The Application of Biomaterials in Ecological Remediation of Land Pollution: Bioremediation of Heavy Metals in Cement Contaminated Soil Using White-Rot Fungus *Pleurotus sajor-caju*

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Research Article

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Abstract

Environmental pollution is a result of cement manufacture. Soil and plant analysis at the Lafarge Cement Factory (LCF) as well as bioremediation of soils contaminated with cement at various concentrations were done. Human-induced heavy metal mobilization in the biosphere has developed into a significant phenomenon. This study used atomic absorption spectrophotometry to determine the levels of Ni, Cr, Pb, and Zn in 15 soil samples and 36 *Synedrella nodiflora* plants that were taken from the area around the Lafarge Cement factory in Sagamu, Nigeria (AAS). The findings of the metal study showed that some metals exceeded the essential limitations for the soil and plants, as well as the natural background levels. The white-rot fungus *Pleurotus sajor-caju* was investigated for its ability to mineralize heavy metals. Five kilograms of soil was carefully weighed and thoroughly mixed with cement to give 0.1%, 0.2%, 0.3% and 0.4% contamination levels, five kilograms of the contaminated soil from the vicinity of the cement factory with an unknown percentage level of contamination was also weighed and a set control was also weighed (0%). Following the addition of the fungus to the soil samples using rice straw as a substrate, the samples were incubated for a month. After a month of the fungus' incubation, the soil's heavy metal level significantly decreased. After a month, P. sajor-caju was able to mineralize the heavy metals and improve the soil's nutrients. In order to initiate the ecological restoration process for soil contaminated by cement, P. sajor-caju can be used as a bioremediation agent.

1. Introduction

The primary component of cement, Cement Kiln Dust (CKD), is a fine, gray or white powder that is typically kept as trash in open pits and landfills. The primary component of concrete used to build modern structures is cement. Cement manufacture, however, produces a lot of cement dust. Short-term contact to cement dust may not be problematic, but ongoing exposure to the substance can harm plants and animals irreparably [1–7]. Air pollution has a great impact on human health, climate change, agriculture and natural ecosystem. Besides health, cement factories are deteriorating environment as shown by series of studies [1–13]. Atmospheric emissions from industrial establishments are one of the major sources of environmental pollution. One type of industry that causes particle pollution is the cement industry [14]. The main inputs of cement activity on the environment are the broadcasts of dust and gases [15]. Cement dust spreads along large areas through wind, rain etc. and are accumulated in and on soils [16–20]. Bioremediation is a biotechnological approach of rehabilitating areas degraded by pollutants or otherwise damaged through mismanagement of the ecosystem using plants and microorganisms to remove or detoxify environmental contaminants [21]. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. In this work, we will focus on the agricultural impact of cement as one of the major soil contaminants in one of the major cities in Nigeria and how its effects can be corrected through the process of bioremediation. This study was conducted to determine the amount of heavy metals deposition by cement dusts on soil and *Synedrella nodiflora* plants. The study also looked into the bioremediation potential of *Pleurotus sajor-caju* on heavy metal content of cement contaminated soil.

Thus, while soil pollution is caused by the presence of man-made chemicals or other toxic material in the natural soil environment. The most common soil pollutants are petroleum hydrocarbons [22–24], solvents, pesticides [25–36], lead and other heavy metals [37–52]. The occurrence of toxic material in most areas correlates directly with the degree of industrialization and intensity of chemical usage in those areas. Cement is made up of four main compounds: tricalcium silicate($3CaO{\cdot}SiO_2$), dicalcium silicate($2CaO{\cdot}SiO_2$), tricalcium aluminate ($3CaO{\cdot}Al_2O_3$), and a tetra-calcium aluminoferrite ($4CaO{\cdot}Al_2O_3Fe_2O_3$). Small amounts of uncombined lime and magnesia also are present, along with alkalis and minor amounts of other elements [53].

Cement dust of sufficient quantities have been reported to dissolve leaf tissues [54]. Other reported effects of cement dust on plants include reduced growth, reduced chlorophyll, clogged stomata in leaves, cell metabolism disruption, interrupt absorption of light and diffusion of gases, lowering starch formation, reducing fruit setting [55], inducing premature leaf fall and leading to stunted growth [56] thus causing suppression in plants and in animals it leads to various respiratory and hematological disease, cancers, eye defects and genetic problems [57–59]. Besides gaseous and particulate pollutants there are also enhanced levels of toxic heavy metals in the environment of cement factory likely cobalt, lead, chromium, nickel, mercury [39–49, 60] posing potential hazard for all living organisms. Increased concentrations of the above pollutants cause progressive reduction in the photosynthetic ability of leaves, mainly a reduction in growth and productivity of plants [61]. Metal toxicity in plants has been reported by various authors [62, 63] Among the heavy metals, mercury, lead, nickel, chromium are most dangerous heavy metals released by cement factories [64] and is responsible for causing various biochemical changes which also includes cyto toxic and mutagenic effects [65, 66] such as chromosomal aberrations, stickiness, c-mitosis, chromosomal bridge, chromosome fragmentation, vagrant chromosomes, DNA fragmentation etc in various plants. Bioremediation has been intensively studied over the past two decades, driven by the need for a low-cost, *insitu* alternative to less expensive engineering-based remediation technologies [63, 67, 68] Bioremediation has been applied to remove crude oil [69, 70], motor oil [71] and diesel fuel [72] from soil but the removal efficiency is highly variable [73]. Plants can indirectly influence degradation by altering the physical and chemical conditions of the soil [74]

While, bioremediation remain a non-invasive technique that leaves the ecosystem intact [75]. It can deal with lower concentration of contaminants where the cleanup by physical or chemical methods would not be feasible but can only be effective where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate [76, 77]. The factors that must be optimized for successful bioremediation are: biostimulation, oxygen and inorganic nutrients, pH, temperature, water availability, and adsorption effects. *Pleurotus sajor-caju* fungi have been well known for metal removalability from aqueous phase. The multilaminate and microfibrillar structure of fungal cell wall along with distinctive aspects of high percentage of cell wall material attributes excellent metal binding properties. *P. sajor-caju* is recognized as a mushroom with distinct properties. It can be cultivated within a wide range of temperatures on different natural resources and agricultural wastes. The sporophores of *P.sajor-caju* have 26.9% protein having high digestibility values and all essential seventeen amino acids in good concentration. [78] found that *P.sajor-caju* and *Pleurotus fabellatus*, when compared with other basidiomycetes of the genus *Pleurotus sp.*, are collectors of heavy metals and also have high rates of degradation of the medium where they are growing. The mycelium of fungi can absorb heavy metals, which can accumulate and move to the other parts of the fungal body in concentrations, and are sometimes far higher than that of the medium [78]. Its high metal absorbing property makes *P. sajor-caju* ideal for bioremediation purposes.

2. Materials And Methods

2.1. Description of the sample site

Lafarge cement factory is located in the southern part of Sagamu a small town in the southwestern part of Nigeria, it lies between latitude 6^{o} 50' North and longitude 4^{o} 00' East. The area is characterized by high annual temperature, high rainfall, and high relative humidity and it is classified as humid tropical region, the soil type is ferralitic. The surrounding vegetation consists of trees, shrubs and herbs. The experiment for this study was carried out in the department of Crop Protection and Environmental Biology, University of Ibadan and at National Horticultural Research Institute Ibadan.

2.2. Collection of samples

The soil sample used for this experiment was collected at various distances from the West African Portland Cement Company- Lafarge, facility, Sagamu, Ogun State, Nigeria. In May, 2014, twelve soil samples and 36 plant samples of (*Synedrella nodiflora*) were collected from designated sampling points. Soil samples were collected from the top soil at not more than 15cm depth and were scrapped into labeled polyethylene bags. The soil used for control was gotten from the crop garden at the department of Crop protection and Environmental biology, University of Ibadan. Spawns of *P.sajor-caju* were obtained from the mushroom unit of the National Horticultural Research Institute (NIHORT), Jericho Ibadan, Oyo State, Nigeria.

2.3. Sterilization of glasswares and treatment of soil samples

Glasswares were washed thoroughly with a solution of detergents and rinsed with clean water before allowed to dry. Sterilization of glasswares was done for 150 mins at 60^{o}C. Soil samples meant for degradation studies was sterilized using an autoclave at 121^{o}C for15 mins after which they are allowed to cool to room temperature.

2.3.1. Experiment 1:

To evaluate the quantity of heavy metals as a result of cement dust deposition in the soil and the flora around the Sagamu cement factory and a nearby farm. In the Laboratory, the collected soil and plant samples were air dried for 2 weeks at room temperature for 27ᴼC, the soil samples were sieved using a 2mm mesh sized sieve to obtain a homogenous distribution of soil contents.

2.3.2. Preparations for Heavy Metals in Soils

One gramme of the sieved soil was accurately weighed and transferred to a 100ml beaker, 20ml of 1:1 of nitric acid (analar grade) was added to the weighed sample, the sample was then boiled gently on a hotplate until the volume of nitric acid was reduced to5ml. 20ml of distilled water was added to the sample and boiled gently again until the volume was 10ml, which was then allowed to cool. The suspension was filtered through a Whattman filter paper. Washing the filter paper and the beaker with small portions of distilled water, a volume of 25ml was obtained, this was then made up to50ml using a 50ml graduated flask. The samples were analysed for heavy metals using the Atomic Absorption Spectrophotometer.

2.3.3. Preparations for Heavy Metals in Plants

The root and shoot of *Telfaria occidentalis* and *Synedrella nodiflora* were air dried for 2weeks at room temperature of 27ᴼC and the plant parts were ground and ashed in a muffle furnace at a temperature of 500–550ᴼC. Heavy metals were extracted using the double acid extraction method.

2.3.4. Soil pH Analysis

The soil pH was determined by weighing 10g of air dried soil samples into beakers, 10ml of distilled was then added, after which the mixture was stirred manually for 5mins with a glass rod and it was allowed to stand for 30mins. The pH was then read using the pH meter [78].

2.4. Preparation of Contamination

5kg of homogenized soil sample was carefully weighed and poured in a clean basin. No cement was added. This served as the CONTROL with a contamination level of 0% and was labeled as A. Four separate samples of 5kg of homogenized soil were carefully weighed and poured into separate clean basins. Cement was then added to each sample in quantities of 5g, 10g, 15g and 20g and mixed thoroughly. This gave a contamination concentration level of 0.1%, 0.2%, 0.3% and 0.4% and each was labeled B, C, D and E respectively with increasing concentration. 5kg of the naturally contaminated soil was carefully weighed and no cement was added and was labeled F.

2.4.1. Experiment 2:

To investigate the effect of *P. sajor-caju* incubation on the heavy metal content of cement contaminated soil. The procedures were according to the method of [80] and modified as follows: 200g of each sample was weighed into transparent polyethylene bags, replicated four times and labeled. 5g of rice straw was added after which 10g of vigorously growing spawns of the fungi was added. Plastic necks were used to support the nylons so that they could be corked with cotton wool and the necks wrapped in foil. The nylons were also doubled to prevent tearing or melting due to heat. They were placed in complete randomized design (CRD). Making a total of another 24 samples. At four weeks after inoculation, mycelia-ramified waste was separated from the spawns and rice straws and the soil was analysed for physicochemical properties and heavy metal content after air-drying.

2.5. Data and statistical analysis

Standard error values were calculated and data was analysed using a one-way Analysis of variance (ANOVA). Fishers Least Significant Difference (LSD) was used to identify where there was significant differences between the treatment means at 95% confidence interval ($P < 0.05$).

3. Results And Discussions

3.1: Nutrient composition of contaminated soils around LCF Sagamu, Ogun state

Table 1 shows the nutrient levels of soils contaminated with cement deposit as a result of the factory activities. An increase in soil pH was observed at a distance closer to the cement factory and the pH decreased as the distance increased. The lowest pH was observed at a distance of 100m where a farm is located, followed by the control and then at 60m, 40m and 20m respectively. There was no significant difference in the Organic matter (O.M.) content of all the soils at the various distances that was tested for including the control. The soils that were used for control was observed to have the highest phosphorus content, this is followed by the phosphorus content in the soils at 60m distance away from the factory, while there was no significant difference in the Phosphorus content in the soils at 20m, 40m and 100m. There was a relatively significant difference in the Calcium content of the soil at 20m and the soil at100m. This is showing a decrease in the Calcium content of the soils at 100m distance away from the factory. The increase in the high content of Calcium in the soil used for control cannot be compared with the Calcium content in the other soils at the varying distances, this is because, the soil used for control was gotten from a non-cement producing area. There was no significant difference in the sodium content of all the soils that were sampled at the various distances but the least quantity was observed at 100m, this was also applicable for Potassium.

3.2: Distribution of heavy metals in soils around LCF, Sagamu, Ogun State

Table. 2 shows the distribution of heavy metals in soils around Lafarge Cement Factory, Sagamu, Ogun state. For the soil samples, the highest level of heavy metal content corresponded to Zinc, at a distance of 60m followed by Lead. The lowest levels of heavy metal were recorded for Chromium and Nickel. A significant decrease in the quantity of Zinc was observed at 100m away from the Lafarge cement factory. According to the present study, the range for Nickel was 2.50 - 25.21 mg/kg. The elemental concentrations in the soil samples are in the decreasing order of Zn > Pb > Cr > Ni.

3.3: Heavy metal content of uncontaminated soil and cement-contaminated soils

Table 3 shows the heavy metal content of uncontaminated soil and different concentrations of cement-contaminated soils before incubation with *Pleurotus sajor-caju* (zero month). A general increase was observed as the concentration levels increases for Lead, Chromium, and Zinc, this increase was significant in all the treatments. Significant differences were also observed for Copper and Nickel at 0% and 0.1%.

Table 4 shows the heavy metal content of uncontaminated soil and different concentrations of cement-contaminated soils at one month after incubation with *Pleurotus sajor-caju.* The result shows that Lead at the concentration of 0.4% had the highest mean of 31.60mg/kg and the lowest mean of 6.70mg/kg at 0% concentration. The 0.4% concentration of Lead was however significantly different from all other concentration levels at $P<0.05$. This trend was also observed with Chromium which had the highest mean of 20.18mg/kg at 0.4% level of concentration and the lowest mean of 1.94mg/kg at 0% concentration. This was significantly different from other concentrations of 0.1%, 0.2%, and 0.3% at $P<0.05$. The highest reduction for Zinc was observed at the contaminated soil obtained from a farm at the site of the Lafarge cement factory with a mean of 12.10mg/kg which was significantly different from all the other treatment used except at 0.1% level of concentration. Nickel had the highest reduction at 0.1% concentration with a mean of 37.7mg/kg and this was significantly different from all the other treatments. Copper had the highest reduction of 12.40mg/kg at 0.4% concentration, this was not significantly different to 0.1% concentration but was significantly different to the other treatments.

Studies on soil have implicated these metals as reaching or exceeding toxicity levels. For example, Bennet *et al.* [81] has shown that the toxic level of Chromium in soil is around 2-50 mg/kg and this in comparison with Chromium measurement in the current situation which is high. The critical level for Nickel in soil have been investigated by many researchers [40, 46, 51, 81-83] and estimated to be in the range of 2-50 mg/kg. According to the present study, the range for Nickel (2.50 - 25.21 mg/kg) is alarming suggesting that Nickel pollution is critical in the investigated area. The study indicated high bioavailability of Zinc in the cement-contaminated soil used in the experiment. This high bioavailability of Zn may be due to the high soil pH, which can increase solubility [84]. The organic matter content of soil can favour bioavailability of Zn, making it more abundant for uptake by vegetables, as metals associated with organic matter have high mobility due to decomposition and oxidation of organic matter with time [85]. There have been reports that high pH increases solubility of Copper in soil, making it phyto-bioavailable [86]. The presence of high concentrations of heavy metals (Zinc, Chromium, Nickel and Lead) in the soil of the study area indicates that it has been affected by anthropogenic activity, in particular the cement factory. The high levels of heavy metals in these soils have increased the accumulation of metals in the flora around the cement factory. As a result of the complicated relationship in levels of individual element in the different set of samples, focusing on their distribution in terms of distance and direction will be futile, instead we find a way of discussing contamination status of the sampling sites. Surface soil and vegetation are considered as major sink of airborne metals. Consequently, the measurement of metals in these media can be useful to establish trends and abundance and their consequences as a result of natural changes and those caused by man [87-94]. Of the two environmental matrices, the soil has largely been used to establish metal contamination in relation to natural or anthropogenic influence on the environment [18, 48]. Having established a good correlation between the soil and vegetation samples under the current investigation, the use of the soil data in explaining sources of environmental contamination will provide good judgment in understanding contamination of heavy metals by atmospheric deposition and industrial activities. This study showed that the mycelia elongation increased progressively up to 12 DAI. However, 0.1% contamination level had the highest vertical elongation level closely followed by 0.2% contamination level with 0.4% contamination level and the contaminated soil from cement factory having the lowest vertical elongation. This ability of the white rot fungus to grow on all the concentration levels agrees with the fact that white rot fungi grow by hyphal extension and thus can reach pollutants in the soil in ways that other organisms cannot [95, 96]. The results obtained from this study showed an increase in the nutrient content of the soil with the increase of the incubation period. Increase in percentage organic carbon and organic matter in soil incubated with *Pleurotus sajor-caju* was also observed with the increase of the incubation period, thus indicating that a degradation must have taken place. This is similar to the work done by Raimi and Sabinus [19]; Adenipekun, and Omoruyi, [97] in bioremediation of cement and battery polluted soil using *Pleurotus pulmonarius*, where higher organic carbon and organic matter were recorded compared to control samples resulting in remediation of the samples. An increase in Potassium and decrease in Phosphorus occurred in both the cement-free and the cement-contaminated soil from 400.0mg/kg to 900.0mg/kg,42.25mg/kg to 17.51mg/kg for the cement-free soil, and from 170.7mg/kg to 200.0mg/kg, 35.55mg/kg to 17.20mg/kg for the cement-contaminated soil. This indicates that a degradation has occurred. The reduction in calcium observed in both soil samples showed that *P. sajor-caju* accumulated the calcium from both soil samples. The reduction is higher in the cement contaminated soil due to the fact that cement is rich in calcium.

Until the current investigation, data on metal levels in the two environmental matrices (soil and vegetation) from the area under the potential influence of the emissions of these pollutants by the cement facility here evaluated were scarce. Therefore, the current results should be of a special interest as reference values in future evaluations of the facility, which was deemed very necessary. From the results of the current investigation, it recommend that future cement production facilities must be set away from settlements and farms, and that our environmental regulations must be strengthened so as to prompt current industrial operators to take precautions and new techniques to protect the environment from hazardous pollutants [87 - 94]. The reason being that the human body is of a complex structure, therefore, the accumulation of metals can cause many toxic effects, which can influence different mechanisms on the body [14].

By way of monitoring the operational influence of the cement facility on the environment, this study underlines the need for replicating periodic studies (two years duration) on the facility on the environment in addition to the evaluation of the facility on human health and more research should also be done on the health implication of vegetables grown in the area on man. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant [73]. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. Studies were conducted on removal of Pb, Zn, Cu and Mn from artificially contaminated soil using *Pleurotus sajor-caju*. The level of heavy metals in the soil reduced drastically after cultivating *Pleurotus sajor-caju* for a period of 30 days to bioabsorb the metals. More than 50% of the metals were removed. *P. sajor-caju* has thus proven its ability to enhance the nutrient content of polluted soils, and can be used as a remediator of heavy metal polluted environment.

4. Conclusions

In conclusion, the reduction in the pH of the soil after the inoculation of *P. sajor-caju* from 6.90 to 6.50 after 4 weeks for the cement-contaminated soil showed that microbial activities actually occurred in the soil. This is similar to the findings of other studies where they observed a decrease from 7.55 to 7.11 for cement polluted soil, and from 5.90 to 5.62 after 2 months of incubation. This study also showed the reduction in the heavy metal content of the different cement-contaminated soil treatments, which indicates that *P. sajor-caju* has accumulated the heavy metals present in the soil after incubation for four weeks. Studies were conducted on removal of Pb, Zn, Cu and Mn from artificially contaminated soil using *Pleurotus sajor-caju*. The level of heavy metals in the soil reduced drastically after cultivating *Pleurotus sajor-caju* for a period of 30 days to bioabsorb the metals. More than 50% of the metals were removed. *P. sajor-caju* has thus proven its ability to enhance the nutrient content of polluted soils, and can be used as a remediator of heavy metal polluted environment.

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Tables

Tables 1-4 are available in the supplementary files section.

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