

## Reduction of Some Heavy Metals in Fibre Cement Roofing Sheet Waste-Contaminated Soil by Consortium of Bacteria and Fungi

Olubunmi Olufunmi Akpomie<sup>1</sup>, Catherine Ese Balogun<sup>1</sup> & Timothy Marhiere Akpomie<sup>2</sup>

<sup>1</sup> Delta State University Abraka, Delta State, Nigeria

<sup>2</sup> Federal University of Lafia, Nasarawa State, Nigeria

Correspondence: Timothy Marhiere Akpomie, Department of Chemistry, Federal University of Lafia, Nasarawa State, Nigeria. Tel: +234-803-3954-992 E-mail: akpomiet@yahoo.com, timothy.akpomie@science.fulafia.edu.ng

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### Abstract

This research was carried out in order to ascertain the effectiveness of microbial remediation (bio-remediation) of environmental pollution by heavy metals from different sources in general and wastes from the manufacturing of fibre cement roofing sheets specifically. The concentrations of Cd, Cr, and Ni in fibre cement soil (0.11, 0.08 & 0.83), in dumpsite soil (4.17, 2.87 & 40.68) and in surrounding soil (2.11, 1.89 & 19.84) and soils outside the pollution area, control, (1.76, 0.89 & 14.17) mg/g respectively were determined using atomic absorption spectrophotometry. Preliminary results showed that the concentration of the heavy metals; Cd, Cr, and Ni were all higher than values recorded by the WHO/FEPA standard. Analysis of the variance of means between the heavy metals showed differences in the respective sampled soils ( $P = 0.209$ ). The minimum inhibitory concentration (MIC) of the heavy metals on the test organisms of *Bacillus sp*, *Rhizopus sp*, *proteus sp* and *microsporium canis* were obtained by the Agar diffusion method from stock culture of isolates from fibre waste dumpsites at the Department of Microbiology, Delta State University Abraka. The MIC values for Ni on the respective test organisms were; 300, 150, 250 & 450, Cr; 250, 400, 350 & 450 while that for Cd was 900, 750, 900 & 700  $\mu\text{g/ml}$ . Sterilized consortia of isolates inoculated with various combinations of bacteria and fungi were used to treat the experimental sampled soils. Concentrations of the respective heavy metals of the sampled soils were evaluated after the 1<sup>st</sup> and 12<sup>th</sup> week of treatments. Furthermore, the concentration of the respective heavy metals reduced in varying percentages between the 1<sup>st</sup> and 12<sup>th</sup> week of treatment and the results were also found to be significantly different statistically ( $t\text{-calculated} > t\text{-critical}$ ). Overall, the percentage reduction in the heavy metal concentrations between the 1<sup>st</sup> and 12<sup>th</sup> week was higher in Cd and Cr (76.25% & 76.25%) respectively than in Ni (52.65%). This was an indication that microbial isolates were very effective in reducing heavy metals in fibre cement roofing sheet-wastes and from the environment.

**Keywords:** Roofing sheets, Bioremediation, Heavy metals, Microbial Isolates, Environment, fibre cement

### 1. Introduction

The anthropogenic activities of man which include the springing up of several industries such as leather, rubber, asbestos, roofing sheets, pharmaceuticals have resulted in an increase of discharge of heavy metals to water bodies and land thus causing undesirable effects which negatively impact on the physicochemical and biological properties of soils and water (Ipeaiyeda et al., 2012, Yuyao et al., 2018).

Fibre cement roofing sheet found to be durable, cool, produce less heat, does not easily rust, heat resistant and light (Zheng & Antonio, 2005) is now in vogue. The wastes from roofing sheet industry are termed inert or nuisance dust and are categorized as "Red" because of the presence of heavy metals such as Ni, Pb, Co, Cr which are hazardous to man, animals and plant (Zeyede et al., 2010). Metals are highly toxic, persistent and not biodegradable so impairs the natural ecosystem and consequently affect human health via the food chain. (Tang et al. 2018).

Fibre cement is composed of hydraulic binder, heavy metals, additives, reinforcing fibre which includes cellulose fibre, polyvinylacrylamide, polyvinylchloride, lignin, viscosity enhancing agent (hydroxyethyl methycellulose) and filler ( $\text{CaCO}_3$ , Chromium, Iron Oxide, Propylene fibre) (Zeyede et al., 2010). These components constitute environment hazards to soil, water and air when discharged indiscriminately. The action of water, sun, ice, wind,

moss, lichens and pollutants such as acid rain, SO<sub>2</sub> may cause corrosion which gradually releases the asbestos fibres and heavy metals in the matrix (Beddoe & Dorner, 2005).

An increase in the toxicity of heavy metals in the environment may eventually reach man and animals through the food chain thereby necessitating its removal from the polluted natural environment (Irma et al., 2013). The impact of the heavy metals in the soil manifests in several ways such as affecting soil respiration (Blagodatskaya et al., 2006), lead to physiological dysfunction and malnutrition in plants and plant seed, can accumulate in the human body causing, in some cases irreversible harm to human health (Singh et al., 2015).

The conventional methods of remediating heavy metals from contaminated sites are excavation, solidification and stabilization and other methods such as adsorption of the chemical materials (Choski & Jozi, 2007; Al – Muhtaseb et al., 2008). These temporarily remove heavy metals and have the disadvantages of being expensive, generation of secondary metabolites which are hazardous and inefficiency (Bahn et al., 2012). Biological techniques on the other hand, address these setbacks since they are easy to operate, cost-effective and do not produce secondary pollutants hence are eco-friendly. They also help in retaining the soil structure and the pollutants and microbes can almost completely be removed from the polluted environment (Yuyao et al., 2018).

The use of microbial remediation is becoming common and is considered promising due to its many advantages (Singh & Prasad, 2015). In bioremediation processes, microorganisms use the contaminants as nutrients or sources of energy (Kumar et al., 2011; Asha et al., 2013). The microbial populations respond to heavy metal contamination based on the concentration and bioavailability of the metals. These are affected by factors such as environmental conditions (Zhang et al., 2019; Du et al., 2018). The microbial survival in heavy metal polluted soils depends on biochemical properties, physiological and/or genetic adaptation which includes morphological, environmental modification of metal speciation (Shanab et al., 2007). Heavy metal reduction by microorganism can occur passively (biosorption) or actively (bioaccumulation). Irma et al. (2013) found that *Aspergillus fumigatus* has good biosorption capacity towards some heavy metals. Vargas et al. (2009) discovered that fungi isolated from compost were able to detoxify metal polluted environments.

In Nigeria, the usage of fibre cement roofing sheet is still in practice and there is not much research on the natural remediation of the environment in which the wastes are disposed so as to ensure a safe environment. This research aims at using indigenous microorganisms present in Eternit fibre cement roofing sheet waste in removing or reducing some heavy metals present in the waste which may constitute hazards to man and the environment. Numerous microbial species such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus*, *Rhizopus* and *Penicillium* have significant heavy metal removal ability. (Wierzeba, 2015; Dasola, 2014).

## 2. Materials and Methods

### 2.1 Collection of Samples

#### 2.1.1 Experimental Organisms

*Proteus sp*, *Bacillus sp.*, were collected on prepared sterile Nutrient Agar plates and *Aspergillus niger* and *Microsporium canis* on Potato Dextrose Agar plates from Microbiology Laboratory, Delta State University, Abraka from stock culture of organisms isolated from roofing sheet waste.

#### 2.2 Fibre Cement Waste and Soil Samples

The samples were collected in polythene bags using auger at different points within the dumpsite at a depth of 0-30cm. Samples were collected from the factory waste soil within the factory, dumpsite and a control from a point where there was roofing sheet production activity. The samples were transported to the Microbiology laboratory, Delta State University, Abraka in icepacks for analyses.

#### 2.3 Microbiological Analysis

##### 2.3.1 Ability of Isolates to Grow on Fiber Cement Waste

Mineral Salt Agar (MSA) incorporated with fibre cement waste (FCW) were used to test the ability of isolates to utilize fibre cement waste. The modified method of Akpomie et al., (2016) was used in preparing fibre cement waste agar. Here, fibre cement waste (100g) was autoclaved at 121°C for 30mins before filtering through glass wool. The filtrate was made up to the litre mark with freshly distilled water incorporated with mineral salts (g/l; CaCl<sub>2</sub>: H<sub>2</sub>O, 0.1 g; ZnSO<sub>4</sub>.0.01g; K<sub>2</sub>HPO<sub>4</sub>, 0.5g; MgSO<sub>4</sub>. H<sub>2</sub>O, 0.1g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. 7H<sub>2</sub>O, 0.01g; KCL, 0.05g; FeSO<sub>4</sub>.H<sub>2</sub>O, 0.1g thereafter 15g of agar powder was added for solidification. This was autoclaved for 30mins, allowed to cool and poured into plates. The plates were inoculated with the bacteria and fungi and incubated at 28±2°C for 24h and 72h respectively.

## 2.4 Treatment of Waste

The ball-milled fibre cement, dumpsite and soil samples were autoclaved at 120°C for 30 mins and treated with standardized inoculum of the isolates (singly and in combinations). The controlled samples were not treated with microorganisms.

## 2.5 Inoculum Development and Standardization

### 2.5.1 Bacterium:

Each bacterium inoculum was prepared by suspending 18h bacterium isolates in sterile normal saline (0.89%NaCl). The turbidity of the bacterial suspension was then adjusted to 0.5 McFarland Standard which is equivalent to  $1.5 \times 10^8$  (fu/ml).

### 2.5.2 Fungi:

The fungal inoculums were prepared by flooding the surface of Potato Dextrose. Agar slants inoculated with each fungus with sterile distilled water. The spores of the aerial mycelia were scraped with a loop and weighed

## 2.6 Determination of Minimum Inhibitory Concentration of the heavy metals on the isolates.

The MIC of the metals were determined by the Agar diffusion methods (Hassan *et al*; 2008) as described by (Vehisamy et al., 2011). Stock solution of the metals (1000 µg/ml) were prepared by weighing  $K_2Cr_2O_7$ ,  $Cd(CH_3CO_2)_2$  and  $NiCl_2$  and dissolved in 1000ml of sterile distilled water. The solution was mixed thoroughly and strengths of different concentrations (Cr: 400, 350, 300, 250 and 200 µg/ml; Cd: 1000, 950, 900, 850 and 800 µg/ml and Ni: 400, 350, 300, 250, 200 and 150 µg/ml) were made by double dilution method.

The microorganisms were subjected to growth in the different concentrations and observations were made after 24h and 72h incubation for bacteria and fungi respectively.

## 2.6 Treatment with Single Isolates

The fibre cement waste samples were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 20mins before inoculation.

Bacteria: Ten millimetre (10ml) mineral salt solution of each bacterium was introduced into polythene bags containing 200g of each sample (in triplicates). They were kept at  $28 \pm 2^\circ C$  for twelve weeks, after which the concentrations of the metals were determined. Samples were mixed intermittently and 100ml of sterile distilled water was added every 48hr.

## 2.7 Treatment with Consortia of Isolates

Sterilized samples were inoculated with various combinations of bacteria and fungi.

### 2.7.1 Combination of *Bacillus* and *Proteus* sp

A 5ml mineral salt suspension of each bacterium was mixed together and poured into 200g of sterilized samples and incubated at  $28 \pm 2^\circ C$  for 12weeks. They were mixed intermittently with 100ml of distilled water every 48hrs.

### 2.7.2 Consortium of fungi

Ten gram of each fungus was weighed and mixed together, thereafter was added to 200g of sterilized samples.

### 2.7.3 Consortium of all bacteria and fungi

This was done by mixing 2.5g of each fungus and 50ml mineral salt suspension of each bacterium. They were mixed and introduced into 200g of sterilized samples. Samples with no inoculum served as control for all the treatments.

## 2.8 Heavy Metal Analysis

Cr, Cd and Ni were analyzed using Atomic Absorption Spectrophotometry (APHA, 2004). The acid extraction was done using the method 3050B (USEPA, 1996). One gram of sample was placed in 250ml flask for digestion. The sample was heated to 95°C in 10ml of 50%  $HNO_3$ . It was allowed to cool then refluxed with repeated additions of 60%  $HNO_3$  until no brown fumes were given off by the sample. The solution was allowed to evaporate until the volume was reduced to 5ml. After cooling, 10ml of 30%  $H_2O_2$  was added slowly without allowing any loss. The mixture was again refluxed with 10ml of 37% HCl for 15minutes. The digestate obtained was filtered through 0.45µm membrane filter, diluted to 150ml with deionized water and stored at 4°C for analyses.

The concentrations of the heavy metal were measured using atomic absorption spectroscopy (AAS machine: Agilent Technology and 55AA Atomic Absorption Spectrophotometer). The respective reduction in the metal concentrations were calculated using the expression;

$$\frac{\text{Initial concentration of metal} - \text{final concentration}}{\text{Initial concentration}} \times 100 \dots\dots\dots (1)$$

The one way anova statistical method was used to analyze the result (appendix E).

### 3. Results & Discussion

Table 1. Concentration of Cadmium, Chromium and Nickel in the Samples (mg/g)

Heavy metals	Fibre	Dumpsite	Soil	Control	WHO/FEPA Standard
Cadmium (Cd)	0.11	*4.17	*2.11	1.76	0.30
Chromium (Cr)	0.08	*2.87	1.89	0.89	2.00
Nickel (Ni)	0.83	*40.68	*19.84	14.17	0.02

\* high concentration compared to WHO/FEPA Standards

Table 1 gives the varying concentrations of the heavy metals (Cd, Cr and Ni) in the respective fibre and soil samples. Concentrations were all high and differed among the samples. These concentrations were also noted to be higher than the WHO/FEPA standard except in the fibre samples (see appendix D). The heavy metals may have been introduced into the samples at various points of processing. The high concentration at the dumpsite may have resulted from accumulation over time and that of the surrounding soil could be from leachate.

Table 2. Minimum Inhibitory Concentrations (MIC) of the Heavy Metals on Test Organisms ( $\mu\text{g/ml}$ )

Isolates	Nickel	Chromium	Cadmium
<i>Bacillus</i> sp	300	250	900
<i>Rhizopus</i> sp	150	400	750
<i>Proteus</i> sp	250	350	1000
<i>Microsporium canis</i>	450	450	700

In Table 2, Ni and Cr showed a more pronounced inhibitory effect on the organisms than Cd. This may be that Ni and Cr possessed a higher antimicrobial activity than Cd. All three heavy metals may have inhibited the microorganisms by interacting with the enzymes directly involved or those involved in general metabolism. Cadmium is known to significantly influence the enzymes of microorganism except when they develop resistance to the metal (Chingching et al., 2008). The organisms exhibited a high resistance to the metals. Many bacteria make metals less toxic thus making organisms that live in heavy metal contaminated site potentially useful in bioremediation. The ability of the organisms to reduce the heavy metals and grow in their presence may be attributed to the fact that microorganisms develop ingenious mechanisms of metal resistance and detoxification which may include electrostatic interaction, ion exchange, precipitation, redox process and surface complexation, metal oxidation, metal effluxes. The degree of utilization of the metals varied among the organisms.

Table 3a. Effect of Treatment on the Cadmium Concentration in Soil, Fibre-cement and Waste Dumpsite after 12 Weeks

Treatment	Cadmium Concentration			Reduction After 12 Weeks (%)
	0 h	1 <sup>st</sup> Week	12 <sup>th</sup> Week	
Fibre waste without inoculum	0.11	0.104	0.100	9.09
Fibre + all organisms	0.11	0.047	0.026	76.36
Fibre + all bacteria	0.11	0.068	0.046	58.18
Fibre + all fungi	0.11	0.081	0.052	52.72
Fibre+ <i>Bacillus</i> sp	0.11	0.094	0.073	33.64
Fibre + <i>Proteus</i> sp	0.11	0.090	0.070	36.36
Fibre+ <i>Microsporium</i> sp	0.11	0.100	0.083	24.50
Dumpsite (control)	4.17	0.097	0.080	27.27
Dumpsite + all organisms	4.17	4.168	4.161	0.24
Dumpsite + all bacteria	4.17	2.103	1.580	62.11
Dumpsite + all fungi	4.17	3.224	2.849	30.70
Dumpsite + <i>Bacillus</i> sp	4.17	3.368	3.315	20.50
Dumpsite + <i>proteus</i> sp	4.17	3.979	3.001	28.03
Dumpsite + <i>Microsporium</i> sp	4.17	4.025	3.750	10.07
Dumpsite + <i>Rhizopus</i> sp	4.17	4.096	4.007	3.91
Soil (control)	2.11	4.084	3.990	4.32
Soil + all organisms	2.11	2.109	2.105	0.20

Soil + all bacteria	2.11	1.442	1.140	45.97
Soil + all fungi	2.11	1.718	1.544	26.82
Soil + <i>Bacillus sp</i>	2.11	1.733	1.590	24.64
Soil + <i>proteus sp</i>	2.11	1.838	1.782	15.55
Soil + <i>Rhizopus sp</i>	2.11	2.004	1.872	11.28
Soil + <i>Microsporium sp</i>	2.11	2.103	1.850	12.32

The above table gives the concentrations of Cd in the various samples before treatment (0 h) and after the 1<sup>st</sup> and 12<sup>th</sup> week of microbial treatments. Generally, the result showed that in all samples, there were varied reductions in the heavy metal concentration between the 1<sup>st</sup> and 12<sup>th</sup> week of microbial treatments. The highest % reduction in Cd (76.36%) was observed in the fiber sample inoculated with all microbial isolates. This was against the 62.11 and 45.97% observed in dumpsite and soil control samples respectively. Statistical treatment of the data suggests that there was significant difference between the 1<sup>st</sup> and 12<sup>th</sup> week of treatment of the waste with the microbial isolates, see appendix A. This maybe indicative of the reliability of the method.

Table 4. Effect of Treatment on Chromium Concentration in Soil, Fibre-cement and Waste Dumpsite after 1<sup>st</sup> and 12<sup>th</sup> Weeks

Treatment	Chromium Concentration			Reduction after 12weeks (%)
	0 h	1 <sup>st</sup> week	12 <sup>th</sup> week	
Fibre (control)	0.08	0.077	0.073	8.75
Fibre + all organisms	0.08	0.062	0.019	76.25
Fibre + all bacteria	0.08	0.065	0.036	55.00
Fibre + all fungi	0.08	0.067	0.043	46.25
Fibre + <i>Bacillus sp</i>	0.08	0.071	0.048	40.00
Fibre + <i>proteus sp</i>	0.08	0.070	0.044	45.00
Fibre + <i>Rhizopus sp</i>	0.08	0.073	0.057	28.75
Fibre + + <i>Microsporium sp</i>	0.08	0.072	0.052	35.00
Dumpsite (control)	2.87	2.862	2.840	1.05
Dumpsite + all organisms	2.87	1.654	1.105	61.50
Dumpsite + all bacteria	2.87	1.738	1.314	54.22
Dumpsite + all fungi	2.87	1.748	1.418	50.59
Dumpsite + <i>Bacillus sp</i>	2.87	1.997	1.463	49.02
Dumpsite + <i>proteus sp</i>	2.87	2.532	2.118	26.20
Dumpsite + <i>Rhizopus sp</i>	2.87	2.724	2.440	14.98
Dumpsite + <i>Microsporium sp</i>	2.87	2.750	2.539	38.05
Soil (control)	1.89	1.888	1.820	3.70
Soil + all organisms	1.89	0.886	0.422	77.67
Soil + all bacteria	1.89	0.995	0.755	60.05
Soil + all fungi	1.89	1.097	0.823	56.46
Soil + <i>Bacillus sp</i>	1.89	1.397	1.049	44.50
Soil + <i>proteus sp</i>	1.89	1.581	1.290	31.75
Soil + <i>Rhizopus sp</i>	1.89	1.745	1.479	21.75
Soil + + <i>Microsporium sp</i>	1.89	1.651	1.365	27.77

Table 4a is the result obtained upon the determination of the % reduction in the concentration of Cr in the various samples after treatment with the microbial isolates and after the 1<sup>st</sup> and 12<sup>th</sup> week. The obtained results showed a similar trend with those of Cd. Between the range of measurement, there were also varying degrees in percentage reduction of the heavy metals when treated with all forms of the various microbial isolates. Though treatment of the fibre and any of the individual microbial isolate was effective in reducing the heavy metal concentration, the treatment with all organisms, 76.25%, was highest when compared to 61.50 and 77.67% for the Dumpsite with all organisms and soil with all organisms respectively. Additionally, statistical treatment, appendix B showed that there was significant difference between the 1<sup>st</sup> and 12<sup>th</sup> week of treatments.

Table 5. Effect of Treatment on Nickel Concentration of Soil, Fibre-cement and Waste Dumpsite after the 1<sup>st</sup> and 12<sup>th</sup> Weeks

Treatment	Nickel Concentration			Reduction after 12weeks (%)
	0 h	1 <sup>st</sup> week	12 <sup>th</sup> week	
Fibre (control)	0.83	0.827	0.823	0.84
Fibre + all organisms	0.83	0.462	0.398	52.65
Fibre + all bacteria	0.83	0.541	0.519	37.47
Fibre + all fungi	0.83	0.697	0.652	21.45
Fibre + <i>Bacillus sp</i>	0.83	0.751	0.698	15.90
Fibre + <i>proteus sp</i>	0.83	0.720	0.706	14.94
Fibre + <i>Rhizopus sp</i>	0.83	0.810	0.766	7.71
Fibre + + <i>Microsporium sp</i>	0.83	0.807	0.785	5.42
Dumpsite (control)	40.68	40.670	40.598	0.20
Dumpsite + all organisms	40.68	37.235	32.598	19.87
Dumpsite + all bacteria	40.68	38.524	34.926	14.14
Dumpsite + all fungi	40.68	38.963	35.986	11.54
Dumpsite + <i>Bacillus sp</i>	40.68	39.752	36.526	10.21
Dumpsite + <i>proteus sp</i>	40.68	46.600	38.265	5.05
Dumpsite + <i>Rhizopus sp</i>	40.68	40.519	38.212	6.07
Dumpsite+ <i>Microsporium sp</i>	40.68	40.680	38.230	6.02
Soil (control)	19.84	18.474	19.800	0.20
Soil + all organisms	19.84	19.003	12.532	36.83
Soil + all bacteria	19.84	19.056	13.210	33.42
Soil + all fungi	19.84	19.512	14.264	28.10
Soil + <i>Bacillus sp</i>	19.84	19.684	15.160	23.59
Soil + <i>proteus sp</i>	19.84	19.752	15.642	21.14
Soil + <i>Rhizopus sp</i>	19.84	19.009	16.321	17.73
Soil + <i>Microsporium sp</i>	19.84	19.752	16.116	18.77

Table 5a is the result for the analysis of Nickel. The trend is similar to those of Cadmium and Chromium. However, the percentage reduction in the concentration of Ni was much lower than that of Cd and Cr. For Ni, the three categories were 52.65, 19.87 and 36.38% respectively. There was also a significant difference between the 1<sup>st</sup> and 12<sup>th</sup> week of fiber sample treatment of this heavy metal with all the microbial isolates. See appendix C.

Table 6. pH of Contaminated Soil, Fibre-cement and Fibre-waste Dumpsite Before and After the Different Treatments

Treatment	fibre-cement		Dumpsite		Soil	
	a	b	a	b	a	b
Control	8.2	8.0	7.8	8.2	8.0	8.5
All organisms	8.2	8.0	7.8	8.0	8.0	7.8
Proteus sp + Bacillus sp	8.2	8.3	7.8	8.2	8.0	7.2
Rhizopus +Microsporium	8.2	8.7	7.8	8.2	8.0	8.5
Bacillus sp	8.2	8.5	7.8	8.2	8.0	8.0
Rhizopus sp	8.2	8.7	7.8	7.9	8.0	7.8
Microsporium sp	8.2	8.3	7.8	7.9	8.0	8.0
Proteus sp.	8.2	8.2	7.8	7.6	8.0	9.0

a=before treatment; b=after treatment.

Table 6 gives the pH of the various samples before and after treatments with the microbial isolates. All pH values were shown to be basic in nature. The difference in activities of the organism may be attributed to the fact that pH affects the activity of enzyme in microorganisms thus affecting the rate of microbial metabolism of heavy metals thus may contribute to microorganisms reacting differently to different pH values. pH, temperature, substrate species, substrate concentration all, affect heavy metal removal by microorganisms (Marchenko et al., 2015; Gola et al., 2016; Park et al., 2016). These may have contributed to the varying degrees of the ability of the microorganisms to remediate the heavy metals in the soil. This may also be due to the bioavailability of the heavy metals and the adsorbed dose (Rasmussen et al., 2000).

Furthermore, heavy metals may break vital enzymatic functions, disrupt ion regulation and/or directly affect the formation of DNA as well as protein (Gauthier *at al.*, 2014; Hildebrandt et al., 2007) which may have contributed to the poor reduction of some of the metals (especially Nickel) by *Proteus sp* and *Aspergillus sp*. The microbial

cells in this study may have been able to reduce the level of the heavy metals especially chromium and Cadmium through bioaccumulation, biosorption and/or through other mechanisms.

Fungal species isolated from industrial wastes have been found to exhibit resistance to heavy metals which are found naturally and become concentrated as a result of human activities (Vidali, 2001). The reduction may further be explained by adsorption which normally does not depend on energy metabolism and occurs exclusively in living cells (Wang, 2001). Microbes adsorb a high amount of heavy metal ions rapidly. *Bacillus* was found to adsorb  $\text{Cu}^{2+}$  (Tebo, 1998). It may also be by complexation or reflux reactions thus changing the valence of the metal which can affect their mobility or toxicity (Gavrilescu, 2007). Kotas and Stkiska (2000) showed that many genera of microbes including bacteria, some yeasts and fungi help in bioremediation of chromium and metal contaminated soils and waste by bio-absorption and bioaccumulation. The organisms that utilized the heavy metals varied, 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 characteristics.

Practically, all the treatments with the organisms reduced the metal levels introduced from the fibre cement activity. The report of Irma *et al.* (2013) showed that the ability of some microorganisms to tolerate heavy metals and to promote transformations may be through adsorption. Several microorganisms like bacteria, fungi and algae have been used to clean up heavy metal contaminated environment (Neha *et al.*, 2013; Srivasta *et al.* 2015). Park *et al.* (2005) isolated *Rhizopus* sp which was able to bioremediate chromium metals. Li *et al.*, (2015) also reported a *Rhizopus* sp which can easily remove ZN, Cu, Cd and Th.

However, the fungi (*Aspergillus niger* and *Microsporium canis*) in this study were not efficient in the heavy metal removal. This may be due to the strain of fungal species used and also the environment from which it was isolated. The treatments with consortia of organisms reduced the metals more than other treatments which may be attributed to microorganisms living in mixed colonies comprising of different species and genera of organisms where there is synergy of different metabolic activities. Many researchers have reported the higher effectiveness of consortia of microorganisms for bioremediation of heavy metals than single organisms (Kader *et al.*, 2007); Mosa *et al.*, 2016; Abioye *et al.*, 2018) which conforms with the findings of this study where the different combinations of the consortia gave a better result than single treatment procedures.

#### 4. Conclusion

All the organisms were able to grow on the fibre cement medium showing their ability to tolerate high metal concentrations induced by the fibre cement. The Minimum Inhibitory Concentrations of the metals on the microorganisms further confirmed the potential of the organisms to grow at high metal concentrations especially Cadmium and Chromium. Hence, the organisms were quite effective in reducing the levels of the metals at varying degrees. The treatment of the fibre, dumpsite and polluted soil with the consortia of all organisms and all bacteria was more effective than all fungi and treatment with individual organisms. The all bacteria and all organisms protocol of treatment significantly reduced the levels of the chromium and Cadmium and to an extent the nickel present in the heavy metal polluted fibre cement samples. The consortium of *Bacillus* sp, *Proteus* sp, *Rhizopus* sp and *Microsporium* sp can be used in the treatment of waste from fibre cement roofing sheet industry and in bioremediating polluted soils from such activities.

Further work is suggested on expanding the focus on genetics of the organisms and other organisms and the specific mechanism of action of the organisms so as to enhance the metal reduction potential in fibre cement which is an area that has not been well researched into.

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#### Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## Appendix A

**Table 3b:** Summary of t-test Analysis of Significant Mean Difference Between the 1<sup>st</sup> Week and 12<sup>th</sup> Weeks with the Controls and Treatments of Cadmium Concentration.

Week	Mean	Std	Variance	Treatment	df	t cal.	t Crit.	P<0.05
1st	1.86	1.54	2.36	24	23	3.46	1.71	0.0001
12th	1.71	1.45	2.11	24				

In Table 3b, since the t-cal. (3.46) higher than the t-critical (1.71) with difference (23) at p<0.05 level of significance, there exist significance difference in the mean cadmium concentration between the 1<sup>st</sup> and 12<sup>th</sup> weeks among the controls and their treatments.

**Appendix B**

**Table 4b:** Summary t-test Analysis to Significant Mean Difference Between the 1<sup>st</sup> Week and 12<sup>th</sup> Weeks with the Controls and Their Treatments of Chromium Concentration.

Week	Mean	Std	Variance	Treatment	df	t cal.	t Crit.	P<0.05
1st	1.24	0.98	0.96	24	23	5.81	1.71	0.0001
12th	1.02	0.89	0.79	24				

In Table 4b, since the t-cal. (5.81) greater than the t-critical (1.71) with difference (23) at p<0.05 level of significance, there exist significance difference in the mean chromium concentration between the 1<sup>st</sup> and 12<sup>th</sup> weeks among the controls and their treatments.

**Appendix C**

**Table 5b:** Summary t-test Analysis of Significant Mean Difference Between the 1<sup>st</sup> Week and 12<sup>th</sup> Weeks with the Controls and Treatments of Nickel Concentration

Week	Mean	Std	Variance	Treatment	df	t cal.	t Crit.	P<0.05
1st	20.12	16.63	276.44	24	23	4.69	1.71	0.0001
12th	17.66	15.32	234.60	24				

In Table 5b, since the t-cal. (4.69) higher than the t-critical (1.71) with difference (23) at p<0.05 level of significance, there was significance difference in the mean nickel concentration between 1<sup>st</sup> and 12<sup>th</sup> weeks among the controls and their treatments.

**Appendix D**

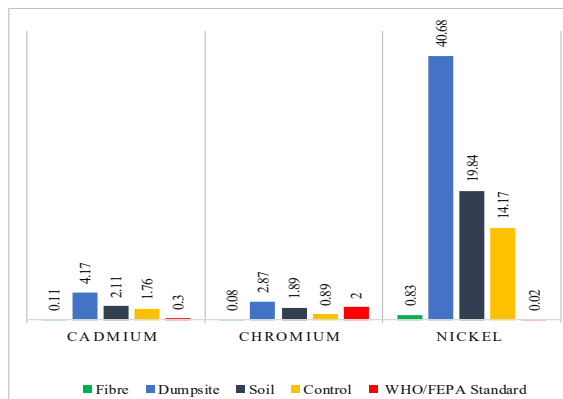


Fig.1: Bar chart showing the concentrations of Cd, Cr and Ni in the various samples as compared to WHO/FEPA standard.

**Appendix E**

**One-way ANOVA: Cr, Cd, Ni**

**Method**

Null hypothesis            All means are equal  
 Alternative hypothesis    Not all means are equal  
 Significance level         $\alpha = 0.05$

*Equal variances were not assumed for the analysis.*

**Factor Information**

Factor	Levels	Values
Factor	3	Cr, Cd, Ni

**Welch's Test**

Source	DF		F-Value	P-Value
	Num	DF Den		
Factor	2	5.35326	2.13	0.209

**Model Summary**

R-sq	R-sq(adj)	R-sq(pred)
14.35%	0.07%	0.00%

**Means**

Factor	N	Mean	StDev	95% CI
Cr	5	10.12	17.12	(-11.14, 31.38)
Cd	5	0.498	0.481	(-0.100, 1.096)
Ni	5	7.61	8.85	(-3.38, 18.59)

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