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Slag of Batteries and Application of Biotechnology Processes in Their Lower Toxicity

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ABSTRACT

Lead slag (Pb slag) is a residue resulting from the recovery of a lead automotive battery. The residue requires a special provision for its content of Pb, As, Ni and Cd. Phytoremediation was tested as an alternative to the removal of Pb, and it was assessed in terms of the transfer, accumulation, stabilization, degradation or neutralization of the metal. Castor bean Ricinus communis L. was used for its ability to grow in diverse environments and to accumulate heavy metals. R. communis seedlings were assessed in sterile soil mixtures with Pb slag 15% and 30%. In further trials, we assessed the inoculation of Auxin-Producing Bacteria (APB) and Siderophore -Producing Bacteria (SPB) into R. communis L. and the extraction of Pb. The data confirmed Pb accumulation in R. communis L. structures, especially after the inoculation of APB and SPB into R. communis L. when grown in soil: Pb-slag 15%.

KEYWORDS Lead (Pb), slag, Ricinus communis, phytoremediation, siderophores

Introduction

In automotive batteries, Lead (Pb) forms the grids (44%) and pulp (56%). The recycling and recovery of Pb also implies effects on the environment through its accumulation, waste dispersion and damage to human health. In Mexico recycled batteries of domestic (International Knowledge & Comunes, 2011; Moguel, 2007) are processed. This process requires rotary drum furnaces (Bourson, 1995), in addition to fluxes (carbonate sodium), reaction (iron [Fe] chips and coal) at a ratio of Pb:sodium carbonate and Fe shavings: carbon.

Pb in the soil causes a reduction in biomass and microbial diversity due to its interference with metalloproteins, respiration processes, and enzymatic activities sensitive species; however, some microorganisms tolerate and persist as the metal (Konopka et al., 1999).

Also, Pb in vegetal tissue causes disorders in enzymes, affecting mineral nutrition water potential, hormonal status, membrane structure, and electron transport (Seregin & Ivanov, 2001). Pb inhibits seed germination and greatly retards plant growth. However, enzymes such as protease, alkaline phosphatase, and acid phosphatase are increased in the presence of the Pb observed. Plant Growth-Promoting Rhizobacteria (PGPR) promote growth through nitrogen fixation, phosphate solubilization, production of the enzyme Amine Cyclopropane-1-Carboxylate (ACC) deaminase, siderophore production and aid in auxin production in plant growth. Achromobacter, Arthrobacter, Azobacter, Azospirillium, Bacillus, Pseudomonas, Serratia, and Streptomyces have been reported as rhizosphere colonizers, with beneficial effects on plants growing.

R. communis L. is a shrub or annual tree with capacity to adapt to different climates and to grow in soil contaminated by heavy metals including As, Cu, Hg, Zn, Pb, and Cd (Rajkumar & Freitas, 2008)respectively, on the basis of their morphological, physiological, biochemical characteristics and 16S rDNA sequences. Assessment of plant growth-promoting parameters revealed the intrinsic ability of the strains for the utilization of 1-aminocyclopropane-1-carboxylic acid as the sole N source, solubilization of insoluble phosphate and production of indole-3-acetic acid (IAA. Additionally, evaluated R. communis L. and Brassica juncea L. in soils artificially contaminated with Pb (200 - 800 mg kg-1 plus EDTA). Both species exhibited accumulation of the Pb level and decrease in roots, stems, and leaves, whereas R. communis L. was not affected. Showed that Pseudomonas inoculation promoted plant growth and aerial and root biomass in R. communis L. and phytoextraction with increased Zn. Pandey (2013) indicated a bioconcentration factor of >1 metal in the root as an indicator of the potential for accumulation, and a translocation factor of <1 metal, demonstrating stabilization at the plant root. This work evaluates R. communis L. for treating slag with Pb and inoculation with APB, SPB and consortium.

Materials and Methods

Lead slag (Pb) was obtained from a recycler of both domestic and imported batteries. In the laboratory, the slag was sieved through no. 14 mesh to remove the larger aggregates.

Analysis of carbonates and bicarbonates of Pb slag was analyzed by methods describe by Romeiro et al. (2006).

Inoculation. Two strains from the collection of Environmental Microbiology Laboratory Unit of CINVESTAV-Irapuato were employed: 1) Bacillus spp. strain 1: Productive Auxin-Producing Bacteria (APB), such as Indole Acetic Acid (IAA), and 2) Streptomyces spp. strain 2: Siderophore-Producing Bacteria (SPB).

Reading by spectrophotometer indole groups. At 3 to 8 days of the growth of the strain of Bacillus spp.EE50 strain, the indole group production by the colorimetric method, and 1 mL of culture medium was centrifuged at 5,000 rpm/|5 min. 100 µL of the supernatant was added to 200 mL of the Salkowski reagent (Barcos Arias, et al. 2015), on the Enzyme-Linked ImmunoSorbent Assay (ELISA) plate (1:2). The plate was covered for 30 min and subsequently a reading in a Spectrophotometer at 540 nm absorbance. A calibration curve was made from a stock solution of Indole Acetic Acid (IAA), which was diluted in methanol (1 mg of IAA in 1 mL of methanol); from this solution, dilutions (10 to 100 µg mL-1 IAA) with Czapek medium. Established production from day 1 with maximal production of 40 µg mL-1 .

Production of siderophores by Streptomyces spp. in CAS medium. With the aim of measuring the orange halo produced in the Chrome Azurol S (CAS agar) medium by the SPB.

Figure 1. The culture is observed of Streptomyces spp. L2 at 3, 9, and 15 days. the orange halo in CAS medium, changing to an orange color in the presence of the siderophores. The latter oxidize the Fe of the Fe-CAS orange complex (Alexander & Zuberer, 1991)we inoculated serial dilutions of root samples onto chrome azurol S (CAS

Results and Discussion

Physico-chemical Characterization of Pb Slag, Pb-Washed Slag and Base Soil

The slag of automotive batteries for recovering Pb, it has high alkalinity and salinity exhibited by the pH, high Electrical Conductivity, a high CO3-2 content, and low values of HCO3- and SO4-2 (Table 1). Barcos et al. (2014a, 2015b) demonstrated that this slag mixed with soil permitted the establishment of plants with extracting the Pb present by phytoremediation processes.

Table 1. Phytochemical characteristics of lead (Pb) slag, washed slag, and soil: slag mixtures

Property	Slag	Washed slag	Base soil	Mixture of soil:Pb slag15%	Mixture of soil:Pb slag 30%
pH	10.1	7.95	6.62	8.81	8.93
EC (mS^{-1})	605.6	42.7	10	137.5	139.4
CO_3^{-2}	1,642	136.8	ND	136.8	276.0
HCO_3^-	59.0	19.6	ND	19.6	37.8
SO_4^{-2}	183.9	18	ND	18	34
O.M. (%)	-	-	0.33	1.16	1.16
[Pb] mg kg-1	-	-	189.0	10,244.0	19,913.0

ND = Not Determined.

In Table 2, the morphological characteristics of R. communis L in the base soil and in the mixtures with soil:slag at 15% and 30%, the immediate effects are evident, above all at the greatest concentration of slag, in which R. communis L. loses height, possesses less foliage, and the effects of photosynthesis.

Table 2.- Morphological characteristics of R. communis L. under different growth conditions harvested on day 35

Variable	Base Soil	Mixture of Soil:Pb slag 15%	Mixture of Soil:Pb slag 30%
Sheets (#)	4 ± 2 a	4 ± 0.5 b	2 ± 1 c
Stem diameter (mm)	5 ± 2 a	5 ± 0.7	2 ± 1 c
Stem height (cm)	8 ± 4 a	7 ± 1 b	4 ± 3 c
Leaf area (cm^2)	167 ± 12 a	82 ± 56 b	10 ± 24 c
Root fresh weight (g)	11 ± 2 a	5 ± 4 b	5 ± 17 c
Air fresh weight (g)	10 ± 21 a	11 ± 31 b	6 ± 2 c
Chlorophyll (SPAD)	31 ± 21 a	47 ± 12 b	10 ± 2 c
Root dry weight (g)	0.6 ± 0.1a	0.3 ± 0.2 b	0.05 ± 0.04 c
Aerial dry weight (g)	1 ± 0.6 a	0.7 ± 0.4 b	0.2 ± 0.1 c

After the exposure of R. communis to soil:Pb slag at 15% and 30% were valuated a negative effect was evidenced, in the plants a growth loss with lesser stem height, dimension and weight, which signifies affectation in the different physiological processes of the plant. The latter was proven with low photosynthesis according to the increase of the content of slag (soil: slag 30%). Chlorophyll exhibited an increase in the soil:Pb-slag assays at 15%, which may have comprised a compensatory process in the face of the changes in the plants in the presence of growth-limiting factors. The effect of the inocula of growth-promoter bacteria individually and in Consortium with the production of indole and siderophores on the development of R. communis L. was found in the lots of Consortium APB-SPB (Table 3), demonstrating in the plant greater vigor and aerial and radicular growth, which suggests an additive effect between the indole and siderophores on R. communis L. in soil with the limiting components depicted.

Table 3. Effect of the different inocula on the morphological characteristics of Ricinus communis L.

Variable	W/out inoculum	APB	SPB	Consortium
Sheets (#)	3 ± 2	4 ± 2	4 ± 2	4 ± 2
Stem diameter (mm)	4 ± 3	4 ± 2	5 ± 2	5 ± 2
Stem height (cm)	7 ± 4	8 ± 3	8 ± 3	8 ± 4
Leaf area (cm^2)	162 ± 82	191 ± 30	138 ± 74	179 ± 19
Root fresh weight (g)	10 ± 11	8 ± 7	13 ± 20	11 ± 10
Air fresh weight (g)	6 ± 5	15 ± 4	11 ± 23	8 ± 7
Chlorophyll (SPAD)	30 ± 23	30 ± 22	34 ± 22	31 ± 17
Root dry weight (g)	0.7 ± 0.07	0.5 ± 0.06	0.5 ± 0.06	0.7 ± 0.07
Air dry weight (g)	1 ± 0.2	1 ± 0.1	1 ± 0.5	1 ± 0.2

APB: Auxin-Producing Bacteria; SPB: Siderophore -Producing Bacteria; Consortium = APB+SPB.

The inoculation of SPB, APB, or of their Consortium exerts a positive effect on the production of chlorophyll compared with the control without inoculation (Table 3), which suggest that the presence of indoles and siderophores limits the entry of Pb into the plant. The latter is the chelating effect, which is more effective than 15% slag.

Conclusions

R. communis L. is a plant that survives in a soil mixed with slag Pb at a concentration of 15%. Inoculation of microorganism-producing auxins favors survival, while the production of siderophores represents a mechanism against the toxicity of lead (Pb). The Consortium treatment appears to favor the aerial tissues, which explains the increase in the value of the photosynthesis.

R. communis L., with inocula of APB, SPB and the Consortium, permit the plant to survive and maintain the processes of Pb accumulation that are functional in the root at a slag concentration of 15%, as well as of 30%.

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