APPLICATION OF MICROORGANISMS ISOLATED FROM FIBER CEMENT ROOFING SHEET WASTE AS A POTENTIAL HEAVY METAL REMOVAL AGENT.

BY

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DELTA STATE UNIVERSITY,

ABRAKA

APRIL, 2016.

TITLE PAGE

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PG/13/14/221634

A DISSERTATION SUMITTED TO THE POSTGRADUTE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE MASTERS OF SCIENCE IN MICROBIOLOGY, DELTA STATE UNIVERSITY, ABRAKA, NIGERIA.

APRIL, 2016.

CERTIFICATION

This dissertation titled “Application of microorganisms isolated from fiber cement roofing sheet waste as a potential heavy metal removal agent” by BALOGUN, Catherine, Ese meets the regulations governing the award of the degree of Master of Science, Delta State University and is approved for its contribution to scientific knowledge and literary presentation.

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DR. (MRS.) O.O. AKPOMIE **DATE**

(Head of Department)

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DR. (MRS.) O.O. AKPOMIE **DATE**

(Supervisor)

DECLARATION

I BALOGUN, Catherine, Ese declare that this dissertation titled **"**Application of microorganisms isolated from fiber cement roofing sheet waste as a potential heavy metal removal agent" is my research work and all the literature used or quoted there in has been acknowledged by complete referencing. This dissertation is an original work and has not been submitted and will not be submitted to any University or institution other than Delta state University, Abraka for the award of an M.Sc. degree

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BALOGUN, Catherine, Ese

DEDICATION

This project work is dedicated to Almighty God for His protection, provision and Love.

ACKNOWLEDGEMENT

I appreciate Almighty God who made it possible for this project work to be completed successfully.

Specially, I must acknowledge my supervisor and the head of department Dr. (Mrs.) O.O. Akpomie for her thorough supervision, patience, advice and support.

I want to express my profound gratitude to Prof. B.O. Ejechi and to all other lecturers in the department who have been there for me.

I am grateful to the various authors and researchers whose work has served as a reliable point of reference for this study.

The contributions of Mrs. Grace Denedo, Blessing Edah, Dr. Aghogho, Mr. Lari Bulouebibo, Odijie Abel, Omoregbe Stanley and Tola all the laboratory staff of the Department of Microbiology and Chemistry Delta State University, Abraka and the administrative staff of Department of Microbiology are greatly appreciated.

My profound gratitude also goes to my lovely husband Engr. Balogun, C.B.N, my children Gbenga Balogun, Gbemisola Balogun and Folashade Balogun, and my siblings Mrs. Josephine Frank and Mr. Avwerosuo Orovwigho for their love, support, encouragement and prayers.

To all who have supported me I say thank you and God bless

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ABSTRACT

Potential of heavy metal removal by indigenous microorganisms obtained from fiber cement roofing sheet waste was examined. The pH, electrical conductivity and metal concentrations were determined using pH meter, electrical conductivity meter and Atomic Absorption Spectrophotometer respectively. Enumeration and isolation of microorganisms were by pour plate method while identification was by standard microbiological protocol (Gram reaction, morphology and biochemical test). The ability of bacteria and fungi isolated to utilize the fiber cement waste as growth medium was tested. A total of fourteen (14) bacterial species and 7 fungal isolates were identified. *Bacillus* and *Proteus* species (bacteria), *Rhizopus* and *Microsporium* (fungi) were selected for the waste treatment tests based on their ability to grow on fiber cement waste medium. The Minimum Inhibitory Concentration (MIC) of the metals on the growth of bacterial and fungal isolates were subsequently determined. The MIC values for *Bacillus*, *Proteus*, *Microsporium* and *Rhizopus* species were 300mg/ml, 250mg/ml, 450mg/ml and 400mg/ml for Nickel; 250mg/ml, 350mg/ml, 450mg/ml and 400mg/ml for Chromium; 900mg/ml, 1000mg/ml, 700mg/ml and 750mg/ml for Cadmium respectively. The percentage reduction of Cadmium in treated samples with single isolates ranged from 5-33%; Chromium, 6-49% and Nickel, 4-23%, percentage reduction in treatment with all bacteria was Cadmium 22-56%; Chromium, 16-60% and Nickel, 5-37% while for all fungi the values were Cadmium 18-50%; Chromium, 17-56% and Nickel, 4-28%. The treatment with consortium of the isolates had the higher efficiency in the heavy metal reduction. Percentage reduction of Cadmium, Chromium and Nickel by the consortium was 31-75%, 20-78% and 7-52% respectively. When compared to the untreated samples, biological treatment with the selected isolates significantly (t-test, $p<0.01$) reduced the heavy metals to varying levels. The efficacy of the treatment was assessed by seed germination (beans and maize) in treated samples using germination index. Although analysis of variance (F 0.06-8.41, $p<0.01$-$p<0.05$) showed that the seeds germinated better in treated waste samples as indicated by one way ANOVA. Germination of the seeds improved and not significantly different from germination in untreated samples. It can therefore, be concluded that a biological treatment consortium comprising indigenous strain of *Proteus Bacillus*, *Rhizopus* and *Microsporium* species emerged from this study to be an effective, ecologically friendly and cost-effective treatment alternative since the consortium demonstrated a higher percentage of heavy-metal removal. This study demonstrates that microorganisms from fiber cement waste, soil without fiber and waste dumpsite have potential to be used as an alternative bioremedial tool for treating fiber cement waste containing contaminated with heavy metals.

CHAPTER ONE

INTRODUCTION

Over the last few decades, there has been an increase in industrialization worldwide with Nigeria not left out. Several industries such as leather, paper, rubber, electroplating, iron, aluminium, steel, asbestos, zinc, fiber cement and steel-related production mills have sprung up resulting in the increase in discharge of pollutants to receiving waters, causing undesirable effects on the aquatic environment (AAC, 2001; Ipeaiyeda *et al.*, 2012). As part of providing shelter for mankind, roofing was developed as a building envelope thus resulting in a growth in the roofing industry. The roof is the covering in the uppermost part of a building or shelter which provides protection from animals and weather, notably rain or snow, but also heat, wind and sunlight. Roofs are made of a variety of materials and most, with the exception of those made from grass/reed, thatch, and potentially toxic materials. Roofs are basically used for beautifying, shelter, safety, comfort and securing buildings against harmful sources. Typical roofing materials are metal sheets, ceramic tiles, rock slate, fiber cement and ferro-cement. Fibre cement is a composite building and construction material, used mainly in roofing and facade products because of its strength and durability. Fiber cement roofing sheet are usually cooler, durable, produce lesser heat, not easily rust and not hot during hot temperature or change in temperature. In fibre cement there is a fibre re-inforcement, which contributes to making the fibre-cement material even stronger for roofing. Together with a carefully planned production process, fibre cement makes it possible to develop strong and long lasting construction materials.

Today fibre cement is considered as a material physically suited for construction products such as cladding and roofing which is primarily due to its function, performance and commercial value. Originally, the reinforcing fibres in

fibre cement were of asbestos and the material was commonly used as siding in buildings due to its low cost, fire-resistance, water tightness, light weight, and other useful properties (Zheng and Antonio, 2005). However, asbestos fibres are inevitably released during machining of the fibre-cement products, and by long-term erosion of the material after it has been exposed to atmospheric weathering and wind, which causes the cement to degrade after disposing in the soil through landfilling.

Waste including roofing sheet waste is enormous in most African countries, with dumping taking place in landfills and sometimes with other hazardous material, and in other instances left on the site, often in the case of smaller construction sites (Salam, 2010). Occupational health concerns and the protection of workers in the fibre-cement factories have finally led to the progressive elimination of asbestos from these products. Hazards attributed to fiber cement include zinc, copper, cadmium and lead being present at quite high levels in roofing sheet waste or fittings (lead and copper flashings) (Gould 1993; Thomas and Greene, 1993). The asbestos fibres are intimately bound to the cement matrix and were considered to be immobilized in the cement and therefore less prone to be released in the environment, suspended in the air, and inhaled in the lung than in other materials or applications (Ipeaiyeda *et al.*, 2012).

Due to high economic activities in several industrial sectors and concomitant increase in projected use in the future, effluents emanating from production industries can also follow the same trend, resulting in increased concentration of heavy metals in effluent discharged into the environment. Heavy metals are present in most roofing materials and having relatively high density in low concentration (Irma *et al*., 2013) and increasing toxicity of heavy metals in the environment may eventually reach human bodies through the food chains;

necessitating the need for its removal. Removal of heavy metals from solution has been carried out mostly by adsorption of chemical materials (Choski and Jozi, 2007; Al-Muhtaseb *et al*., 2008). Conventional methods to remediate heavy metals contaminated site are excavation and solidification/ stabilization, these temporarily remove heavy metals and have the disadvantages (Bahi *et al.*, 2012) of cost-effectiveness limitations, generation of hazardous by-products or inefficiency. Biological methods solve these drawbacks since they are easy to operate, do not produce secondary pollution. Microorganisms, plants and algae are usually used for the removal of heavy metals in a process known as bioremediation. This process which involves the use of biological agents such as yeast, fungi or bacteria is increasingly considered for clean-up of metal contaminated and polluted ecosystem (Helena, 2003). The isolation of heavy metal resistant microorganisms and the understanding of the mechanisms (redox interaction, Van der Waals forces, electrostatic interaction, covalent bounding and extra cellular precipitation or combination these process). The negatively charge groups (carboxyl, hydroxyl, phosphoryl) of the bacterial cell wall adsorb metal cations which are then retained by mineral nucleation which may contribute to the development of improved bioremediation processes. The microorganisms involved in the removal of fiber cement in roofing sheet include *Bacillus pseudifirmus, Bacillus cohnii, Sporosarcina pasteurii, Bacillus pasteurii, Arthrobacter, Crystallopoietes, Lysinibacillus fusiformis, Micrococcus sp.* and *Pseudomonas putida.* Rod shaped bacteria were found embedded in calcite crystals which proved that bacteria act as the source of nucleation (Surajana *et al*., 2009).

Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated environment. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Kumar *et al.*, 2011; Asha *et al*., 2013).

General objectives

The general objective of this research is to isolate indigenous microorganisms that are capable of removing or reducing some heavy metals present in waste generated from Eternit roofing sheet, Sapele.

Specific objectives are to;

a) isolate and identify microorganisms associated with fiber cement roofing sheet wastes
b) select the isolates that are capable of growing on the roofing sheet waste medium
c) determine the physical parameters and heavy metal concentration of the waste
d) determine minimum inhibitory concentration of the metals against the isolates
e) determine the effect of treatment with selected single and combined microbial isolates on the level of heavy metals in the waste
f) determine the effect of microbial treatment of waste on seeds germination of selected plant crops (Maize and beans).

Hypothesis

a) Microorganisms are associated with roofing waste
b) Indigenous microorganisms can reduce the level of metal pollutants
c) Microbial consortium treatment yield a better removal of waste than single microbial treatment.

Justification of study

Roofing sheet waste (fiber cement waste) consist of the materials by their volume ratio. 40% bonding agent, 11% addictive, 2% reinforcing fibers, 5% process fibers, 12% waste and 30% air. The bonding agent in the product is cement which is synthesized from lime stone and clay mari is proportionally the most

significant of the material. Cement is made up of four major compound tricalcium silicate, dicalcium silicate, Tricalcium aluminate and Tetracalcium aluminoerrite and other metals, some of which are heavy metals. These metals are toxic to the environment and to human, when they enter the food through the food chain and are consumed by man and animal (Malanic, 2004). When humans inhales air contaminated with these metals or consume fish, fruit and vegetables that have accumulated metals from soil and water or drink metal contaminated water, it result in serious health effects such as reduced growth and development, causes cancer, nervous system damage and brain damage.

Unfortunately many industries do not treat the waste before discharging into water bodies and soil because of the cost of chemical treatment. This serves as stimulus for researches into alternative less costly and ecofriendly treatment, This study is justified because of the common problems associated with conventional treatment namely the difficulty encountered in treating solid waste. Microorganism that can render metal innocuous by transformation exit in nature. These organisms can be employed in the treatment of solid waste since biological treatment break the waste into smaller volume which are subsequently easier to dispose of appropriately.

Significance of the study

Since contamination of soil and ground water by indiscriminate discharge of industrial waste has become a significant problem today, a number of technologies has been investigated to remedy the situation. Treatment processes have incorporated chemical, physical and biological methods or a combination of them. Treatment options include excavation, fixation, leaching, landfill disposal, surfactant application and a host of others that are expensive, environmentally unfriendly and only transfer the contaminants from one place to another.

Bioremediation technology is inexpensive, naturally and environmentally friendly. The use of microorganisms in the removal of heavy metal from fiber cement roofing sheets waste can help minimize the prohibitive cost associated with soil remediation, prevent soil texture alteration, prevent the transfer of pollutants from one medium to another and ensure a healthier technique for remediating heavy metals. Not much work has been carried out in the area of bioremediation of fiber cement waste.

CHAPTER TWO

LITERATURE REVIEW

2.1 Roofing sheet

Shelter is the basic need of all human beings and shelter needs roof and wall cladding for protection hence the establishment of roofing sheet industries. Different roofing sheet companies use different materials for the manufacturing of roofing sheets such as Aluminum, Zinc, Asbestos, Metals, steel, fiber cement, copper. Roofing provides the main protection against direct solar radiation. Cost, durability, aesthetics and environmental impact are put into consideration when selecting a roof. Eternit limited is one of Nigeria's major producer of fiber cement building material which is a roofing sheet material.

The roofing sheet manufacturing company initially uses asbestos for their roofing sheets but was discontinued in the last 14 years because it was acknowledged that exposure to asbestos is harmful to health, being directly related to a number of life threatening diseases including asbestosis, pleural mesothelioma lung (lungs cancer) (Barker *et al*., 2006). Waste from eternit roofing sheet are termed inert or nuisance dust and are classified under the red category because they contain heavy metals such as Nickel, lead, cobalt, chromium that are hazardous to man, animals and plant (Zeyede *et al.*, 2010).

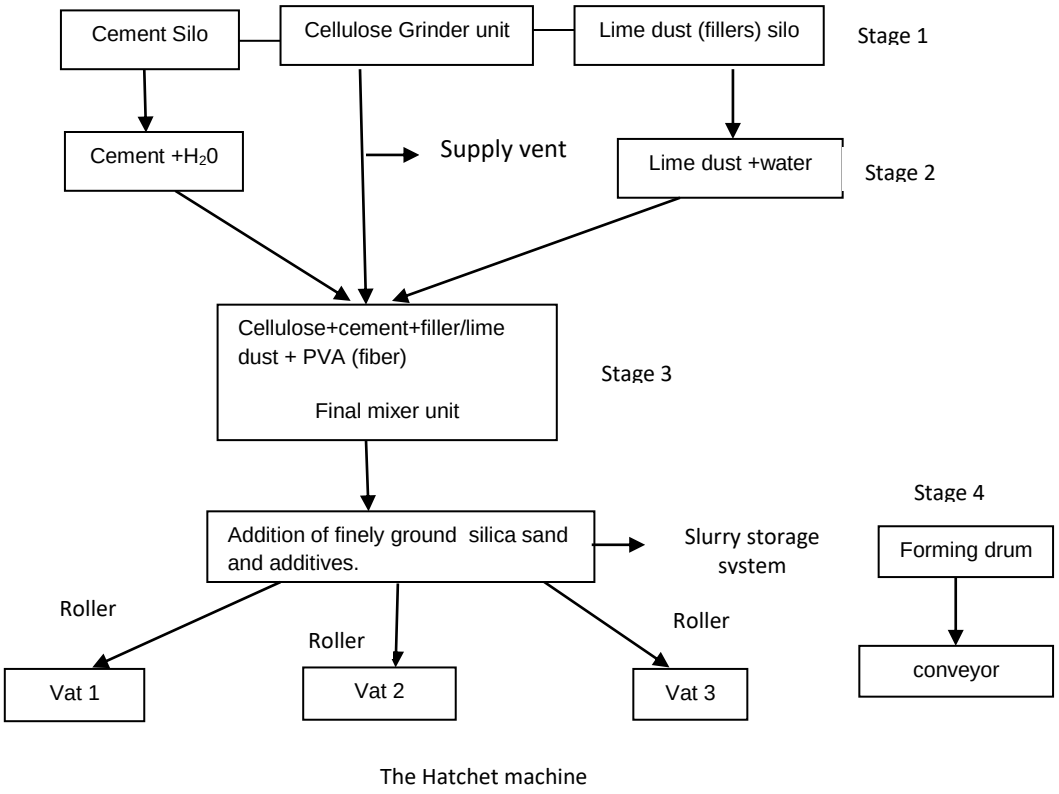

Figure 1:Production of fiber cement roofing sheet

Source**:** (Zeyede *et al.*, 2010)

2.1.1 Waste

Waste is any substance, article or goods that their owners cannot use or want to get rid off. Waste may be categorized according to its origin (domestic, industrial, commercial, construction or institution), according to its contents (such as organic materials, glass, metal, plastic paper), or according to hazard potential (toxic, non- toxic, flammable, radioactive, infectious) (Ray, 2008). Industrial waste is defined as waste generated by manufacturing or industrial processes. It may be solid, liquid or gases. These three categories of waste are closely interrelated, both as they impact on the environment. Solid waste disposed of in soil can influence the quality of ground water and surface waters by way of leachate entering the

ground, and travelling with it through the ground, then entering a surface water body (Woodard, 2001).

2.1.2 Roofing sheet waste

Waste generation in quantity and variety has increased due to acceleration of urban population growth and increase in spontaneous settlements (Achankeng, 2003). Much building waste made up of materials such as bricks, concrete and wood damaged or unused for various reasons during construction can be as high as 10 to 15% of the materials that go into a building. Since considerable variability exists between construction sites, there is much opportunity for reducing this waste (Bogner *et al.*, 2007). According to Ferguson *et al*. (1995), over 50% of the waste in a typical United Kingdom landfill could be construction waste. Craven *et al*. (1994) reported that construction activity generates 20 to 30% of all waste deposited in Australian landfills. Direct dumping of untreated building/construction wastes in rivers, seas, and lakes, result in the accumulation of toxic substances in the food chain through the plants and animals that feed on them (Medina, 2002) which seriously affects the health of residents located closer to dumpsites.

Studies have shown that soil and groundwater system can be polluted due to poorly designed waste disposal facilities, leakage from underground storage tanks and agricultural wastes. Soil and groundwater acidification and nitrification have been linked to roofing waste dumps (Bacud *et al*., 1994) as well as microbial contamination of soil and groundwater system (Awomeso *et al*., 2010). Sia Su (2008) attributed cancer, heart diseases and teratogenic abnormalities to groundwater contamination via leachate from waste dumps. Increase in population and rapid expansion of cities has resulted to generation of huge waste and the

improper method of disposal of these wastes constitutes serious health and environmental problems.

Table 1: Composition of Fiber Cement

Percentage composition of fiber cement	Chemicals component
Hydraulic binder (Portland cement)	CaO, SiO_2, Al_2O_3, Fe_2O3, MgO, So_2, NO_2, Na_2O_2, K_2O, Ca_2SiO_5
Trace element in Portland cement	Cd, Cu, Zn, Lead, Ni, Cr
Additives	Ca_2SO_4, Fly ash, ground silica, Calcium silica hydrates Silica fume.
Reinforcing fiber	Fiber ash
fiber (short and long)	Cellulose fiber, PVA, polyacrylonitrile, PVC, polyacrylamide, lignin, hemicelluloses, pectin, fly ash, ground silicon sand
Hydrophobe	Zinc Stearate, Silans siloxans
Viscosity enhancing agent	Hydroxyethyl methycellulose
Filler	Calcium carbonate, Chromium oxide, Iron oxide, polypropylene fiber

Source: (Zeyede *et al.*, 2010)

2.1.3 Heavy metal in roofing sheet waste

A toxic heavy metal is any relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts. The term has particular application to cadmium, mercury, lead and arsenic, all of which appear

in the World Health Organisation's list of 10 chemicals of major public concern. Toxic heavy metals are found naturally in the earth such as CaO, SiO_2,Al_2O_3, Fe_2O3, MgO, So_2, NO_2, Na_2O_2,K_2O, Ca_2SiO_5, and become concentrated as a result of human caused activities. Contamination of soils, groundwater, sediments, surface water, and air with hazardous heavy metals and toxic chemicals is one of the major problems facing the world today. The need to remediate these natural resources (soil, water and air) has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal because of their potential to enter the food chain (Asha *et al*., 2013). Heavy metals found in fiber cement waste include Calcium carbonate, Chromium oxide, Iron oxide, lead, nickel, cadmium and mercury. Lead influences the nervous system and slowing down nerval response. In the environment lead is known to be toxic to plants, animals and microorganisms (APHA, 1992).

Mercury; the primary focus is on methyl mercury originating from the diet in particular through the consumption of fish and fish products. Cadmium accumulates especially in the kidneys leading to dysfunction of the kidney with increased secretion of proteins in urine (proteinuria) and other effects. An increase in the content of cadmium in agricultural soil will result in an increased uptake of cadmium by plants. In the environment cadmium is reported toxic to especially animals and microorganisms. Cadmium is known to significantly influence leaf litter decomposition by microorganisms.

2.1.4 Hazards of Fiber cement Roofing Sheet Waste

In a worldwide sense, heavy metal contaminated environments represent a common environmental problem constituting a major hazard for ecosystems and human health with expensive clean-up costs. The input of heavy metals by industry and agriculture has led to the release and improper disposal of enormous amounts

of heavy metals (Ansari and Malik, 2007). Heavy metals can be found in soils as free cations, as complexes (e.g. $CdCl^{3-}$, ZnCl+) with organic and inorganic ligands, and associated with soil colloids (Wang *et al.*, 2010), they can accumulate in biological systems finding their way into the food web via different mechanisms. The properties of soil being a complex mixture of materials of mineral (e.g. clay) and organic (e.g. humic substances) origin, aqueous and gaseous components and dynamic system with variations in moisture content, pH and redox conditions. Soil and heavy metal interactions can be understood on the basis of ion exchange, surface adsorption and/or chelation reactions. These contaminated soils and sediments harbour organisms, both prokaryotes and eukaryotes, able to deal with pollution (Zettler *et al*., 2002; Baker and Banfield, 2003). Microorganisms are key elements for recycling nutrients and heavy metals imposes a chronic stress upon the decomposer subsystem, and a variety of experimental systems and regimes have been investigated. Some of these organisms have the ability to modify the physicochemical conditions of their surrounding environment either by detoxification, metal homeostasis, precipitation or solubilization, redox transformations or by metabolic exploitation (Bruneel *et al*., 2006; Hetzer *et al*., 2006; Guiné *et al*., 2007)

The environmental stress caused by heavy metals, generally decreases the diversity and activity of soil bacterial populations leading to a reduction of the total microbial biomass, decrease in numbers of specific populations such as Rhizobia and a shift in microbial community structure (Sandaa *et al*., 1999; Wang *et al*., 2010). Soil microbial population responses to heavy metal contamination provide a relevant model for ecological studies to assess the influence of environmental characteristic. Several studies have demonstrated that metals influence microorganisms by affecting their growth, morphology and biochemical activity (Tsai *et al*., 2005; Pérez-de-Mora *et al*., 2006) and diversity. The response of the

bacterial populations to heavy metal contamination depends on the concentration and bioavailability of metals itself and is dependent by multiple factors such as the type of metal and microbial species. High concentrations of metals (both essential and non-essential) harm the cells by displacing the enzyme metal ions, competing with structurally related non-metals in cell reactions and also blocking functional groups in the cell bio-molecules. Microbial survival in heavy metal polluted soils depends on intrinsic biochemical properties, physiological and/or genetic adaptation including morphological, as well as environmental modifications of metal speciation (Abou-Shanab *et al*., 2007). Studies on the effects of metals on soil bacteria have been conducted showing that short term contact causes the selection of resistant bacteria within weeks. A more prolonged exposure to metals slowly selects resistant bacteria. On the other hand long term exposure to metals leads to the selection/adaptation of the microbial community which then thrives in polluted soils (Pérez-de-Mora *et al*., 2006; Chihching *et al*., 2008). The presence of different metals together may also have greater adverse effects on the soil microbial biomass/activity and diversity than those caused by single metals at high concentrations (Renella *et al*., 2005).

Study of the adaptive microbial responses usually focuses on the phenotypic changes observed. Adaptation can also be accessed via the characterisation of the molecular mechanisms of resistance. Different techniques can be employed to investigate these mechanisms such as PCR, DNA hybridisation and subsequent analysis of restriction fragment length polymorphism (RFLP), or amplified ribosomal DNA restriction analysis (ARDRA) (Guo *et al*., 2009). A great advantage these techniques have over the more traditional techniques is that they can be targeted specifically to genes revealing the molecular mechanisms of adaptation. The ability of some microorganisms to tolerate heavy metals and the ability of some to promote transformations that make some metals less toxic, make

organisms that live in heavy metal contaminated sites potentially useful in bioremediation. Bioremediation strategies are dependent on the knowledge of the *in situ* microbial diversity targeting the most resistant strains. By characterising the microbial communities, the taxa able to survive and remain active in the extreme environments can be identified and potentially targeted for bioremediation purposes (Akob *et al*., 2007). In order to optimize and develop remediation processes, more studies about the bacterial populations that inhabit these sites are required.

2.1.4.1 Chemical hazards

Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. Contamination of soils, groundwater, sediments, surface water, and air with hazardous fiber cement roofing sheet waste and toxic chemicals is one of the major problems facing the world today. The migrating metals are intercepted and immobilized by precipitation with biologically produced H_2S (Asha *et al.*,2013).Toxic metals readily bind to sulfhydryl group of proteins. Production of sulphur dioxide which reacts with the atmosphere and falls back as acid rain. Production of carbon monoxide which is a product of incomplete combustion. Production of carbon dioxide in the atmosphere which reacts with methane resulting in the promotion of global warming and depletion of the ozone layer which can result in skin cancer, eye cataracts (Woodard, 2001). Silica when it is released as fine particles and inhaled causes potentially fatal lungs diseases. Exposure to cellulose fiber dust can lead to inflammation or scaring of the lungs in humans.

Heavy metals such as mercury, lead, chromium cobalt which are found in the waste are disastrous.

Lead: Absorption of lead may have a severe danger to public health. Effect of lead include colic, constipation, anaemia, and harm to central nervous system. Causes problems in the synthesis of haemoglobin (Woodard, 2001).

Cadmium: Cd has no essential biological function and is thus highly toxic to living organisms. Chronic exposure to cadmium in humans has several toxic effects, such as high blood pressure, kidney, lung, liver and testes damage (Manahan, 2004; Baird and Cann, 2005). Cd is also associated with a disease called Itai-Itai, meaning "it hurts" in Japanese (Baird and Cann, 2005), and it is characterised by bone pain, pathological fractures and signs of renal impairment (Marazioti, 1998).

Nickel: Higher contact with nickel result in lungs disorder (Al- Othman *et al*., 2011).It prevents plants growth, uptake of nutrients, physiological as well as metabolic processes. This also affect chlorosis, harm to root tips, minimize water and uptake of nutrients and impairment to enzymes (EC, 2006).

Chromium: The most widespread human effect is chromium allergy caused by exposure to chromium (especially Cr(VI) compounds) in the working environment. Chromium compounds are also assumed to cause cancer. Environmentally Cr(VI)-compounds are generally considered the most toxic. The content of heavy metals in waste is primarily a consequence of the intended use of heavy metals in industrial products. At the end of their useful life all products will end up in waste to the extent they are not attractive for recycling (Valls and de Lorenzo, 2002). Heavy metals may, however, also be lost to waste during production and use phases. Losses in the manufacturing process are often disposed of as manufacturing waste, while products may be exposed to wear and tear inclusive corrosion during the use phase.

2.2 Interactions of microorganisms with heavy metals

Low concentrations of certain metals such as zinc, copper, cobalt and nickel are essential for the metabolic activity of bacterial cells. Other metals like Pb, Cd, Hg and Cr have no known effects on cellular activity and are cytotoxic (Chen *et al*., 2005a; Abou-Shanab, 2007). It is known that microbial activity plays an important role in the metal speciation and transport in the environment. In high concentrations, heavy metal ions become toxic to cells. Due to the fact that some heavy metals are necessary for enzymatic functions (Zn) and growth, the cell has different mechanisms for metal uptake, this can be accomplished by bioaccumulation or biosorption.

Bacterial surface structures are of extreme importance to understand their interactions with the surrounding environment, especially with metals. Some of the cellular structures of microscope interact with metal ions, some of the mechanisms used in the removal of heavy metals include bioaccumulation, biosorption, bioaumentation and biostillmulation. Bioaccumulation is a substrate specific process, driven by ATP (Spain and Alm, 2003; Errasquin and Vazquez, 2003) and is an active process of heavy metal uptake. Three mechanisms of metal transport into the bacterial cell are known: passive diffusion, facilitated diffusion and active transport. Some of the active transport systems are metal selective. However, there are some exceptions, Cd can be transported by the same transporters as Zn. A disadvantage of bioaccumulation is the recovery of the accumulated metal which has to be done by destructive means leading to damage of the biosorbent structural integrity (Ansari and Malik, 2007).

Biosorption refers to other mechanisms that are driven by the chemiosmotic gradient across the cell, not requiring ATP and it is primarily controlled by physico-chemical factors. These include adsorption, ion-exchange and covalent bonding and may occur either in living or dead biomass and is considered as an

alternative to conventional methods of metal recovery from solutions (Spain and Alm, 2003; Chen *et al*., 2005), being a passive metal uptake system. Both Gram-negative and Gram-positive bacteria have their cell wall charged with a negative charge. This is due to carboxyl, hydroxyl and phosphyl groups, thus in the presence of positive heavy metal cations these groups are very important in cation sorption. Biosorption has a possible application as a process for the removal and concentration of heavy metals from wastewater (Errasquin and Vazquez, 2003). However, the cost of the biomass plays an important role in determining the cost of a biosorption process, thus a low-cost biomass is an important factor when considering practical application of biosorption (Chen *et al.*, 2005b).

Biosorption is another promising biochemical process, which can be applied for the removal of low concentrations of heavy metals in water (Mulligan *et al*., 2001). It involves the removal of heavy metals by passive binding to non-living biomass (Chen *et al.*, 2005a; 2005b). This is a passive process and is independent of metabolic control in which heavy metals are deposited in cell walls by means of ion exchange reactions and complexation with determined functional groups of the cell wall components. Biosorption has some advantages such as, it is independent from metabolism; rapid and independent of temperature; substrates for biosorption are readily available and are easily regenerated (Negishi, 2000). Biosorption has been found to be very selective depending on the typical binding profile of biosorbents (Ansari and Malik, 2007). Bioremediation techniques, used as an *in situ* treatment, offer several advantages over the conventional chemical and physical treatment technologies, particularly for diluted and widely spread contaminants (Radhika *et al*., 2006).

Various microorganisms show a different response to toxic heavy metal ions that confer them with a range of metal tolerance (Valls and de Lorenzo, 2002). Bacteria may achieve this in different ways either through biological, physical or chemical

mechanisms that include precipitation, complexation, adsorption, transport, product excretion, pigments, polysaccharides, enzymes, and specific metal binding proteins. From a metabolic point of view a group of metal-chelating proteins called metallothioneins, are very important in bacterial metal tolerance (Marazioti 1998; Valls and de Lorenzo, 2002). Metallothioneins are small cystein-rich polypeptides that can bind essential metals (e.g. Zn), and non-essential metals (e.g. heavy metals). Other resistance mechanisms include active efflux, complexation, reduction and sequestration of the heavy metal ions to a less toxic state. These tolerance mechanisms are generally plasmid driven, which greatly contributes to dispersion from cell to cell (Valls and de Lorenzo, 2002), chromosome resistance was also related in some bacterial species (Spain and Alm, 2003; Abou-Shanab *et al*., 2007).

Environmentally isolated metal resistant bacteria have been studied in more detail in bioremediation processes. *Cupriavidus metallidurans* CH34 has been shown to bioremediate heavy metal polluted soils and water. This strain has the capacity to accumulate Selenium (Se), Gold (Au) and to volatilize Hg through reactive processes (Sarret *et al*., 2005; Reith *et al*., 2006). *Pseudomonas stutzeri*, isolated from a foundry soil, was shown to be resistant to the toxic effect of chromium up to 1 mM and anaerobically reduce Cr (VI) up to 100 μM (Tsai *et al*., 2005). The interest in heavy metal uptake by bacteria has increased in recent years, especially because of the biotechnological potential that microorganisms have for the removal and/or recovery of metal contaminants (Valls and Lorenzo, 2002; Errasquin and Vazquez, 2003). Bacteria are good biosorbents and with the proper R&D may be in the near future a good alternative for the removal of metals from the environment (Errasquin and Vazquez, 2003).

The processes by which microorganisms interact with metals are diverse and their locations in the bacterial cell is shown in Figure 1.1

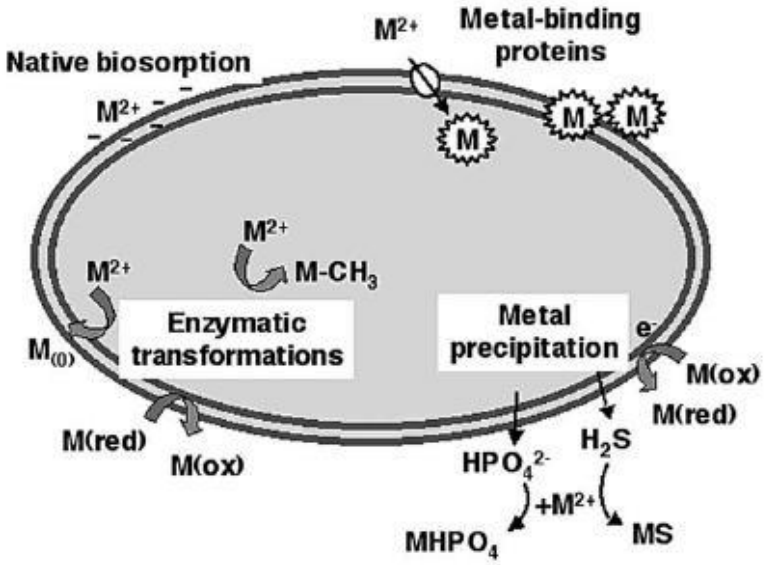

Figure 1.1 Bacterial interaction with heavy metal ions (M2+) in the environment, with reference to the cellular compartment where bacterial response happens
Source: (Valls and de Lorenzo, 2002)

Gram-negative bacteria show more resistance to Cd than Gram-positive ones. This difference can be attributable to the complex cell wall structure of Gram-negative microorganisms (Jjemba, 2004). Microbial resistance is probably linked to the presence of metallothionein-proteins that bind and detoxify several heavy metals. Cd resistance in microbial cells is mostly attained by active efflux via an energy-dependent mechanism (active transport) to pump out cadmium cations through specific efflux pumps (Jjemba, 2004). The Cd adsorption capacity of *Chlorella pyrenoidosa* was induced by light exposure and probably the same happens for some groups of bacteria.

Microorganisms have to maintain the metal concentration in their cells carefully, and Zn is no exception. Bacterial cells maintain a delicate balance between the Zn requirements and their toxicity in several ways: storage mechanisms via metabolic pathways (e.g. metallothioneins) in which Zn is detoxified and stored in the cell, Zn is expelled out of the cell by different low and

high affinity exporters and high and low affinity uptake systems that regulate Zn uptake at different levels of transporter synthesis and activity (Hantke, 2005). Zn in synergy with other metals may also enhance toxic effects in anaerobic and aerobic microorganisms. High concentrations of Zn may reduce protein and ATP content, interact with nucleic acids and enzyme active sites, altering the membrane and leading to cell death (Vega-López *et al*., 2007).

2.2.3.2 Fungi

Some heavy metals such as Zn are essential for the fungal metabolism while others like Pb and Cd do not have any known role in metabolism. When in excess, essential and non-essential metals become toxic, and this toxicity could be many times greater than required (Baldrian, 2003). Heavy metals present in the environment can interact with extracellular enzymes of fungi, but to cause a physiological response heavy metals have to be taken up by fungi. The metal uptake systems are usually present in the cell membrane. Heavy metals can be co-transported using the same transporters of essential metals because of their low specificity (Gadd *et al*., 1990; Baldrian, 2003). The uptake depends on the membrane potential and it is co-transported with calcium (Ca). At a subcellular level, 50% of the metal is bound to the cell wall, 30% stays in the cytoplasm and the remaining 20% is transported to vacuoles. Studies made with *Paxillus involutus*, have shown that Cd uptake involves a rapid binding to the cell wall and a carrier mediated transport more slowly in the cell. Fungi can also take a preventive approach that reduces the uptake of heavy metals. This involves the reduction in the availability of heavy metals via various extracellular precipitation and complexation strategies which could also involve enhanced impermeability to heavy metals.

Fungal cell walls contain basically cellulose, chitin, glucan, and mannan. In addiction to this the cell wall of fungi is composed by proteins, lipids, pigments and polyphosphates (Negishi, 2000). Passive uptake of heavy metals my occur onto the cell wall via ion exchange reactions and complexation with the functional groups of the cell components, without any type of metabolic control. Similarly to what happens in bacteria it is supposed that functional groups like phosphoryl, carbonyl and hydroxyl may be involved in this mechanism also called biosorption (Chen *et al*, 2005a; 2005b). Like in bacteria, biosorption using fungal biomass seems to be a very promising technique for the removal of heavy metals. Work conducted by Puranik and Paknikar (1997) have demonstrated that *Streptoverticillium cinnamoneum* biomass was effective as a biosorption substrate for Pb and Zn.

After entering the fungal cell, heavy metals interfere with the individual reactions and metabolic processes. When heavy metals are present at a toxic level the fungal growth rate decreases and this is often accompanied by changes in the mycelium. These changes could be in the fungal colour, *Trametes versicolor* produces a brown pigment when in the presence of Cd, *Schizophyllum commune* normally has a creamy mycelium but in the presence of Pb gives origin to a black mycelium (Baldrian, 2003).

Figure 1.2 illustrates some of the mechanisms involved in the uptake of heavy metals by fungi.

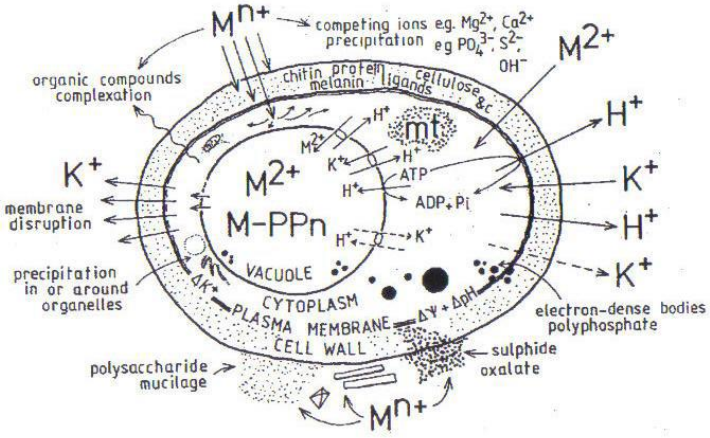

Figure 1.2 Mechanisms involved in heavy metal uptake by fungi

Source: (Gadd, 1986)

Heavy metals are toxic to fungi because of their role as enzyme inhibitors and protein denaturers. Metals like Hg bind to SH groups that are responsible for the regulation of enzyme sites causing irreversible inactivation. Cd has the capacity to bind aromatic amino acid residues in enzymes and cause oxidative damage to proteins (Stohs and Bagchi, 1995). Heavy metals interfere with the reproduction of many fungi, where the spore formation and germination are very sensitive when compared with mycelial growth (Baldrian, 2003; Pawlowska and Charvat, 2004).

Fungi have developed some resistance mechanisms against the toxicity caused by heavy metals. The first line of defence is metal immobilisation by means of extracellular and intracellular chelating compounds. Heavy metals can be chelated by small peptides like phytochelatins or metallothioneins (Negishi, 2000; Baldrian, 2003). One common chelator in fungi is oxalate which provides a way of immobilization of soluble metals in insoluble oxalates, decreasing their availability (Sayer and Gadd, 1997). Another type of metal binding compound is called melanin which is associated with the fungal cell wall. Melanin and related proteins are able to absorb some metals. Work by Rizzo *et al*., 1992, discovered that in *Armillaria* sp. melanin was able to take up Zn, Al, Cu and Fe (Baldrian, 2003). The

relationship of heavy metals and fungi are not yet fully understood but experiments made with some basiodiomycetes have delivered promising results. They have a cell wall mainly composed of polysaccharides and peptides that have a good capacity for heavy metal binding (Baldrian, 2003).

Table 1.2 depicts some values obtained from work using *Phanerochaete chrysosporium.* Like bacteria some fungi could be a good alternative for removal of toxic heavy metals from the environment, even more when it is suggested that fungi are more tolerant of heavy metals as a group than bacteria (Rajapaksha *et al*., 2004).

Table 1.1 Maximum sorption capacities of *Phanerochaete chrysosporium* mycelia with different heavy metals.

Metal	**Sorption capacity (mg g-1 dry weight)**
Cd	110
Cu	60
Hg	61
Pb	108

Source: (Baldrian, 2003)

Dead cells accumulate heavy metals to an equal or even greater extent than living cells. This is because cell surfaces are ionic due to the presence of ionised groups in the cell polymers, “attracting” heavy metals. Ion exchange is believed to be the principal mechanism for metal uptake (Hawari and Mulligan, 2006). Waste biomass is readily available from industry (e.g. penicillin production). The use of dead biomass eliminates any nutrient requirements. Work by Niu *et al.,* 1993, has shown an uptake of 116 mg/g of Pb when using *Penicillium chrysogenum* biomass (Bailey *et al*., 1999).

2.4 Roles of Microorganisms in the Removal of Heavy Metal

Microorganisms play an important role in the remediation of metals in soil. Microbial metal uptake can either occur passively (biosorption) or actively (bioaccumulation). Studies carried out by Irma *et al*., (2013) revealed that *Aspergillus fumigantus* isolated from contaminated site has good biosorption capacity towards selected heavy metals. Vargas *et al*., (2009) showed efficient detoxification of multi-polluted heavy metals by fungi isolated from compost.

Hadis *et al*. (2011) studied bioremediation by isolating arsenite resistant bacteria from arsenic contaminated soil and the investigation of arsenite bioremediation efficiency by the resistant isolates. The isolate is able to remove 95% of arsenic that was present. Narayannarn *et al.* (2011) studied the bioremediation of effluent from magnasite and bauxite mines using *Thiobacillus* sp and *Pseudomonas* spp. The result of biosorption process showed that *T. ferroxidans* reduced and absorbed some heavy metals from mines (Cd, Ca, Zn, Mn and Pb) and *P. aeruginosa* absorbed most of Ca, Zn followed by Pb than *ferrooxidan*. Both species effectively absorbed Cd. Microorganisms can be isolated from almost any environment. Bioremediation process is influenced by soil type, pH, temp. nutrient amendment and oxygen. Bioremediation has the capacity to detoxify inorganic pollutants like metals by methods of adsorption, accumulation by microbes or changing their speciation. It is know that microorganisms regularly exposed to pollutants develop resistance that can be exploited for bioremediation.

Metals have been known to play a major role either directly or indirectly in almost all metabolic processes, growth and development of microorganisms. However, increasing concentrations of metals beyond tolerance levels have forced these organisms to adapt to various biological mechanisms to cope with this condition. Hence, microbes have developed mechanisms like metal efflux systems,

complexation, reduction of metal ions or utilization of the metal as a terminal electron acceptor in anaerobic respiration to tolerate heavy metal accumulation (Patton *et al.*, 2001). Bacteria that are resistant to such heavy metals and have the ability to grow in high concentrations of these metals play an important role in their biological cycling which has great potential in bioremediation of poorly cultivable roofing waste soil high in heavy metal content. Heavy metal tolerance has been observed in the *Enterobacteriaceae* member, *Serratia marcescens* and has been thought to be attributed to plasmid-borne resistant genes. Eight isolated strains of this microorganism have been used for heavy metal tolerance testing against various metal salts in order to identify specific strains that can be used for removal of particular metals from environments such as soil and water where they are present as pollutants.

2.5 Biological treatment of industrial waste

Industrial waste treatment is a process of removing contaminants from waste water. It includes physical, chemical and biological methods. Physical methods of waste water treatment accomplish removal of substances by use of naturally occurring forces such as gravity where no gross chemical or biological changes are carried out such as sedimentation, filteration flocculation. Chemical method consists of using some chemical reaction to improve water quality. This includes ion exchange, coagulation, adsorption. There are many conventional methods of removing heavy metals from waste water such as chemical precipitation, flotation, adsorption, ion exchange and electro chemical deposition (Wang *et al.*, 2004). But all these methods are expensive, need skilled technicians, incomplete removal of heavy metals, high energy requirement and production of toxic sludge (Eccles, 1990).

Microorganisms can be isolated from almost any environmental conditions. Microbes can adapt and grow at subzero temperatures, extreme heat, desert conditions, in water with an excess of oxygen and in anaerobic condition in presence of hazardous components or in any waste stream. Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions. Other metals have no biological role cadmium, (Cd), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Soil micro-organisms have been shown to bioaccumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil. *Oscillatoria* spp. (a bluegreen algae), *Chlorella vulgaris* and *Chlamydomonas* spp. (green algae).

2.6. Microbial Remediation and its Application

Microbial bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade the hazardous contaminants to environmentally safe levels in soil, subsurface materials, water, sludge and residues. Microbial activity is proved to play an important role in remediating metals in soil residues. Studies on interaction of microorganisms with heavy metals have an increasing interest in recent years. Microbial metal uptake can either occur actively (bioaccumulation) or passively (biosorption). Study carried out by Irma *et al*., (2013) revealed that *Aspergillus fumigatus* isolated from contaminated site has good biosorption capacity towards selected heavy metals. Vargas *et al*. (2009) showed efficient detoxification of multi polluted heavy metals by fungi isolated from compost.

2.6.1 Microbes for metal remediation

The mechanisms by which metal ions bind to the cell surface include electrostatic interactions, Van der Waals forces, covalent bonding. redox interactions and extracellular precipitation, or combination of these processes 32.The negatively charged groups (carhoxyl, hydroxyl, and phosphoryl) of the bacterial cell wall adsorb metal cations, which arc then retained by mineral nucleation33. Biosorption studies of U. Zn. Ph, Cd, Ni, Cu, Hg. Th, Zn, Cs, Au, Ag, Sn and Mn, showed that the extent of sorption varies markedly with the metal as well as with the microorganisms (Table 3).

Surfactants such as rhamnolipid produced by P. aeruginosa show specificity for certain metals such as Cd and Ph. Higher molecular weight (—$l0^6$) bioemulsifiers such emulsan, can also aid in metal removal 35, Studies of Sand et al.36 revealed that *Theobacillus ferrroxidans* and *Leptospirillum ferroxidans* are capable of oxidizing iron and sulfur. Joerger et al37 reported that metal accumulating bacterium *Psedomonas stutzeri* AG 259 is capable of producing silver based single crystals, which can reduce the toxicity of metals.

2.7 Remediation techniques for removal of heavy metals from fiber cement soil

Different techniques are used to contain and/or remove heavy metals from contaminated soil. Although several technologies are available for the treatment of contaminated sites, the selection depends on contaminant and site characteristics, regulatory requirements, costs and time constraints (Riser-Roberts, 1998).

2.7.1 Physico-chemical techniques

(i) Isolation and containment

Heavy metal contaminants can be isolated and contained, to prevent their movement and reduce their permeability. To accomplish this physical barriers made of different materials are used for capping, and vertical and/or horizontal containment. Capping has been used with good results to reduce the water intake. Vertical barriers are used to reduce the movement of groundwater. Solidification/stabilization techniques are very common in the United States, because they contain the contaminants, lowering the labour and energy costs (Mulligan *et al*., 2001).

(ii) Mechanical separation

This technique aims at the removal of larger clean particles from smaller polluted particles. This method has been used in mineral ore processing and now in remediation of heavily contaminated soils (Mulligan *et al*., 2001).

(iii) Chemical treatments

Chemical reactions such as oxidation and reduction can be used to decrease the mobility of heavy metal contaminants. This is commonly used in treatment of contaminated soil. This method involves the addition of chemicals such as potassium permanganate, hydrogen peroxide, or chlorine gas. Chemical treatments have the advantage of being performed in situ, but also may add a new source of contamination (Mulligan *et al.,* 2001).

(iv) Electrokinetics

This technique involves passing low intensity electric currents between a cathode and an anode inserted in the contaminated soil. An electric gradient generates movement by electromigration, and electrophoresis. The metals can be removed by electroplating or precipitation or recovering the metals by pumping the

waste from which it originated. This technique has been used in Europe (Mulligan *et al*., 2001).

(v) Soil washing

Heavy metals can be removed from soils adding different chemicals to soil. This can be done in reactors. These chemicals can be inorganic or organic acids such as sulphuric acid as acetic acid respectively; chelating agents like ethylenediaminetetraacetic acid (EDTA) can also be used. The cleaned soil is then returned to its former location. The effectiveness of this technique depends on the soil characteristics (Mulligan *et al.*, 2001).

The feasibility of using biodegradable biosurfactants of bacteria and yeasts origin have been tested *in situ* with some promising results for the removal of heavy metals (Mulligan *et al*., 1999).

(vi) Ion exchange

This is one of the more common techniques for heavy metal removal. In this process ions of a given species are displaced from an insoluble exchange material by different ions in solution. The materials used for the exchange include zeolites, chelating resins, microbial and plant biomass. Ion-exchange techniques are highly pH dependent. A drawback to this technique is the high operating costs (Metcalf and Eddy, 2003).

(vii) Biological Techniques

Removal of heavy metals using living organisms is still in its infancy. These methods include bioleaching, oxidation/reduction reactions and biosorption. Bioleaching may involve either fungi or bacteria. *Thiobacillus* sp are responsible for the oxidation of inorganic sulphur compounds (Mulligan *et al*., 2004), forming sulphuric acid. This can be utilised for desorbing the metals in the contaminated site by substitution of protons. *Aspergillus niger* offers also a promising alternative

due to its citric and gluconic acid production, these particular acids can act as chelating agents for heavy metal removal (Mulligan *et al.*, 2001; 2004).

(viii) Mechanism of tolerance

Organisms respond to heavy metal stress using different defense system (fig. 1) such as exclusion compartmentalization, formation of complexes and synthesis of binding proteins like metallothioneins (MTs) and phytochelatins (PCs). Ochari 2008 has divided general toxicity mechanism for metal ions into three categories. 1. blocking the essential biological functional groups of biomolecules especially proteins and enzymes. 2. displacing the essential metal ion in biomolecules and 3. modifying the active conformation of biomolecules resulting the loss of specific activity. Microorganisms can affect heavy metal concentrations in the environment because they exhibit a strong ability for metal removal from solution: this can be achieved through either enzymatic or non-enzymatic mechanisms '. Avoidance restriction of metal entry into the cell, either by reduced uptake/active efflux or by the formation of complexes outside the cell and sequestration reduction of free ions in the cytosol either by synthesis of ligands to achieve intracellular chelation or by compartmentalization are the two major strategies of organisms to protect themselves against heavy metal toxicity. The general mechanisms of metal tolerance in microbes are shown below.

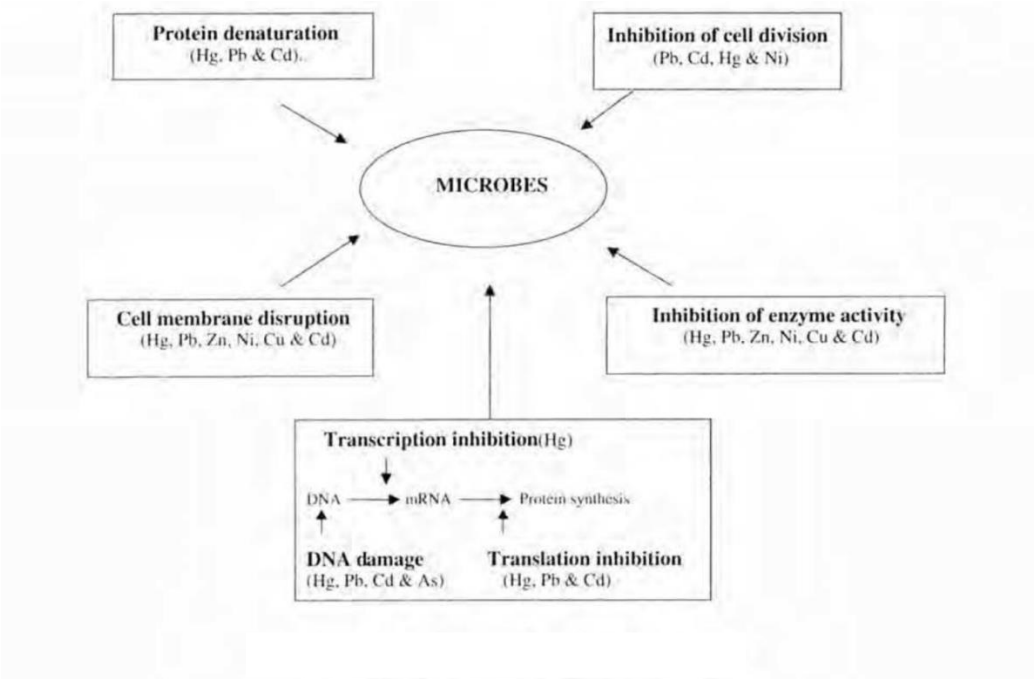

Figure 2: Heavy metal toxicity mechanism to microbes

Table 2: Mechanism of metal tolerance in Microorganisms

Metal	Mechanism of tolerance
$As0_2^{-}$, $As0_4^{3-}$, Sb^{3+}	Anion efflux (ATPase)
$Cd0_2^{+}$, Zn^{2+}, Sb^{3+}	Efflux (ATPase)
Hg^{2+}	Reduced
Co^{2+}, Ni^{2+}	Efflux
CrO_4^{2+}	Decreased uptake
Cd^{2+}, $Cd2^{+}$, Zn^{2+}	Cation efflux
CrO^{2+}	Decrease uptake
Cu^{2+}	DNA damage

Source: (Ochari, 2008)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The area from which samples were collected is shown in Figure 1 (Map of study area showing location of roofing and ceiling sheet manufacturing company's factory and sampling stations in Sapele Local Government Area). The company has produced a wide range of roofing and ceiling sheets. This roofing sheet company is located in Sapele, Delta State, Nigeria. The factory is closed to commercial and residential building since part of the town developed around the factory.

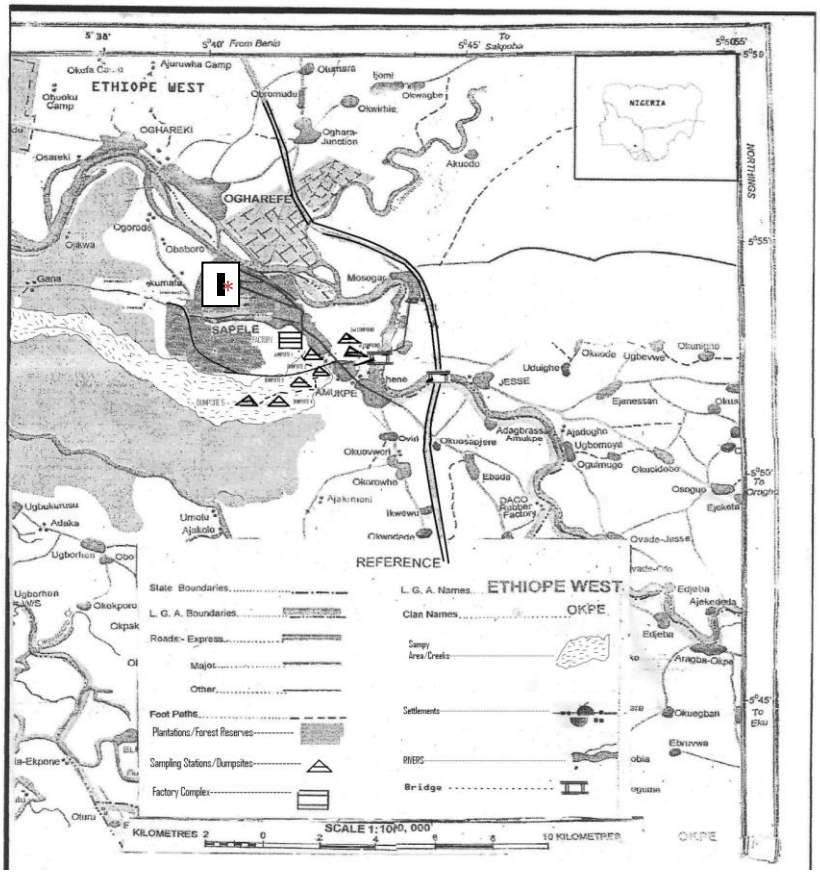

Figure 3. Map of study area showing location of Eternit PLC and sampling stations in Sapele Local Government Area.

3.2 Collection of Samples

Samples (fibre cement waste) were collected in polythene bags using auger at three different points within the dumpsite at a depth of 0-30cm. Three samples from the factory, three samples from soil within the dumpsite and one control from a point where there is no roofing sheet activity. Samples were transported to the Microbiology Laboratory in ice packs for analyses.

3.3 Media and Reagents

The chemical reagent used included, kovoc's reagent, hydrogen peroxide, sodium chloride solution, safranin, ethanol, lugol iodine, crystal violet stain, Nutrient agar, Deoxycholate citrate agar (DCA) and Potato dextrose agar (PDA), spirit, Triple sugar iron, simmon citrate agar, peptone water, citrate reagent, sucrose, glucose, physiological saline and lactose.

3.4 Microbiological Analysis

3.4.1 Isolation and enumeration of microorganisms

This was done according to the method of Collins and Lyne (2007). One gram of each of the fiber and soil was first measured and dissolved in 10mL of sterile distilled water prior to serial dilution. Isolation and enumeration were done by spread plate on Nutrient agar for bacterial count and potato dextrose agar for fungal count. All the plates in duplicate were incubated at 37^{o}C for 24h for bacteria isolates, while the plates for fungi were incubated at 28±2^{o}C for 72hrs.

3.4.2 Enumeration and identification of isolates

Colonies that developed on the plates after incubation were counted, recorded and expressed as standard number of colony forming unit per gram (cfu/g) for bacteria and spore forming unit per gram (sfu/g) for fungi. The discrete colonies that grew on the plates were sub cultured on fresh medium to obtain pure

culture by streak plate method. The pure culture was maintained at 4^{o}C as stock culture for further test.

3.5 Identification of isolates

This was done in accordance with the methods reported by Holt *et al*. (2004). Gram staining reactions, motility test, and the various biochemical tests which include catalase test, Endospore test, Triple sugar iron, Citrate, Indole, oxidase test.

3.6 Gram staining

A young culture of each isolate was used. Smears of isolates were made on a clean grease-free slide with a loopful of normal saline, heat fixed by passing the slide over flame several times and air dried. The slide was flooded with crystal violet and allowed to stay for one minute and then washed off with water, Lugol's iodine was applied to the smear and allowed to stay for another one minute. The iodine was drained and the slide washed with water, and then flooded with alcohol to decolorize the smear for 30 seconds The slide was then washed with water. The counter Stain (safranin) was then added and allowed to stay for one minute before washing off with water. The slide was air dried and then observed under the microscope using x100 objective lens. Gram positive cell appeared purple while gram negative cell appeared pink in colour.

3.7 Motility Test

Nutrient agar slant was prepared in McCartney bottles as prescribed by the manufacturer in (appendix 1). A well isolated colony was picked using a sterile straight wire and the medium was stabbed within 1cm to the bottom of the tube and was incubated at 37°C for 24 hours. A positive result is indicated by a turbid area growing away from the line of inoculation and negative result is indicated by no growth along the inoculation line.

3.8 Biochemical test

3.8.1 Catalase Test

An inoculum was picked from the sub-cultured plate and placed on a glass slide and hydrogen peroxide (H_2O_2) was dropped on it. Immediate production of bubbles indicates positive result and absence of bubbles shows negative result.

3.8.2 Citrate Utilization Test

Slants of the Simmon's Citrate Medium were prepared in McCartney bottles as prescribed by the manufacturer which is shown in appendix 1. Using a sterile straight wire-loop, the test organism was stabbed and incubated at 37°C for 24 hours. The change in colour from green to blue indicated positives result while negative is observed when it retained its green colour.

3.8.4 Oxidase Test

A filter paper was placed on a clean petridish and inpregnated with 1% aqueous solution of Nitrotetramethyl-p-phenenolin-diamine-dihydrochloride. A loopful of inoculum from the pure culture was picked and smeared over the area of the filter paper containing oxidase reagent. Organism indicated positive when it retained the purple colouration within five to ten seconds.

3.8.5 Triple Sugar Iron Test (TSI)

Triple sugar iron test agar was prepared and rested in a slant position, the bacterial isolate was stabbed to the bottom of the tube and was rubbed on the surface as well. It was incubated at 37^{o}C for 24 hours. The change in colour at the bottom from light pink to yellow indicate glucose positive, changes from light pink to yellow throughout the medium indicated glucose and lactose positive. The presences of bubbles or crack in the medium indicated gas production while change of colour from light pink to black in the medium indicated hydrogen sulphide production.

3.8.6 Indole test

Glucose peptone phosphate broth was prepared and were inoculated with a loopful of test organism using sterile inoculating wire-loop and incubated at 37°C for 48 hours. 0.5m1 of Kovac's reagent was added to the broth culture and shaken gently and laid on the bench. A positive test is indicted by a red ring in the upper layer, while negative is observed when it retained its yellow colour or no change.

3.8.7 Coagulase test

The coagulase test method that was used for this study was the slide method. A looful of 24hrs old broth culture was emulsified in a drop of distilled water on a clean slide, and a drop of plasma was placed on the slide, a positive result will show immediate clumping while negative test showed no clumping.

3.9 Identification of fungal isolates

With a sterilized inoculating needle, a pin head size of the mycelium was picked from the young culture (after 72hours of incubation). The mycelium was placed on a clean, grease free glass slide and smeared. A drop of lacto- phenol blue was added to the slide and covered with cover slip inclined at an angle of 45°C to avoid air bubbles. The stained slides were then observed under ×10 and ×40 magnifications of the light microscope. The organisms were identified using cultural characteristics, color, pattern, shape, hyphae and reproductive structures when viewed under the microscope.

3.10 Ability of Organisms to grow on roofing sheet Waste

Fibre cement waste agar (FCWA) were used to test the ability of the isolates to utilize fiber cement waste. The modified method of Prakasam and Dondero (2004) was used in preparing fiber cement waste agar. The fiber cement waste were autoclaved at 121^{o}C for 30mins before filtering through glasswool. The filtrate were made up to 1liter with freshly distilled water incorporated with mineral (g/l: $CaC1_2$. $2H_2O$, 0.lg; $ZnSO_4$. $7H_2O$, 0.01g; K_2HPO_4, O.5g;

$MgSO_4$7H20, 0.lg; $(NH_4)_2SO_4$, 1.0g. KC1, 0.05g; $FeSO_4.7H_20$,0.lg). thereafter 15g of agar powder was added for solidification. This was autoclaved again for 30mins. On cooling, the fiber cement waste agar plate was inoculated with the bacterial and fungal isolates and incubated at 28°C for 24h and 72h for bacteria and fungi respectively. Appearance of colonies on the plates indicate the ability of the isolate to utilize and grow on fiber cement waste.

3.11 Inoculum Development

In order to get a standard inoculum, individual bacterium was grown for 18h on tryptone soy broth (Labtech) at 37ºC on an orbital shaker at 150rpm. The cells were harvested by centrifugation and rinsed three times in sterile saline. Each was re-suspended in sterile mineral salt medium to yield an absorbance reading of 0.5 at 540nm using a spectrophotometer. A ten milliliter suspension of each developed inoculum in the mineral salt suspension was introduced into 50g of sterilized fiber cement waste. Fungal inocula were developed by transferring the fungal colonies from PDA plates into physiological saline and centrifuging at 150rpm. The mycelial cells were subsequently rinsed and filtered using oven- sterilized Whatman No 1 filter paper and dried to a constant weight in a dessicator at 30°C. Survival test for each was done on fresh PDA plates incubated at 28°C for 72h.

3.12 Treatment of waste with single and combined isolates

Sterilized samples were treated with various combinations of bacterial and fungal isolates. Thereafter the physical and heavy metal concentration were determined at 4weeks interval for a period of 12 weeks. The control samples was not treated with any organism.

3.13 Determination of minimum inhibitory concentration of the bacterial and fungal isolates

Minimum inhibitory concentrations of (MICs) of the metals were determined by the Agar diffusion methods (Hassan *et al*., 2008) as described by Velusamy *et al*.,(2011). The metals Cr, Cd and Ni were used as salts respectively. Stock solution of the metals (100μg/ml) were prepared by weighing Cr, Cd acetate and $NiCl_2$ and dissolved in 10ml of sterile distilled water. The solution was mixed thoroughly and strengths of 1000 μg/ml 500 and 250 μg/ml were made by double dilution method. These three different concentrations (1000 μg/ml 500 and 250 μg/ml) of the respective metals were tested on the bacterial isolates and growth was observed after 24 hrs of incubation.

3.14 Treatment with Single Isolates

The samples (fiber cement waste) were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 20mins before inoculation. l0ml mineral salt suspension of each of the bacterial isolates (inoculum) was singly introduced into bags containing 50g of sample in 3 replicate. They were set aside at 28± 2°C for a period of twelve weeks, thereafter the physical and heavy metal concentration were determined every four weeks for period of 12weeks. The physical parameters and heavy metals concentration were determined. Samples were mixed intermittently and l00ml of sterile distilled water were added every 48 hours.

Ten grams of dried mycelia cells of each fungal isolate was weighed and mixed with 50g of each of the sterilized sample. They were left to stand at room temperature (28± 2°C) and kept under the same condition as for bacterial isolates. Samples with no inoculum serve as control. The effect of the growth of the isolates were determined by appropriate physical, heavy metals and microbiological analyses of the soil at 4weeks intervals.

3.15 Treatment with Consortium of Isolates

The samples were inoculated with various combinations of bacterial and fungal isolates. Thereafter the physical and the chemical characteristics were determined every four weeks for a period of 12 weeks. The control sample were not treated with any organism.

All bacteria: 5m1 mineral salt suspension of each bacterial isolate were mixed together and poured into 200g of sterilized samples and incubated at room temperature 28±2°C for a period of 12 weeks. They were mixed intermittently with l00ml of distilled water every 48 hours.

All fungi: The same procedure was followed but was carried out with 5g of each fungus mixed together and added to 200g sterilized samples.

All organisms: This were done mixing 2.5g of each fungal isolate and 50ml mineral salt suspension of each of the bacterial isolates. They were mixed together and the consortium introduced into 200g sterilized samples. Samples with no inoculum serves as control (Akpomie, 2014).

3.16 Phytotoxicity Assay

Germination index (GI) was used to evaluate the phytotoxicity of treated waste to maize and beans. Water was added to the samples to obtain moisture content of 85%. After 2h, the sample extracts were separated by centrifugation(6000rpm) and filtered with a 0.8nm pore size filter paper. The filtrate was diluted with distilled to give to 20%, 40%, 60% 80% and 100% (extract to water (v/v)). Ten milliliter of each dilution were distributed in five petri dishes with 10 seeds each of maize and beans. The seeds was incubated at 27°C for 24h in the dark. The number of germinated seed and the root elongation of the

sample compared to the control (distilled water) were used to calculate the GI according to the following equation (Akpomie, 2014).

$$GI(\%)=\frac{GsLs}{GcLc}x100$$

Gs and Ge are the average number of germinated seeds in the sample and in the control replicates respectively

Ls and Lc-average root elongation in the sample and in control replicates respectively.

3.17 Physicochemical Analysis

A. Physical analysis

pH

10g of samples were stirred in 20m1 distilled water and allowed to settle for 30mins. The pH meter with glass electrode of a digital pH meter were dipped into it and the pH value read on the digital display.

Conductivity

A conductivity meter (Hatch 4600) was used to determine the conductivity of the samples. An electrode connected to a meter was immersed into the sample of water so that the water covered a sensitized electrode. Values on the display kept varying until a stabilized value was obtained and recorded.

B. Chemical analysis

Heavy Metal Analysis

Heavy metals (Cr, Cd and Ni) were analysed using Atomic Absorption spectrophotometry (AAS) (APHA, 2004).

Digestion Procedures

2.3.1 EPA Method 3050B

One gram (1g) of sample was placed in 250 ml flask for digestion. The first step was to heat the sample to 95^0C with 10 ml of 50% HNO_3not allowing to boiling. After cooling the sample, it was refluxed with repeated additions of 65 % HNO_3 until no brown fumes were given off by the sample. Then the solution was allowed to evaporate until the volume was reduced to 5 ml. After cooling, 10 ml of 30% H_2O_2 was added slowly without allowing any losses. The mixture was refluxed with 10 ml of 37% HCl at 95^0C for 15 minutes. The digestate obtained was filtered through a 0.45 μm membrane paper, diluted to 100 ml with deionized water and stored at 4^0C for analyses. The total extraction procedure lasted for 180-200 min.
Digestion solution = Nitric acid and Sulphuric acid 1:1 ratio

The concentration of the heavy metal was measure using AAS

(AAS machine: Agilent Technology

55AA Atomic Absorption Spectrophotometer)

Statistical Analysis

The data was analysed with descriptive statistics (mean and standard deviation) while the effect of the treatment process were by t-test when compared with the untreated samples. Graphical illustrations were also used for comparisons. Analysis of variance were also used to analyzed the growth performance of the test crops in normal (uncontaminated) soil, contaminated untreated soil and treated contaminated soil while Post-hoc test were used to determine specific differences within the three sets.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.0 Results

The total bacterial and fungal counts of soil collected from eternit production factory Sapele, Delta State is presented in figures 4 and 5. It was observed that the total bacterial count of sample site 5.61, 5.72 and 5.80 log10(cfu/g) was higher than the fungal count 5.05,5.07, and 5.20 log10(cfu/g) as well as the control 5.23 and 5.68 log10(cfu/g) for factory dumpsite, soil and fiber cement waste.

The identities of bacteria isolated from factory dumpsite, soil and fiber cement waste based on their cultural and biochemical test characteristics are presented in Table 3. A total of fourteen (14) bacterial species were identified. The gram negative isolates were *Pseudomonas* sp, *Flavobacterium* sp, *Enterobacter* sp, *Citrobacter* sp, *Acinetobacter* sp, *Proteus mirabilis, Escherichia coli* and *Alcaligene* sp. while the gram positive included *Enterococcus* sp. *Kurthia* sp, *Micrococcus* sp, *Arthrobacter* sp, *Staphylococcus aureus,* and *Bacillus species* Table 4 also present the identities of fungi isolated from factory dumpsite, soil and fiber cement. The 7 fungal isolates showed more diversity and these include *Aspergillus niger, Microsporium* sp, *Aspergillus flavus Rhizopus* sp, *Cladophilophora corrionii,* and *Trichophyton terrestre.* Yeasts were also isolated (Table 4).

The ability of bacteria and fungi isolated from the fiber cement waste, dumpsite and soil to utilize the fiber cement waste as growth medium was tested (Table 5). *Proteus*, *Bacillus*, *Acinetobacter*, *Arthrobacter*, *Rhizopus* and *Microsporium*, *Kurthia* and *Micrococcus* species growth well in fiber cement waste.

The minimum inhibitory concentrations of heavy metal are presented in Tables 6. Two bacterial and fungal species that grew best on the fiber cement waste agar were selected for further use. The MIC of the metals on the bacterial and fungal isolates were subsequently determined. The value for *Bacillus, Proteus, Microsporium* and *Rhizopus* species was 300mg/ml, 250mg/ml, 450mg/ml and 400mg/ml for Nickel, for chromium were 250mg/ml, 350mg/ml, 450mg/ml and 400mg/ml while for Cadmium was 900mg/ml, 1000mg/ml, 700mg/ml and 750mg/ml respectively.

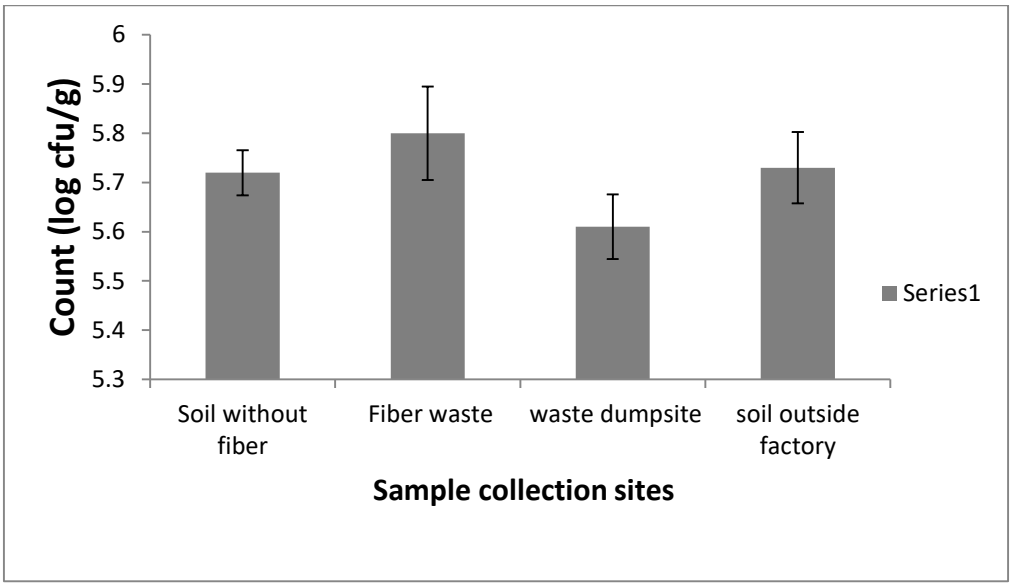

Figure 4: Bacterial count of samples

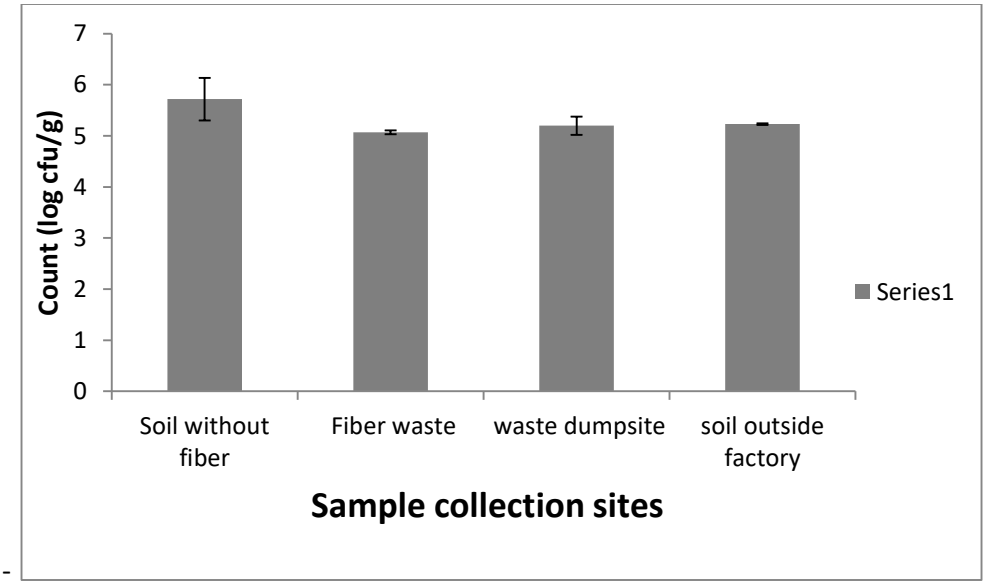

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Figure 5:Fungal count of samples

Table 3: identification of bacterial isolates

S/N	Cultural Characteristics	Morphological Characteristics	BIOCHEMICAL TESTS													Identified isolates
			Gram stain	Coatgulase	Catalase	Indole	Citrate	Oxidase	Glucose	Lactose	Sucrose	H_2S	Gas	Urease	Motility	
1	Colonies were pink smooth, and mucoid.	Rods	-	-	+	+	-	-	+	+	+	-	+	-	+	*Escherichia coli*
2	Colonies were slightly raised on agar plate	Cocci	+	+	+	+	-	-	+	+	-	-	+	-	+	*Staphylococcus aureus*
3	Colonies were white, flat, and entire on nutrient agar.	Rods	+	-	+	-	-	-	+	+	-	-	-	-	-	*Bacillus* sp
4	Colonies were green and raised on N.A	Rods	-	-	+	-	-	+	+	-	-	-	-	-	-	*Pseudomonas aeruginosa*
5	Creamy, smooth edges & convex colonies	Rods	+	-	+	-	+	+	+	-	-	-	-	-	-	*Kurthia* sp
6	Flat irregular colonies	Rods	-	-	+	+	-	-	+	-	+	-	+	+	+	*Proteus mirabilis*
7	Creamy, smooth edges and convex colonies	Rods	-	-	+	-	-	-	+	-	-	-	+	-	-	*Acinetobacter sp*
8	Colonies were cream, entire on nutrient agar.	Rods	+	-	+	-	+	-	+	-	-	-	-	-	-	*Arthrobacter sp*
9	Colonies were, pinkish, entire on MacConkey agar.	cocci	+	-	+	-	-	-	-	-	-	-	-	-	-	*Micrococcus sp*
10	Orange, smooth edges & convex colonies	Rods	-	-	+	-	-	+	+	-	-	-	+	+	-	*Citrobacter sp*
11	Colonies were white, entire on nutrient agar.	Rods	+	-	+	-	+	-	+	+	-	-	-	-	-	*Enterobacter aerogenes*
12	Colonies were white, entire on nutrient agar.	Rods	+	-	+	-	+	-	+	+	-	-	-	-	-	*Enterococcus* sp.
13	Flat entire ovoid colonies	Rods	-	-	+	-	-	-	+	-	-	-	-	+	-	*Flavobacterium* sp.
14	Colonies were pale pink smooth, and mucoid on, NA.	Rods	-	-	+	+	-	-	+	+	-	-	+	-	+	*Alcaligene* sp.

Key: += Positive, -=Negative

Table 4: Identification of fungal isolates

Characteristics	1	2	3	4	5	6	7
Cultural characteristics	Black, wooly with profused growth	Dark grey non-luxicrant with concentric rings	White wooly with profuse growth	Green non-luxicrant with concentric ring	green, wooly with profused growth	Cream non-luxicrant with concentric ring	Brown, wooly with profused growth
Colour of isolates	Dark	Orange	White	Green	Brown	Cream	Brown
Hypae	Septate	Septate	Non septate	Septate	Septate	Septate	Septate
Conidiospore	Non-septate upright	Septate arise from a mycellium slightly branched near apex	Non-septic upright teminating in globose swelling	Septate`` arise from a mycellium slightly branched apex	Non-septate upright	Septate arise from a mycellium slightly branched near apex	Non-septate upright
Conidia	Present, one-cell globose in dry basipetal chains	Present, one-celled hyaline originally colour basipetally	Present one-celled globose and dry, ovoid borne in small term minal cluster	Present one-two celled hyaline globose brightly colour basipetally	Present, one-cell globose in dry basipetal chains	Present 2-4 celled hyaline globose brigatly color basipetaly	Present, one-cell globose in dry basipetal chains
Stolon	Absent	Absent	Present	Absent	Absent	Absent	Absent
Rhizoid	Absent	Absent	Absent	Absent	Absent	Absent	present
Spore colour	Dark	Grey	White	Green	Green	Cream	Brown
Spore attachment	Bear phiatides at the apex with conidia at the top	Phiatides which pries off conidia in dry chains at the top	Bear sporangla at the top containing cluster of light spores	Phiatides which price off conidia in dry chains at the tops	Bear phiatides at the apex with conidia at the top	Phiatides prich off conidia in dry chain at the top	Bear phiatides at the apex with conidia at the top
Tentative organism	*Aspergillus niger*	*Trichoderma* sp	*Microsporium* sp	*Aspergillus flavus*	*Cladophilophora corrionii*	*Trichophyton terrestre*	*Rhizopus spp*

Table 5: Ability of isolates from each sample to grow on the fiber cement waste medium

Organisms	Soil Without roofing sheet waste	Dump site	Factory	Soil
Bacillus	+ ++	+ + + +	+ +	+ + +
Pseudomonas	-	+ + +	-	+ + +
Kurthia	-	+ +	+	+ + + ++
Citrobater	-	+ +	-	-
Acinetobacter	-	+ + + +	+ + +	-
Micrococcus	-	- -	+ + + +	+++ + +
Enterobacter	-	+ +	+ +	-
Enterococcus	-	+ + +	-	+ + +
Alcaligene	-	-	+	++++
Arthrobacter	-	+ +++	+	-
E.coli	-	-	-	++
Flavobacterium	-	-	-	++
Staphylococcus	-	-	-	++
Proteus	+++++	-	-	-
Aspergillus niger	++	++	-	-
Aspergillus Flavus	-	-	-	+
Rhizopus	-	++++	-	-
Trichoderma	-	-	-	-
Trichophyton	-	+++	-	-
Microsporium canis	-	+++++	-	-
Cladophialophora	-	++	-	-
Yeast	-	-	-	-

Key: +++++=heavy growth, ++++=moderate growth, +++=scanty growth, -= no growth

Table 6: Minimum inhibitory concentration (MIC) of heavy metals against test organisms in mg/ml

Isolates	Nickel	Chromium	Cadmium
Bacillus	300	250	900
Proteus	250	300	1000
M. canis	450	550	750
Rhizopus	150	400	700

The result in table 7 present the initial concentration of heavy meetals in fiber cement waste, dumpsite and soil samples.

The pH level by bacterial and fungal isolates after 12weeks in fiber cement waste, dumpsite and soil are presented in figure 6 to 8. The contaminated soil and surrounding soil pH did not change significantly after the various mixed culture treatment.

The electrical conductivity of the treated samples was following a regular pattern in all treatments and in the three samples (Table 8).

The heavy metals removal from fiber cement waste, dumpsite and soil are presented in figure 9 to 11. The consortium and single treatment with isolates reduced the concentration of cadmium (figure 9). Although the reduction in chromium levels were significant, they were not as high as was observed for nickel (Figure 10). The results in figure 11 show that all the test experiment produced a reduction in the nickel levels. It was also observed that among the microorganisms, consortium were more effective when compared with single bacterial isolates, followed by all bacteria and all fungi.

The effect of cadmium, chromium and nickel removal by bacterial and fungal isolates for the period of 12weeks are presented in figure 12 to 14. It was observed that among the microorganisms, consortium were far better than single

isolates, followed by all bacteria and all fungi in the samples experiment. It was also detected that chromium and cadmium were more affected among the metals.

Table 7: The concentration of cadmium, chromium and nickel in samples dumpsite and normal soil

	Concentration (mg/l)				
Parameters	Fiber	Dumpsite	Soil	Control	WHO (S)/FEPA
Cadmium	0.11	4.17	2.11	1.76	0.3-0mg/l
Chromium	0.08	2.87	1.89	0.89	2.00
Nickel	0.83	40.68	19.84	14.17	0.02

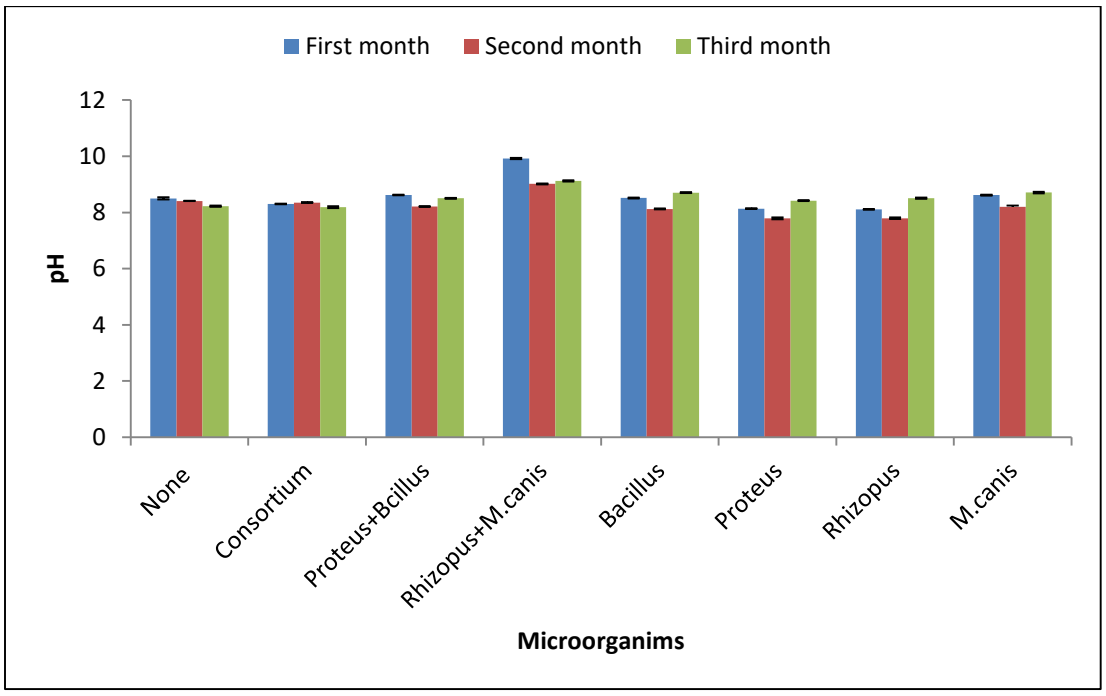

Figure 6: pH of fiber cement waste treated with isolates

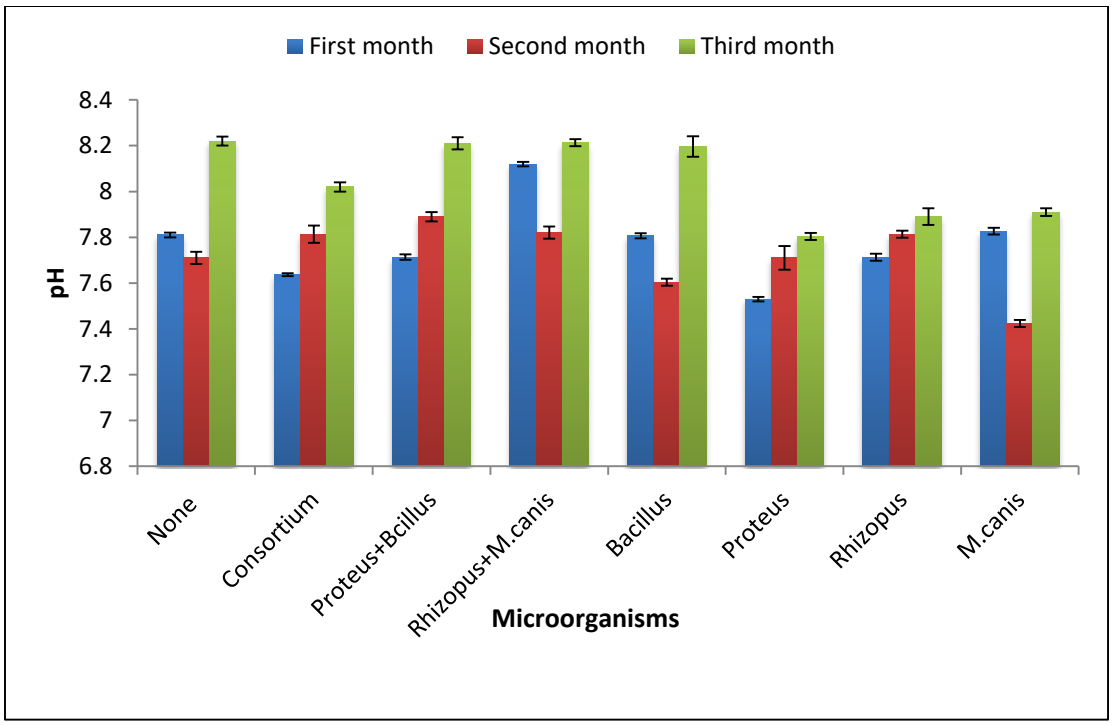

Figure 7: pH of Dumpsite treated with isolates

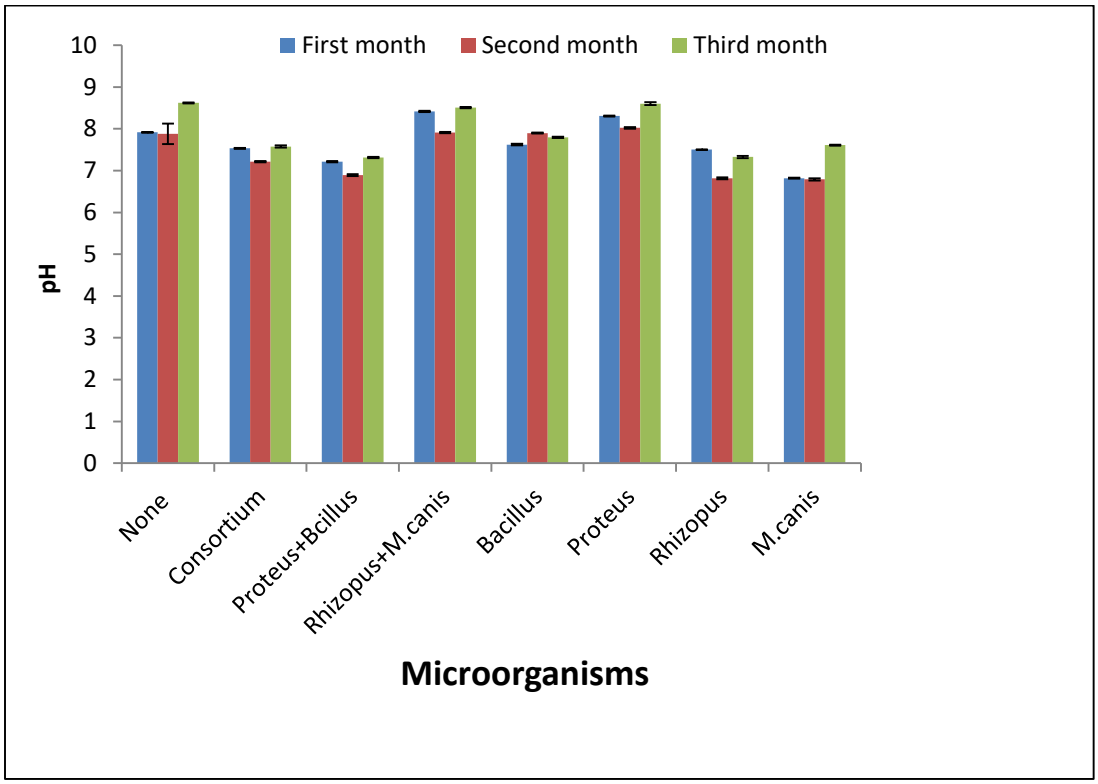

Figure 8:pH of Soil treated with isolates

Table 8. Effect of single and combined microbial treatment on electrical conductivity of samples

Sources of sample	Microbial treatment agents	EC (Mean± SD) (S/M) First month	Second month	Third month	Significant Diff (P)
Fiber	None	925.0±2.0	838.6±1.52	693.6±1.52	>0.05
	Consortium	868.3±1.52	1140.3±1.52	1543.3±1.52	>0.05
	Bacillus+Proteus	950.3±1.53	1025.3±2.88	1283.0±2.00	>0.05
	Microsporium + Rhizopus	630.6±2.00	890.0±1.00	969.3±1.52	>0.05
	Bacillus	630.3±1.52	830.0±1.00	1045.0±1.00	<0.05
	Proteus	1356.3±1.53	1124.0±1.00	740.0±1.00	<0.05
	Rhizopus	1002.3±1.5	931.0±1.00	516.0±1.00	<0.05
	Microsporium	623.0±2.00	630.3±2.61	583.6±1.52	<0.05
Dumpsite	None	1913.6±1.52	1686.0±0.57	1462.0±0.577	<0.05
	Consortium	2103.0±1.00	1931.6±0.88	916.0±0.577	<0.05
	Bacillus+Proteus	1831.6±1.52	1429.0±0.57	1619.0±0.577	<0.05
	Microsporium + Rhizopus	1773.3±2.08	2130.6±0.66	1691.60.88	<0.05
	Bacillus	2934.0±1.00	2189.0±0.57	2521.0±0.57	<0.05
	Proteus	1433.6±1.52	1991.6±0.88	2322.3±0.88	<0.05
	Rhizopus	2230.0±1.00	839.0±0.57	2635.0±0.577	<0.05
	Microsporium	2431.3±1.15	1996.6±0.88	1821.0±0.57	<0.05
Soil	None	282.6±1.52	351.0±1.00	190.0±0.57	<0.05
	Consortium	413.3±1.52	336.6±1.52	221.3±0.88	<0.05
	Bacillus+Proteus	221.3±1.52	521.3±1.52	253.3±0.33	<0.05
	Microsporium + Rhizopus	311.0±1.00	221.0±1.00	250.0±0.57	<0.05
	Bacillus	421.3±1.52	317.0±1.00	253.0±0.57	>0.05
	Proteus	333.6±1.52	273.0±1.00	108.3±0.88	>0.05
	Rhizopus	301.0±1.00	280.0±1.00	221.0±0.57	<0.05
	Microsporium	423.6±0.57	321.0±1.00	109.0±0.57	<0.05

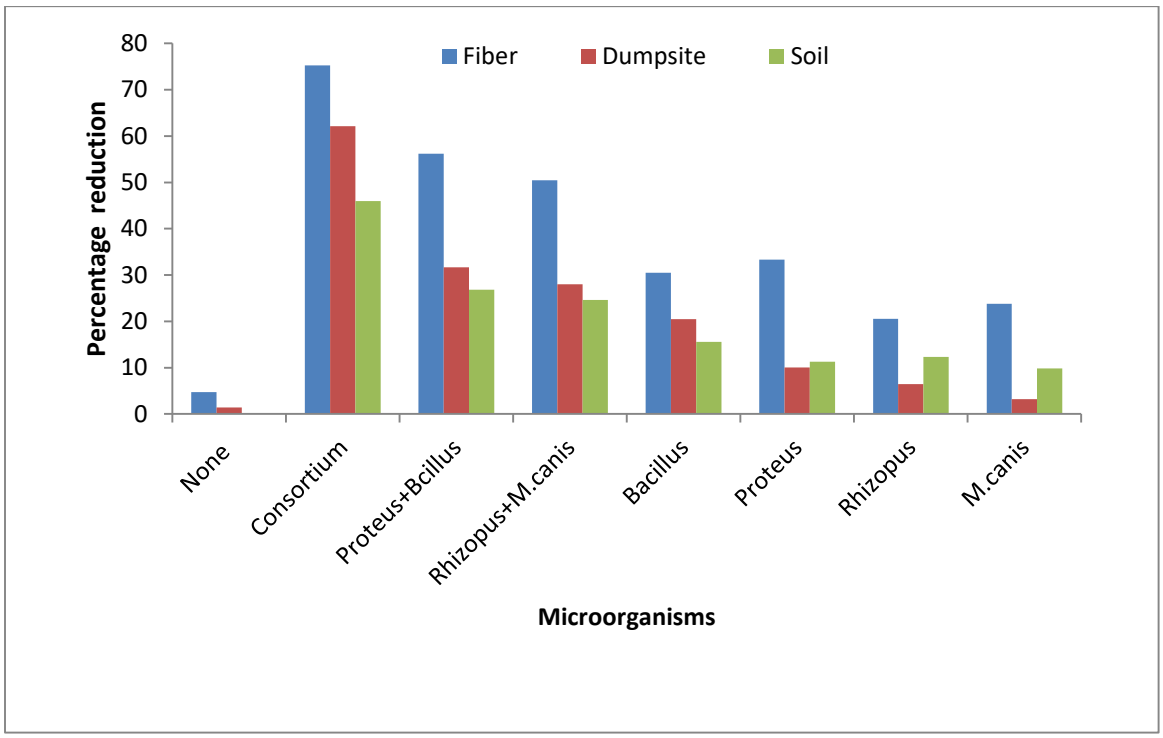

Figure 9:Percentage reductuion of Cadmium in the samples after treatment with isolates

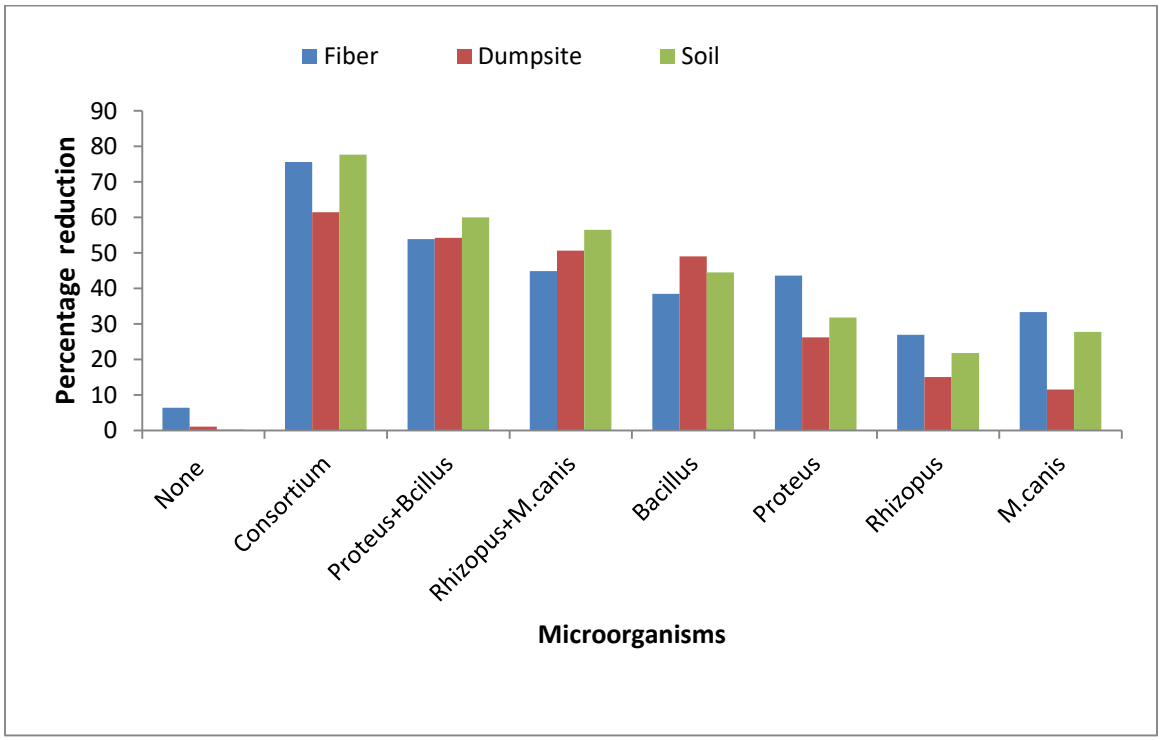

Figure 10:Percentage reduction of Chromium in the samples after treatment with isolates

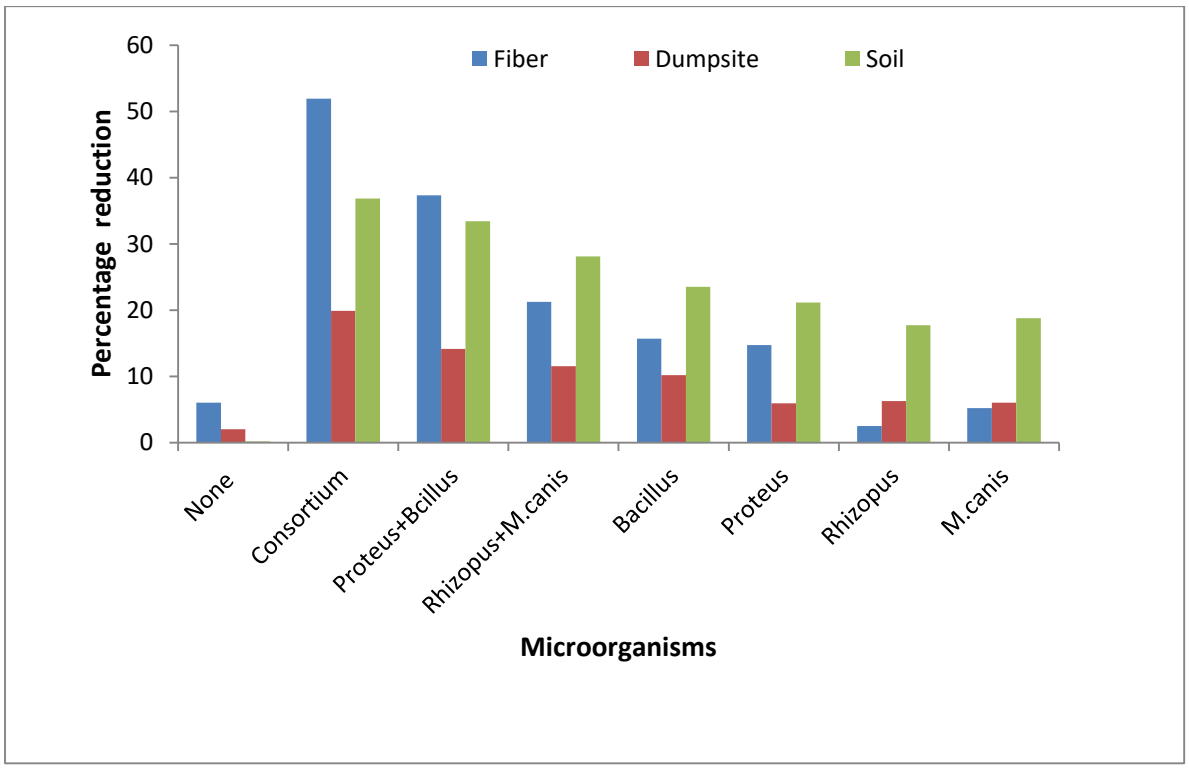

Figure 11: Percentage of Nickel in the samples after treatment with isolates

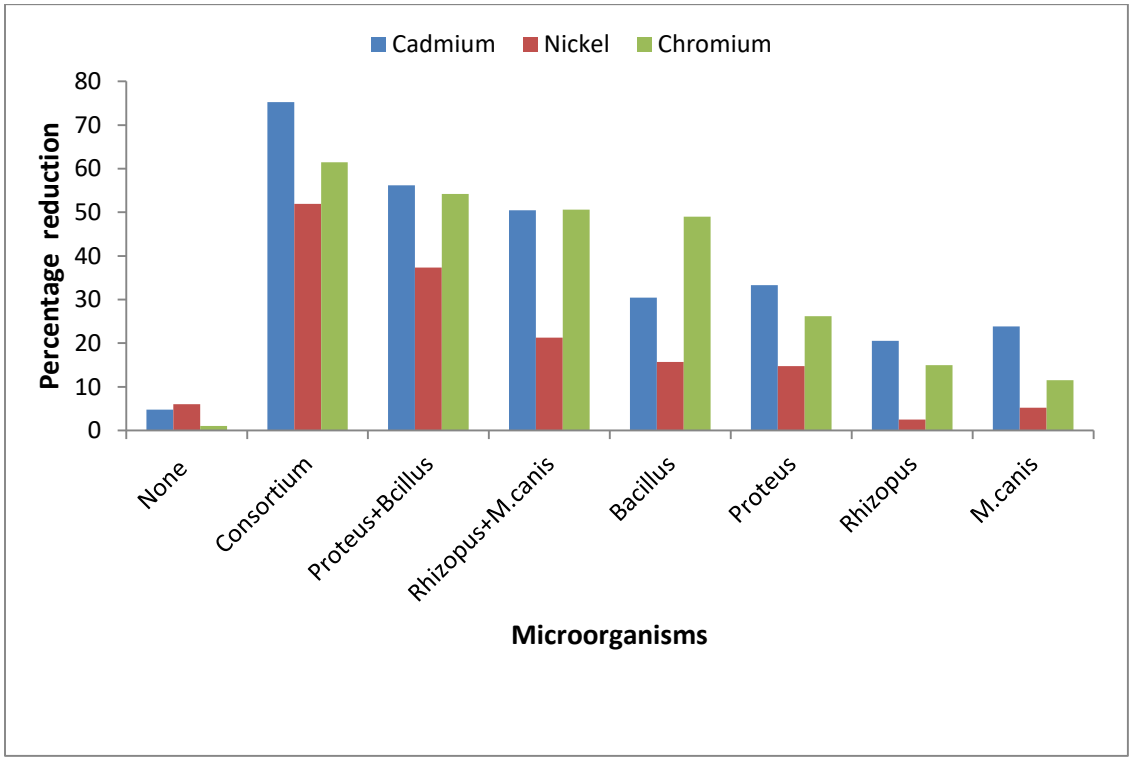

Figure 12:Summary of Heavy metal removal in fiber cement waste after 12 weeks

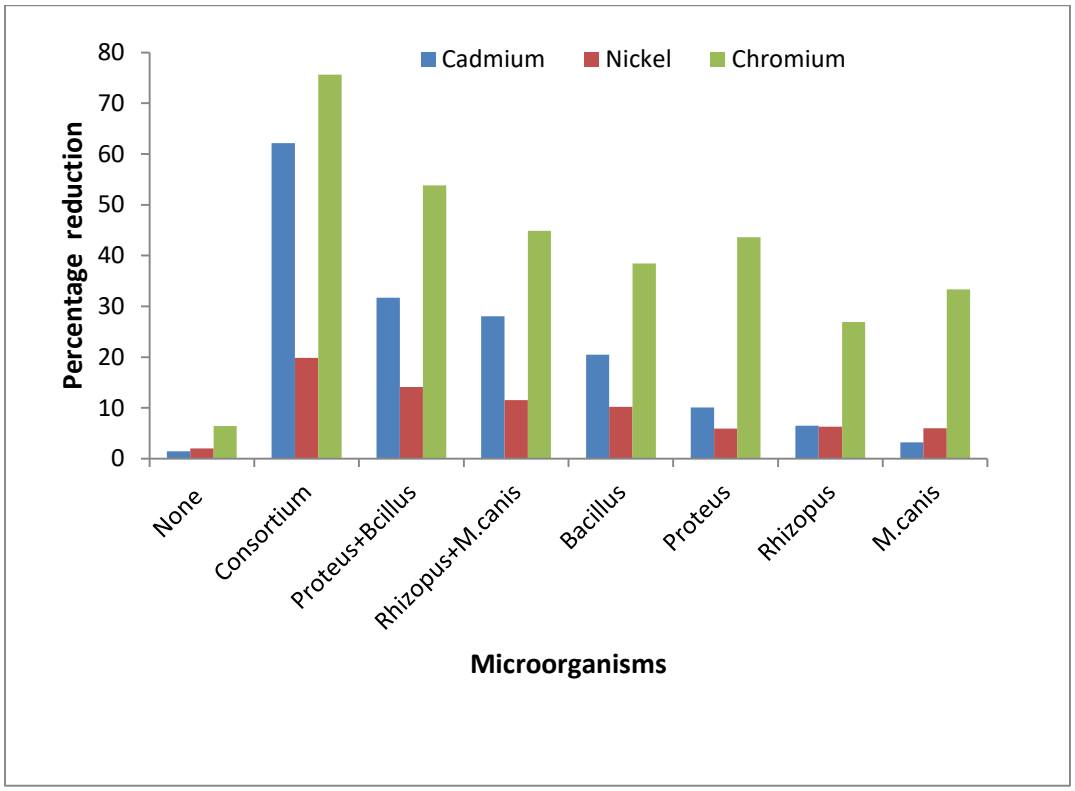

Figure 13:Summary of Heavy metal removal from Dumpsite after 12 weeks

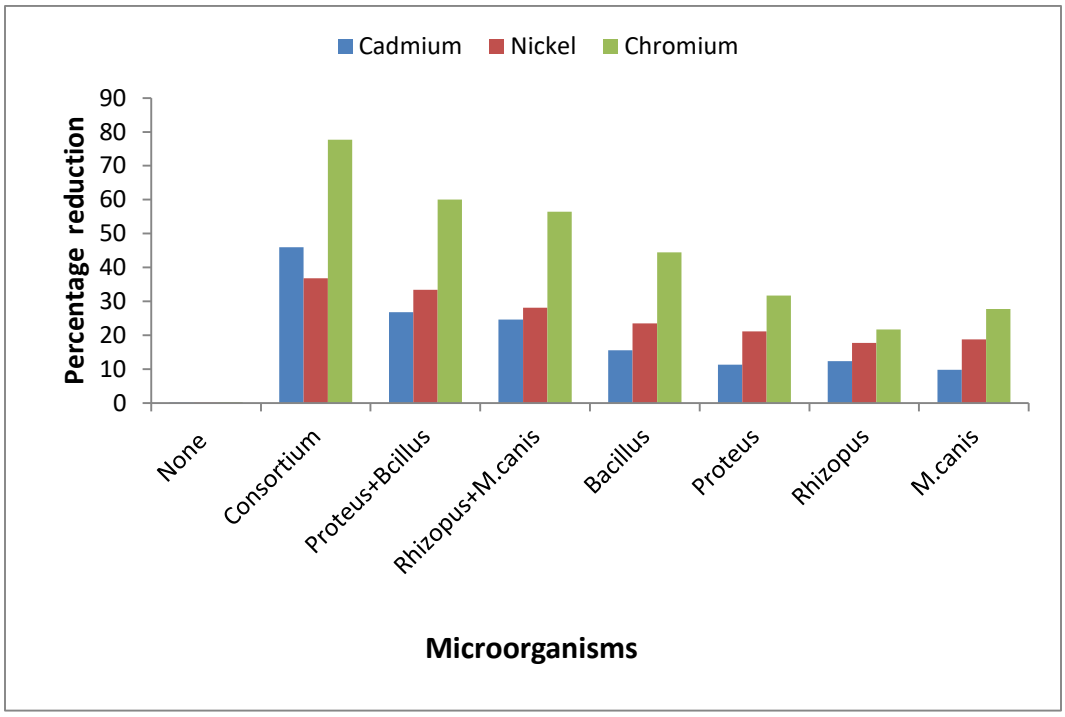

Figure 14:Summary of Heavy metal removal from soil after 12 weeks

The phytotoxicity of the microbially treated samples on the growth of beans and maize are presented in figure 15 to 20. The consortium treatment produced better growth of beans than the single treatments. The growth indices were generally higher than single in fiber cement waste (figure 15). The result in the figure 16 further shows that the growth of maize in fiber cement waste was higher with consortium, mixed bacteria and fungi treatment when compared to treatment with single isolates. It was observed that there was no mark different from growth in mixed bacterial and fungal culture treatment.

Figure 17 shows that the growth of maize in dumpsite was higher with consortium, mixed bacteria and fungi treatment when compared to single isolates treatment. The result in figure 18 observed that among the microorganisms, consortium treatment supported the growth of beans more than single isolates. It was also revealing that constant growth was observed in the percentages of bacterial.

A similar trend was observed with consortium treatment which produced better growth of beans and maize than the single isolates. The growth of consortium, bacterial and fungal were generally higher in normal soil (figure 19 and20). Comparison by t test showed that there was no significant difference in the growth indices in samples ($p>0.05$).

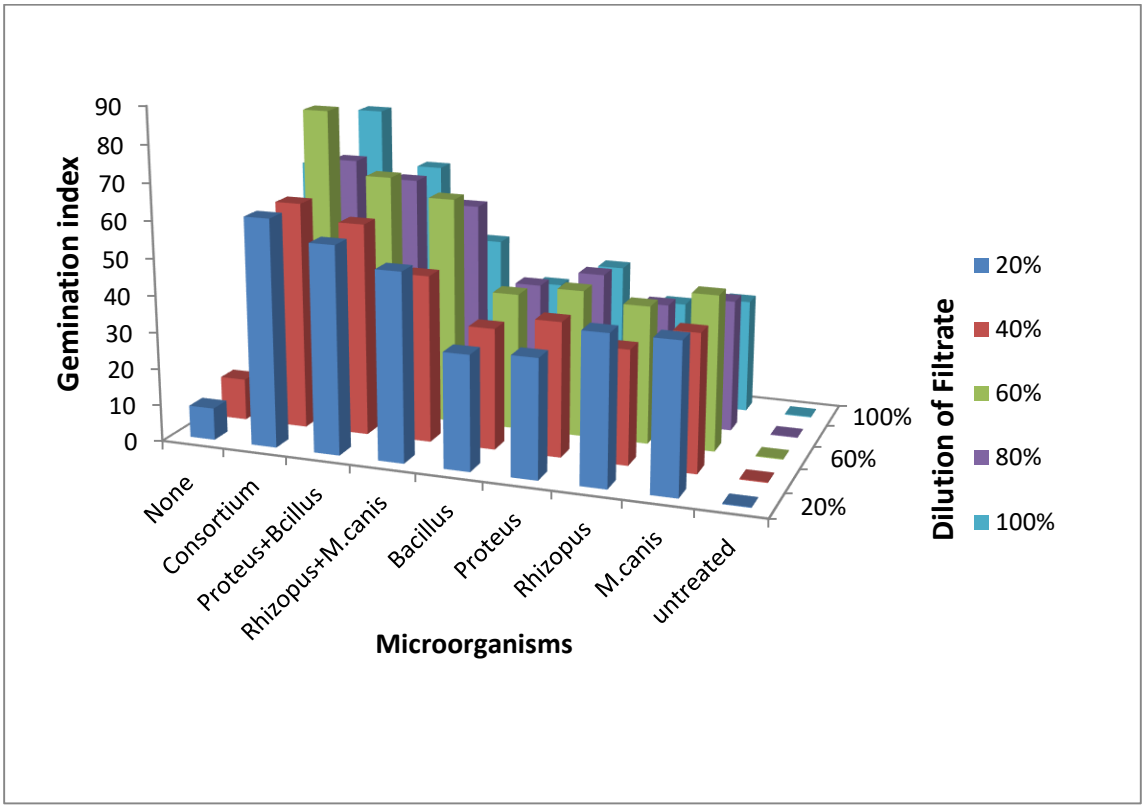

Figure 15: Phytotoxity of treated fiber cement waste on growth of Beans

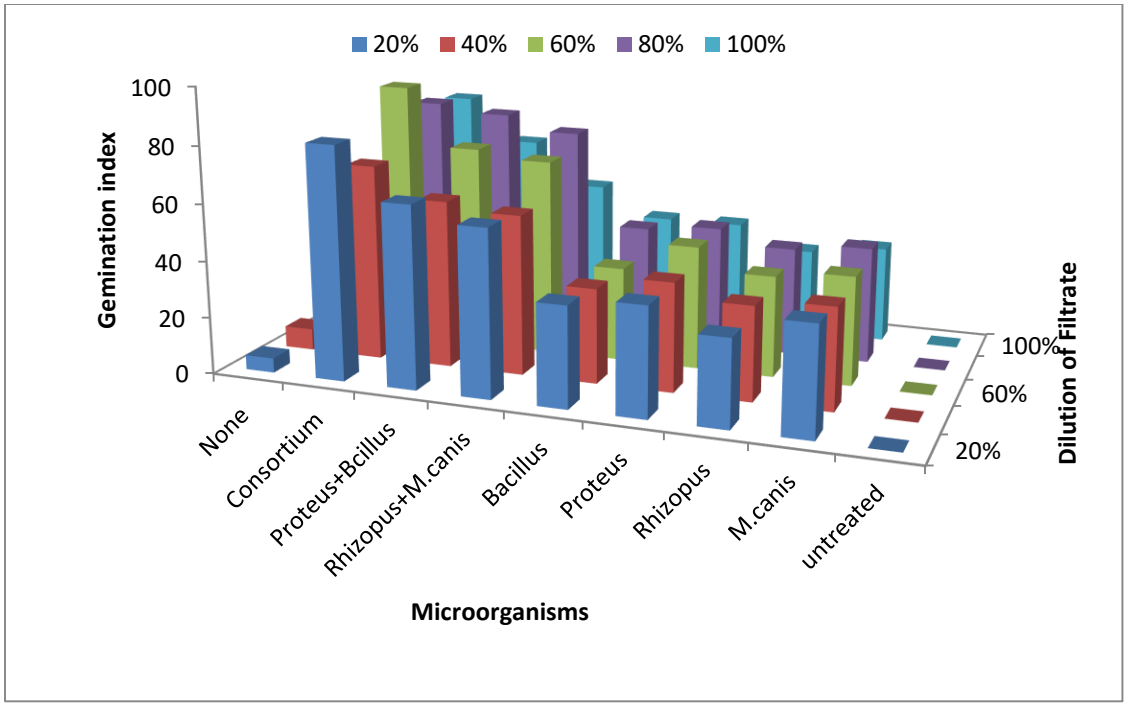

Figure 16: Phytotoxity of treated fiber cement waste on growth of maize

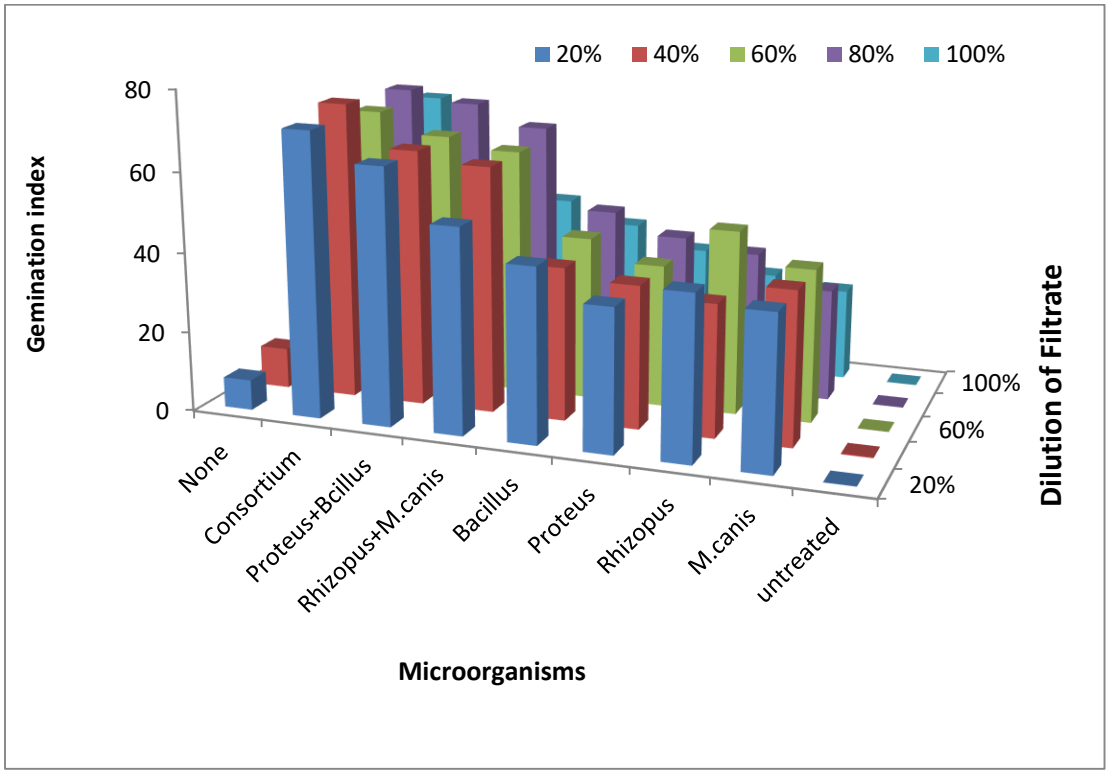

Figure 17: Phytotoxity of treated waste dumpsite on growth of maize

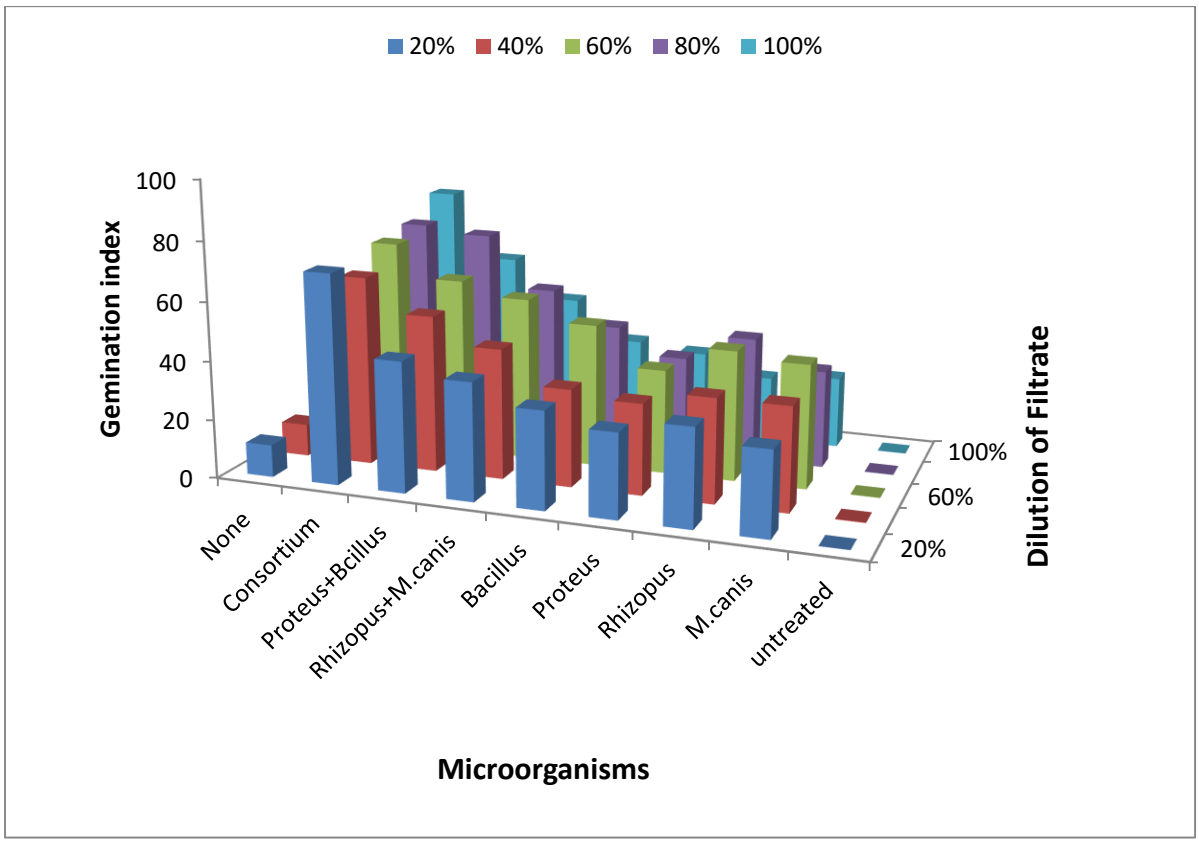

Figure 18: Phytotoxity of treated waste dumpsite on growth of Beans

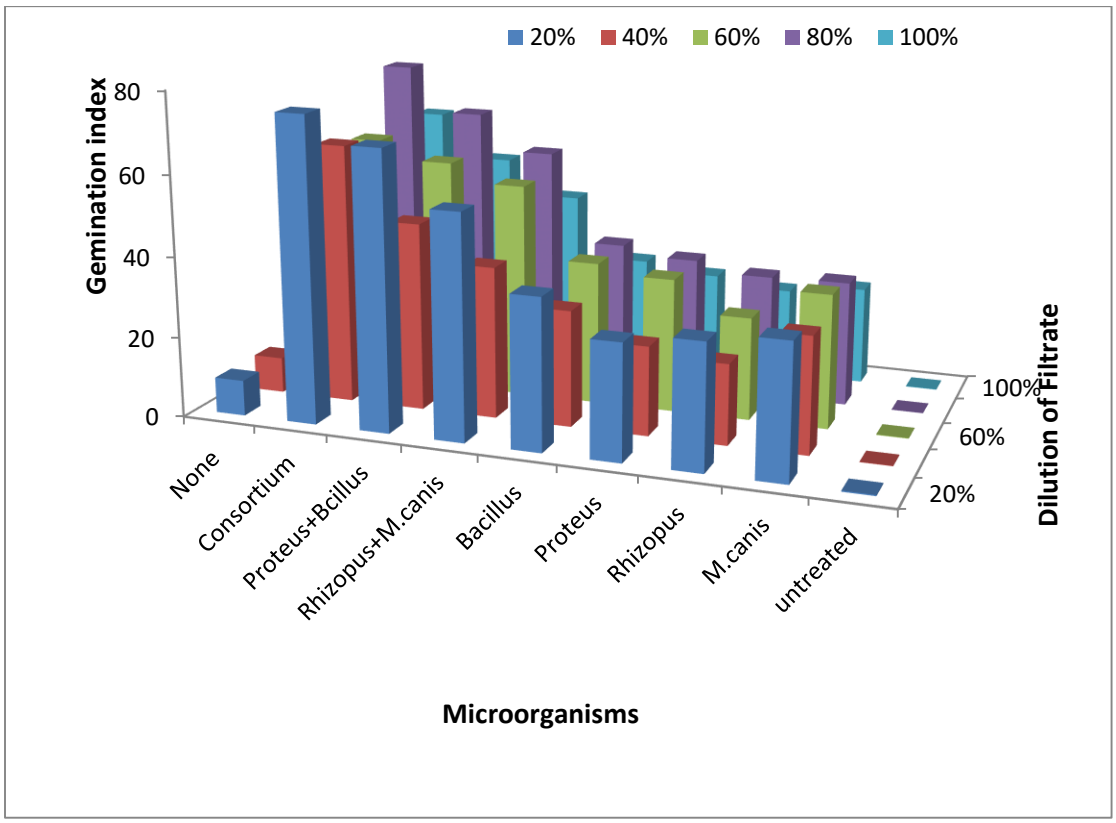

Figure 19: Phytotoxity of treated soil without fiber **on growth of beans**

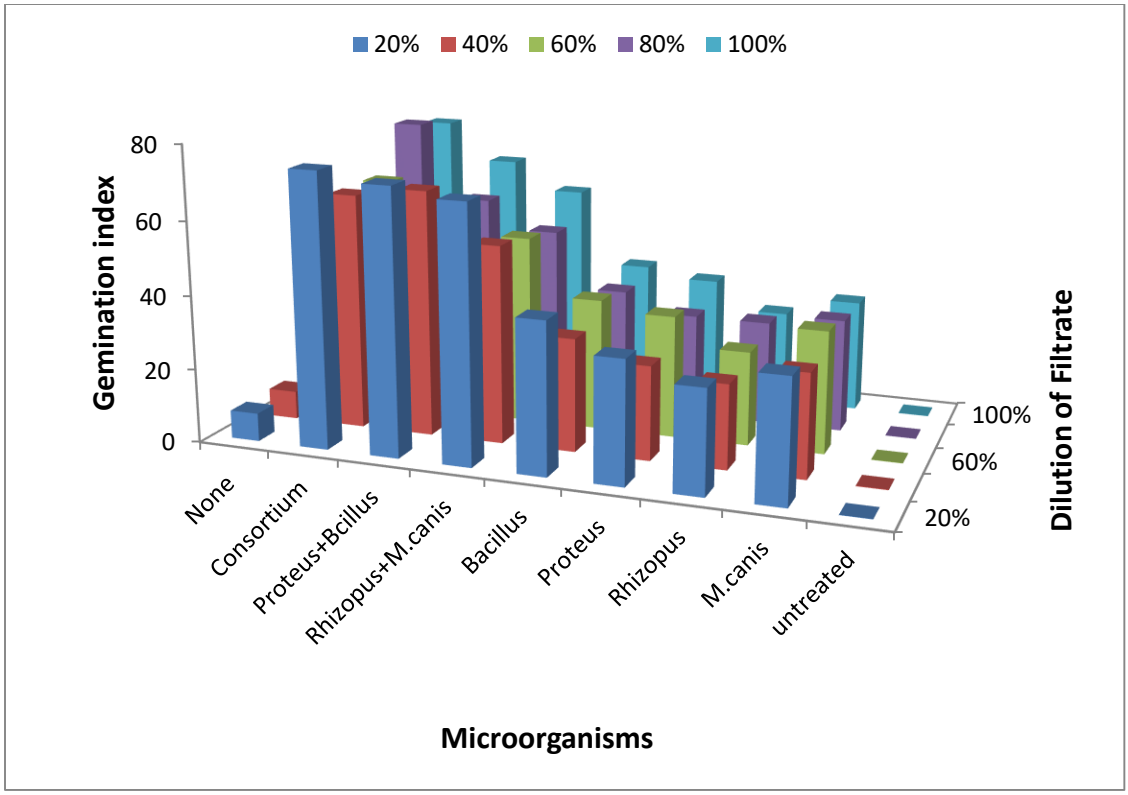

Figure 20: Phytotoxity of treated soil without fiber on growth of maize

4.2 Discussion

In this study, the microbial population observed from fiber cement waste, waste dumpsite and soil without fiber samples obtained indicated that the mean bacterial count of the fiber cement waste was higher (5.80Log10 cfu/g) than waste dumpsite, soil outside factory and soil without fiber (5.61 5.72 and 5.23 Log10cfu/g) while the mean fungal count of the soil without fiber cement waste was higher 5.68 Log10(cfu/g) than fiber cement waste, waste dumpsite and soil outside factory 5.20, 5.05 and 5.07 Log10(cfu/g). The microbial load observed in fiber cement waste, waste dumpsite and soil outside factory samples indicated that microorganisms can be found in these samples. Bacud *et al.,* (1994) and Awomeso *et al.* (2008) reported that microorganisms are found in roofing sheet waste through processing and disposal system. The increase in bacterial count of fiber cement waste, could be as a result of development of resistance of the bacteria ability to utilize chemicals while the other organisms that could not grow may have been inhibited by high toxic chemical contamination. Habi and Daba, (2009) reported that bacteria exposed to high concentrations of toxic heavy metals may have developed resistance against the elevated level of this metals. The decrease of bacteria in the samples may probably be as a result of environmental stress or metal present. Li *et al*. (2004) reported that at high concentration heavy metals exert inhibitory action on bacteria by blocking essential functional group or modifying active conformation of biological molecules. The increase of fungal count in samples could be due to the fact that fungi can grow well in low toxic soil than high toxic soil while the decrease of fungi in other samples could be as a result of morphology and physiological change which affect the growth rate, reproduction process and enzyme production.

The bacteria and fungi isolated from the fiber cement waste, fiber cement waste dumpsite, soil without fiber and soil outside factory indicated that the organisms are associated with the samples and they could have gained entry into the samples through water used in production from sewage and run-offs from agricultural lands. The microorganisms isolated from the samples tend to be similar to those from fiber cement waste waste in other parts of the world. Abou-Shanab, (2007) isolated similar bacteria, but *Pseudomonas* and *Bacillus* sp. were the major isolates while *Proteus* sp. was found to be dominant. Habi and Daba, (2009) reports show that some organisms like *Bacillus* and *Pseudomonas* sp are always encountered. Mathyprakash and Jayanthi (2011) isolated *Aeromonas* sp, *Alcaligenes* sp, *Bacillus* sp, *Staphylococcus* sp and *Pseudomonas* sp from fiber cement waste. Thus similar groups of microorganisms tend to be associated with fiber cement waste and waste dumpsite in many parts of the world. However, strain variations with adaption to different geographical habitats exit.

It was not all the organisms isolated from the samples that were able to grow on the fiber cement waste medium. Only *Bacillus* sp, *Proteus* sp, *Rhizopus* sp and *Microsporium* sp were able to grow well on the medium thereby suggesting that some of them were just transient microorganisms. Ogbonna *et al*., (2012) showed that the content of heavy metals in waste is primarily a consequence of the intended use of heavy metals in industrial products. The growth of *Bacillus* sp, *Proteus* sp, *Rhizopus* sp and *Microsporium* sp could be attributed to the potential of the isolates to use fiber cement waste as source of growth and or ability to absorb metals. Tsai *et al*., (2005) reported that organic pollutant in fiber cement waste are also useful as carbon source for microorganisms for metabolism in order to survive. Choski and Jozi, (2007) and Al-Muhtaseb *et al*. (2008) have demonstrated that metals influence microorganisms by affecting their growth, morphology and

biochemical activity. It suggests therefore that the organisms able to survive have acquired a variety of mechanisms such as exclusion, compartmentalization, formation of complexes and synthesis of phytochelatin for for adaptation to the presence of these toxic constituents.

The determination of minimum inhibitory concentration of the heavy metals on the test organisms, showed that Nickel and chromium were more effective on the tested organisms than cadmium, this may probably show that Nickel and chromium possesses antimicrobial activity. Cadmium inhibited the growth of *Proteus* and *Bacillus* but the concentration varied. The high MIC of Cadmium on *Proteus*, *Rhizopus* and *Microsporium* sp can be attributed to antimicrobial activity of the cadmium. However, heavy metal can inhibit microorganisms by interacting with the enzymes directly involved or those involved in general metabolism. Cadmium is known to significantly influence the enzymes of microorganisms except when they develop resistance to the metal (Chihching *et al*., 2008). Toxic heavy metals are found naturally in the earth, and become concentrated as a result of human caused activities. The study showed a high incidence of metal resistance to microorganisms. Many bacterial and fungi species isolated from industrial wasted had shown to develop resistance to heavy metals.

The physio-chemical characteristics of the fiber cement waste and waste dumpsite and soil without fiber samples were determined prior to treatment. It was observed that the level of the physical and heavy metals concentrations differed among the three samples studied. This is a reflection of natural variation as indeed organisms capable of growth at various pH values occur. Various microorganisms show different response to toxic heavy metal ions that differentiate them with a range of metal tolerance (Valls and de Lorenzo, 2002). Presence of weakly positive

and neutral ions at the biomass surface best interact with soil and fiber cement Hemambika *et al*. (2011). pH range for bioremediation is between 6.0 to 8.9. The finding of this research indicated that pH level fell within a suitable range which support microbial growth in treatment categories.

The electrical conductivity of the samples indicated that some of the values of the microorganisms were quite high in samples. This suggest that the ionic constituents of the fiber cement waste and waste dumpsite favors the growth of organisms. High EC may be due to increase in the concentration of some soluble salt. However it shows that organic pollutants in the samples are also used as carbon source by microorganisms for metabolism in to other survive. While others that are low, reduced as result of higher ionic present in the samples. The reduction of EC might be due to use of ions by microorganisms for their growth and survival. Some microorganisms that live in soil and dumpsite naturally use certain chemicals that are harmful to human (Colberg, 1995). Some of the values reported in this study are consistent with the finding of a study by Thassitou and Arvanitoyannis, (2001) who reported that EC in fiber waste usually accumulate high values in land surface.

The effect of microorganisms in removing cadmium, chromium and nickel from fiber cement waste, waste dumpsite and soil without fiber was tested. The organisms that utilized heavy metals varies, depending on the chemical nature of the agents, because microorganisms cannot destroy metals but can influence their mobility in the environment by modifying their physical and chemical characteristic. Practically, all the tested isolates reduced the three metal concentrations to levels much lower than acceptable limits in fiber cement waste. This suggests that the four selected test microorganism have the capacity to reduce cadmium, chromium and nickel concentration which may be by biosorption,

bioaccumulation or enzymatic reduction as indicated by Vidali, (2001). The test organisms in this study, *Proteus, Bacillus, Rhizopus and Microsporium canis* have been reported to be involved in the removal of cadmium, chromium and nickel concentration from fiber cement waste, waste dumpsite and soil without fiber. The report of Irma *et al*. (2013) shows that the ability of some microorganisms to tolerate heavy metals and the ability of some to promote transformations that make some metals less toxic, make organisms that live in heavy metal contaminated sites potentially useful in bioremediation.

The variation in the ability of the test organism to utilized fiber cement waste were repeated when the soil obtained from the dumpsite and surrounding was treated with the test isolates. However the level of reductions and the heavy metals reduced were identified with that of the fiber cement waste. The result showed that Nickel was not so reduced significantly in the fiber cement waste by some of the organisms, but were significantly reduced in the dumpsite as well as the soil by the same organisms and vice versa. The reduction pattern were much observed in the consortium culture treatment of waste dumpsite. On the other hand there was much reduction in cadmium and chromium (46 and 78% respectively) in soil without fiber by the organisms when compared with initial metals concentration after 12 weeks. The nature of the soil environment may be responsible for the consistency. The soil contain organic and inorganic compounds that may inhibit or promote microbial metabolism. Bacteria may achieve this in different ways either through biological, physical or chemical mechanisms that include precipitation, complexation, adsorption, transport, product excretion, pigments, polysaccharides, enzymes, and specific metal binding proteins. From a metabolic point of view a group of metal-chelating proteins called

metallothioneins, are very important in bacterial metal tolerance (Marazioti 1998; Valls and de Lorenzo, 2002).

Generally there were better reductions in the fiber cement waste, waste dumpsite and soil without fiber with consortium microbial treatment than with single application of the organisms. This might have been encouraged by the fact that microorganisms rarely live in colonies comprising only of same species or genera. They live in mixed colonies where the different metabolic capabilities may be of mutual advantage to the members of the colony. Feedback inhibition may reduced or entirely absent there by promoting faster degradation. This is the reason for the application of consortium of microorganisms in biological treatment. The finding in this study with respect to the treatment of the samples with mixed culture provide support for the use of consortium of microorganisms for bioremediation strategy. The combined effect of bioadsorption, biotransformation and co-metabolism result in an enhanced degradation. Microbial survival in heavy metal polluted soils depends on intrinsic biochemical properties, physiological and/or genetic adaptation including morphological, as well as environmental modifications of metal speciation (Abou-Shanab *et al*., 2007).

Specifically there was better growth in the beans and maize planted in fiber cement waste, waste dumpsite and soil without fiber with consortium treatment than with single application of the organisms as observed in the study. The procedure with the untreated samples in germination of the test seeds did not produce good germination like the second process of germination by the seeds at the same time with microbial treatment.

The single microbial treatment of samples weakly supported the germination of maize and beans seeds in phytotoxicity test. This suggests that the single

treatments strategies failed to detoxify the samples to a levels suitable for germination of seeds. Expectedly the consortium treatment provided better conditions for the germination of beans seeds than maize grain. This finding further endorses the superiority of consortia treatment approach. Pérez-de-Mora *et al*., (2006) have demonstrated that metals influence plants by affecting their growth, morphology, nutrients uptake and diversity.

However, high germination of plants was observed in beans when compared with the germination of maize in fiber cement waste and waste dumpsite with consortium treatment than with single treatment of the organisms. Generally, beans grew better than maize both in the single and consortium treated soil without fiber. The soil environment contains many chemical compounds that may inhibit the activity of the appropriate enzymes needed for the germination of maize. Thus the treatment of these waste in the soil may not be as effective as treating the waste before discharge, although it was observed that some of the chemical constituents were better reduced in the waste dumpsitem. Naaz and Pandey (2010) reported that maize plants produce visible symptoms of toxicity and germination retardation due to high accumulation of heavy metals when exposed to fiber cement waste. Although the results showed that metals concentration in treated soil was significantly reduced. The better germination of beans seeds might be attributed to the ability of the plant to tolerate the soil conditions better than the maize plant. Samples may still contain some chemicals that may inhibit the activity of the appreciate enzymes needed for the germination of maize. However, this needs to be substantiated by longer periods of exposure to microbial treatment before germination in future works. Evidence in support of this hypothesis came from the outcome of a laboratory trial where no significant difference in the germination of the test crops was found in treated soil Bello *et al.*, (2008).

CHAPTER FIVE
SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

The waste generated from the production of fiber cement roofing contains heavy metals that are harmful to the environment. The fiber cement waste is classified as red category because of its toxic nature. These toxic wastes are often discharged into land with little or no treatment, because of the cost of physical and chemical treatment technology, especially in developing countries including Nigeria. Indigenous microbial strains are therefore considered for biological treatment of waste, because of their adaptation to their environment. The aim of the study was to remove heavy metal from waste generated from fiber cement roofing sheet company using microbial species isolated from fiber cement contaminated soil.

Samples were collected from the factory waste, dump site and soil outside factory and analyzed for pH, electrical conductivity and heavy metal concentration using pH meter, conductivity meter and atomic absorption spectrophotometry respectively.

The microbiological analysis included the enumeration and isolation of microorganisms by pour/spread plate method on Nutrient Agar for bacteria and potato dextrose Agar for fungi. The identification of the isolates was by standard microbiological protocols. Isolates capable of growth on fibre cement waste agar (FCWA) were used for subsequent experiments.

The effect of single and consortium treatment of the isolates on the levels of the physical and chemical parameters of the fiber cement waste and contaminated soil levels were investigated under laboratory conditions for a period of 12 weeks. The efficacy of the isolates to remove the heavy metals was determined using germination index (plant growth) of Beans and maize seed after treatment. Statistical analysis was done using descriptive means, graphical illustration and ANOVA.

Bacillus, Proteus, Pseudomonas, Citrobacter, Acinetobacter, Micrococcus (bacteria) and *Aspergillus, Trichophyton, Trichoderma, Rhizopus, Microsporium canis* (fungi) were among the organisms isolated.

Analyses of the fiber waste and fiber contaminated soil before treatment showed the heavy metal levels to be higher than the recommended standard (WHO, 1998) indicating that the waste were not or improperly treated. The percentage reduction of cadmium in treated samples with single isolates ranged from 5-33%, chromium 6-49% and Nickel 4-23%, percentage reduction in treatment with all bacteria was cadmium 22-56%; chromium 16-60% and Nickel 5-

37% while for all fungi the values were cadmium 18-50%, chromium 17-56% and Nickel 4-28%. Percentage reduction of cadmium, chromium and Nickel by the consortium was 31-75%, 20-78% and 7-52% respectively. The treatment with consortium of the isolates had the highest efficiency in the heavy metal reduction.

The germination index results showed that:

i. Single microbial treatment was not as good as consortium
ii. There was better germination of beans and maize seed with consortium treated samples
iii. Germination of beans seed was better than that of maize after the microbial treatment

The seeds grew better in treated samples than in untreated samples. The germination of the seed improved and not significantly different from germination in untreated samples ($P<0.05$).

5.2 Conclusion

The study identified the strains of bacteria and fungi that had the desired characteristics for the detoxification of the heavy metals from fiber cement waste. The research findings indicate that bioremediation using growing microorganisms present in fiber cement waste can reduce the concentration of heavy metals in fiber cement waste. Ability to grow and utilize cells sourced from fiber cement waste also promises environmental eco-friendly viable option. The presence of consortia microorganisms improved the metals properties and ensured a continuity in the process irrespective of the external environment. By developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting laboratory trials of new bioremediation techniques which are cost effective, and dedicating sites which are set aside for long term research purpose, these opportunities offer potential for significant advances. The selected organisms significantly reduced the level of the toxic constituents of fiber cement waste and waste dumpsite to acceptable levels thereby rendering them harmless as indicated by the phytotoxicity test. Effectiveness of single isolates also lead to the observation of consortium in treating fiber cement waste. Consequently a consortium of the indigenous strains of bacteria and fungi with potential application in fiber cement wastes treatment was identified. The consortium significantly detoxified the pollutants better than the single isolates and supported the growth of beans and maize crops in the

laboratory trials. This results may have the effect of encouraging fiber cement roofing sheet industry to embrace treatment of fiber cement waste using microorganisms, because of its eco-friendly, easy to operate do not produce secondary toxic substance and not expensive. The procedure is natural and hence the ecosystem is not likely to be negatively impacted.

5.3 Recommendation

- ❖ Microorganisms associated with fiber cement waste can be used as bioremediation of pollutants.
- ❖ The fiber cement industry in Sapele, Sapele local government area contain high level of pollutants that continues to threaten the environment if not controlled with appropriate treatment. It has been stated earlier that the cost of the conventional, physical and chemical treatment technology is discouraging to Fiber cement industries. However, this can be overcome by embracing and promoting biological treatment as this study has shown.
- ❖ Research and development in the environment of operation of the Fiber cement industries can identify microorganisms that can act in consortium to detoxify Fiber cement waste indicated by the findings of this study. This requires appropriate support from the Government agencies charged with the responsibility of maintaining the environment.
- ❖ Fiber cement industries are encouraged to embrace treatment of fiber cement waste using microorganisms, because of its eco-friendly, easy to operate, do not produce secondary toxic substance and not expensive.

5.4 Contribution To Knowledge

• Indigenous bacteria and fungi in roofing sheet waste were isolated.

• Organisms capable of utilizing the roofing sheet waste were identified.

• A protocol of organisms capable of reducing heavy metals in the waste was identified.

• An eco-friendly soil treatment sequence were developed and substantiated with germination of beans seeds and maize grain using phytotoxicity assay.

REFERENCES

Abou-Shanab, R.A.I., Van Berkum, P. and Angle, J.S. (2007). Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphre of *Alyssum murale*. *Chemosphere* 68:360-367.

Abou-Shanab, R.I., Angle, J.S., Delorme, T.A., Chaney, R.L., Van Berkum, P., Moawad, H., Ghanem, K. and Ghozlan, H.A. (2003). Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. *New Phytologist* 158: 219-224.

Achankeng, E. (2003). *Globalization, Urbanization and Municipal Solid Waste Management in Africa*: *the case of Bamenda and Yaounde.* University of Adelaide. 22p.

Ainsworth, R. (2004) *Safe, piped water: Managing microbial water quality in piped distribution systems*. IWA Publishing, London, for the World Health Organization, Geneva. 98p.

Akhtar, K., Akhtar, M.W. and Khalid, A.M. (2007). Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum*. *Water Research* 41:1366-1378.

Akob, D.M., Mills, H.J. and Kostka, J.E. (2007). Metabolically active microbial communities in uranium-contaminated subsurface sediments. *FEMS Microbiology Ecology* 59:95-107.

Aksu, Z., Egretli, G. and Kutsal, T. (1998). A comparative study of copper (II) biosorption on Ca-alginate, agarose and immobilized *C. vulgaris* in a packed bed colum. *Process Biochemistry* 33: 393-400.

Alef, Y. and Nannipieri, P. (1996). Methods in Applied Soil Microbiology and Biochemistry. *Academic Press*. London. Pp 19-22.

Alloway, B.J. (1990). Heavy metals in soils. Glasgow & London: Blackie and Son Ltd. 14p.

Ansari, M.I. and Malik, A. (2007). Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. *Bioresource Technology* 98: 3149-3153.

APHA (1998), American Public Health Association, "Standard Methods for the Examination of Waste and Waste water 20th (ed) APHA Washington D.C. 56p.

Asha L.P. and Sandeep R.S. (2013) Review on Bioremediation- Potential Tool for Removing Environmental Pollution, *Intl J. Basic and Appl. Chem. Sci.* **1**: 2277- 2073

Awomeso, W., Winner, A and Fombe, L. F. (2010). Solid Waste Dumping and Collection Facilities in Douala: Indications for a Sustainable Management Policy in Loyola. *Journal of Social Sciences*, 9(1): 1-9.

Baby, S., Kumar, S.S., Singh, N.A., Preeti, S., Deepali, S and Nath, S.R. (2008) Impact of Dust Emission on plant vegetation in the vincinity of cement plant. *Env. Eng. Mgt. J*. **7**(20):540-600.

Bahi, J. S., Radziah, O., Samsuri, A. W., Aminudin, H. and Fardin, S. (2012). Bioleaching of Heavy Metals from Mine Tailings by .*Bioremediation Journal*, **2**:57-65.

Baker, F. and Banfield, J. (2003) *Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health*. WHO Emerging Issues in Water and Infectious Disease Series. London, IWA Publishing. Pp. 16-18

Baldrian, P. (2003). Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology* 32: 78-91.

Barker, B.J., Ipeaiyeda, F. and Banfield, J. (2006). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology* 44: 139-152.

Barnett, H.C. and Hunter, B.B. (1999). llustrated General of Imperfect fungi 4th (ed), Burger Publishing Company, Mineapolis, Minnesota.

Belimov, A.A., Kunakova, A.M., Safronova, V.I. and Stepanok, V.V. (2004). Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. *Microbiology* (Moscow) 73: 99–106.

Berg, J.M., Tymoczko, J.L. and Stryer, L. (2002). Biochemistry. 5th Ed.. New York: *W.H. Freeman and Company* Pp. 81-98.

Bernardet, J.F., Nakagawa, Y. and Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *International Journal of Systematic and Evolutionary Microbiology* 52:1049-1070.

Bernhard, A.E., Colbert, D., McManus, J. and Field, K.G. (2005). Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. *FEMS Microbiology Ecology* 52:115-128.

Bogner, J., Abdelrafie Ahmed, M., Diaz, C., Faaij, A., Gao, Q., Hashimoto, S., Mareckova, K. and Zheng, T. (2007). *Waste Management*, *In Climate Change*: *Mitigation, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. In B. Metz, O. R., Davidson, P. R., Bosch, R. Dave, & Meyer, L. A. (Eds.). Cambridge: Cambridge University Press. Pp. 586-618.

Boothman, C., Hockin, S., Holmes, D.E.,Gadd, G.M. and Lloyd, J.R. (2006). Molecular analysis of a sulphate-reducing consortium used to treat metal-containing effluents. *BioMetals* 19: 601–609

Boswell, C.D., Dick, R.E., Eccles, H. and Macaskie, L.E. (2001). Phosphate uptake and release by *Acinetobacter johnsonii* in continous culture and coupling of phosphate release to heavy metal accumulation. *Journal of Industrial Microbiology Biothecnology* 26:333-340.

Brehem-Stecher, B.F. and Johnson, E.A. (2004). Single-cell microbiology: tools, technologies, and applications. *Microbiology and Molecular Biology Reviews* 68:538-559.

Bruneel, O., Duran, R., Casiot, C., Elbaz-Poulichet, F. and Personne, J.-C. (2006). Diversity of Microorganisms in Fe-As-Rich Acid Mine Drainage Water of Carnoulès, France. *Applied and Environmental Microbiology* 72: 551-556.

Casarett and Doull's, Toxicology: The Basic Science of Poisons (2001). Klaassen, K.D., 6th Ed. New York: *McGraw-Hill Higher Education*, Pp. 20-28.

Chang., C.C. and Tseng, S.K. (1998). Immobilization of *Alcaligenes eutrophus* using PVA crosslinked with sodium nitrate. *Biotechnology Techniques* 12:865–868.

Chee-Sanford, J.C., Aminov, R.I., Krapac, I.J., Garrigues-Jeanjean, N. and Mackie, R.I. (2001). Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Applied Environmental Microbiology* 67:1494-1502.

Chen, J.Z., Tao, X.C., Xu, J., Zhang, T. and Liu, Z.L., (2005a). Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeroginosa* in a column. *Process Biochemistry* 40: 3675-3679.

Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X. and Chen, Y.X., (2005b). Biosorption of copper(II) and zinc(II) from aqeous solution by *Pseudomonas putida* CZ1. *Colloids and Surfaces* 46: 101-107.

Chihching, C., Yumei, K., Changchieh, C., Chunwei, H., Chihwei, Y. and Chunwei, Y. (2008). Microbial diversity of soil bacteria in agricultural field contaminated with heavy metals. *Journal of Environmental Sciences* 20:359-363.

Collins, E. and Lyne, P. (2007) Microbiological Methods 5th (ed). Butterworth Publishing Company London. 92p.

Craven, D. J., Okraglik, H. M. and Eilenberg, J. M. (1994). Construction waste and a new design methodology. In Kilbert, C. J. (Ed.), *Sustainable Construction*. Centre for Construction and Environment, Gainsville, FL. Pp. 89-98.

Crowley, D.E., Wang, Y., Reid, C.P.P. and Szaniszlo, P.J. (1991). Mechanisms of Fe acquisition from siderophores by microorganisms and plants. In: Chen, Y., Hadar, Y. (Eds.), Iron Nutrition and Interactions in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, Pp. 213–232.

De las Heras Alarcón, C., Twaites, B., Cunliffe, D., Smith, J.R. and Alexander, C. (2005). Grafted thermo- and pH responsive co-polymers: Surface-properties and bacterial adsorption. *International Journal of Pharmaceutics* 295: 77–91.

Dell'Amico, E., Cavalca, L. and Andreoni, V. (2005). Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. *FEMS Microbiology Ecology* 52: 153-162.

Dell'Amico, E., Cavalca, L. and Andreoni, V. (2008a). Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biology & Biochemistry* 40: 74–84.

Dell'Amico, E., Mazzocchi, M., Cavalca, L., Allievi, L. and Andreoni, V. (2008). Assessment of bacterial community structure in a long-term copper-polluted ex-vineyard soil. *Microbiological Research* 163:671-683.

Dhalkephalkar, P.K. and Chopade, B.A. (1994). High-levels of multiple metal resistance and its correlation to antibiotic-resistance in environmental isolates of *Acinetobater*. *Biometals*. 7:67-74.

Eccles, F.L. (1999) Treatment of Metal- contaminated Waste: Why select a biological process. *Trend Biotechnol* **17**:462-485.

Ehrlich, H.L. (1994) "Inorganic Hazardous Amenable to biological transformation" In Biotechnology for the treatment of Hazardous waste (D.L. Storner, ed) Lewis Publisher, Boca Rotan- Ann Arbor- London- Tokyo. Pp 27-44.

Eiler, A. and Bertilsson, S. (2004). Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. *Environmental Microbiology* 6, 1228-1243.

Ekop, A.S. and Eddy, N.O. (2009). Adsorption of Pb2+, Zn2+ and Ni2+ from Aqueous Solution by Helix aspera Shell. *E-Journal of Chemistry* 6:1029-1034.

Errasquin, E.L. and Vazquez, C. (2003). Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. *Chemosphere* 50: 137-143.

European Communities (2006) Commission regulation (EC) No. 188 1/2006 Regulation of setting Maximum levels for certain contaminants in food stuffs. *Official J. European Union* **1**:364-424.

Ferguson, J., Kermode, N., Nash, C. L., Sketch, W. A. J., & Huxford, R. P. (1995). *Managing and minimizing construction waste, A Practical Guide.* London, Institution of Civil Engineers. Pp. 79-98.

Gadd, G.M. & Griffiths, A.J. (1978). Microorganisms and heavy metal toxicity. *Microbial Ecology* 4: 303-317.

Gadd, G.M. (1990). Microbiology of Extreme Environments. Milton Keynes UK. Open Universty Press. 89p.

Gadd, G.M. (1992). Microbial control of heavy metal pollution. Microbial control of pollution, *48th Symposium of the Society for General Microbiology, J.C. Fry et al.*, Cambridge University Press, Pp 59-88.

Gadd, G.M., Gray, D.J. and Newby, P.J. (1990). Role of melanin in fungal biosorption of tributyltin chloride. *Applied Microbiology and Biotechnology* 34: 116-121.

Giller, K.E., Witter, E. and McGrath, S.P. (2009). Heavy metals and soil microbes. Soil Biology & Biochemistry 41: 2031-2037.

Giovannoni, S.J. and Stingl, U. (2005). Molecular diversity and ecology of microbial plankton. *Nature* 437: 343-348.

Glick, B.R., Penrose, D.M. and Li, J. (1998). A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. *Journal of Theoretical Biology* 190: 63-68.

Gomori, G. (1990). Preparation of Buffers. In *Methods of Enzymology*. Edited by N. A. John & I. S. Malvin., San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: Academic Press, Inc. Pp138-146.

Goris, J., De Vos, P. and Coenye, T. (2001). Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp.nov., *Ralstonia metallidurans* sp. Nov.,

and *Ralstonia basilensis* Steinle *et al*. 1998 emend,. *International Journal of Systematic Bacteriology* 51: 1773-1782.

Gouveia, N. and Do prado, R.R. (2009) Health Risk in Area close to solid waste landfill sites. *J. Microb*. **44**: (5) 1-8.

Guiné, V., Martins, J.M.F. and Gaudet, J.-P. (2003). Facilitated transport of heavy metals by bacterial colloids in sand columns. *Journal de Physique. IV*. 107:593–596.

Guiné, V., Martins, J.M.F., Causse, B., Durand, A., Gaudet, J.-P. and Spadini, L. (2007). Effect of cultivation and experimental conditions on the surface reactivity of the metal-resistant bacteria *Cupriavidus metallidurans* CH34 to protons, cadmium and zinc. *Chemical Geology* 236: 266-280.

Guo, Z., Megharaj, M., Beer, M., Ming, H., Rahman, M. M., Wu, W. and Naidu, R. (2009). Heavy metal impact on bacterial biomass based on DNA analysis and uptake by wild plants in the abandoned copper mine soils. *Bioresource Technology* 100:3831-3836.

Habi, K. and Daba, H (2009). Bacterial zinc uptake and regulators. *Current Opinion in Microbiology* 8:196-202.

Hadis, G., Mehran, H., Arezoo, T. and Mohammad, M.G. (2011). Investigation of bioremediation of arsenic by bacteria Isolated from contaminated soil, *Afr. J. Microb. Res.* **5** (32): 5889- 5895.

Hantke, (2005). Cadmium Ion Biosorption by the Thermophilic Bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*. *Applied and Environmental Microbiology* 72:4020-4027.

Hassen, A., Saidi, N., Cherif, M. and Boudabous, A. (1998). Resistance of environmental bacteria to heavy metals. *Bioresource Technology* 64: 7-15.

Helena, C.S. (2003). Management and recycling of demolition waste in Hong Kong. *Proceedings of the 2nd International Conference on Solid Waste Management*, Pp. 433-442.

Hiroki, M. (1992). Effects of heavy metal contamination on soil microbial populations. *Soil Science and Plant Nutrition* 38:141-147.

Horner-Devine, M.C., Carney, K. and Bohannan, B.J.M. (2004). An ecological perspective on bacterial biodiversity. *Proceedings of the Royal Society B: Biological Sciences* 271: 113-122.

Hugenholtz, P., Goebel, B.M. and Pace, N.R. (1998). Impact of culture independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* 180: 4765-4774.

Ipeaiyeda, S., Guerreiro, A., Villa-García, M., Rendueles, M., Díaz, M. and Piletsky, S. (2012). Synthesis of 2-(diethylamino)ethyl methacrylate-based polymers: Effect of crosslinking degree, porogen and solvent on the textural properties and protein adsorption performance. *Reactive and functional polymers*. 70: 890-899.

IramShazia, U., Gul Rukh, S. and Ara T. (2013) Bioremediation of Heavy Metals Using Isolates of Filamentous Fungus *Aspergillus fumigatus* Collected from Polluted Soil of Kasur, Pakistan, *Intl. Res. J. Biol. Sci*. **2**(12), 66-73

Irwin, I.J. and Baird, A.W. (2004). Extremophiles and their applications to veterinary medicine. *Irish Veterinary Journal* 57: 348-354.

Ista, L.K., Perez-Luna, V.H. and Lopez, G.P. (1999). Surface-Grafted, Environmentally Sensitive Polymers for Biofilm Release. *Applied and Environmental Microbiology* 65:1603-1609

Jin. H., Park, D., Lamb, P., Paneerselvam, G., Nanthi, B. and Jae-Woo, C. (2011) Role of organic amendments on enhanced bioremediation of heavy metalloid contaminated soils, *J. Haz. Mater.*, **2–3:** 549–574

Jjemba, P.K. (2004). Envirnomental Microbiology Principles and applications. Enfield, NH. Science Publishers. Pp 38-46.

Kumar, A., Bisht, B.S., Joshi, V. D and Dhewa, T. (2011). Review on Bioremediation of Polluted Environment : A Management Tool. *Intl. J. Env. Sci.,* 1: 6-8.

Li, P., Guo, Y., Zhu, N., Zhu, S. and Deng, C. (2007). Molecular phylogenetic diversity of bacteria and its spatial distribution in composts. *Journal of Applied Microbiology* 103: 1344–1354

Madhaiyan, M., Poonguzhali, S. and Sa, T. (2007). Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). *Chemosphere* 69:220–228.

Madigan, M.T., Martinko, J.M. and Parker, J. (2003). Brock Biology of Microorganisms 10th Ed.. Upper Sadle River, USA. Pearson Education, Inc. Pp 13-14.

Manahan, S.E. (2004). Environmental Chemistry 8th Ed. Boca Raton: CRC Press LLC 138p

Marazioti, C. (1998). Heavy Metal Tolerance and Uptake by Soil Bacteria. *Institute of BioScience and Technology: Cranfield University.* 46p.

Mathyprakash, K. and Jayanthi, N.R. (2011). Adsorption of chromium from aqueous solution by activated alumina and activated charcoal. *Bioresource Technology* 98: 954-957.

Mayak, S., Tirosh, T. and Glick, B.R., (2004a). Plant growth-promoting bacteria that confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* 42: 565-572.

Medina, M. (2002). *Globalization, Development, and Municipal Solid Waste Management in Third World Cities*, El Colegio de la Frontera Norte, Tijuana, Mexico, 38p.

Metcalf and Eddy Inc. (2003). Wastewater Engineering: treatment and reuse / revised by George Tchobanoglous, Franklin L. Burton and H. David Stensel. 4th Ed. New York: *McGraw-Hill Higher Education*.

Moffet, B. F., Nicholson, F. A., Uwakwe, N. C., Chambers, B. J., Harris, J. A. and Hill, T. C.J. (2003). Zinc contamination decreases the bacterial diversity of agricultural soil. *FEMS Microbiology Ecology* 43:13-19.

Mulligan, C.N., Kamali, M. and Gibbs, B.F. (2004). Bioleaching of heavy metals from a low-grade mining ore using *Aspergillus niger*. *Journal of Hazardous Materials* 110:77-84.

Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (1999). On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. *Environmental Progress* 18:50-54.

Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology* 60, 193-207

Narayanan, M and Devarajan, N. (2011). Bioremediation of Effluents from Magnetite and Bauxite Mines using *Thiobacillus* sp and *Pseudomonas* sp, Department of Biotechnology, Peryar University, Salem, Tamilnadu, India. Pp 21-24

Ochiai, E, (1997) Bioinorganic chemistry: Introduction Align and Bacon Bostoni 45p.

Ogbonna, M., Piotrowska-Seget, Z., Cycon, M. and Kozdroj, J. (2005). Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. *Applied Soil Ecology*. 28: 237–246.

Panswad, T., Doungchai, A. and Anotai, J. (2003). Temperature effect on the microbial community of enhanced biological phosphorus removal system. *Water Research* 37:409-415.

Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P. and Schloter, M. (2006). Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biology & Biochemistry* 38: 327-341.

Prakasam, T.B.S. and Dondero, N.S. (2004). Aerobic Heterotrophic Bacterial Population of Effluent and solid waste. *Appl. Microb.* **115**:1122-1127

Prasad, M and Freitas, V. (1999), Removal of Toxic Metals from Solution by Leaf, Stem and Root Phytomass of Quercus Ilex L. (Holly Oak), *J. Env. Poll*. **110**(2000):277-283.

Rajapaksha, R.M.C.P., Tobor-Kapłon, M.A. & Bååth, E. (2004). Metal Toxicity Affects Fungal and Bacterial Activities in Soil Differently. *Applied and Environmental Microbiology* 70: 2966-2973.

Riser-Roberts, E. (1998). Remediation of Petroleum Contaminated Soil: Biological, Physical and Chemical Processes, *Lewis Publishers*, Boca Raton, Florida, USA. 27p.

Rosen, P.B. (1996). Bacterial resistance to heavy metals and metalloids. *The Journal of Biological Chemistry* 1:273–277.

Sag, Y., Nourbakhsh, Z., Aksu, Z. and Kutsal, T. (1995). Comparison of Ca-alginate and immobilized *Z. ramigera* as sorbents for copper (II9 removal. *Process Biochemistry* 30: 175-181.

Salam, A. (2010). *Environmental and Health Impact of Solid Waste Disposal at Mangwaneni Dumpsite in Manzini, Swaziland. Journal of Sustainable Development in Africa*, *12*(7): 64-78.

Salt, D.E., Prince, R.C., Pickering, I.J. and Raskin, I. (1995). Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiology* 109:1427–1433.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual, 2nd ed. Cold Harbour Laboratory, Cold Spring Harbour, N. Y. 131p..

Sandaa, R.A., Torsvik, V. and Enger, O. (2001). Influence of long-term heavy-metal contamination on microbial communities in soil. *Soil Biology & Biochemistry* 33, 287-295.

Sandaa, R.-A., Torsvik, V., Enger, Ø, Daae, F.L., Castberg, T. and Hahn, D. (1999). Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. *FEMS Microbiology Ecology*. 30: 237-251.

Sandifer, R.D. and Hopkin, S.P. (1996). Effect of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* 33:2475–2486.

Sarret, G., Avoscan, L., Carriere, M., Collins, R., Geoffroy, N., Carrot, F., Covès, J. and Gouget, B. (2005). Chemical forms of selenium in the metal-resistant bacterium Ralstonia metallidurans CH34 exposed to selenite and selenate. *Applied Environmental Microbiology* 71: 2331–2337.

Selatnia, A., Bakhti, M.Z., Madani, A., Kertous, I., Mansouri, Y. (2004). Biosorption of Cd2+ from aqueous solution by a NaOH treated bacterial dead *Streptomyces rimosus* biomass. *Hydrometallurgy* 75: 11-24.

Spain, A. and Alm, E. (2003). Implications of microbial heavy metal tolerance in the environment. *Reviews in Undergraduate Research* 2: 1-6.

Stohs, S.J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of heavy metals. *Free Radical Biology and Medicine* 18: 321-336.

Stokke, B., Smidsrod, O., Bruheim, P. and Skjak-Braek, G. (1991). Distribution of urinate residues in alginate chains in relation to alginate gelling properties. *Macromolecules* 24: 4637-4645.

Suranjana, A.R. and Manas, K.R. (2009)Bioremediation of Heavy metals Toxicity – with special Reference to Chromium, special. Pp 57-63.

Tsai, Y.P., You, S.-J., Pai, T.Y. and Chen, K.W. (2005). Effect of cadmium on composition and diversity of bacterial communities in activated sludges. *International Biodeterioration & Biodegradation* 55: 285-291.

Valls, M. and de Lorenzo, V. (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiology Reviews* 26:327-338.

Van Hullebush, E.D., Zandvoort, M.H. and Lens, P.N.L. (2003). Metal immobilization by biofilms: mechanisms and analytical tools. *Reviews Environmental Science Biology Technology* 2:9-33.

Van-de-Peer, Y., Chapelle, S. and de Wachter, R. (1996). A quantitative map of nucleotide substitution rates in bacterial rRNA. *Nucleic Acids Research* 24: 3381-3391.

Vega-López, A., Amora-Lazcano, E., López- López, E., Terrón, O. and Proal-Nájera, J.B. (2007). Toxic effects of zinc on anaerobi microbiota from Zimapán reservoir (Mexico). *Anaerobe* 13: 65-73.

Vidali, M. (2001). Bioremediation. An Overview, *Pure Appl. Chem*., **73**(7):1163 - 1172

Wang, F., Yao, J., Si, Y., Chen, H., Russel, M., Chen, K., Qian, Y., Zaray, G. and Bramanti, E. (2010). Short-time effect of heavy metals upon microbial community activity. *Journal of Hazardous Materials* 173:510-516.

Wang, J. and Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnology Advances* 27:195-226.

Wang, J., Shammas, N.K. and Hung N.K. (2004). Physicochemical treatment. Human Press, New Jessey Pp 141-198.

Woodard, F (2001) Industrial waste treatment Handbook 2nd ed Woodard and Curran Inc. Published by Butterworth. Pp 256-348.

Zettler, L.A.A., Gomez, F., Zettler, E., Keenan, B.G., Amils, R. and Sogin, M.L. (2002). Eukaryotic diversity in Spain‟s river of fire. *Nature*. 417:137.

Zeyede, A.I., Loukidou, M.X. and Matis, K.A. (2010). Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal polluted soils. *Process Biochemistry* 39:909-916.

Zeyede, K., Moen, B.E and Bratveit, M. (2010) Cement dust exposure and acute lung function. A close shift study, *BMC Pulmo. Med.* **10**: 19-23.

Appendix I

COMPOSITION AND PREPARATION OF MEDIA

NUTRIENT AGAR

Peptone	50g
Beef extract	1.50ml
Sodium chloride	5.0g
Agar	15.0g
Distilled water	1000ml
pH	7.3±0.2

Direction

Dissolve 28.0g in 1000 ml of distilled water. Gently heat to dissolve the medium completely. Sterilize by autoclaving at (121^{o}C) for 15 minutes. Dispense the medium as desired.

PEPTONE WATER

Peptic digest of animal tissue	10.00
Sodium chloride	5.00
pH	7.2 +/- 0.2 at 25^{0}C

Dissolve 15g in 1000 ml of distilled water. Sterilize by autoclaving at (121 ^{0}C) for 15 minutes. Cool to room temperature before use.

SIMMON CITRATE AGAR

Composition	Gram Litre
Magnesium sulphate	0.20
Ammmonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar No. 2	50.00

pH 6.9±0.2

Directions

Suspend 24g in 1000ml of distilled water. Heat to dissolve completely and sterilize by autoclaving at 121°C for 15 minutes. Allow to cool and dispense.

REAGENT COMPOSITION

Crystal Violet

Crystal Violet	20g
Ammonium Oxalate	9g
Ethylakohol	200ml
Distilled water	1000ml

Safranin Solution

Safranin	2.5g
Ethylacohol	100ml
Distilled water	1000ml

Distilled water	1000 ml

Potato Dextrose Agar

Potato Extract	4.0 g
Dextrose	20.0 g
Agar No.1	15.0 g

Potato dextrose agar

Thirty-nine gram (39g) of potato dextrose agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was the placed in an autoclave to sterilize it for 15 minutes at 121 ^{0}C. After sterilization, the flask was allowed to cool.

Appendix II

Table 1:Bacterial count of samples

Samples	soil Dumpsite	Fiber cement factory	Factory dumpsite	control
A	5.68	5.71	5.55	5.73
B	5.77	5.90	5.60	5.55
C	5.71	5.80	5.68	5.76
Mean	5.72	5.80333	5.61	5.68

Table 2:Fungal count of samples

Samples	soil Dumpsite	Fiber cement factory	Factory dumpsite	control
A	5.11	5.11	5.30	5.23
B	5.04	5.07	5.32	
C	5.00	5.04	5.00	
Mean	5.05	5.07	5.20	

Table 3 Result of heavy metal from fiber cement waste samples

Parameters	Results
Chromium	0.078
Lead	0.001
Zinc	0.659
Magnesium	1.161
Nickel	0.828
Cadmium	0.105
Cobalt	0.054
Copper	0.171

AAS reduction of treated samples (1st week)

S/N	Treated samples	Cd Mean	% reduction	Cr Mean	% reduction	Ni Mean	% reduction
1	Fiber control	0.104	0.95	0.077	1.23	0.827	0.12
2	Fiber all organisms	0.047	55.24	0.062	20.51	0.462	44.20
3	Fiber all bacterial	0.068	35.24	0.065	16.67	0.541	34.66
4	Fiber all fungi	0.081	22.86	0.067	14.10	0.697	15.82
5	Fiber *Bacillus*	0.094	10.48	0.071	8.97	0.751	9.30
6	Fiber *Proteus*	0.090	14.27	0.070	10.26	0.720	13.04
7	Fiber *Rhizopus*	0.100	4.76	0.073	6.41	0.810	2.17
8	Fiber *Microsporium* canis	0.097	7.62	0.072	7.70	0.807	2.54
9	Dumpsite control	4.168	0.05	2.862	0.28	40.670	0.025
10	Dumpsite all organisms	2.103	49.57	1.654	42.37	37.235	8.47
11	Dumpsite all bacterial	3.224	22.67	1.738	39.44	38.524	5.30
12	Dumpsite all fungi	3.368	19.23	1.748	39.09	38.963	4.22
13	Dumpsite *Bacillus*	3.979	4.15	1.997	30.42	39.752	2.28
14	Dumpsite *Proteus*	4.025	3.48	2.532	11.78	40.600	1.49
15	Dumpsite *Rhizopus*	4.084	2.06	2.724	5.09	40.519	1.97
16	Dumpsite *Microsporium* canis	4.096	1.78	2.750	4.18	19.860	0.3
17	Soil control	2.109	0.05	1.888	0.06	18.474	0.05
18	Dumpsite all organisms	1.442	31.66	0.886	53.12	19.003	6.89
19	Soil all bacterial	1.718	18.58	0.995	47.35	19.056	5.20
20	Dumpsite all fungi	1.733	17.87	1.097	41.96	19.512	4.22
21	Soil *Bacillus*	1.838	12.89	1.397	26.08	19.684	3.95
22	Soil *Proteus*	2.004	5.02	1.581	16.35	19.752	1.65
23	Soil *Rhizopus*	2.103	3.32	1.745	7.67	19.009	0.79
24	Soil *Microsporium* canis	2.046	3.03	1.651	12.64	19.752	0.45

(12weeks)

S/N	Treated samples	Cd Mean	% reduction	Cr Mean	% reduction	Ni Mean	% reduction
1	Fiber control	0.100	4.76	0.073	6.64	0.823	6.04
2	Fiber all organisms	0.026	75.23	0.019	75.64	0.398	51.93
3	Fiber all bacterial	0.046	56.19	0.036	53.85	0.519	37.32
4	Fiber all fungi	0.052	50.47	0.043	44.87	0.652	21.26
5	Fiber *Bacillus*	0.073	30.47	0.048	38.46	0.698	15.70
6	Fiber *Proteus*	0.070	33.33	0.044	43.58	0.706	14.73
7	Fiber *Rhizopus*	0.083	20.57	0.057	26.92	0.766	7.49
8	Fiber *Microsporium* canis	2.080	23.81	0.052	33.33	0.785	5.20
9	Dumpsite control	4.161	1.43	2.840	1.05	40.598	2.02
10	Dumpsite all organisms	1.580	62.11	1.105	61.49	32.598	19.88
11	Dumpsite all bacterial	2.849	31.68	1.314	54.21	34.926	14.13
12	Dumpsite all fungi	3.315	28.03	1.418	50.59	35.986	11.52
13	Dumpsite *Bacillus*	3.001	20.50	1.463	49.02	36.526	10.20
14	Dumpsite *Proteus*	3.750	10.07	2.118	26.20	38.265	5.92
15	Dumpsite *Rhizopus*	3.990	6.48	2.440	14.98	38.212	6.29
16	Dumpsite *Microsporium* canis	4.007	3.91	2.539	11.53	38.230	6.02
17	Soil control	2.105	0.2	1.820	0.27	19.80	0. 2
18	Dumpsite all organisms	1.140	45.97	0.422	77.67	12.532	36.83
19	Soil all bacterial	1.544	26.82	0.755	60.05	13.210	33.42
20	Dumpsite all fungi	1.590	24.64	0.823	56.46	14.264	28.10
21	Soil *Bacillus*	1.782	15.54	1.049	44.49	15.16	23.49
22	Soil *Proteus*	1.872	11.28	1.290	31.75	15.6422	21.16
23	Soil *Rhizopus*	1.850	12.32	1.479	21.74	16.321	17.73
24	Soil *Microsporium* canis	1.903	9.81	1.365	27.77	16.116	18..77