Phytoremediation of Contaminated Soil Using Maize (*Zea mays*) and Mycorrhiza Inoculation

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Abstract- The phytoextracting capacity of maize (*Zea mays*) on soil contaminated with brewery waste was determined. The method used was based on the responses of the maize plants grown on four different soils (inoculated and uninoculated, with and without brewery waste) tagged M^+B^+, M^-B^+, M^+B^- and M^-B^-. These were analysed for mid-rib growth, neurosis, and heavy metals uptake in the plant in addition to soil and pH analysis. Results showed that maize (*Zea mays*) planted on soil of type M^+B^+ had a rapid increase in mid-rib size (55.3cm) while the plant grown on the control sample had the lowest size (47.0cm). There were initial increases in plant with neurosis in the inoculated samples which either stabilised or increased while the number in the uninoculated samples reduced with time. The plants grown on inoculated soil had greater heavy metal uptakes of 54–83% except for Cd where the uptake was 33–40% while those grown on uninoculated soil had metal uptakes of 19–52% except for Zn where the uptake was 80–81%. The investigation concluded that maize has the capacity of removing heavy metals from brewery waste and suggested revegetation of the soil to reduce wind and water erosions.

Keywords- arbuscular, biodegradation, inoculum, metabolise, phytotechnology, remediation

1 INTRODUCTION

The increase in industrialisation has created many sites contaminated with heavy metals. The contaminated land is toxic to plants and animals, which creates considerable public interest in remediation. The commonly used remedies are *ex situ*, which poses an expensive dilemma and an even greater threat. Phytoremediation offers the prospect of a cheaper and healthier way to deal with this problem (Wei *et al*., 2010; Wuana and Okieimen, 2010; Ibrahim *et al*., 2013). The science of phytoremediation arose from the study of heavy metal tolerance in plants in the 1980s (Chaney, 1983; Adriano and Strojan, 1999). Advantages and disadvantages of phytoremediation have been reported elsewhere (Gerhardt *et al*., 2009; Suchkova *et al*., 2010; Wei *et al*., 2010; Wuana *et al*., 2010; Afzal *et al*., 2014).

Experimental studies using native and tame grasses and leguminous forbs including big bluestem (*Andropogan geradi* Vi.) and (*Festuca arundinacea* Schreb) have revealed the importance of mycorrhizae (Gao *et al*., 2011). Enhanced rhizosphere biodegradation (or rhizodegradation) takes place in the soil immediately surrounding plant roots. One laboratory study raised the possibility that transpiration due to alfalfa plants drew methane from a saturated methanogenic zone up into the vadose zone where the methane was used by methanotrophs (Narayanan *et al*., 1995).

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Lin and Mendelssohn (1998) indicated that the salt marsh grasses *Spartina alternifioral* and *S. patens* could potentially increase subsurface aerobic biodegradation of spilled oil by transporting oxygen to their roots. Phytoextraction applies to metals (e.g., Ag, Co, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn), metalloids (e.g., As, Se), radionuclides (e.g., B) (Jamil *et al*., 2009; Chandra *et al*., 2010; Suchkova *et al*., 2010; Anukwa *et al*., 2021; Wang *et al*., 2021) as these are generally not further degraded or changed in form within the plant. Metals within the root zone can be stabilised by changing from soluble to insoluble oxidation states through root-mediated precipitation. Roots have been used to mediate the precipitation of lead as insoluble lead phosphate (Salt *et al*., 1995; Agunbiade *et al*., 2009; Ji *et al*., 2011; Ha *et al*., 2011).

The formation of a lead phosphate precipitate in a hydrophobic solution was identified by Dushenkov *et al*. (1995). Although lead is not usually accumulated in plants under natural conditions (Reeves and Brooks, 1983), it has been removed from soil using three crops of Indian mustard in one growing season, with a decrease in soil concentrations of lead to acceptable levels (Blaylock *et al*, 1999; Menhas *et al*., 2021). Soil Pb and Cr^{6+} contents may be alternatively inactivated by plants and soil amendments (phyto-stabilization).

The dependency of plants on micorrhizae is determined in large part to the extent of its root system. Plants with a system of well-developed fine, dense root such as grasses are dependent on micorrhizae only in poor nutrient soils that are known as optimal micotrophic plants (Suresh *et al*., 2004; Arthur *et al*., 2005; Etim, 2012). The root system may be colonised typically by more than one fungal species and mutual exclusion can be observed. Success in

occupancy varies and is not necessarily related to host response (Lopez-Aguillon and Mosse, 1987). Host response differs from fungal species (Carling and Brown, 1980) and with geographical isolates within the species (Berthlenfalvasy, 1992). The response range may be due to changing efficiencies of different fungi for different elements (Mange *et al.*, 1982) or even no changes in the soil environment itself during the season (Bazin *et a*l., 1990). Maize (*Zea mays*) has been used to carry out phytoremediation in a variety of applications (Wuana *et al*., 2010; Ibrahim *et al*., 2013; Garcia *et al*., 2013; Xu *et al*., 2013; Kosnar *et al*., 2018; Menhas *et al*., 2021; Wang *et al*., 2021). The work investigated the suitability of using maize to remediate a brewery-contaminated land and to measure its efficacy and cost effectiveness.

2 Materials and Methods

2.1 Source of Material

Maize seeds of yellow variety (*Zea mays*) were purchased from Sabo Market in Ogbomoso. Brewery waste was collected from the Nigerian Brewery Plc, Ibadan. Sawdust was collected from Oja Tuntun Sawmill, Ogbomoso. Fertile topsoil was collected from the poultry site of the Animal and Health Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.

2.2 Inoculum Preparation

The vesicular arbuscular mycorrhizae employed was *Glomus mosseae*. The inoculum used was prepared from the inoculum stock of the Department of Pure and Applied Biology of LAUTECH, Ogbomoso. The inoculum was supplied in 10 kg plastic pots filled with sterile soil. About 50 kg of the certified stock of inoculum was first placed in a hole dug in the sterile soil and three seeds of maize were planted in each hole. The pot was kept in the laboratory and watered regularly for three months.

The male inflorescence of the matured plant was removed to prevent cob formation and subsequently to direct most of the carbon assimilated into *mycorrhizal* fungus formation. At the end of three months, watering was stopped to allow the maize plant to dry for another four weeks. After proper drying, the portion of the maize above the soil was removed and the soil, together with the root fragments, was left with a potential amount of *Glomus mosseae*. The maize roots were chopped into tiny fragments and mixed thoroughly with the sterile soil to allow even distribution of potential inoculi that essentially consist of spores, hyphae and infected root fragments.

2.3 Nursery Preparation

Twelve plastic pots were used for the nursery. Each pot was perforated at the base to allow for water drainage. Each was filled with sawdust and was watered prior to seed planting. Dense planting for the seeds was done in the pots, and were allowed to cover the entire surface of the sawdust. Dust was spread on the seeds to cover them. The seedlings were transplanted ten days after planting. The transplanting was carried out using twelve plastic pots, each filled with sterile soil. There were four treatments in all with three replicates for each type. The treatments were as follows:

A) Inoculated soil plus brewery waste: M^+B^+
B) Uninoculated soil plus brewery waste: M^-B^+
C) Inoculated soil only: M^+B^-
D) Uninoculated soil only: M^-B^-

2.4 Transplanting

Before transplanting all the planting pots were watered. The seedlings were transferred in the evening in order to give them enough time to acclimatise to their new environment before sunrise to prevent transpiration shock. The transplanting was gently done by pulling off the maize seedling out of the nursery pots. The soil was scooped to make holes of few centimetres on the surface of the planting pots. About 50g of inoculum was placed in the hole of the planting pots for treatments A and B (i.e., inoculated soil + brewery wastes and inoculated soil only for treatments A and C). The seedlings were placed in the holes with their roots completely buried in the soil at 2cm below the soil level of the planting pots and were supported with soil.

2.5 Growth Measurement

The mid-rib of each plant leaf was measured using a metre rule. Negative growth effects like 'necrosis' (i.e., dead plants per pot) and 'die back' (i.e., number of dead leaves from tip per pot) were determined by direct counting at weekly intervals. This continued till the third month when the plants were terminated and thus marked the end of the field experiment.

2.6 Harvesting, Soil Analysis and Spore Counting

Harvesting was done by uprooting few plants from each pot. The plants were then tagged and oven-dried at 70°C for 46 hours. They were then blended using a blending machine and taken for plant tissue analysis. Soil samples from each pot were collected. Fine root hairs were also collected from each pot and preserved in 50% ethanol for spore counting.

2.7 Quantification of Arbuscular Mycorrhizal Infection In Plant Roots

Zea mays fine root samples were collected in three replicates with a hand trowel from the soil at 10cm depth. The fine roots were placed in clean McCartney bottles and labelled accordingly. In the laboratory, the roots were washed, cleaned of soil particles and were fixed and stored in 50% ethanol. A sub-sample of 2g was taken from the field sample and cleaned in 10% potassium hydroxide solution and heated by auto-clave at 120°C for 15 minutes. The roots were rinsed in water several times to remove the potassium hydroxide.

3 Results and Discussion

The results obtained are presented in the figures and tables. Fig. 1 shows the number of maize plants that had neurosis when grown on soil contaminated with brewery waste. The number on inoculated soil with brewery waste became constant after the fourth week. This showed that

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the introduction of mycorrhizal inoculation reduced incidence of neurosis in the maize plant whereas for uninoculated soil with brewery waste the number peaked at the fourth week and started reducing thereafter. For maize planted only on inoculated soil without brewery waste, the number kept on rising from three in the second week, to five in the fourth week and eight in the sixth week. This is in direct contrast to the control sample grown on uninoculated soil that rose to nine before dropping to eight in the sixth week.

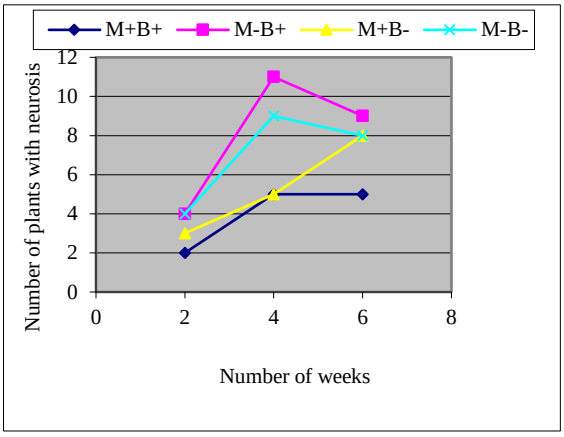

Fig. 1: Effect of micorrhizae inoculation on growth on contaminated soil

Fig. 2 showed that the maize plants inoculated with micorrhizal had lower dieback rates compared to the uninoculated plants. The inoculated samples with brewery waste had almost similar pattern with the control samples while the inoculated samples without brewery waste had lower rates of dieback. All the samples had similar growth patterns with samples that had *Micorrhizal* inoculation having better growth (Fig. 3).

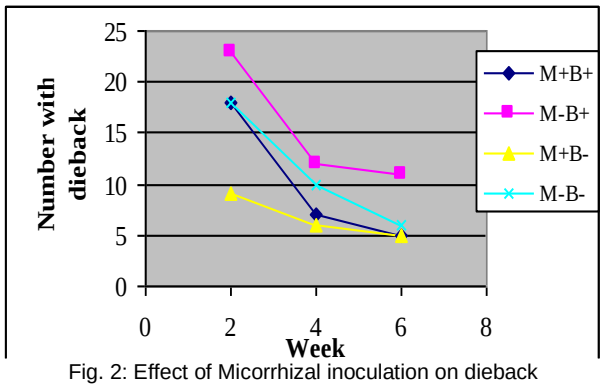

Fig. 2: Effect of Micorrhizal inoculation on dieback

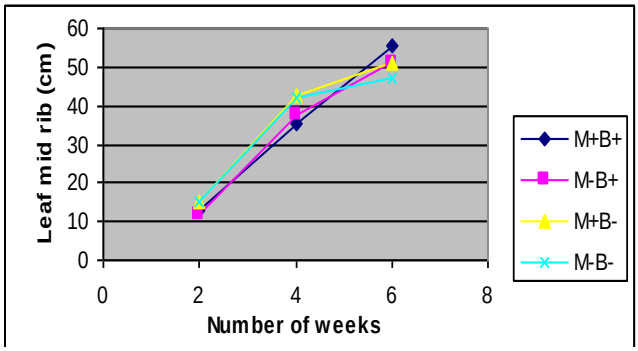

Fig. 3: Effect of Micorrhizal inoculation on plant size from mid rib

The samples with micorrhizal inoculation had very good responses to metal uptake compared to the uninoculated and control samples (Table 1). The general response of plants to metal contaminants is a reduction in leaf biomass. This indicates that the plant is experiencing a toxic response to contamination. As the plants matured, as shown by the increase in their midrib size (Fig 3), there was improved water use and contaminant removal. Since the plants exude chemicals that provide carbon and energy for microbial growth, the combination of plants and microorganisms increase the biodegradation of compounds.

Table 1. Effect of *micorrhizal* inoculation on heavy metals uptake in biomass of maize soil contaminated with brewery waste

	Micro-nutrient treatment	Zn	Fe	Mn	Cu
A	M^+B^+	41.25	104.25	82.50	4.75
B	M^-B^+	31.50	72.50	38.75	0.01
C	M^+B^-	75.25	137.25	71.50	4.75
D	M^-B^-	52.00	105.25	44.25	ND

Table 2 showed the result of soil analysis. Heavy metals concentrations present in the soil before planting and after the addition of brewery waste before planting are indicated as S_1 and S_1B^+ respectively. It could be seen that the contaminated soil samples on which were grown maize (*Zea mays*) that were inoculated with micorrhizal (M^+B^+ and M^+B^-} had better uptake of heavy metals.

Table 2. Contribution of *mycorrhizal* inoculation on micronutrient in soil contaminated with brewery waste

Nutrient treatment	Zn	Fe	Mn	Cd	pH
S_1	12.98	15.33	13.29	0.29	7.1
S_1B^+	69.13	23.25	13.47	0.42	7.0
M^+B^+	12.21	11.29	6.16	0.28	8.1
M^-B^+	13.38	17.36	7.47	0.34	8.1
M^+B^-	11.16	10.05	5.49	0.25	8.2
M^-B^-	12.95	13.61	6.40	0.32	8.1

The metal uptake ranged from 54.3 (59.4%) for Mn, 82.3 (83.9%) for Zn, 33.3 (40.5%) for Cd, and 51.4 (52.5%) for Fe. For the non-inoculated soil sample the metal uptake were between 44.5 (52.5%) for Mn, 80.6 (81.3%) for Zn, 19.1 (23.8%) for Cd, and 25.3 (41.5%) for Fe. These results showed the ability of the *Zea mays* plant to translocate the heavy metals from root to shoot as shown by previous investigations (Anukwa *et al*., 2021; Brown *et al*., 1994; Vigliota *et al*., 2016).

4 Conclusion

The successful application of phytoremediation in treating brewery waste has been demonstrated. The effect of *micorrhizal* inoculation on the extracting capacity of maize (*Zea mays*) has shown the applicability of this plant in the removal of these four heavy metals that are present in brewery waste.

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This technology of using plant processes to remove, degrade or render harmless hazardous materials present in the soil may offer a cost-effective, non-intrusive and safe alternative to commercial soil clean-up techniques. In many cases, even the physical presence of a plant can improves the condition of the soil, giving it structure and stability and altering hydrology by enhancing water retention and preventing erosion. Reclamation and revegetation of these soils will reduce wind and water erosion and subsequent dispersal of contaminated soil as well as promote the restoration of the local ecosystem. The corn cob has been recommended for further investigation to determine whether it has any traces of the heavy metals.

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