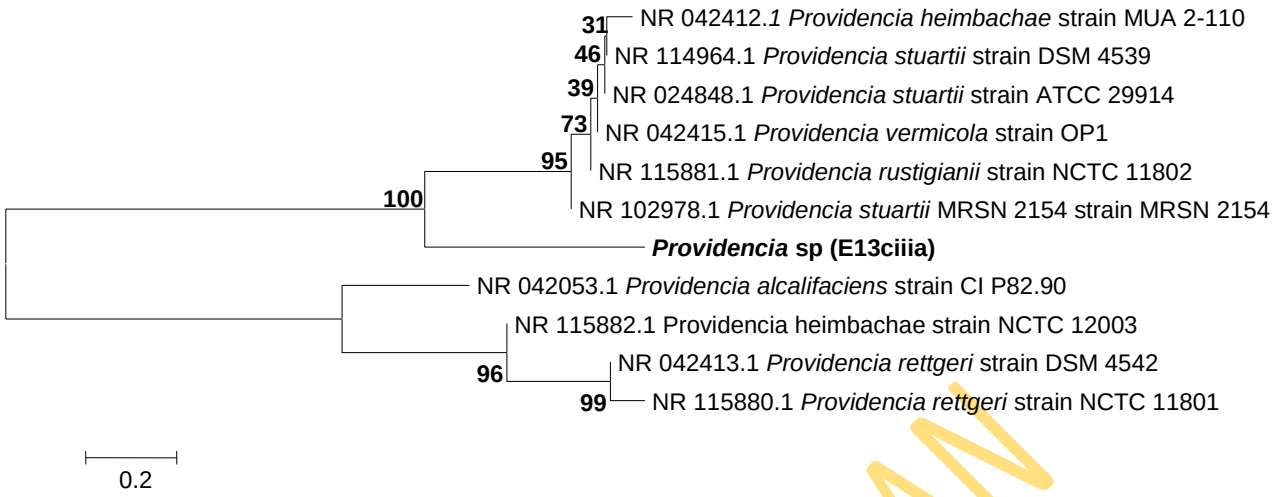

Figure A 17a: Evolutionary relationship of *Providencia* sp (E13ciia)

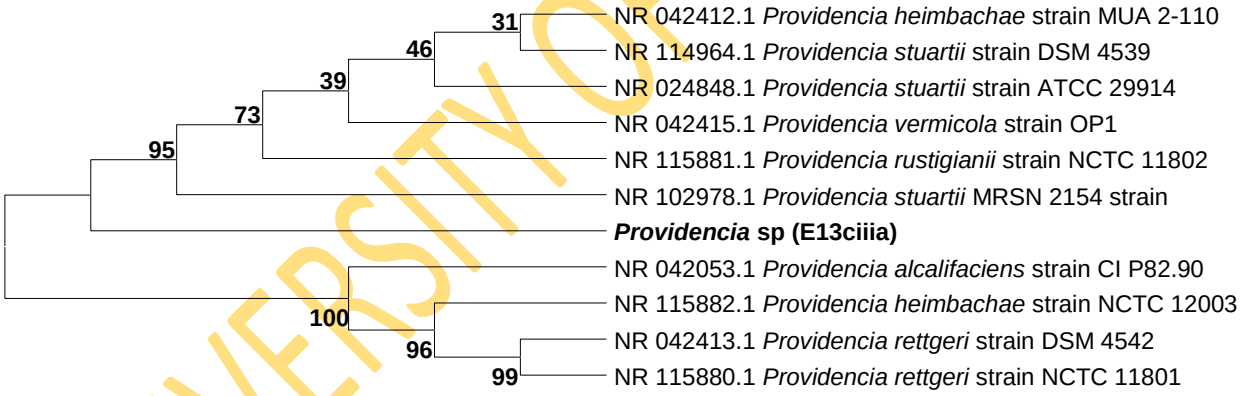

Figure A 17b: Evolutionary relationship of *Providencia* sp (E13ciiia) showing topology

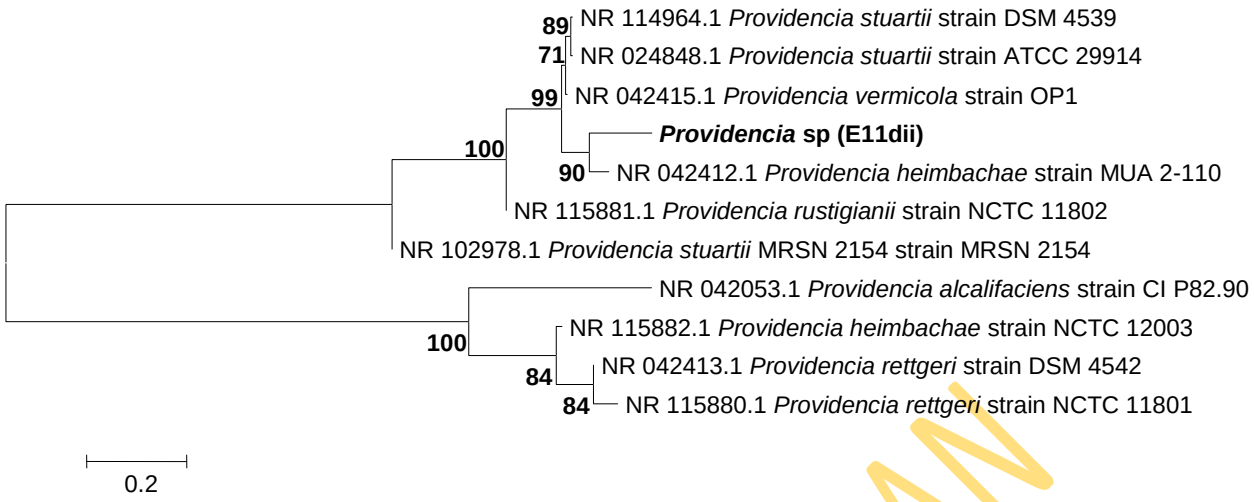

Figure A 18a: Evolutionary relationship of *Providencia* sp (E11dii)

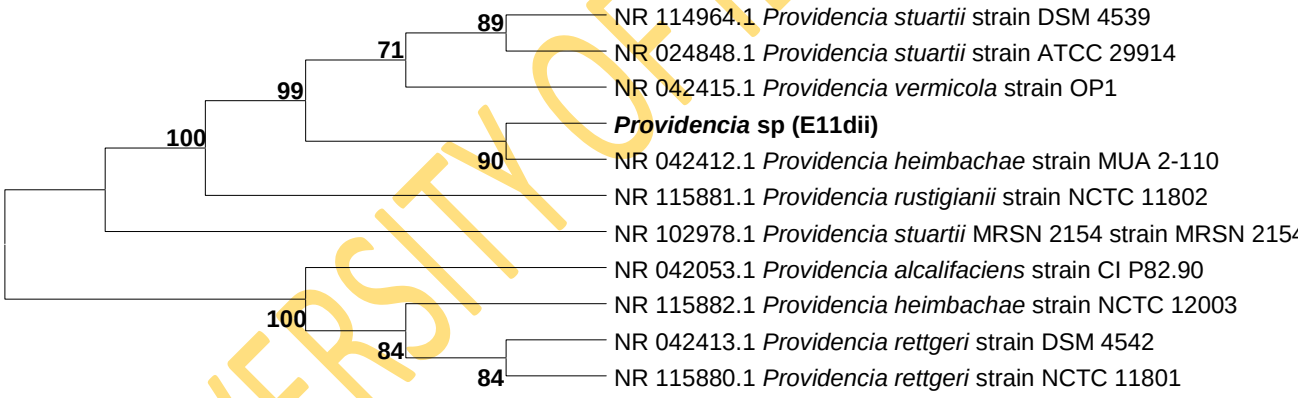

Figure A 18b: Evolutionary relationship of *Providencia* sp (E11dii) showing topology

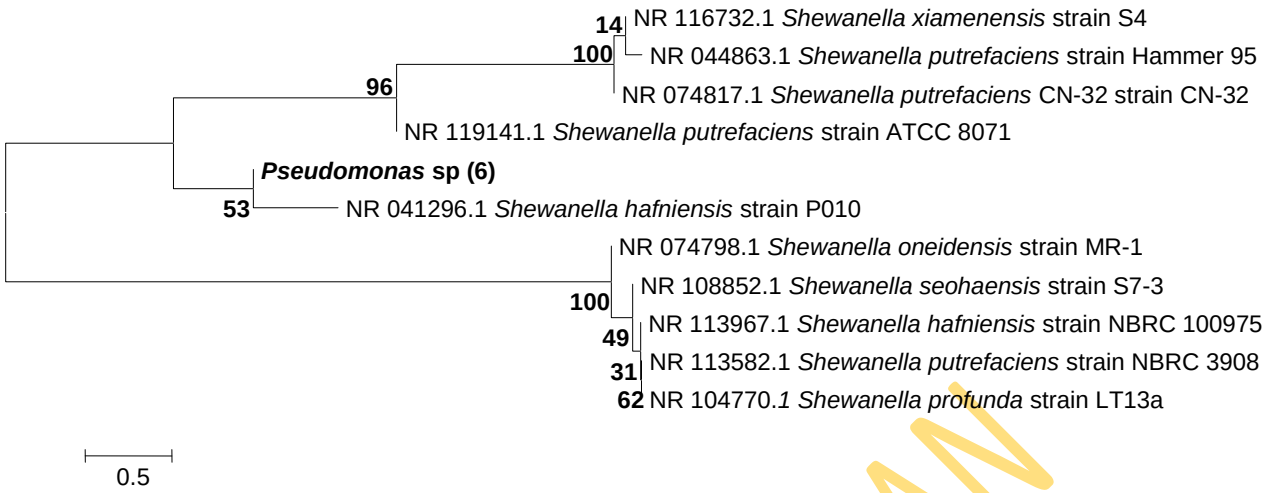

Figure A 19a: Evolutionary relationship of *Pseudomonas* sp (6)

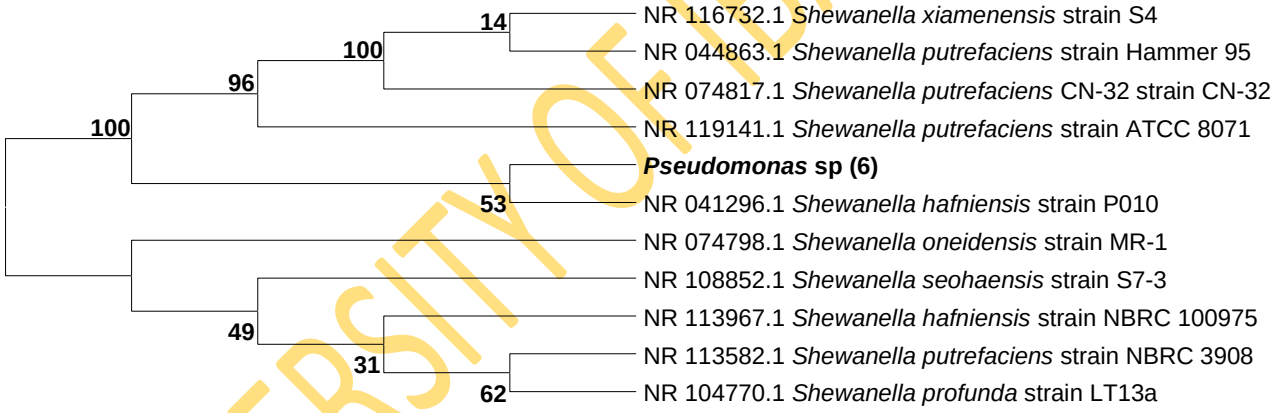

Figure A 19b: Evolutionary relationship of *Pseudomonas* sp (6) showing topology

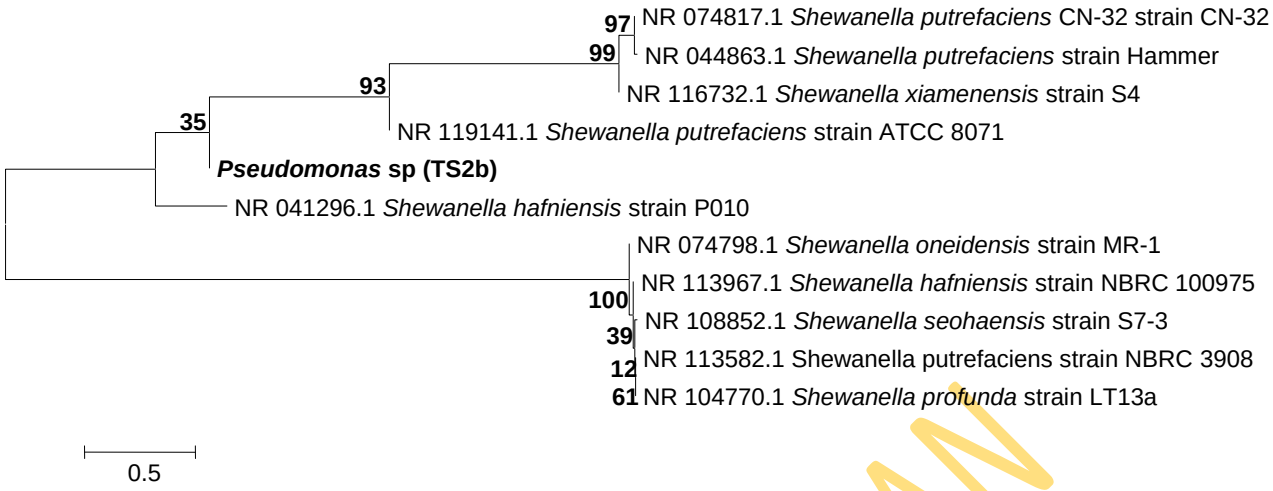

Figure A 20a: Evolutionary relationship of *Pseudomonas* sp (TS2b)

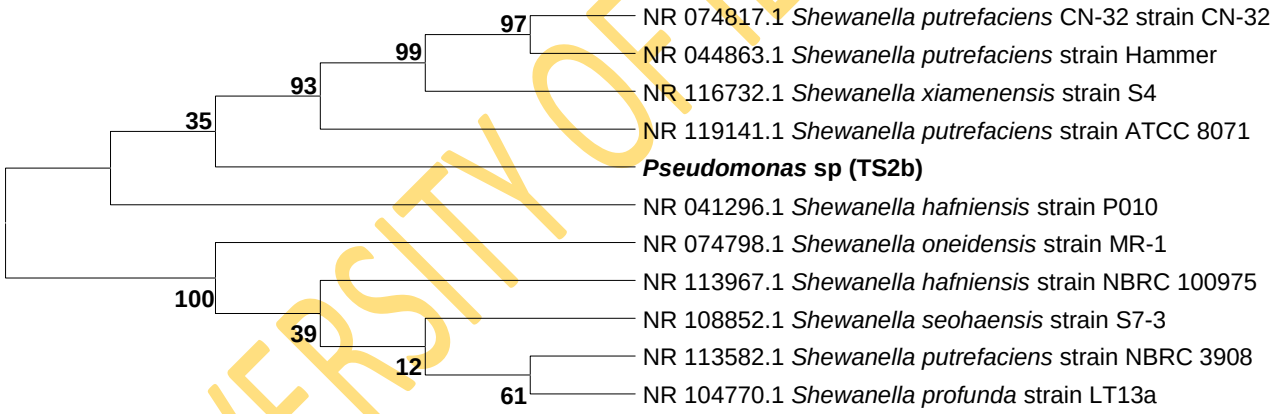

Figure A 20b: Evolutionary relationship of *Pseudomonas* sp (TS2b) showing topology

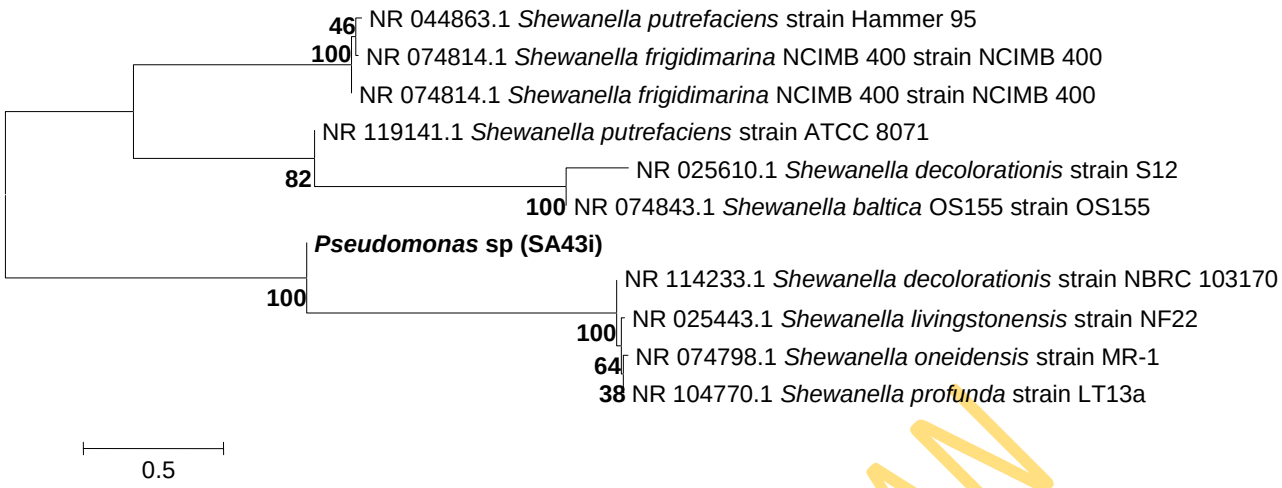

Figure A 21a: Evolutionary relationship of *Pseudomonas* sp (SA43i)

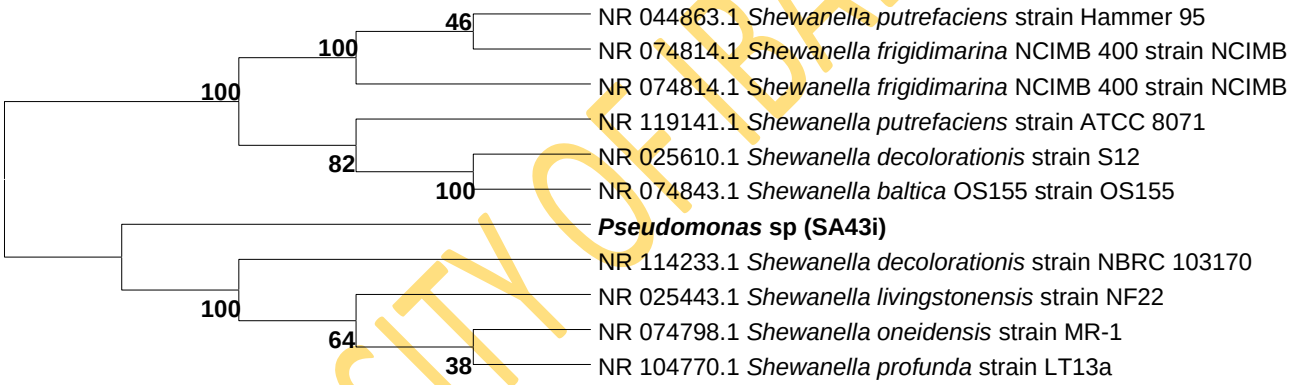

Figure A 21b: Evolutionary relationship of *Pseudomonas* sp (SA43i) showing topology

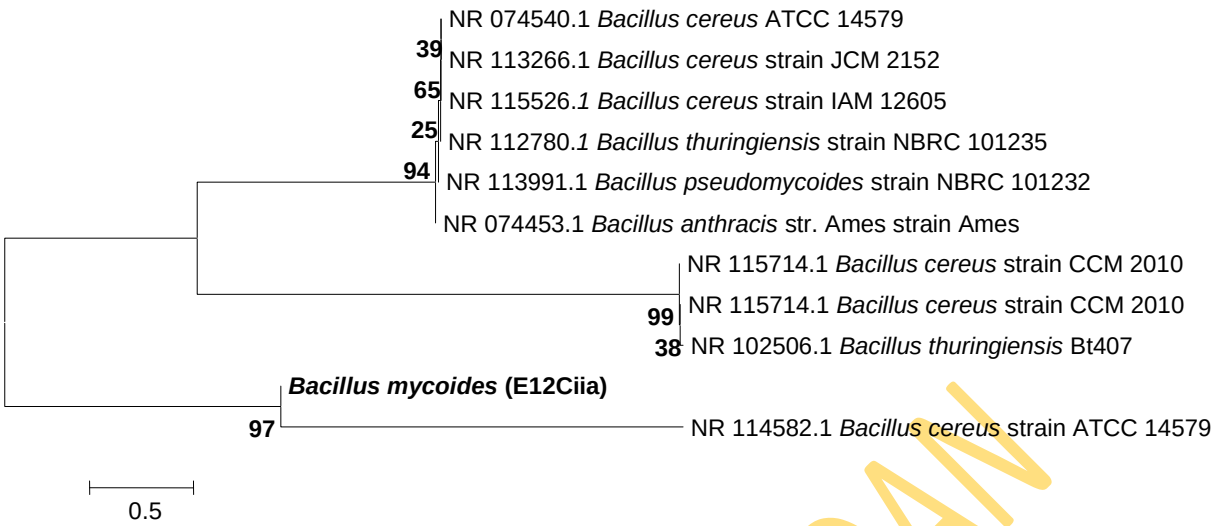

Figure A 22a: Evolutionary relationship of *Bacillus mycoides* (E12Ciia)

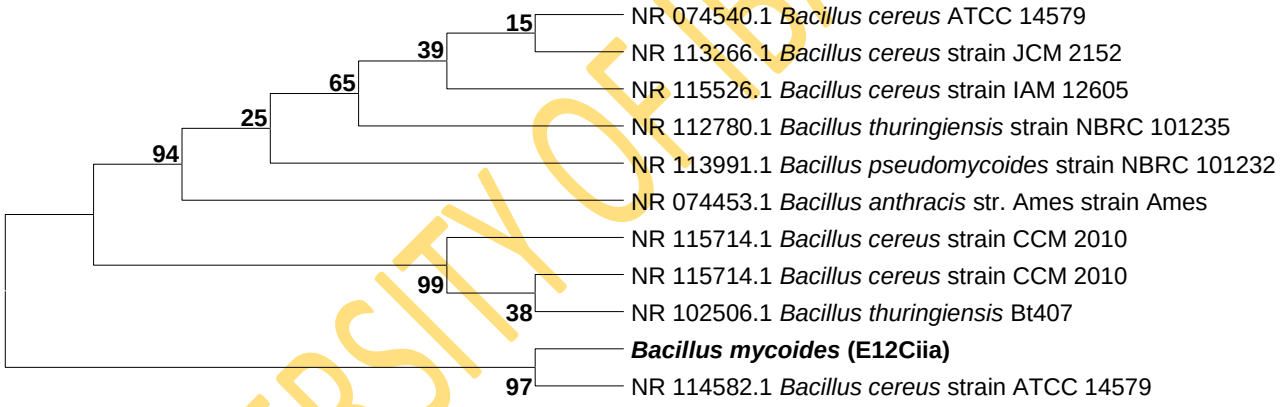

Figure A 22b: Evolutionary relationship of *Bacillus mycoides* (E12Ciia) showing topology

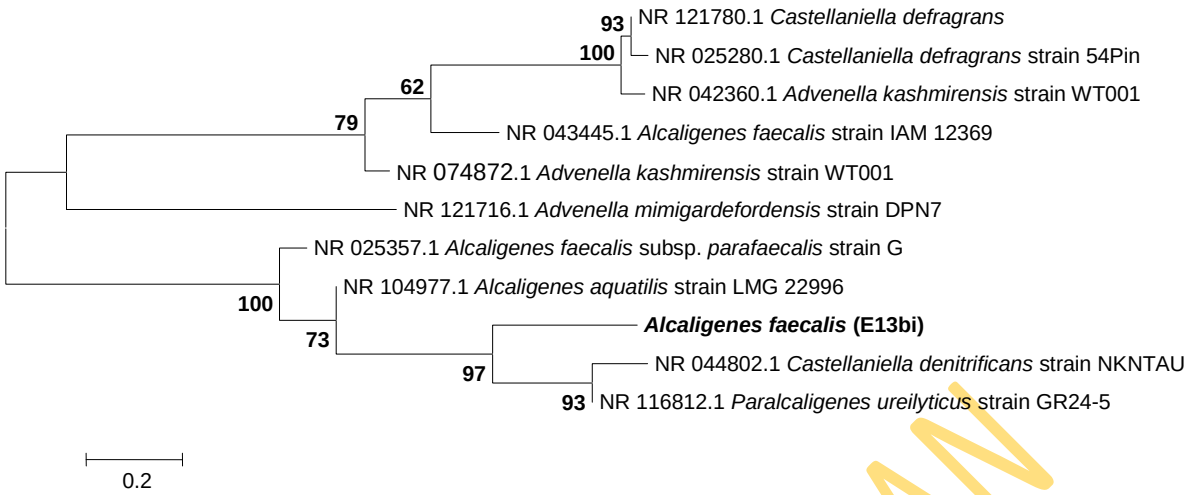

Figure A 23a: Evolutionary relationship of *Alcaligenes faecalis* (E13bi)

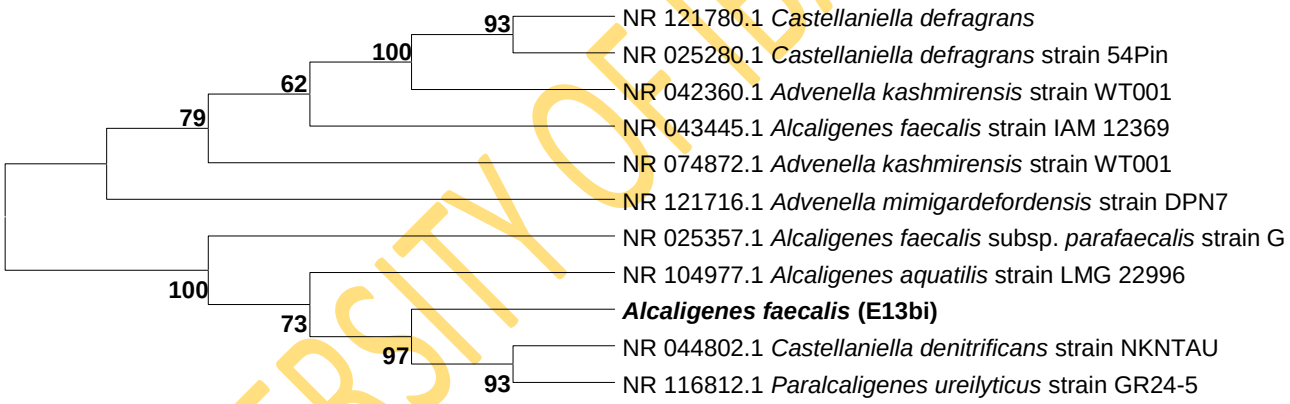

Figure A 23b: Evolutionary relationship of *Alcaligenes faecalis* (E13bi) showing topology

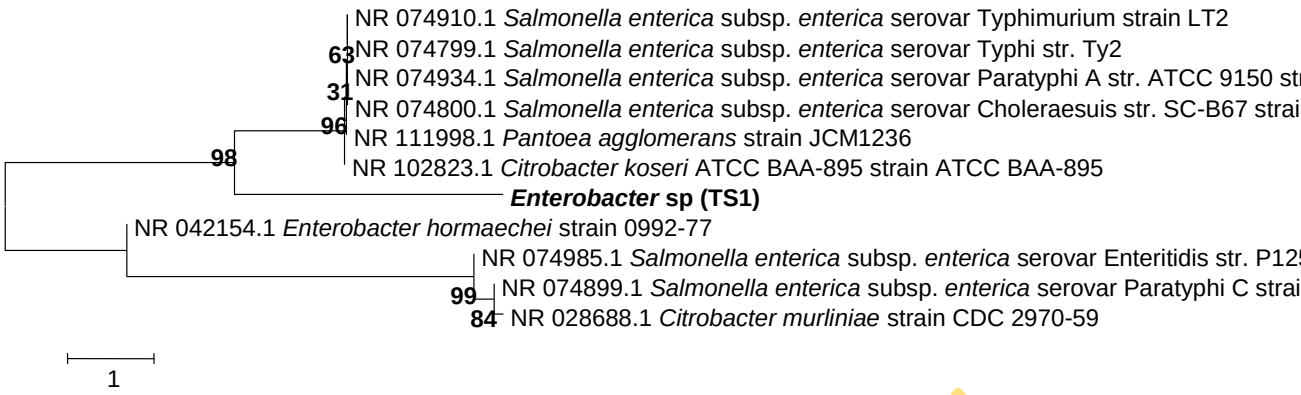

Figure A 24a: Evolutionary relationship of *Enterobacter* sp (TS1)

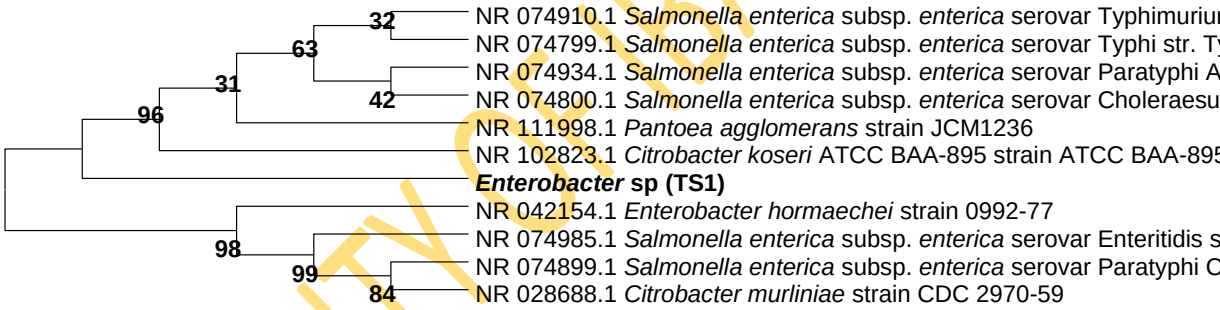

Figure A 24b: Evolutionary relationship of *Enterobacter* sp (TS1) showing topology

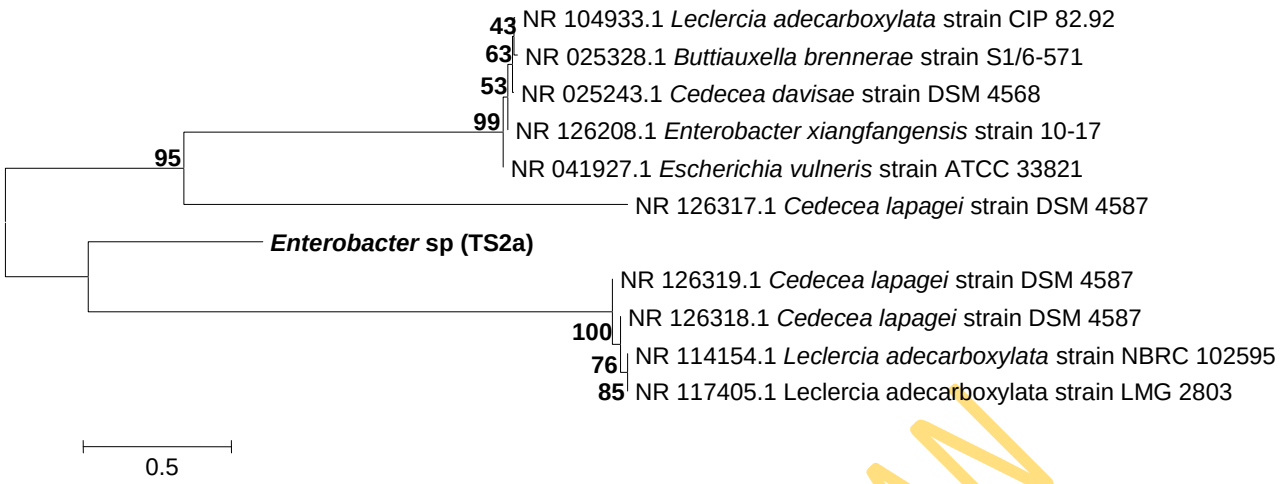

Figure A 25a: Evolutionary relationship of *Enterobacter* sp (TS2a)

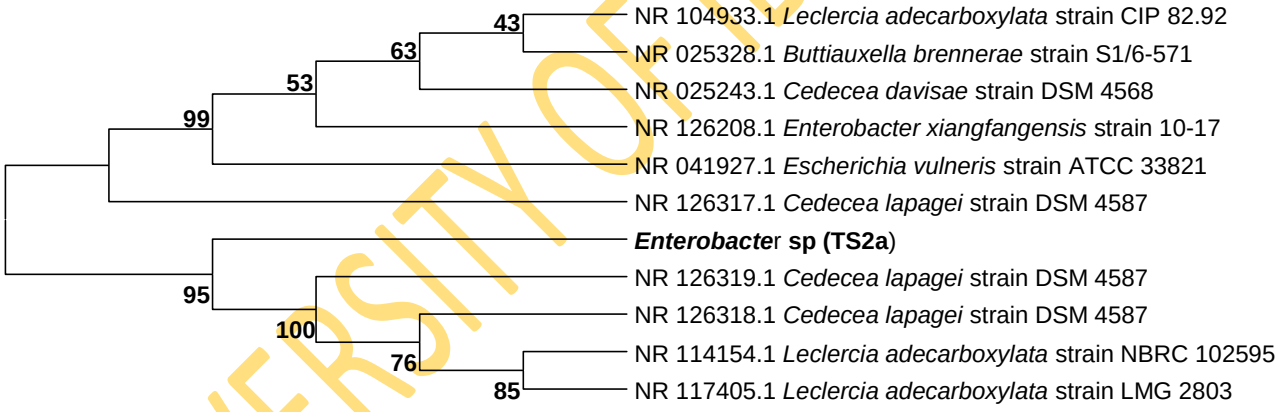

Figure A 25b: Evolutionary relationship of *Enterobacter* sp (TS2a) showing topology

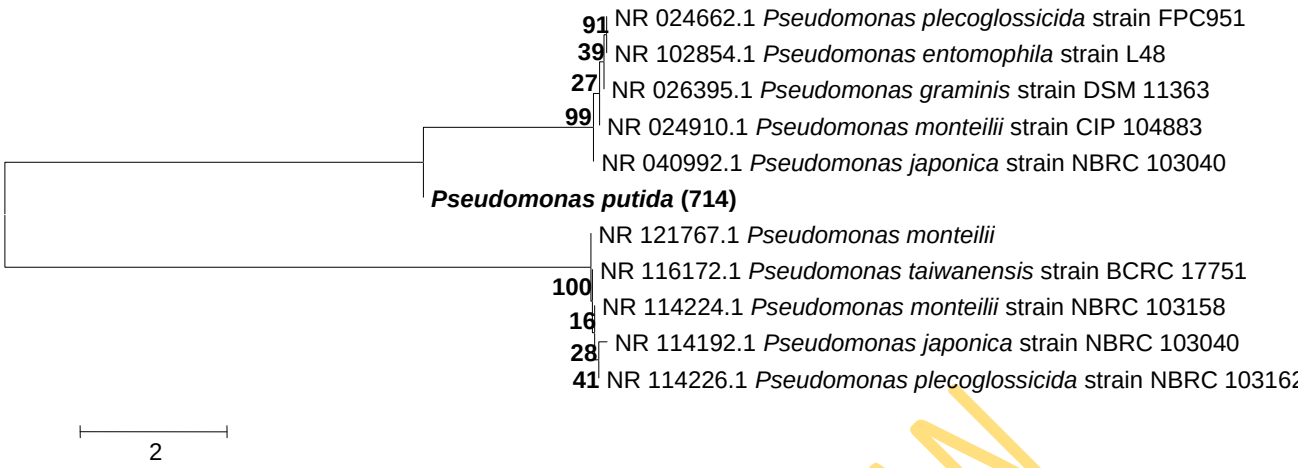

Figure A 26a: Evolutionary relationship of *Pseudomonas putida* (714)

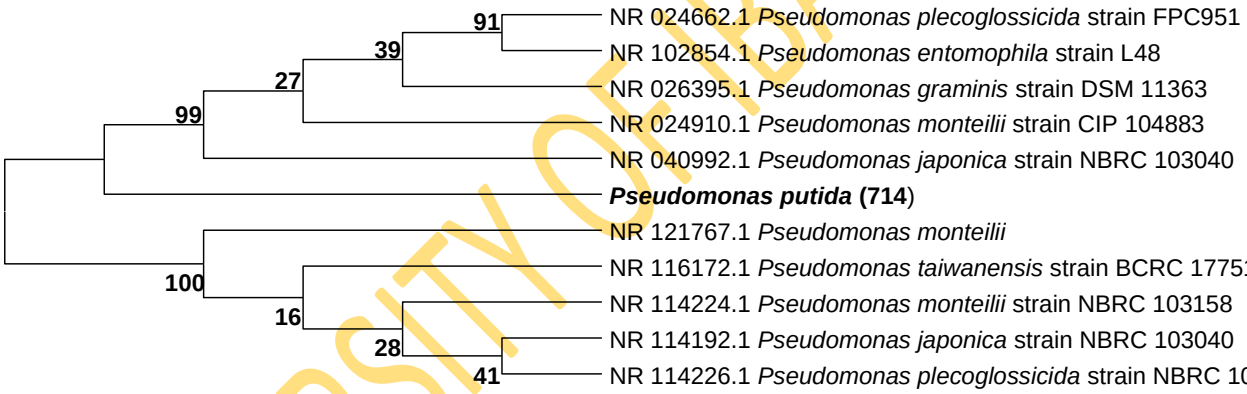

Figure A 26b: Evolutionary relationship of *Pseudomonas putida* (714) showing topology

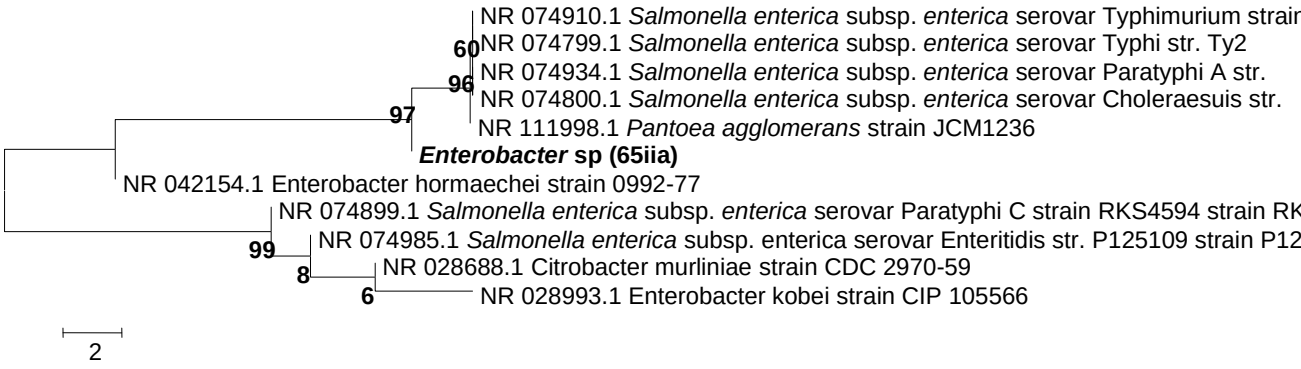

Figure A 27a: Evolutionary relationship of *Enterobacter* sp (65iia)

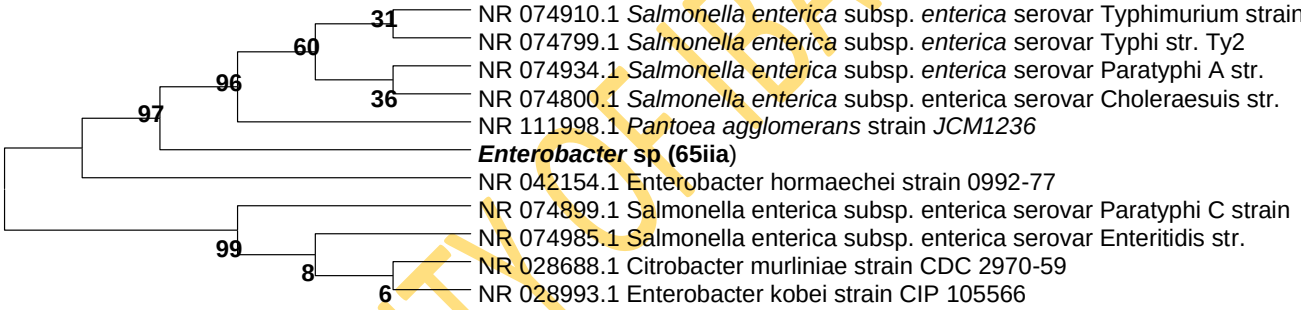

Figure A 27b: Evolutionary relationship of *Enterobacter* sp (65iia) showing topology

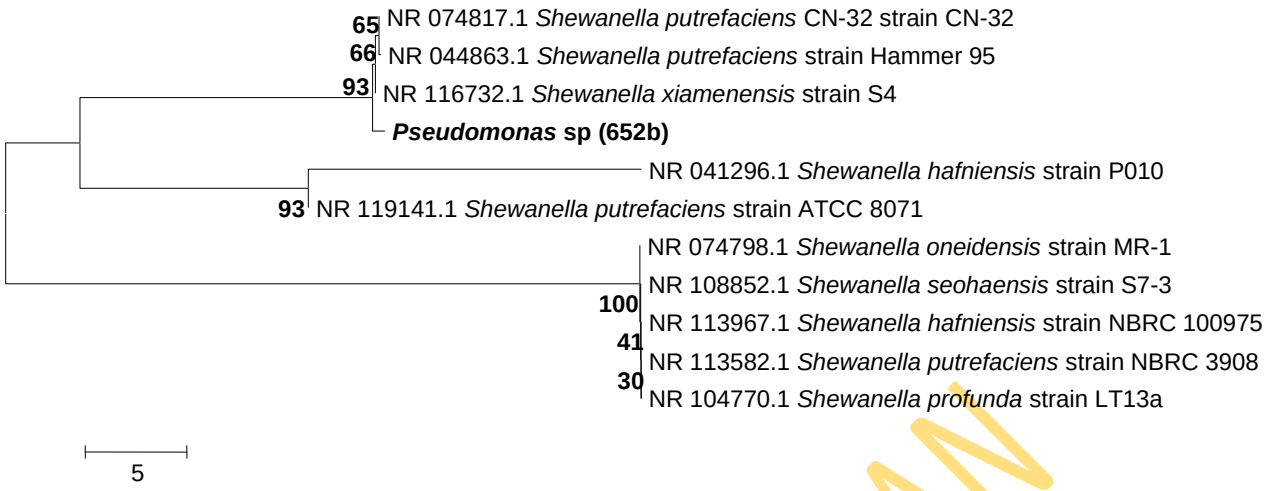

Figure A 28a: Evolutionary relationship of *Pseudomonas* sp (652b)

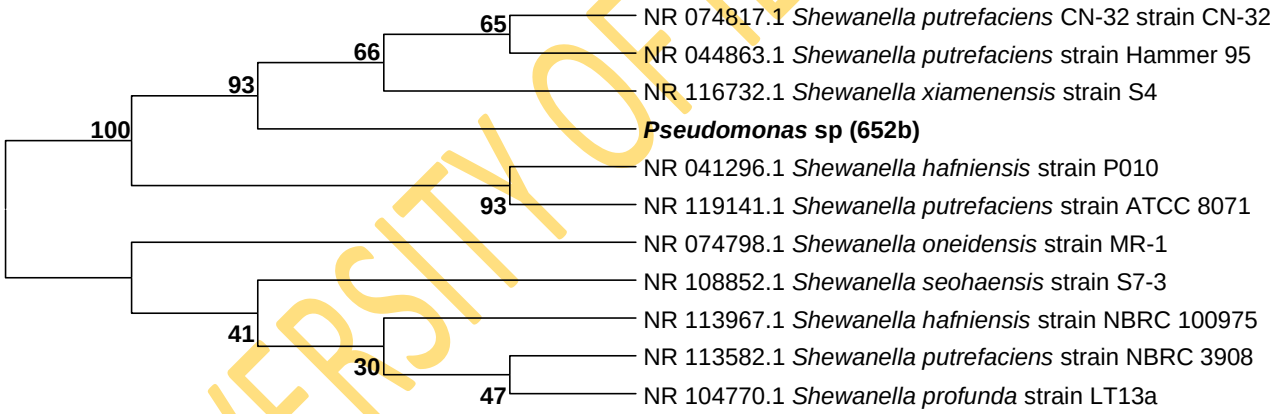

Figure A 28b: Evolutionary relationship of *Pseudomonas* sp (652b) showing topology

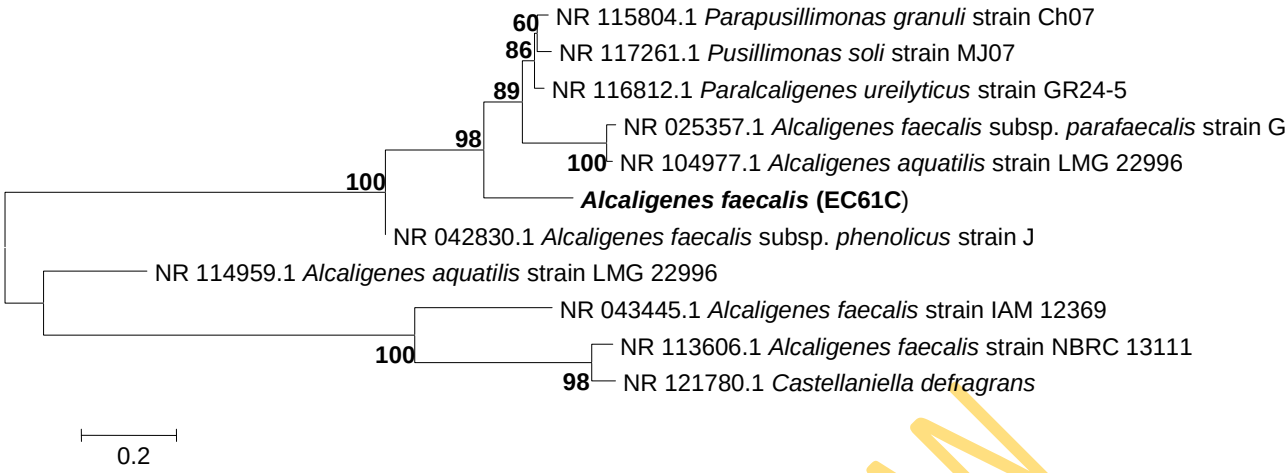

Figure A 29a: Evolutionary relationship of *Alcaligenes faecalis* (EC61C)

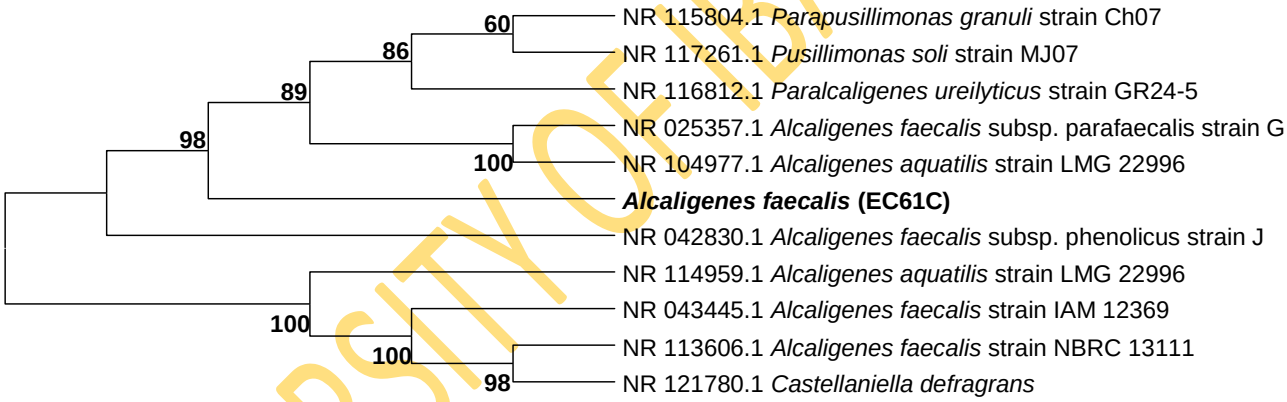

Figure A 29b: Evolutionary relationship of *Alcaligenes faecalis* (EC61C) showing topology

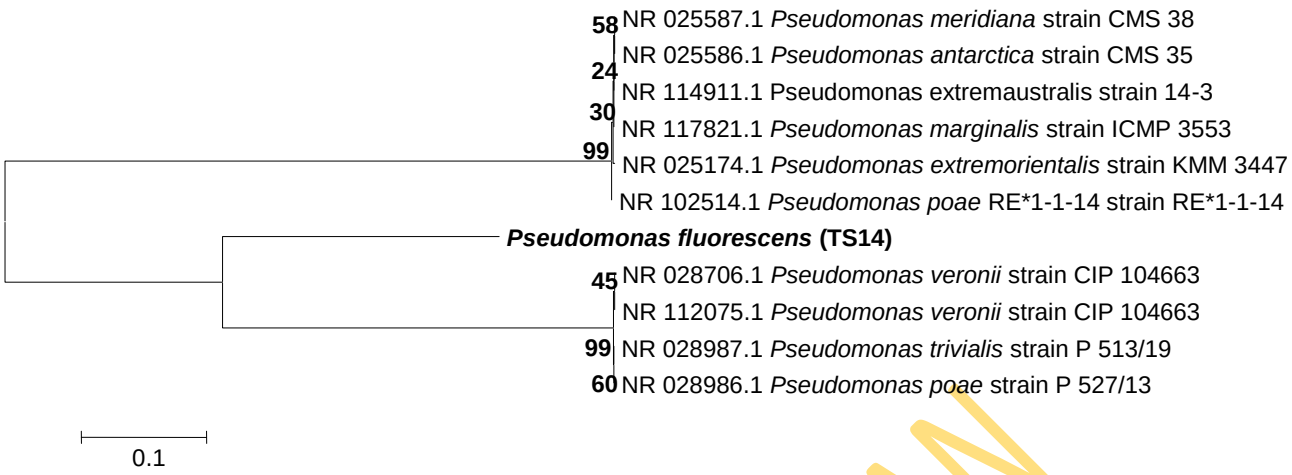

Figure A 30a: Evolutionary relationship of *Pseudomonas fluorescens* (TS14)

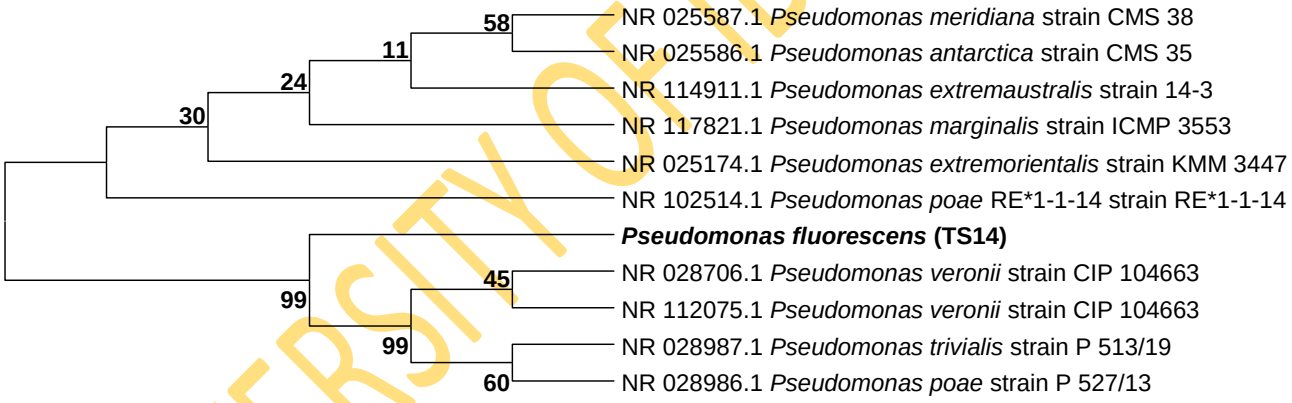

Figure A 30b: Evolutionary relationship of *Pseudomonas fluorescens* (TS14) showing topology

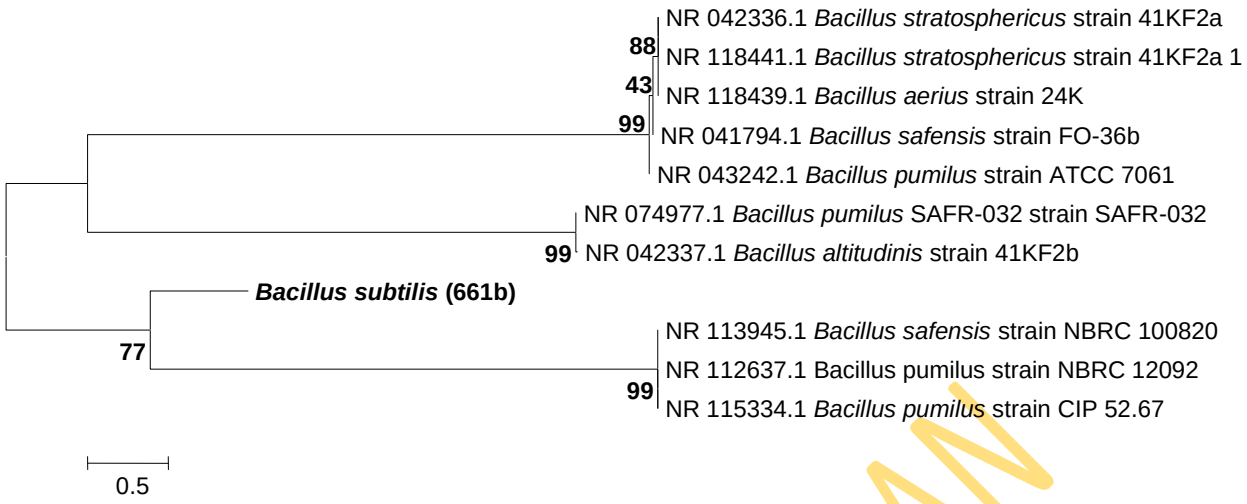

Figure A 31a: Evolutionary relationship of *Bacillus subtilis* (661b)

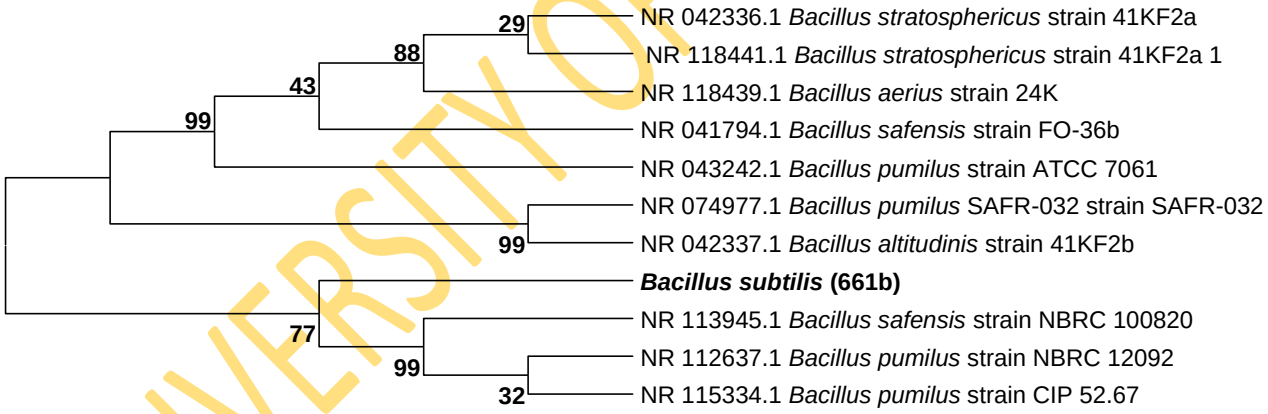

Figure A 31b: Evolutionary relationship of *Bacillus subtilis* (661b) showing topology

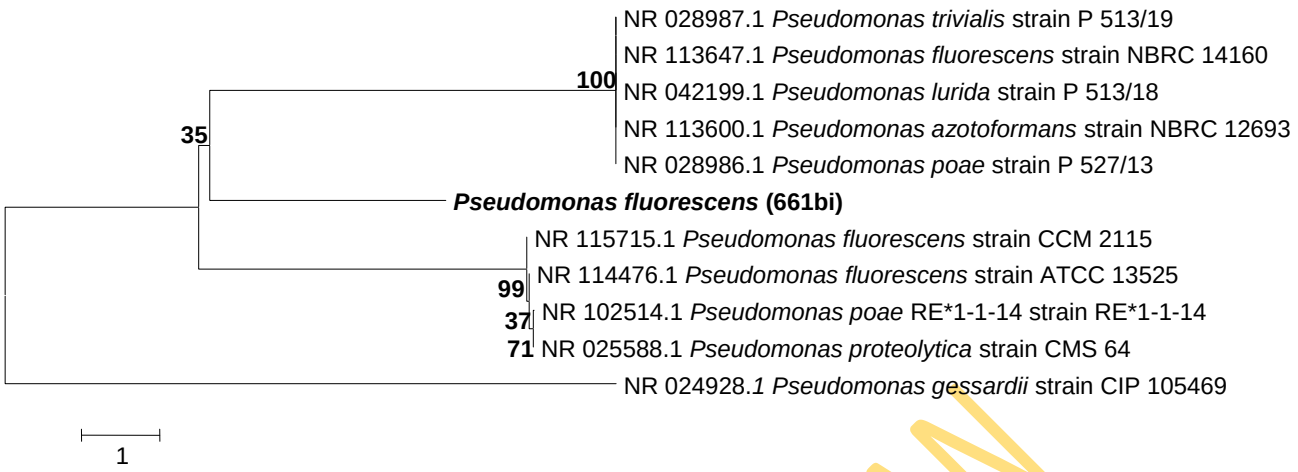

Figure A 32a: Evolutionary relationship of *Pseudomonas fluorescens* (661bi)

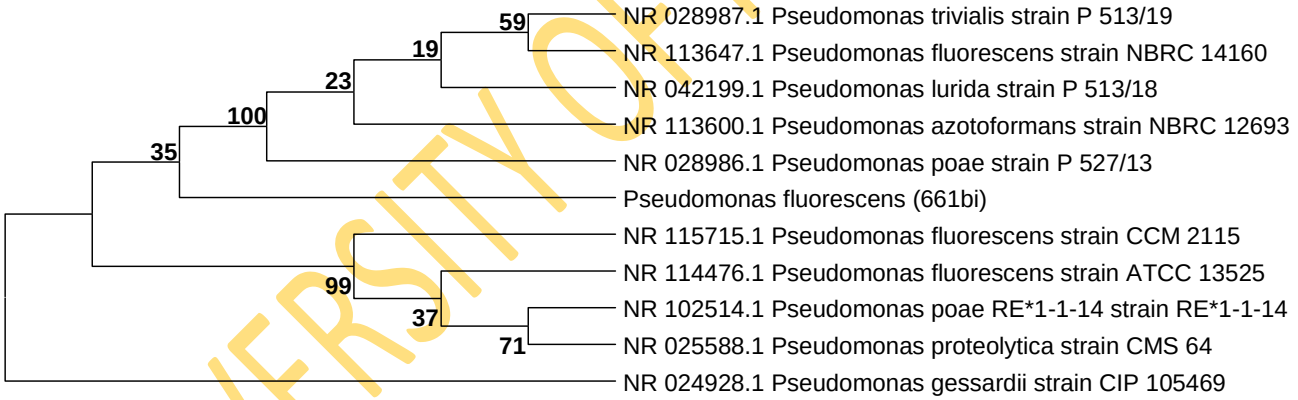

Figure A 32b: Evolutionary relationship of *Pseudomonas fluorescens* (661bi) showing topology

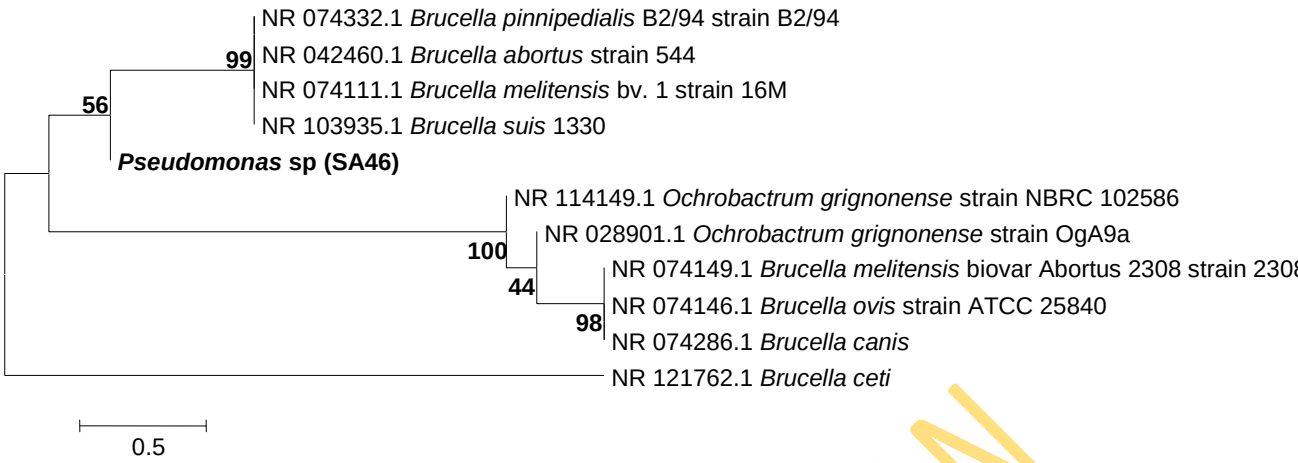

Figure A 33a: Evolutionary relationship of *Pseudomonas* sp (SA46)

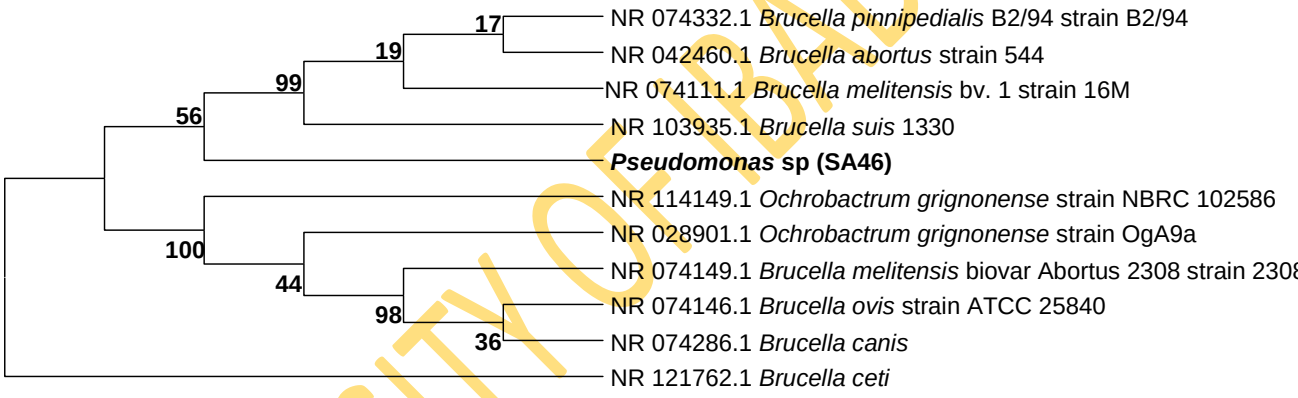

Figure A 33b: Evolutionary relationship of *Pseudomonas* sp (SA46) showing topology

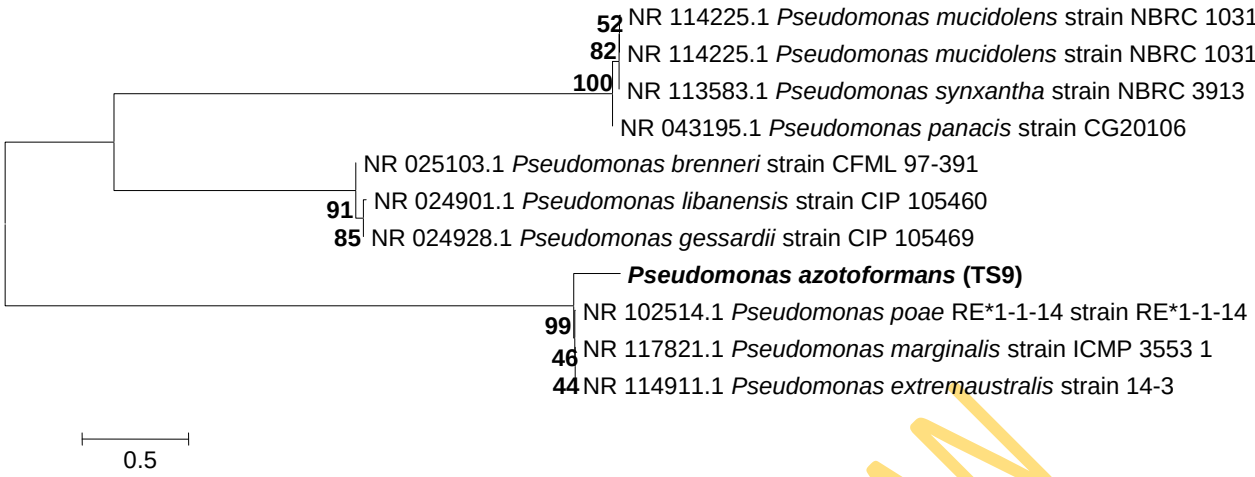

Figure A 34a: Evolutionary relationship of *Pseudomonas azotoformans* (TS9)

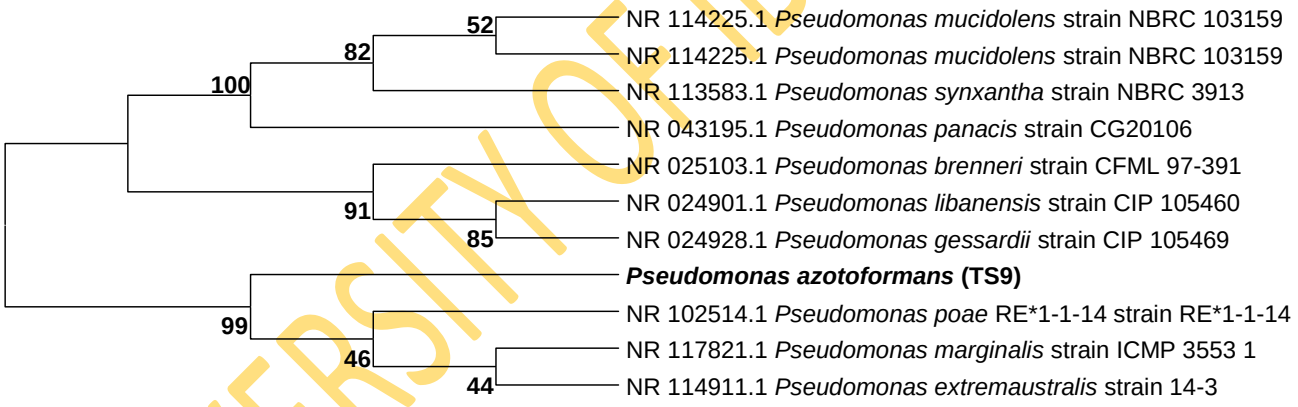

Figure A 34b: Evolutionary relationship of *Pseudomonas azotoformans* (TS9) showing topology

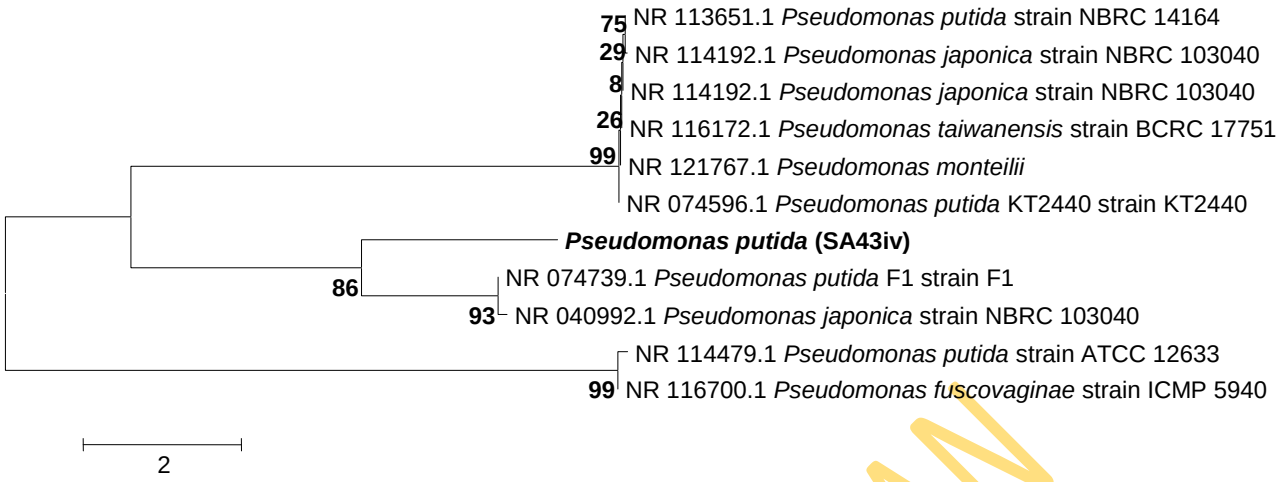

Figure A 35a: Evolutionary relationship of *Pseudomonas putida* (SA43iv)

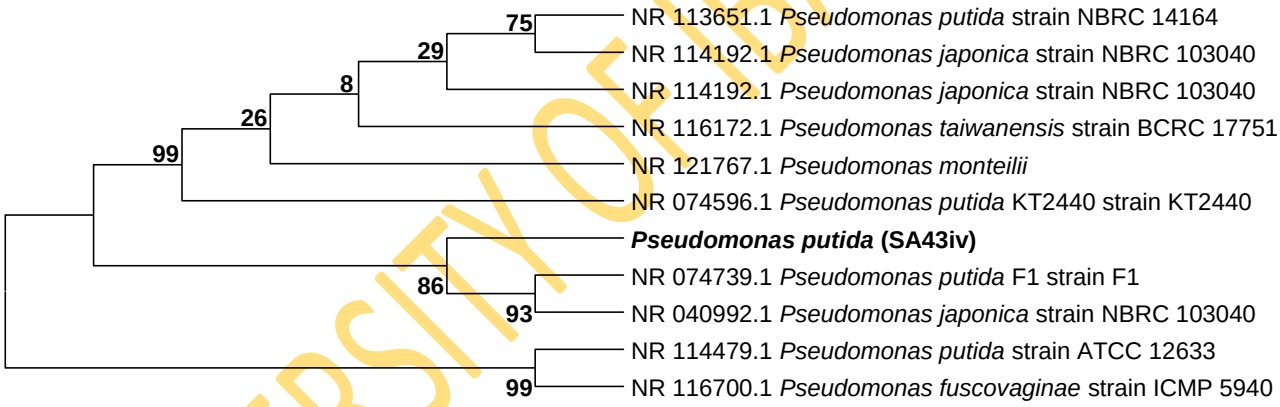

Figure A 35b: Evolutionary relationship of *Pseudomonas putida* (SA43iv) showing topology