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IMPACT OF ORGANIC SOIL AMENDMENTS ON FUNGAL POPULATION AND GERMINATION OF MAIZE SEEDS IN LEAD-CONTAMINATED SOIL

[*]Aduramigba-Modupe, A.O., Fajemisin, O. A. and Adejumo, S.A.
[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan
*Corresponding Autor's Email: fyaduramigba@yahoo.com

ABSTRACT

Proliferation of industrial and other anthropogenic activities has led to an increase in heavy metal contamination of agricultural soil, elevating the risk of heavy metal (especially, lead) toxicity to all life forms. It is therefore imperative to develop effective bioremediation techniques for soil remediation. Cow dung and compost (*Tithonia rotundifolia* and poultry droppings) were added at 30 t/ha to lead contaminated soil. Abundance of heavy metal associated fungi in the contaminated soil was determined through pour plate isolation method using Potato Dextrose Agar. The effect of lead contamination and soil amendments was also determined on maize seed germination in the laboratory using Petri dishes in three replications. Heterotrophic fungal count in lead-contaminated soil decreased as Pb concentration increases. The abundance of soil associated fungi declined with an increase in lead concentration. It ranged from 3.2×10^3 to 0.00 CFU/mL in soils containing 0.36 g/kg lead and 63.01 g/kg lead, respectively both amended with cow dung. *A. fumigatus, A. flavus, A. nudulans, A. tamarii, A. uvarum and A. terreus* were the fungi species isolated from contaminated soil samples. *A. fumigatus and A. flavus* were the most prominent. *A. fumigatus* was able to survive at 100% concentration of lead amended with compost, 75% lead concentration amended with cow dung, 25% lead concentration amended with cow dung, and 100% lead concentration without amendment. Percentage germination of maize seeds also decreased with higher concentrations of lead in contaminated soil. High percentage germination (81.81 and 80.00) was observed in the control soil, and on 0.36 g/kg lead-contaminated soil, amended with cow dung, respectively. Organic fertilizers could be adopted to develop an efficient, cost-effective, and readily accessible bioremediation strategy for soil remediation, especially for the production of maize.

Keywords: Bioremediation, Lead contamination soil, Maize, Fungi, Compost, Cow dung

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important annual cereal crops in the world. It is a source of food and nutrition for several households in Nigeria (Ogunniyi *et al*., 2021). Maize contributes more to food security, poverty eradication and the Gross Domestic Products of Nigeria than other cereal crops, such as sorghum and millet (Adeagbo *et al*., 2021). In addition, maize is easily processed into several forms of products for both human and livestock consumption (Ogunniyi *et al.*, 2021). The value chain of maize (especially, postharvest processes) significantly contributes to job creation and overall economic development of the Country. However, maize production is affected by several environmental factors, especially, the nature and conditions of soil (Riccetto *et al.*, 2020), which determines the yield potentials of specific soil types. Contamination of soil with heavy metals reduces maize-yield potential and limits the economic significance of the crop.

The overall quality of the soil environment influences the quality of crop production. Pollution of the biosphere by heavy metal contamination, through domestic, agricultural and industrial activities has resulted in a serious problem thereby posing threat on safe or healthy utilisation of soil (Igwe *et al.*, 2005; Gravand *et al.*, 2021). Industrial waste, fertilizer application, pesticide utilization and the disposal of untreated metal-contaminated sewage continually contribute to heavy metal

accumulation and cytotoxic tendencies of soil (Herland *et al.*, 2000). Although, some biological heavy metals like Cu, Zn, Fe and Ni could be tolerated in minute quantities by plants, however, excessive or accumulated amount of these metals pollute the soil, which in turn becomes a threat to all forms of life, including plants, microorganisms, and animals, at varying degrees of toxicity (Gravand *et al.*, 2021; Mirhosseini *et al.*, 2021).

Lead is one of the potent environmental contaminants of all the heavy metals found in soil, disrupting or inhibiting several ecological, environmental and evolutionary processes in the microsphere. This alters the balance of microbial community, influences their niche functionality and affects the overall productivity of contaminated soil (Sofy *et al.*, 2020; Sun, X., Sun, M., Chao, Y. *et al.* 2023). Lead is one of the raw materials used in the production of ceramics, fertilizers, writing materials and batteries. It is also found in different products like paints, dyes, rubber products, gasoline, paper-prints and pesticides. Due to lead's ubiquity, soil is unwittingly contaminated by lead products, accumulating to toxic levels and significantly limiting the potentials of soils for healthy crop production (Sangeetha *et al.*, 2021).

Lead toxicity has been associated with poor agronomic performance of maize (Sofy *et al.*, 2020), especially in developing countries like Nigeria, where waste management systems are inefficient. This limits the yield and economic potential of the crop. Several heavy metal bioremediation techniques have been proposed over the years, these include bioslurping, bioventing, biosparging, phytoremediation, biofiltration, bioaugmentation, bio-stimulation and compost-remediation, which involves the use of organic fertilizers (da Silva *et al.*, 2020). Fertilization with manure could improve the microbial quality of soil and encourage the growth of lead-degrading or solubilising microorganisms. This research work therefore investigates the effect of cowdung and compost on bioremediation of lead contaminated soil for the purpose of maize production.

MATERIALS AND METHODS

Collection and preparation of soil samples

Lead contaminated soil samples were collected from the Crop Physiology section of Environmental Biology unit of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Oyo State, Nigeria. The uncontaminated soil samples was used to prepare the different soil mixtures for germination test. This was collected from Crop garden Department of Crop Protection and Environmental Biology, University of Ibadan. The soil samples were sieved to remove extraneous materials like, stone, plant residues and other non-soil materials. Samples were thereafter pre-mixed at different levels ; 0%, 25%, 50%, 70%, and 100 % with lead contaminated soil. Mixed soil samples were subsequently analysed for lead concentration.

Determination of lead concentration

Compost samples were taken from both contaminated and uncontaminated soils for analysis. They were crushed to generate fine particles. Subsequently, 1 g of soil sample from each treatment was weighed into labelled glass bottles; 10 mL nitric acid (65% HNO3) was added to each soil sample. Thereafter, 7.5 mL Hydrochloric acid (37% HCl) was added to the initial digested sample and the mixture was refluxed for 2 hours (Hurdebise *et* al., 2015; Castro-Bedriñana *et al.*, 2021). The resulting product was allowed to cool, then each of the samples was sieved using whatmann No. 1 filter papers. The retained solute was made up to 100 mL in Erlenmeyer flask and the level of lead contamination therein was determined using an Atomic Absorption Spectrophotometer (VGP210 BUCK Scientific Model). Standard blank method, duplicate samples

and control standard were used to ensure analytical precision. Lead concentration (expressed in mg/kg) in the samples were extrapolated from standardised lead (Sigma-Aldrich) calibration curve (Castro-Bedriñana *et al.,* 2021). The Pb concentrations were between 360.20 - 63,006 mg/kg in the soil mixtures.

Organic amendments and isolation of fungi from treated soil

Two organic fertilizers: cow dung and compost (*Tithonia rotundifolia* and poultry droppings), also obtained from Crop Physiology section of CPEB were added to soil mixtures at 30 t/ha each as soil amendments. It was a factorial experiment with five treatments.

Fungi associated with the soil samples were cultured in the laboratory to determine population at different levels of lead contamination. The microbial populations in the samples were enumerated using serial dilution and subsequent pour plate method following the procedure of Asadu *et al*., (2015). To reduce the propagules, 1 g of each soil sample was added to 9 mL of sterile distilled water to generate a 10^{-1} dilution. The resultant mixtures, in each case, was thoroughly mixed and further diluted up to 10^{-4} with sterile distilled water. One milliliter aliquot of the 10^{-2} and 10^{-4} dilutions of each sample was thereafter introduced into Petri dishes and 15 mL sterile Potato Dextrose Agar was dispensed into the plates and swirled gently to evenly distribute the sample. Inoculated plates were incubated at 28±2°C for 3-4 days. Thereafter, the growing colonies were counted and expressed as colony forming units per gram (CFU/g) of the samples (Monda *et al*., 2020). Microbial colonies were then sub-cultured to obtain pure cultures.

Fungi identification

To characterise the morphological properties of the isolates, fungal cultures on plates were examined for their cultural characteristics, strains were also prepared for microscopic observations. Growing tip (mycelia strands) of each strain was picked with a sterile inoculating needle and place in a drop of lactophenol cotton blue stain on a slide. A second, sterile inoculating needle was used to tease the mycelial strands, the preparation was then covered with a cover slip. Prepared slides were viewed under a compound microscope at (x400 magnification). Fungal strains were thereafter identified based on the presence and characteristics of typical structures, such as conidia or spores, conidiophore, hyphae and phialide, and compared with standard reference descriptions of fungi (Barnett and Hunter, 2010).

Maize seed germination test

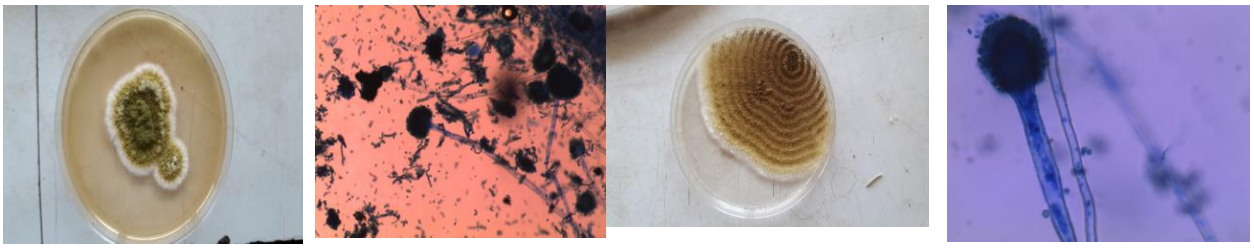
To determine the effect of soil contamination with lead on seed germination, maize seeds (Ife hybrid 3) were surface sterilised with 1% sodium hypochlorite for two minutes and subsequently washed (thrice) with sterile distilled water to eliminate the exogenous microbial contamination (Varpe, 2021). Seeds were blotted dry and arranged (10 seeds per plate) in amended lead contaminated soil samples. The experiment was laid out in a completely randomised design with three replicates. Petri plates were kept at room temperature (25±2°C) in the Laboratory and seeds were observed daily for germination. Percentage seed germination was recorded 10 days after planting. The seeds were considered to have germinated when a radicle length of 2 mm protrudes through the seed coat (Nciizah, *et al.,* 2020). Data were analysed (analysis of variance) using DSAASTAT, 2012 version and means were separated using the Least Significant Difference (LSD) at 95% level of probability.

RESULTS AND DISCUSSION

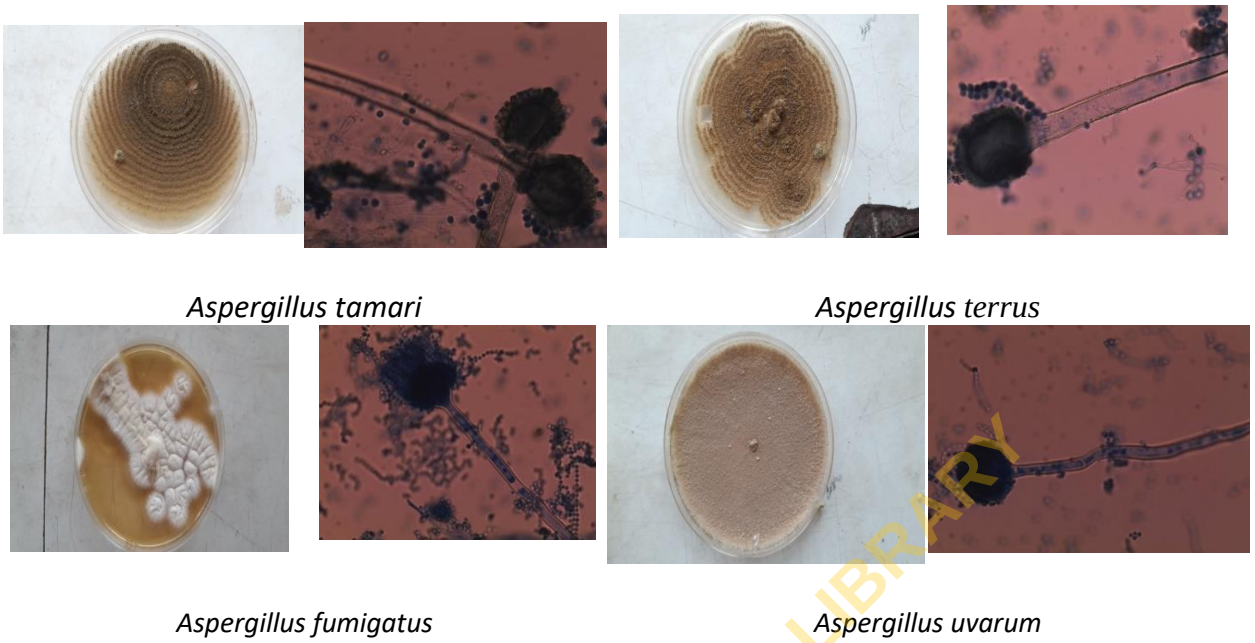
Effect of lead contamination on fungal load

Table 1 shows the effect of different concentration of lead on soil associated fungi. The heterotrophic fungal count in lead contaminated soil ranged from 3.2×10^3 to 0.00 CFU/mL. The lowest fungal count (0.00 CFU/mL) was observed in the unamended 100% Pb contaminated soil with 63.01 mg/kg Pb concentration, while 0% Pb contaminated soil with 0.36 mg/kg Pb concentration and amended with cow dung (30 t/ha) had the highest fungal count of 3.2×10^3 CFU/mL. Abundance of soil associated fungi declined with increase in lead concentration. Fungal count of 2.90×10^3 CFU/mL was recorded in lower level of lead contaminated soil (at 0.36 g/kg lead concentration) fertilized with compost; this was significantly higher than fungal abundance observed in other compost-fertilized contaminated soil samples. Control (uncontaminated sample with no fertilizer) soil had an abundance of 1.72×10^3 CFU/mL, this was however not significantly different from the abundance of fungi isolated from 21.55 g/kg lead contaminated soil fertilized with either 30 t/ha cow dung or compost.

Jan *et al.* (2020) investigated the dynamics of microbial communities in the soil, as well as their distribution and abundance in indigenous and artificially cultured microbial-based soil amendments, they reported an abundance of soil fungal population at a range of 2×10^4 to 8×10^4 CFU/g of prepared soil samples. The spectrum of fungal load generally varies in different soils. Concentrations on the surface of commercial soil, sub-surface potting soil, and compost range between 9.5 × 104 and 5.5 × 105 CFU/g (Haas *et al*., 2016). Di Piazza *et al*. (2020), in their report on the thermotolerant and thermophilic mycobiota in different steps of compost maturation, also attributed the complex biochemical processes resulting in the breakdown of organic matter to the presence and abundance of fungal species. The fungal load reduction observed in contaminated soil could be associated with the inhibitory effects of lead on the microbial community associated with each soil sample. High lead concentrations can disrupt several key metabolic processes or structures required for survival and growth, such as the electron transport chain, integrity of organelles, membrane stability index, metabolism of mineral nutrients, and cellular enzymatic activities (Aslam *et al.*, 2021). *Aspergillus fumigatus, A niger, A. flavus, A. nudulans, A. tamarii, A. uvarum and A. terreus* were the fungi species isolated from contaminated soil samples amended with organic fertilizers (Plate 1). Lead contamination could have adverse effects on the growth and proliferation of fungal species associated with the soil samples (Aslam *et al.*, 2021). This could negatively influence fungal diversity in contaminated soil, selecting for the growth of lead-resistant or Pb-metabolizing genera, such as *Aspergillus*. Toxic effects of lead contamination (resulting from the anthropogenic activities within the soil environment) on plants, animals, and microbial life forms were also reported by Sangeetha *et al.* (2021).



Aspergillus flavus *Aspergillus terrus*



Aspergillus tamari *Aspergillus terrus*

Aspergillus fumigatus *Aspergillus uvarum*

Plate 1: Culture plates and photomicrograph (x400) of *Aspergillus* species isolated from lead-contaminated soil

Influence of lead contamination on maize germination

Germination of maize seeds was significantly affected by lead concentration in the soil, percentage germination decreased with increment in the level of contamination (Table 1). Highest percentage germination (81.81) was observed in the control soil. However, this was not significantly different from the percentage germination on 0.36 g/kg lead contaminated soil, amended with cow dung (30 t/ha). Percentage germination recorded for other contaminated soil samples fertilized with cow dung were not significantly different. Of all the compost fertilized soil samples, soil with 63.01 g/kg Pb had the lowest germination percentage (0.00%), this was not significantly different from the germination rate recorded on 63.01 g/kg lead contaminated soil without organic amendment.

Table 1: The effects of soil amendments on germination of maize and abundance of soil fungi in Pb contaminated soil

Organic fertilizer application (30 t/ha)	Lead contamination (g/kg)	Fungal abundance ($\times 10^3$ CFU/mL)	Maize germination (%)
Cow dung	0.36	3.20±0.37^{a‡}	80.00±5.80^{a}
	9.38	2.00±0.12^{bc}	73.30±3.30^{ab}
	21.55	1.30±0.15^{d}	70.00±0.10^{ab}
	44.63	0.73±0.88^{ef}	60.00±10.01^{bc}
	63.01	0.27±0.89^{fgh}	0.00±0.00^{d}
Compost	0.36	2.90±0.30^{ab}	76.70±6.70^{ab}
	9.38	1.90±0.88^{c}	63.30±12.01^{bc}
	21.55	1.30±0.12^{d}	63.31±3.30^{bc}
	44.63	0.67±0.89^{efg}	56.7±6.70^{c}
	63.01	0.17±0.12^{gh}	0.00±0.00^{d}
NFA†	0.36	2.70±0.36^{b}	80.01±5.80^{a}
	9.38	0.87±0.67^{de}	60.00±15.31^{bc}
	21.55	0.23±0.66^{fgh}	66.7±3.30^{bc}
	44.63	0.13±0.33^{h}	66.7±3.31^{bc}
	63.01	0.00±0.00^{h}	0.00±0.00^{d}
Control	0.00	1.72±0.44^{cd}	81.81±5.04^{a}

† NFA: No fertilizer application.

‡ Means followed by the same letter(s) within a column are not significantly different at 5% probability level according to Least Significant Difference (LSD) test.

A very high concentration of lead was observed in the contaminated soil sample used in this study. Soil contamination with lead due to anthropogenic activities has significantly increased in recent time; therefore, remediation is of prime importance due to its recalcitrant properties and toxicity effects (Sangeetha *et al.*, 2021). Several techniques have been proposed towards heavy metal remediation, however, bioremediation is considered the safest, readily available and affordable group of methods to reduce the toxicity effects of heavy metal contamination (da Silva *et al*., 2020). Cow dung and compost improved the soil microbial properties and their ability to support the emergence of maize. Organic matter contents have been described to possess high variability of structures, diverse microbial communities, and chemical functional groups with bioremediation potentials (Coutinho *et al.*, 2021). Organic fertilizer application in this study appeared to have reduced the toxicity of lead to maize seeds in the contaminated soil samples. In addition to the possibility of adsorbing heavy metal contaminants, da Silva *et al*. (2020) also reported manure application as a means of augmenting the microbial complexity of different soil types. Organisms associated with manure include plant growth-promoting microbes, as well as several organisms associated with heavy metal bioremediation.

The use of plant growth-promoting microorganisms has been proposed as an inexpensive strategy for remediating lead

contaminated soils. Aslam *et al*. (2021) reported the use of Ascomycetes for successful bioremediation, especially, in cereal crops. The fungi were observed to establish a symbiotic relationship with host cereal, improving lead-tolerance by immobilising Pb ions. *Aspergillus* species isolated from lead contaminated soil samples could also be associated with lead bioremediation. Bala *et al.* (2020), in their study on biosorption potentials of lead tolerant fungi, also reported *Aspergillus* species with lead removal capacities of 0.67 ppm, 3.11 ppm and 3.79 ppm in lead-contaminated sterile, Saboraud Dextrose Broth. According to this report, organisms involved in plant's resistance to heavy metals are capable of influencing transcription factors, metal tolerance protein (MTP), natural resistance-associated macrophage protein (NRAMP), and heavy metal ATPase. Sangeetha *et al.* (2021) also reported the production of biosurfactants by soil associated microorganisms, which are capable of adsorbing between 96%–99.6% of lead in contaminated soil, using the Langmuir isotherm model assay.

CONCLUSION

Cow dung and compost (*Tithonia rotundifolia* and poultry droppings) were used as soil amendments in lead contaminated soil samples. These organic fertilizers reduced lead toxicity on maize seeds, thereby improving their percentage germination. However, microbial abundance and the rate of maize germination decreased with increase in lead concentration. Lead contamination also appeared to have inhibited the growth of soil-associated fungi, reduced their diversity and possibly accounted for the proliferation of *Aspergillus* species isolated in this study. Cow dung had better effect (than compost) in mitigating lead toxicity on soil fungi and maize seeds. Organic fertilizers are cheap, easily accessible agricultural waste, which could be considered in the development of efficient lead bioremediation strategies, especially, in the production of maize. In addition to their potentials to reduce the sensitivity of maize to heavy metal contamination, these manures could simultaneously serve as sources of essential nutrients required for crop production.

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