Frequency of Endophytic Bacteria in Roots and Foliage of Brachiaria Humídicola Cv. Humidicola (Rendle) Schweick in Soil with Arsenic Presence

Alexander Pérez Cordero[1], Donicer E. Montes Vergara[1], Yelitza Aguas Mendoza[2]

[1]Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia
[2]Universidad de Sucre, Facultad de Ingeniería, Colombia
Email: alexander.perez@unisucre.edu.co

Abstract

The objective of this study was to correlate the presence of arsenic in roots and stems of Brachiaria humidicola cv. humidicola (Rendle) Schweick grown in cattle areas of the San Jorge subregion and the population density of endophytic bacteria in roots and foliage with the presence of this metal. Samples of soil, roots and stems of B. humicola grass were collected and characterized for arsenic concentration in roots and foliage and the presence of endophytic bacteria (CFU/g tissue). The results show the presence of arsenic concentrations in roots and stems above the values established at national and international level. Likewise, the presence of endophytic bacteria was found in the roots and foliage of B. humidicola, which may be contributing to the remediation and reduction of arsenic concentration from the roots to the foliage. This is the first study correlating the presence of arsenic and endophytic bacteria on cattle farms with B. humicola and the possible role of these bacteria in contributing to bioremediation of this metal in the soil and within the tissues of the pasture species.

Keywords: Arsenic, tissues, pasture, bacteria, remediation.

1. Introduction

According to Martínez-Reina (2013), the region of La Mojana suffers recurrent flooding that occurs mainly due to the overflowing of rivers, including the Cauca, as a result of the collapse of the embankment at the site known as La Mejicana, in San Jacinto del Cauca, department of Bolívar. This situation also affects the municipalities of Guaranda, Majagual, San Benito Abad, San Marcos, Sucre and Caimito in Sucre; Ayapel in Córdoba; Achí, San Jacinto del Cauca and Magangué in Bolívar; and Nechí in Antioquia. This has happened repeatedly, mainly in 2005 and 2010 due to the La Niña phenomenon.

According to the DANE census (2005), the municipality of San Benito Abad has an area of 1592 km² (1428 according to other measurements), which makes it the largest municipality in the Department of Sucre, comprising 14.6% of its area. Its surface is made up of low-lying areas, especially marshlands, with the exception of a small region to the north, which develops a savannah landscape. This municipality is located in the south of the department of Sucre, on the western bank of the San Jorge River and the Ciénaga de Machado. The largest part of the territory of San Benito Abad extends over an alluvial plain drained by the fluvial systems of the San Jorge, Magdalena and Cauca rivers, the first being the only one that passes through the municipality, flowing from south to north. This extensive region is known as the Momposina Depression and has altitudes of no more than 33 m above sea level, which makes it a flood zone for most of the year. The climate is humid tropical, with temperatures that can exceed 32 °C. The flora and fauna is varied, and the wealth of fish is great. Its economy depends largely on livestock, agriculture and fishing.

According to (Escobar, M. & Rúa, 2017), the grass known by its botanical name Brachiaria humidicola cv. humidicola (Rendle) Schweick, originally African (native), is distributed from South Sudan and Ethiopia, which are north of South Africa, to Namibia, which is to the south. It is found especially in areas with high rainfall. It is cultivated in Brazil, Ecuador, Venezuela and other countries of tropical and subtropical South, Central and North America (including the United States). It was introduced to Colombia in 1973 by ICA and CIAT with CIAT accession number 679 and has been evaluated there in different ecosystems such as the savannahs of the Eastern Plains, and also in the plains and Amazonian foothills of Colombia.

However, several studies (Argumedo et al., 2015; Marrugo et al., 2018; Negrete et al., 2019) have recorded the contamination of this ecosystem with high levels of heavy metals such as: As, Cr, Cd, Hg, Pb, Ni, in various ecosystem components of La Mojana. Concentrations of heavy metals have been recorded in sediments and organic matter such as Cu (48400 ng/g), Zn (79200 ng/g), Ni (58100 ng/g), Pb (3200 ng/g), Cd (560 ng/g), Mn (41100 ng/g) and Hg (100 ng/g) (5), HgT concentrations of 0.097±0. 049 µg/g in sediments, 0.191±0.017 µg/g in macrophytes, 17.05 ng/g in rice, in fish such as Caquetaia kraussi (150±57 ng/g), Hoplias malabaricus (239±67 ng/g), Plagioscion surinamensis (578±392 ng/g) and Pseudoplatystoma fasciatum (568±224 ng/g).

Returning to what was stated by (Calo and Marrugo, 2013), in Colombia, mining is an activity of great economic importance, however, in most cases it is carried out with very little environmental monitoring. One of the consequences of the activity associated with the exploitation of metal resources is the production of large amounts of waste that generate emission sources of pollutants that may contain potentially toxic elements, such as heavy metals, according to the parameters of the Environmental Protection Agency (EPA) of the United States. According to Berkowitz, et al., 2008, the danger is greater because they have not been degraded or destroyed, so that, once emitted, they can remain in the environment for many years, increase their concentration in living beings and accumulate.

According to the study carried out by (Abad et al., 2018) at the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA, a CSIC centre) in an area affected by the former tungsten mining in the Salamanca town of Barruecopardo shows that animals grazing in this mining area could ingest higher than recommended amounts of arsenic. Scientists believe that restrictions should be put in place in these grazing areas to preserve food quality.

Taking into account the impact of arsenic as a carcinogenic element and its capacity to biocuamulate in plant tissues and reach humans through the ingestion of animal protein, the present study was carried out with the aim of determining the presence of arsenic in soil, roots and leaves of the pasture species Brachiaria humicola and the presence of endophytic bacteria associated with this pasture located in cattle farms in the San Jorge sub-region.

2. Materials and Methods.

Identification of areas contaminated with arsenic in the department of Sucre. For the identification, a survey was made of the bibliographic information that reports environments contaminated with this heavy metal in the Colombian Caribbean. The selected site corresponded to cattle farms located in the San Jorge sub-region in the department of Sucre.

Characterization of soil and plant tissues with arsenic. Samples of soil, roots and foliage of B. humicola were collected from cattle farms in the municipality of San Benito de Abad in the San Jorge sub-region of the department of Sucre. The samples were labelled, stored and preserved until transport to a specialized and certified laboratory for the characterization of the levels of arsenic present in soil, roots and foliage.

Isolation of endophytic bacteria. This was carried out in the microbiological research laboratory of the University of Sucre. For this, samples of plant material from the Brachiaria humidicola cv. humidicola (Rendle) Schweick pasture were subjected to a surface disinfection process, as described by Pérez et al. (2016). After this process, each tissue was macerated in a porcelain mortar with liquid nitrogen until a homogeneous mixture was obtained. From each homogenate, serial dilutions were prepared and seeded by diffusion technique on R2A agar surface and incubated at 28ºC for 72 h. The population density of endophytic bacteria per tissue, CFU/g tissue, was estimated by direct colony counting on plates (Pérez et al. 2016).

3. Results and Discussion

Figure 1 shows the geographical map of the Mojana sub-region, showing all the departments and municipalities that are part of the sub-region. It also shows the municipality of San Marcos immersed in the sub-region. As expressed by Buelvas-Soto et al., (2022) and in accordance with what is stated by (Marrugo et al., 2007; F Carlos et al., 2016; Marrugo et al., 2018; Ramsar, 2021), the ecosystem of the Mojana sub-region represents one of the most important wetland systems of the Colombian Caribbean, which originates from the interaction of the basins of three rivers: Cauca, Magdalena and San Jorge. These wetlands occupy 30,781,149 hectares of the country, of which 760,340 hectares are recognized as RAMSAR territories, ecosystems with a vast biodiversity that are fundamental in the ecological cycles, as well as providing ecosystem services to the human populations associated with them.

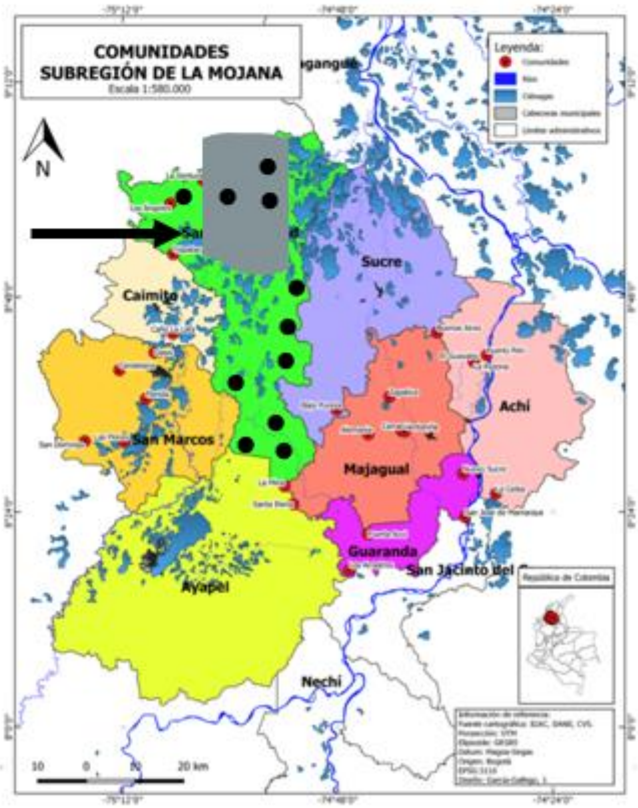

Figure 1. Geographical distribution of soils belonging to the Mojana physiographic sub-region, Colombia. Source: https://www.undp.org/es/colombia/news/suelos-mojana-economia-campesina-cambio-climatico

In figure 2, the arsenic concentration in rhizosphere soil of cattle farms planted with Brachiaria humicola pasture is shown. The arsenic range varied from 4.27 to 7.1 mg/L in soil.

According to studies reported by Heinrich-Salmeron et al. (2011), arsenic (As) 20 is the 20th most abundant element in the earth's crust and is distributed non-uniformly globally, depending on the geographical region, geochemical characteristics of the soil and industrial activity. About one third of the arsenic in the atmosphere is considered to come from natural sources such as environmental reactions, biological activity, volcanic emissions, and the rest comes from a wide range of anthropogenic activities (Alam et al., 2014).

Several studies carried out by (Bhattacharjee et al., 2013; Cohen et al., 2013; Brocato et al., 2015), arsenic (As) is a carcinogenic element (inorganic species (AsIII and AsV) being more toxic than organic species as shown by studies carried out by (Bundschuh et al., 20 08). However, the As present in both irrigation water and soil are exposed to As.

The inorganic forms are absorbed through different transporters and are transformed and mobilized to other plant tissues and to the photosynthesis process. Several studies by (Finnegan & Chen, 2012; Farnese et al. 2014; Pérez Carrera & Fernández Cirelli 2014) concluded that as affects plant growth and germination, causes oxidative stress, decreases phosphorus uptake, inhibits root elongation and decreases both cell proliferation and biomass production. Decreases in leaf number, plant height and germination percentage, various morphological changes and altered photosynthesis were also observed in plant species.

Figure 3 shows the distribution of arsenic from rhizospheric soil to foliage of Brachiaria humidicola cv. humidicola (Rendle) Schweick. The range in roots was from 4.27 to 7.1 mg/L in soil, while in foliage it was from 1.47 to 2.90 mg/L.

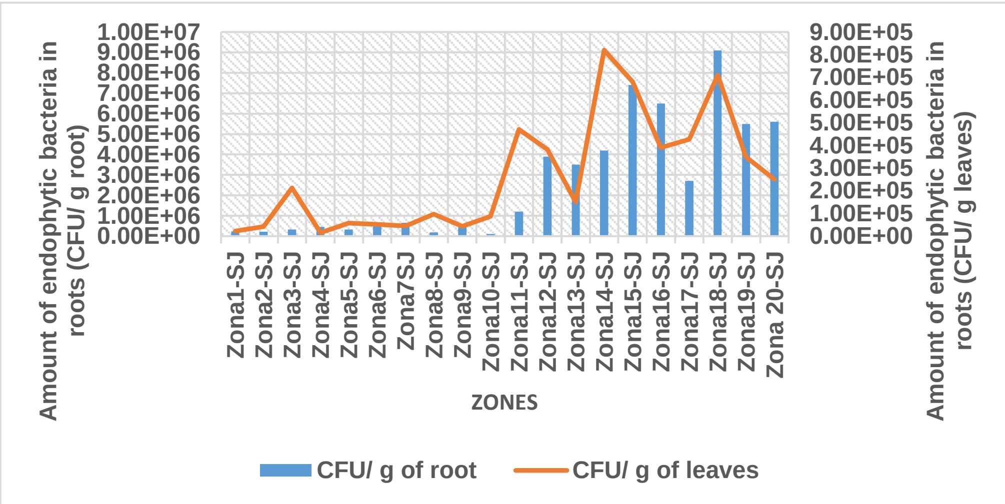

Figure 3. Distribution of arsenic in rhizospheric soil and foliage of the pasture Brachiaria humidicola cv. humidicola (Rendle) Schweick present in cattle farms in the sub-region of San Jorge in the department of Sucre.

In vitro studies using alfalfa species (Pérez Carrera & Fernández Cirelli, 2014) have shown that increasing the concentration of As in the soil also increases its concentration in leaves, stems and roots, and that it varies between harvests, demonstrating its transfer to the forage. Another study by Mascher et al (2002) on another legume, red clover, showed that there was a significant reduction in shoot growth when exposed to high concentrations of As and that this effect was greater in the hypocotyls than in the radicles.

Figure 4 correlates the presence of endophytic bacteria associated with roots of the grass Brachiaria humidicola cv. humidicola (Rendle) Schweick with the concentration of arsenic present in the roots of this grass species.

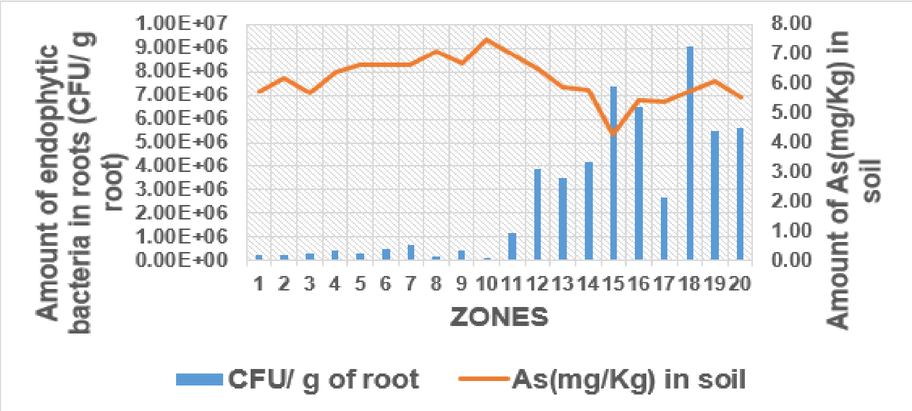

Figure 4. Presence of arsenic in roots and quantity of endophytic bacteria associated with the pasture Brachiaria humidicola cv. humidicola (Rendle) Schweick present in cattle farms in the sub-region of San Jorge in the department of Sucre.

According to the results found, it was observed that the amount of endophytic bacteria in roots ranged from 1.1 X 10^5 to 9.1 X 10^6 CFU/g root. Likewise, a correlation was found between the quantity of endophytic bacteria and arsenic concentration. The results show a lower presence of bacteria when the arsenic concentration was at the highest level and a higher presence of these bacteria when the arsenic concentration was at the lowest level.

Figure 5 correlates the presence of endophytic bacteria associated with the foliage of Brachiaria humidicola cv. humidicola (Rendle) Schweick with the concentration of arsenic present in the foliage of this pasture species.

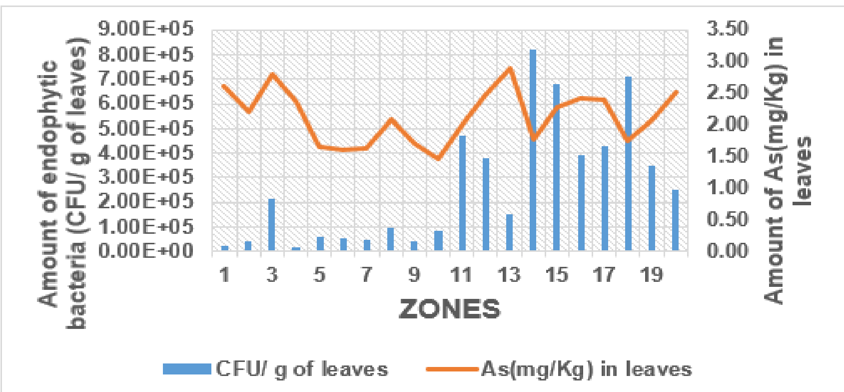

Figure 5. Presence of arsenic in foliage and quantity of endophytic bacteria associated with the pasture Brachiaria humidicola cv. humidicola (Rendle) Schweick present in cattle farms in the sub-region of San Jorge in the department of Sucre.

Interpreting the results, it was observed that the amount of endophytic bacteria in foliage ranged from 1.5 X 10^4 to 8.2 X 10^5 CFU/g of foliage. Also, a correlation was found between the amount of endophytic bacteria and arsenic concentration. The results show a lower presence of bacteria when arsenic concentration was found at higher levels and a higher presence of these bacteria when arsenic concentration was lower.

Another important aspect found in the present study was that less arsenic translocation from the roots to the foliage of Brachiaria humidicola cv. humidicola (Rendle) Schweick was observed, possibly due to the presence of endophytic bacteria contributing to the bioaccumulation of arsenic to aerial parts of the grass.

However, in vitro studies using alfalfa species (Pérez Carrera & Fernández Cirelli, 2014) have shown that increasing the concentration of As in the soil also increases its concentration in leaves, stems and roots, and that it varies between harvests, demonstrating its transfer to the forage. Another study by Mascher et al (2002) on another legume, red clover, showed that there was a significant reduction in shoot growth when exposed to high concentrations of As and that this effect was greater in the hypocotyls than in the radicles.

Das Sharma et al., 2016; Dzionek et al., 2016, consider that, with the development of new and intensified remediation methodologies in recent years, a new branch of more cost-effective and less harmful removal of xenobiotics from the environment has emerged: bioremediation. Bioremediation is based on the natural ability of some microorganisms to incorporate pollutants into their metabolic processes and use them as a source of energy or carbon (Mosa et al., 2016; Dzionek et al., 2016).

Similarly, as expressed by Kruger et al. (2013), microorganisms have been isolated from arsenic-contaminated environments that are capable of assimilating arsenic through their metabolic pathways, achieving a detoxifying effect. According to Lorenzo et al., 2016, recent studies have focused on increasing the ability of microorganisms to resist arsenic through the use of genetic engineering, so that more efficient microorganisms could be designed as a bioremediation strategy. Although there are bacteria with the ability to metabolize arsenic, genetically modified organisms can detoxify arsenic in a more efficient and environmentally friendly way, according to Rangel-Montoya and Balagurusamy, 2015; Yang et al., 2016.

As recommended by Rodríguez et al. (2017), arsenic is a very dangerous metalloid that causes damage to the environment and to the health of living beings, so its detoxification is of vital importance due to the risk it represents. Bioremediation is considered an efficient, economical and feasible solution for the remediation of heavy metals. Due to the great diversity of bacteria that naturally possess genes related to arsenic detoxification and the efficiency they have demonstrated, genetic engineering is a promising tool to enhance this natural activity.

Doncel et al., 2016 and collaborators state that the interaction between plants and tolerant bacteria favors the efficiency of phytoremediation processes, given the ability of bacteria to enzymatically transform pollutants, converting them to their less toxic form.

Bolan et al., 2014, argue that bacteria play an important role in the transformation of heavy metals as they influence their bioavailability and remediation, can alter the toxicity, water solubility and mobility of the element.

Likewise, the root organ of plant species is the main route of entry and accumulation of the heavy metal, therefore, the bacteria present in this tissue are those that possess the biochemical or genetic machinery that allows them to survive these pollutants (Marrero et al., 2012).

On the other hand, as expressed by Luo et al. (2011), who argue that it is possible that endophytic bacteria adapt to living under constant stress by the presence of heavy metals, gaining tolerance. This hypothesis is probably based on the fact that when a cell is faced with large 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).

4. Conclusion

According to the results obtained in the present study concerning the presence of endophytic bacteria in roots and foliage of Brachiaria humidicola cv. humidicola (Rendle) Schweick grass present in cattle farms located in the San Jorge region in the department of Sucre indicate the presence of arsenic associated with roots and foliage of this pasture, which are found in values higher than those established in international legislation.

According to the recommendations given by the scientists, ‘it would be necessary to establish restrictions on the use of arsenic-contaminated pastures that have high arsenic content in their soils and in which plants capable of absorbing arsenic and transferring it considerably to their aerial parts grow’. The presence of high concentrations of arsenic in the environment and pasture ecosystems is a worldwide problem that has a negative impact on human health and is increasing every day due to different anthropogenic activities.

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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.

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

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