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Influence of Bioaugmentation on the Decontamination of Heavy Metal Impacted Landfill Soil

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Abstract

The research is aimed to decontaminate heavy metal polluted landfill soil using consortia of fungi as bioaugmentation agents. Two consortia namely highly metal tolerant fungi (HMTF) and moderately metal tolerant fungi (MMTF) were used for the bioaugmentation. The experiment was conducted at day 0, 20, 60, and 100. Soil physicochemical parameters (pH, conductivity, redox potentials, and metal concentrations) were found to decline along the duration of the experiment. The maximum metal removal (48%) was achieved in soil treated with HMTF. Both treatments were efficient than the control ($P < 0.05$). The bioremediation efficiency was significantly influenced by the bioaugmented fungi. Therefore, the technique can be utilized for future treatment of metal impacted soil.

Keywords: Bioaugmentation; Fungal consortia; Soil contamination; Landfill; Heavy metals

Introduction

Soil is a valuable component of the environment as it does not only serve as a sink for chemical contaminants but also serves as a natural buffer that control the movement or transfer of substances and chemical elements to the biosphere, atmosphere, and hydrosphere [1]. Soil contamination with heavy metals can only occur when there is transport of the metal from the source to the soil and the pollution can either be localized or extensive [2]. The concentrations of some metals (e.g. Cd) in the anthropogenically impacted soil can be up to 32 times higher than that of the background soil, meanwhile, for others such as Pb and Cu, the difference maybe 10 times higher [3]. Metal toxicity varies with the type of metals, with some being more toxic than others. For instance, Ag is more toxic to a freshwater fish than Hg [4]. Many toxic metals have been reported as mutagenic, carcinogenic, and teratogenic to different organisms depending on the contact duration and dosage [5]. As a result of their toxicity effects, heavy metals need to be removed from the contaminated soil. The research is aimed to decontaminate heavy metal polluted landfill soil using consortia of fungi as bioaugmentation agents. Current research focuses on bioaugmentation and the main purpose of bioaugmentation is to increase the catabolic capability of the microorganisms for a better remedial action. Fungi are capable of degrading various environmental contaminants including recalcitrant compounds. This is largely as a result of their inherent ability to release various extracellular enzymes [6]. The ability of fungi to carry out diverse metabolic activity is an important factor in the detoxification and removal of toxic metals from the environment. Unlike bacteria and other organisms, fungi are stronger and have the capacity to withstand extreme environments including high concentration of heavy metals [7].

Materials and Methods

Sample Collection, Microbial Isolation and Identification

The soil sample was collected from the Taman Beringin landfill. The collection of the soil was done according to EPA [8]. The collected soil was kept in sterilised plastic bags and transported to the laboratory for analysis. Serial dilution technique was used for the isolation of the microbes. 1 g of soil sample was dispensed in 10 mL distilled water (10^{-1}) and shaken for 10 minutes. 1 mL was drawn from 10^{-1} and serially diluted in 9 mL until 10^{-7}. 0.1 mL of 10^{-7} was inoculated onto plates of potato dextrose agar (PDA) (Friendemann Schidt, Parkwood, WA, Australia). The plates were incubated at 28 °C for 6 days. The colonies were isolated using physical observation. The identification was carried out using microscopic and molecular techniques [9,10].

Formulation of Fungal Consortia and Experimental Design of Bioremediation

Thirteen species of the identified fungi were used to make the consortia based on their tolerance attributes. The consortia were highly metal tolerant fungi (HMTF) and moderately metal tolerant fungi (MMTF). Each strain was grown individually in potato dextrose broth (PDB) (Friendemann Schidt, Parkwood, WA, Australia) at 28°C for 4 days. On reaching 3 x 10^{9} spore/g, equal volume of the inoculum of each representative isolate was drawn and combined to make the inoculum of consortium which was used for bioaugmentation. The bioremediation setup involves treatment (with fungal amendment) and control (with no fungal amendment). The experiment was run for 100 days. Monitoring was carried out on day 0, day 20, day 60, and day 100. The parameters monitored were physicochemical parameters, organic acids, enzymes activity, and microbial population. The experiment was conducted according to Jayanthi [11].

Results and Discussions

From the initial stage (day 0) to the final stage (day 100) of the bioremediation, the pH of the soil declined continuously. The range of the pH was 6.4 – 7.9 and this applies to both the treatments and the control. There was no significant pH variation between the treatments and the control ($P > 0.05$). The redox potentials occurred in 2 states. At the commencement of the experiment (day 0), the redox potentials were in oxidised state, however, starting from day 20 to day 100, the values for all the treatments including the control changed to a reduced state. This decline can be related to microbial activity during the remediation process. This is because, in the course of biodegradation or bioremediation of contaminants, microbes make use of the available dissolved oxygen to carry out the metabolic activities [12]. This shows the influence of the inoculated fungi in reducing the inorganic content in the soil. The initial fungal population was low (1.5 x 10^{9} CFU/g soil), however, after the addition of the fungal inoculum, the population has skyrocketed. Meanwhile, as the duration lengthened, a gradual decrease in population was observed. The highest population was 1.39 x 10^{10} CFU/g soil. On the contrary, a continuous decease in population was noticed in the control soil right from day 0 up to day 100. Moreover, the population in the control was significantly lower than those of the treatments ($P < 0.05$). Similar trend was observed in the case of bacterial population however, lower bacterial count was recorded relative to that of fungi ($P < 0.05$). This can also be linked to the influence of the bioaugmentation. The soil enzymes activity proceeded in a similar pattern with that of the microbial population. All enzymes activity in the treated soil were above those in the control soil. The maximum were recorded at day 60 and all were for soil treated with HMTF. The highest urease, dehydrogenase, invertase, and acid phosphatase were 1.6 mg NH4-N g-1 dry soil 3h^{-1}, 1.32 mg TPF g^{-1} dry soil 24 h^{-1}, 1.45 mg glucose g^{-1} dry soil h^{-1}, and 0.83 µmol PNP g-1 dry soil h^{-1}, respectively (Figure 1).

In terms of metal removal efficiency, the soil treated with HMTF had the best (48%) removal of Pb followed by Zn (42%), meanwhile, the maximum removal (41%) of Ni was achieved in soil treated with MMTF (Table 1). Both treatments showed outstanding performance over the control which had a maximum removal as 9% for Ni ($P < 0.05$). In terms of residual metals, control soil had the highest residual for all the metals. The trend for the highest residual in control soil is Zn (805.15 mg/kg) > Pb (770.09 mg/kg) > Ni (383.85 mg/kg). The high bioremoval of the metals in soil bioaugmented with HMTF can be associated with the ability of the fungi to survive the toxic effects of the metals. Although different mechanisms are employed by fungi for tolerance and bioremoval of metal contaminants, the survival of the fungi is of immense importance as to the metabolic activity during the bioremediation. The removal of the metals observed in the present study is lower than that of Achal, et al. [13] who witnessed 94% removal of Cr using brown-rot fungus (*Gleophyllum sepiarium*) from Cr contaminated soil collected from Bokaro, Jharhand, India. The likely reason for the disparity between the current results and those of Achal, et al. [13] might be the difference in experimental conditions.

This is because, a small amount of contaminated soil (1 g) was used against large fungal biomass (5 g), large nutrient supplement (25 g of rice husk) and extended experimental duration (6 months). Similarly, the removal efficiency achieved in the current study is lower than those of Hassan, et al. [14] and Hassan, et al. [15]. The possible reason for the variation might be the differences in experimental settings. Such variation in setting includes consortia, number of organisms that make up the consortia, metal concentration, and type of metal contaminants.

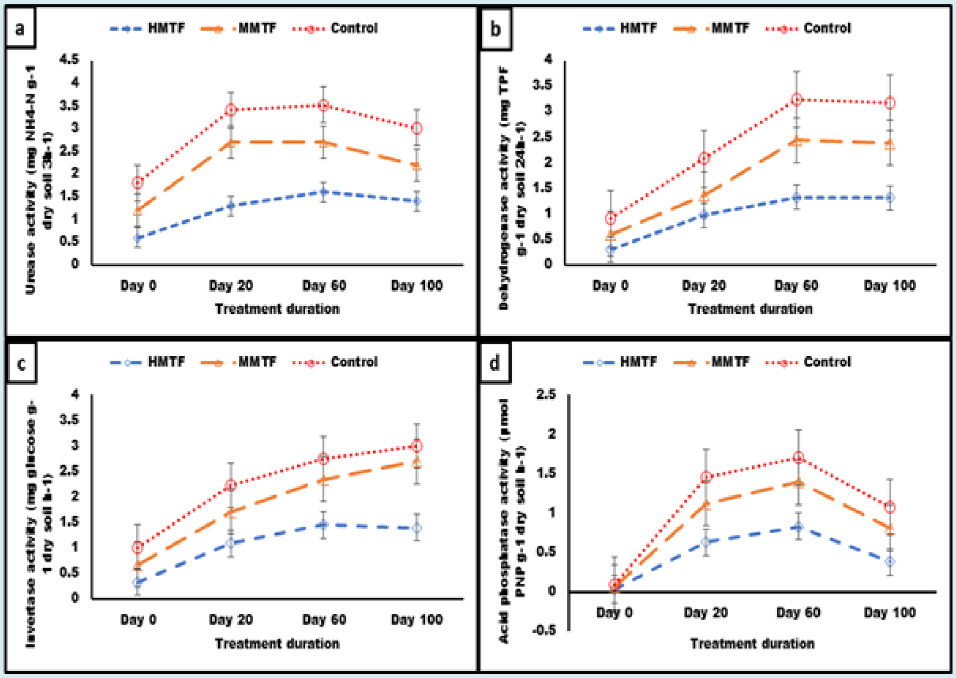

Figure 1: a) Soil urease activity, b) Soil dehydrogenase activity, c) Soil invertase activity, d) Soil acid phosphatase activity.

Heavy metals	Consortia and metal removal efficiencies		
	Highly tolerant fungi	Moderately tolerant fungi	Control
	% removal	% removal	% removal
Ni	40	41	9
Pb	48	34	4
Zn	42	36	7

Table 1: Heavy metal removal efficiencies at day 100 of bioremediation.

Several mechanisms might have played key roles in the bioremoval of the metals. Considering the initial alkaline nature of the soil and its subsequent decrease to acidic condition, some of the likely mechanisms involved during the process might have include immobilization, solubilization, redox reactions, and biosorption. For instance, the production of organic acids which might have resulted in the continuous decline of the pH might have improved the bioavailability and hence enhanced bioabsorption. Also, the acids and the presumed released siderophores might have served as natural chelants which might have solubilized some of the metals into stable complexes [16]. The fungal anionic functional groups coupled with the early alkaline pH might have led to the passive uptake of the metals by fungi through biosorption [17]. Furthermore, bioaccumulation might have also taken place largely as a result of the increased release of protons due to low pH [18].

Conclusion

The results noted that the soil treated with consortia of fungi had significant metal removal over the control ($P < 0.05$). The technique can be utilized by environmental engineers and managers for designing and treatment of metal polluted lands.

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