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ENDOPHYTIC BACTERIA ASSOCIATED WITH DICHANTIUM ARISTATUM BENTH WITH IN VITRO LEAD REMEDIATION CAPACITY IN LIVESTOCK FARM SOILS

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ABSTRACT
The present study consisted of isolating endophytic bacteria from roots associated with *Dichanthium aristatum* Benth from cattle farms in the municipality of Tolú, department of Sucre, Colombia. A total of 10 cattle farms were sampled, from which 10 soil samples with roots were taken per farm at a depth of 20 cm. Endophytic bacteria were isolated from each sample, isolates were separated, lead tolerance and siderophore production were evaluated. The multifactorial ANAVA between the variables density (CFU/g root) as a function of site showed highly significant differences. The results obtained indicate that 97% of the isolates found grew in the high Pb concentrations found in the soils of the cattle farms analysed. This preliminary study carried out in the Colombian Caribbean shows that the strain identified as C63RLIM showed the capacity to tolerate up to 500 mg/L of lead in the form of $PbCl_2$ and to produce siderophore. In this study we found isolates of endophytic bacteria associated with *Dichanthium aristatum* Benth adapted to the soils of cattle farms with the presence of the metal Pb and with the capacity to tolerate in vitro up to 500 mg/L of $PbCl_2$ and to produce siderophore.

Keywords. Endophytic bacteria, pasture, livestock farm, heavy metal, remediation.

1. INTRODUCTION
The main pasture species cultivated in the northern Caribbean region of Colombia are: *Botriochloa pertusa* (Colosoana or kikuyo); *Branchiaria mutica* (Admirable); *Dichanthium aristatum* (angleton) and *Pennisetum* sp (King grass). Angleton grass (*Dichantium aristatum* Benth) represents the third species with the largest area planted in Sucre, reaching an area of approximately 56,200 ha, distributed in 19 municipalities. The municipality of Santiago de Tolú-Colombia, whose main economic activity is semi-intensive livestock farming, has the second largest area planted (9,400 ha) with this species in the department. *Dichantium aristatum* Benth is a perennial grass that has stood out for being resistant

to drought, as well as to burning and trampling, it provides a high nutritional contribution to livestock, but does not tolerate waterlogging (Pérez and Peroza, 2013).

Despite the existence of soils suitable for agricultural activities, rapid industrial growth has introduced enormous amounts of waste, including heavy metals, into the environment (Naik and Dubey 2013). In recent decades, concentrations of non-essential metals (e.g. Pb, Cd and Hg) have increased in water, sediments rapidly and agricultural soils as a consequence of industrial activities. Heavy metal contamination can affect soil fertility, decrease microbial activities, biodiversity, crop yields and be risky for human health due to contamination of food of animal origin (Ma, 2019).

According to Volke et al. (2005), lead (Pb) appears on the list of the most important heavy metal pollutants, which originate from smelting, metal processing, recycled batteries, gasoline, mining and air pollution.) In addition, ATSDR, (2007), state that particles of this mineral can reach water and soils, adhering to and remaining in the surface layer. The half-life for Pb is 740 to 5900 years, with levels depending on soil type, topography, physicochemical characteristics of surfaces and climate (Dantu, 2009). There are Pb compounds that can be transformed to other chemical species; however, elemental Pb does not degrade.

As stated by Schnaas, (1998), the effects that the metal Pb can have on production are related to cattle mortality and agro-ecosystem imbalance. The routes of absorption of Pb include ingestion, inhalation or dermal contact, being the time of exposure and the concentration of Pb in blood, indicators that influence the process of intoxication of the tissues. According to Fincheira et al. (2018), the concentration of Pb in soils used for agricultural production has a direct implication in the contamination of plants, because heavy metals can reach lower soil layers, mixing with groundwater and being absorbed by plant roots.

It has been shown that Pb can cause oxidative damage to genome-DNA (Xu et al., 2018), damage to proteins, lipids and can also substitute essential metal ions such as Zn, Ca and Fe, present in some enzymes (Naik and Dubey 2013). Exposure to lead over long periods of time leads to anaemia, reproductive impairment, kidney failure, cancer and neurodegenerative damage (Kang et al., 2015), therefore, the US Environmental Protection Agency (EPA) has listed lead as an inorganic hazardous waste (Naik and Dubey 2013).

Studies have shown that there is a high diversity of metal-resistant bacteria that have been isolated from metal-contaminated sites. Contaminated soil or water exposes the bacteria present to heavy metals, which leads them to develop tolerance to them (Oves et al., 2017). Among the bacterial genera reported are Gram-positive bacteria such as Bacillus cereus, Arthrobacter sp. and Corynebacterium sp. and Gram-negative bacteria such as *Burkholderia* sp; *Pseudomonas* spp and *Ralstonia* sp. (Jarosławiecka and Piotrowska 2014; Oves et al., 2017). Bacteria of the genus Pseudomonas spp. are present in most soil and water ecosystems, they are able to metabolize a wide variety of organic and inorganic compounds and are well known for their ability to tolerate and resist toxic molecules, including antibiotics, heavy metals, detergents and organic solvents (Chien et al., 2013).

Given that there are high levels of contamination and its repercussions in different geographical regions worldwide and especially in Colombia, new strategies need to be developed to provide solutions in short periods of time. At present, it is considered urgent to monitor the concentrations of heavy metals as contaminants in areas dedicated to agricultural activity, so the present work focused on determining the levels of Pb in soils dedicated to livestock production cultivated with Angletón grass in the municipality of Tolú in the department of Sucre-Colombia.

2. MATERIALS AND METHODS

Soil sampling of livestock farms: Initially, bibliographic information on areas contaminated with metals was collected. Once the areas with soils contaminated with heavy metals had been identified, site visits were made and a diagnosis was made of the current state, crop sown, number of hectares and vulnerable area. Next, the contaminated soil sampling stage was carried out on livestock farms sown with *Dichantium aristatum* Benth grass.

The sampling consisted of random sampling. At each selected site, three samples were taken at random. These samples were taken specifically in the rhizosphere zone of *Dichantium aristatum* Benth, covering the first 20 cm depth. Approximately 3000 g of sample will be taken per site. These will be divided into two sub-samples of 1500 g each (one for heavy metal concentration and the other for microbiological analysis). Subsequently, the collected soil samples were carefully packed in labelled and identified plastic bags, stored and preserved for transport and processing.

Heavy metal concentration in soil samples. The measurement of heavy metal levels in soil samples was carried out using the technological services provided by the Analytical Chemistry certified laboratory with ISO/IEC 17025:2017 accreditation granted by ONAC.

Isolation of endophytic bacteria from roots. The endophytic bacteria were isolated at the Microbiological Research Laboratory of the University of Sucre-Colombia from root, stem and leaf tissues, following the protocol proposed by (Perez et al., 2014), which consists of performing a superficial disinfection of roots. The tissues were then macerated and transferred to 9 mL of peptone broth and incubated at 30 ± 2 °C for 24 h. Serial dilutions to 108 were then made, inoculated 100 µL on R2A agar and incubated at 30 ± 2 °C for 72 h. The population density of endophytic bacteria per tissue (CFU/g root) was estimated by direct colony counting, selecting them according to their shape, surface appearance, color and size. The selected isolates were purified and maintained on R2A agar.

In vitro evaluation of the tolerance capacity of endophytic bacteria to different Pb concentrations. The obtained endophytic bacterial isolates were seeded on the surface of nutrient agar supplemented with PbCl2 at concentrations of 100, 300, 500, 500, 700 and 900 mg/L and incubated at 32°C for 7 days (Sorkhoh et al. 2010).

Siderophore production. Qualitative assessment of siderophore production was carried out by direct seeding of each morphotype on the surface of chromium azurol-S (CAS) medium proposed by Schwyn and Neilands (1987). They were incubated for 7 days at 30°C. The ability of the bacteria to produce siderophores was evidenced by halo formation.

3. RESULTS AND DISCUSSION

The analyses of Pb characterization in soil of cattle farms in the municipality of Tolú-Colombia sown with *Dichantium aristatum* Benth oscillated between 13.01 ± 16.4 mg/kg, according to the European Union limit values for Pb in agricultural soils is 0.3 mg/kg and 0.1 mg/kg for Australia, Codex Alimentarius and South Africa). The results infer that the values found in the soils of grasslands sown with *Dichantium aristatum* Benth grass are above the values established at international level (figure 1).

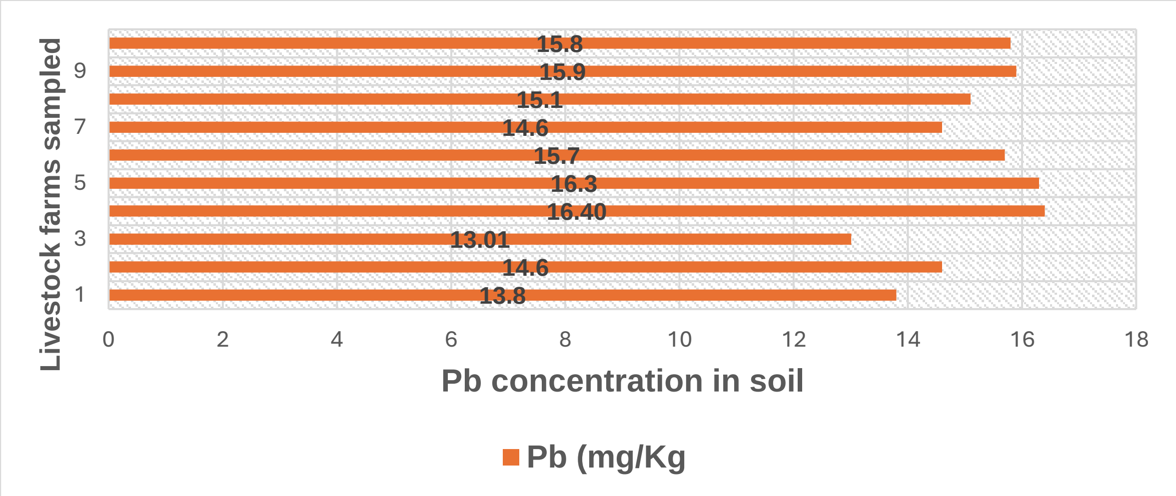

Figure 1. Pb concentration (mg/Kg) in soil of cattle farms established with *Dichantium aristatum* Benth in the municipality of Tolú, department of Sucre, Colombia.

According to Marrero et al. (2012), the root of plants is the main route of entry and accumulation of metal, therefore, the bacteria that are present in this tissue are those that possess the biochemical or genetic machinery that allows them to survive these contaminants. Studies by (Luo et al. (2011), argue that it is possible that bacteria adapt to living under constant stress by the presence of heavy metals, gaining tolerance. This may be due to the fact that when a cell is confronted with high concentrations of a heavy metal, it transports them to the cytoplasm in the form of cations via transporters of the cellular non-specific system; in addition, other mechanisms are activated such as the use of membrane transporters that expel these ions into the environment, enzymatic modifications to change the redox state and the incorporation of metal ions into the cells; however, it is possible that mutations are generated due to the pressure exerted by the contaminated environment (Marrero et al, 2010; Martínez et al., 2010).

Based on the above hypothesis, the present study found a population density of endophytic bacteria associated with *Dichantium aristatum* Benth grass roots as shown in Figure 2. The range of endophytic bacteria was between 3.90 X 105 ± 6.70 X 105 CFU/ g of root. The presence of these endophytic bacteria suggests a fundamental role of these microorganisms in contributing to the remediation of the lead present in the livestock farms analyzed. There is a wide variety of bacteria that improve the nutritional status of plants through nitrogen fixation (NNF), phosphate solubilisation (PS) and production of siderophores (PS) necessary for the uptake of essential nutrients (Sessitsch et al., 2013); the ability of the bacteria to produce siderophores.
Among the most important utilities of siderophores is to help plants reduce the toxicity generated by heavy metal in contaminated media and thus optimize phytoremediation processes by participating in the reduction of the inhibitory effect of some PM on auxin synthesis (Rajkumar et al., 2009).

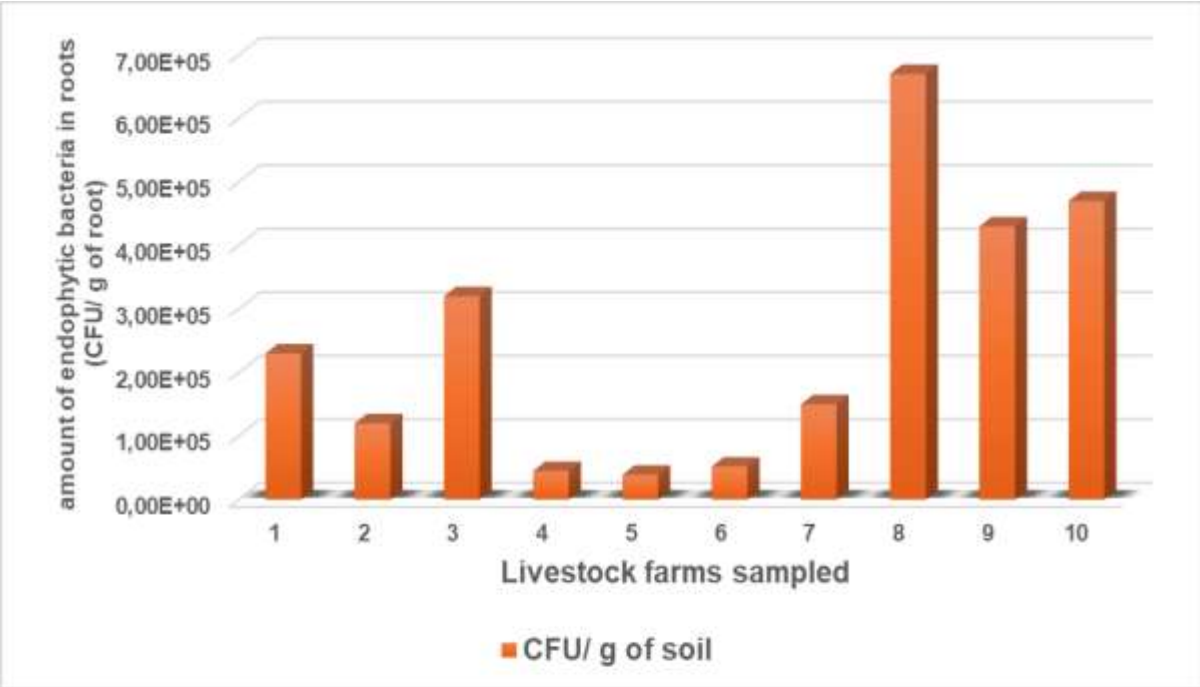

Figure 2. Population density of endophytic bacteria associated with Dichantium aristatum Benth roots and lead concentration in soil from cattle farms in the municipality of Tolú, Sucre, Colombia.

There is a wide variety of bacteria that improve the nutritional status of plants through nitrogen fixation (NF), phosphate solubilization (PS) and production of siderophores (PS) necessary for the uptake of essential nutrients (Sessitsch et al., 2013); the ability of bacteria to produce siderophores. Among the most important utilities of siderophores is to help plants reduce the toxicity generated by heavy metal in contaminated media and thus optimize phytoremediation processes by participating in the reduction of the inhibitory effect of some PM on auxin synthesis (Rajkumar et al., 2009).

In the present study, the ability of the isolated strain C63RLIM to produce siderophore at a concentration of 500 mg/L was found as shown in figure 3.

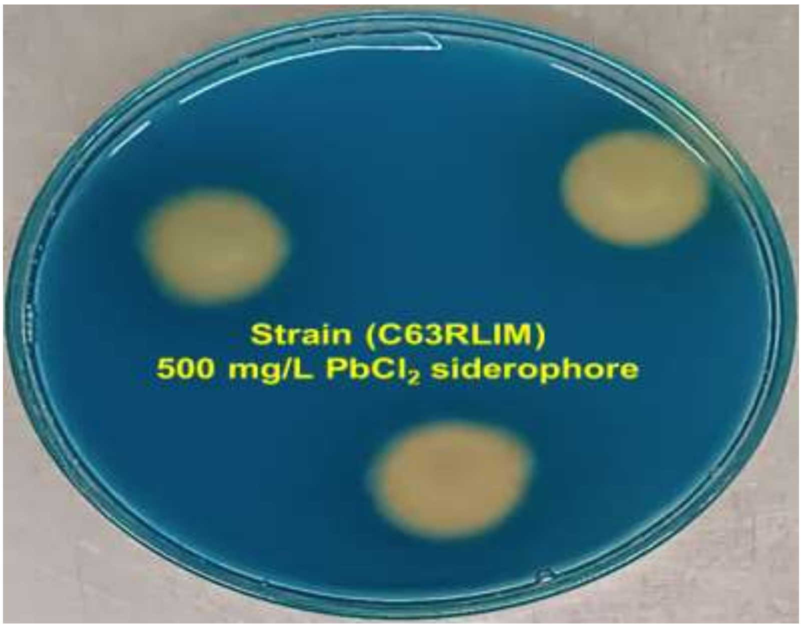

Figure 3. Production of siderophore in chromium azurol-S (CAS) medium by strain C63RLIM at 500 mg/L Pb in the form of $PbCl_2$.

4. CONCLUSION

The results found in the present study suggest that the strain identified as C63RLIM showed the ability to tolerate up to 500 mg/L lead in the form of $PbCl_2$ and to produce siderophore at the same concentration of the metal. The isolates of endophytic bacteria associated with *Dichanthium aristatum* Benth adapted to the soils of livestock farms with the presence of Pb metal and with the capacity to tolerate in vitro up to 500 mg/L $PbCl_2$ and to produce siderophore can be considered as a possible biological alternative to contribute to reduce Pb toxicity in the soil.

5. ACKNOWLEDGEMENTS

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6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

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