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# Synergistic effect of Lead Resistant Bacteria and *L. macroides* US3 biostimulant in ecorestoration of lead-treated soil

**Chioma Bertha Ehis-Eriakha, Esther Omoye Oshiomane Momodu, Fred Coolborn Akharaiyi**
**Department of Microbiology, Edo State University Uzairue, Edo State, Nigeria.**

## INTRODUCTION & AIM

Lead pollution poses a formidable threat to agriculture, bioaccumulating in crops, and ultimately harming human health. Even low-level exposure can significantly reduce crop yields, diminish nutritional value, and precipitate economic losses and food insecurity.

Various kinds of molecular defenses are available to bacteria in order to combat lead toxicity. Some microbes have a group of genes that become active when lead is present. These genes called pbrTRABCD aid in developing bacteria lead resistant by turning on different lead detoxification systems (Li et al 2020).

Microbial-based remediation of lead pollution offers a promising approach to environmental management and sustainability. This eco-friendly technique leverages microorganisms to remove or immobilize lead contaminants, mitigating their harmful effects on ecosystems. However, while microbial remediation can effectively reduce lead concentrations, it may not necessarily eliminate the ecotoxicity posed by lead pollutants.

To address this limitation, regenerating the soil system using Plant Growth-Promoting Rhizobacteria (PGPR) is essential. PGPR can enhance soil fertility, promote plant growth, and reduce lead toxicity, thereby restoring ecosystem balance. This integrated approach combines microbial remediation with PGPR-mediated soil regeneration, providing a comprehensive strategy for managing lead pollution and promoting environmental sustainability. This study highlights the efficacy of PGPR Lysinibacillus macroides US3 as biostimulant to restore lead polluted soil treated treated individually with four resistant bacteria

## METHOD

**Experimental design for maize crop cultivation:** Post bioremediation of lead treated soil with four lead resistant bacteria, the soil was cultivated with *Lysinibacillus macroides* US3. Planting of seeds was carried out using the technique of Ju *et al.* (2020). Seedlings were transferred into each planting pot (diameter 12cm and depth 10 cm, with 4 kg of soil inoculated with *Lysinibacillus macroides* strain US3 per pot and the control pot wich was uninnoculated). The pots were irrigated with 250 ml of water weekly under green house condition. This experiment lasted for 28 days and plat growth attributes such as toot length and shoot length were monitored at weekly interval. Table 3.3 shows the experimental design for maize crop cultivation which was done in dublicates.

**Table 1. Experimental design for maize crop cultivation**

| Control Pot 1 | Pot 2 | Pot 3 | Pot 4 | Pot 5 |
|---|---|---|---|---|
| 4 kg of lead spiked soil + 3 maize seedling | 4 kg of *Pseudomonas* spp lead treated soil + 3 maize seedling + *Lysinibacillus macroides* strain US3 | 4 kg of *Bacillus infantis* lead treated soil + 3 maize seedling + *Lysinibacillus macroides* strain US3 | 4 kg of *Lysinibacillus fusiformis* lead treated soil + 3 maize seedling + *Lysinibacillus macroides* strain US3 | 4 kg of *Halopseudomonas xiamenensis* lead treated soil + 3 maize seedling + *Lysinibacillus macroides* strain US3 |

**Seed inoculation:** Thirty five (35) maize seedlings were subjected to a one-minute surface sterilization with 5% sodium hypochlorite, followed by three thorough washes with sterile distilled water. After being air-dried, the seeds were soaked in a bacterial solution, and the mixture was stirred repeatedly for five minutes. Bacterized seeds were spread out gently on a Petri plate and allowed to air dry for a whole night at room temperature. Serial dilutions were used to count the bacterial cells, and the result was estimated to be $10^6$ CFU/seed (O.D. = 0.8) (Li *et al.,* 2020).

**Examination of plant development characteristics:** Seed germination and vigor index, the lengths of the shoots and roots, as well as their fresh weights and dry weights were all recorded as aspects of plant growth. Until the components' internal cell water was gone, the shoot and root dried out. The percentage was calculated using the formula provided below of germination (Cowdhury *et al*., 2020): Germination rate (%) is equal to 100 divided by the number of seeds that germinated. Vigor index = total plant length x percentage of germination.

**Estimation of the pigment used in photosynthesis:** The old method for measuring leaf chlorophyll in the laboratory was used involved extracting the chlorophyll from a sample of leaves using acetone, followed by the determination of the chlorophyll concentration using a spectrophotometer that measured absorbance at a wavelength of 645 nm (Kalaji *et al*., 2017).

**Determination of lead accumulation in soil and the roots of maize crops:** The maize young plants were gathered, they were divided into distinct roots and shoots, dried separately for 48 hours in an oven set to $65^0$ C. Following the Allen, *et al*. (2020) procedure, the oven-dried materials were powdered and dissolved. To do this, 200 mg of the sample's dust was obtained and dissolved using beakers and a hot induction plate in aquaregia ($H_2SO_4$: $HNO_3$: $HClO_4$, v/v) in the ratio 1:3:1. After allowing the dissolved samples to cool, they were filtered using nylon syringe filters with a 0.22 m pore size. Double distilled water was used to dilute the samples, and the total volume was 50 ml. Following that, these digested samples were kept at room temperature while an atomic absorption spectrometer was used to calculate the concentration of heavy metals in the plant's roots and shoots (Shimadzu 6200).

## RESULTS & DISCUSSION

**Table 2 Molecular identification of Lead resistant bacteria**

| Isolate code | Closest relative ascension no (bases compared) | Similarity (%) | Phylum | Bacteria identification |
|---|---|---|---|---|
| **Pbr A** | MH734834 | 100 | Proteobacteria | *Halopseudomonas xiamenensis* strain B13 |
| **PbR B** | KU922247 | 98 | Firmicutes | *Bacillus infantis* strain K66 |
| **Pbr C** | MN759656 | 100 | Firmicutes | *Lysinibacillus fusiformis* KAF67 |
| **Pbr D** | MT250857 | 95 | Proteobacteria | *Pseudomonas* spp |

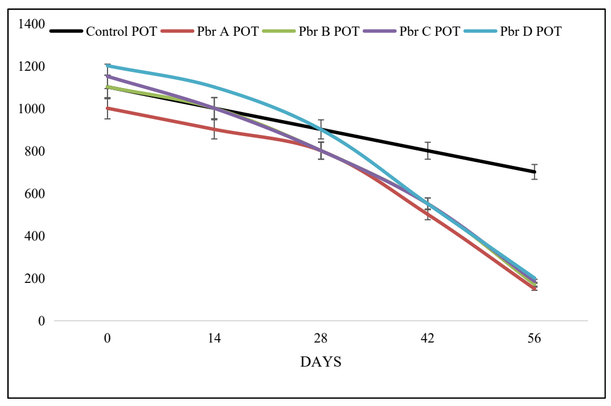


**Fig. 1. Dynamics of lead reduction for treatments and control groups (Day 0 - 56).**

*All isolates significantly reduced lead concentration compared to control soil. Bacillus infantis* strain K66 demonstrated the highest percentage reduction in lead levels, closely followed by *Lysinibacillus Fusiformis* strain KAF67, *Pseudomonas* spp. strain A27, and *Halopseudomonas Xiamenensis* strain B13 while control pot exhibited the lowest percentage of lead reduction (Fig. 1). This observation underscores the effectiveness of the selected microbial strains in mitigating lead contamination, with *Bacillus infantis* strain K66 emerging as particularly promising in lead remediation efforts.

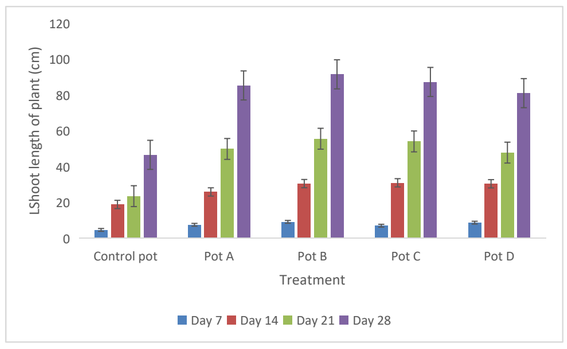


**Fig. 2. Shoot length of maize plant at day 7, 14, 21 and 28 (cm).**

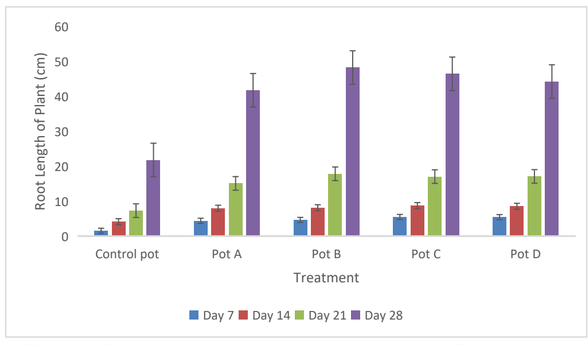


**Fig. 3. Root length of maize plant at day 7, 14, 21 and 28 (cm)**

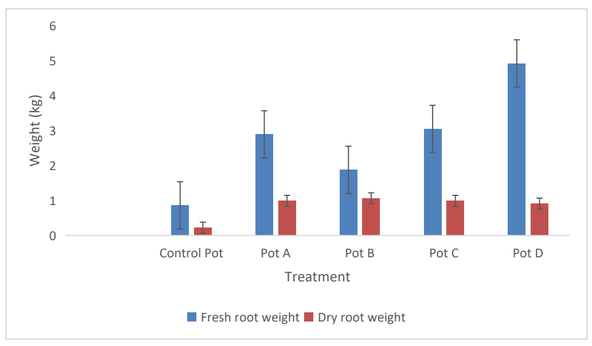


**Fig. 4. Fresh and dry root weight of the maize plant after cultivation (cm)**

Inoculating maize seeds with *Lysinibacillus macroides* strain US3 in lead-treated soil significantly enhanced maize growth under lead stress. The treated plants showed increased root length, shoot length, chlorophyll content, and dry and wet weights compared to the control. Notably, the absence of US3 resulted in minimal growth enhancement, highlighting the bacterium's crucial role in mitigating lead toxicity in maize cultivation.

**Table 3. Lead uptake in maize root (%) post cultivatio for 28 days**

| Treatment | Percentage (%) |
|---|---|
| Control pot | 48 |
| Pot1 | not detected |
| Pot2 | not detected |
| Pot3 | not detected |
| Pot4 | not detected |

**Table 4. Residual lead content present in soil after cultivation of maize (%)**

| Treatment | Percentage (%) |
|---|---|
| Control | 53 |
| Pbr1 | not detected |
| Pbr2 | not detected |
| Pbr3 | not detected |
| Pbr4 | not detected |

## CONCLUSION

The synergistic application of lead-resistant bacteria and US3 biostimulant effectively ecorestored lead-stressed soil, demonstrating a promising approach for sustainable lead mitigation. This study highlights the potential of microbial-based solutions for environmental remediation and agricultural sustainability.

## FUTURE WORK / REFERENCES

Cowdhury, E., O'Brien, S., Tromas, N., Bayer, F., Luján, A. M., van Veen, E. M., Hodgson, D. J., & Buckling, A. (2020). Ecological selection of siderophore-producing microbial taxa in response to heavy metal contamination. *Ecology Letters,* 21(1), 117–127. https://doi.org/10.1111/ ele.12878

Ehis-Eriakha, C. B., Ezeanya-Bakpa, C. C., Akemu, S. E., & Yahaya, S. O. (2020). Assessment of Indigenous rhizospheric soil microbes from zea mays and manihot esculenta for plant growth promoting (PGP) traits. Archive of Science and Technology. 1, 88–97.

Das, A., & Osborne, J. W. (2019). Monitoring the stress resistance of Pennisetum purpureum in Pb (II) contaminated soil bioaugmented with Enterobacter cloacae as defence strategy. Chemosphere, 210, 495–502.https://doi.org/10.1016/j.chemosphere.2018.07.050