CHARACTERIZATION OF CULTIVABLE CADMIUM TOLERANT MICROORGANISMS PRESENT IN COCOA SOILS OF CUNDINAMARCA, COLOMBIA

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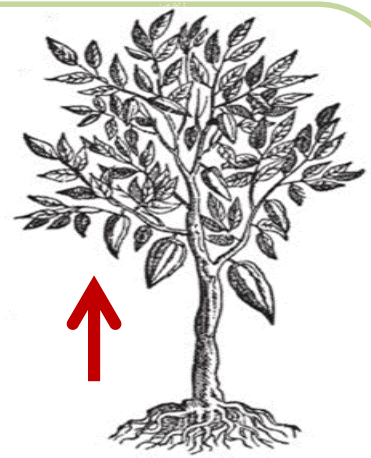
INTRODUCTION: Key aspects

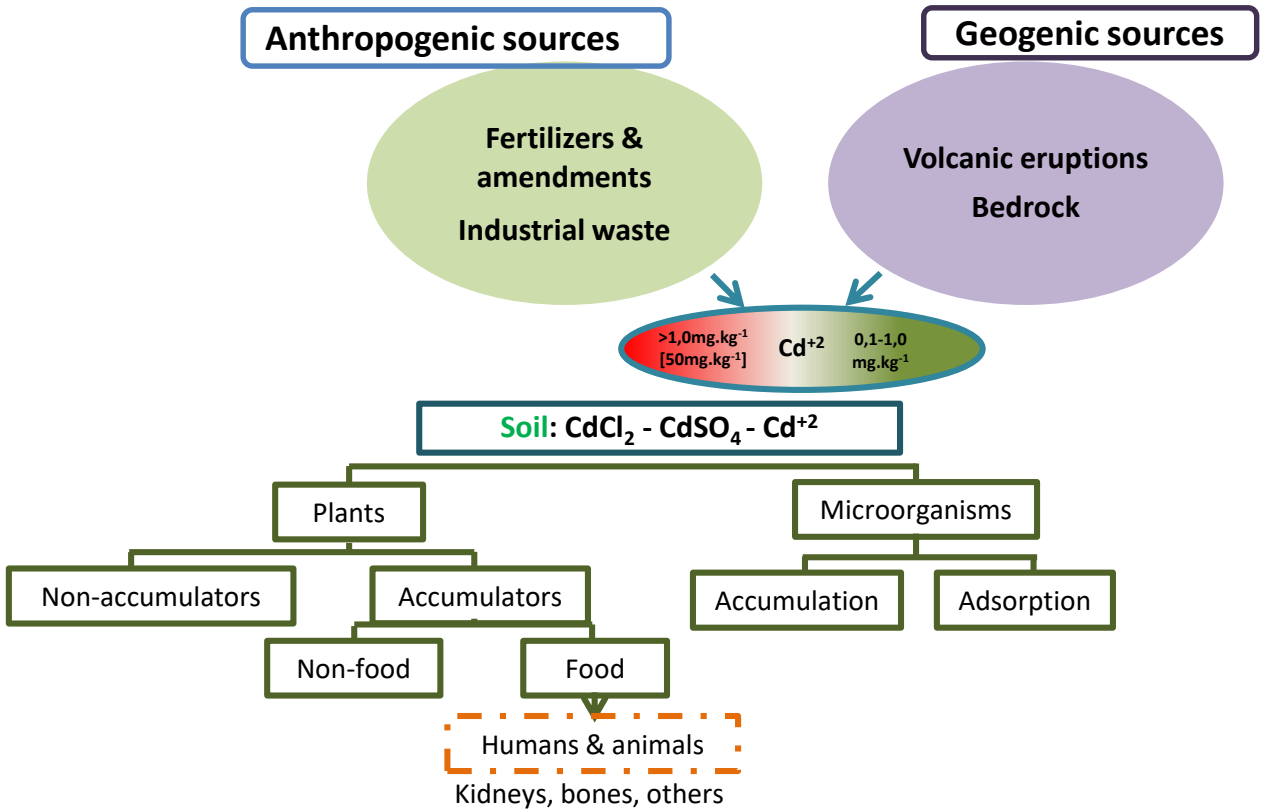
COLOMBIA

2016 → *56,785 ton dry grain*

Cocoa flavor and quality (76%)

Cd accumulation in cacao leaves, fruits and beans

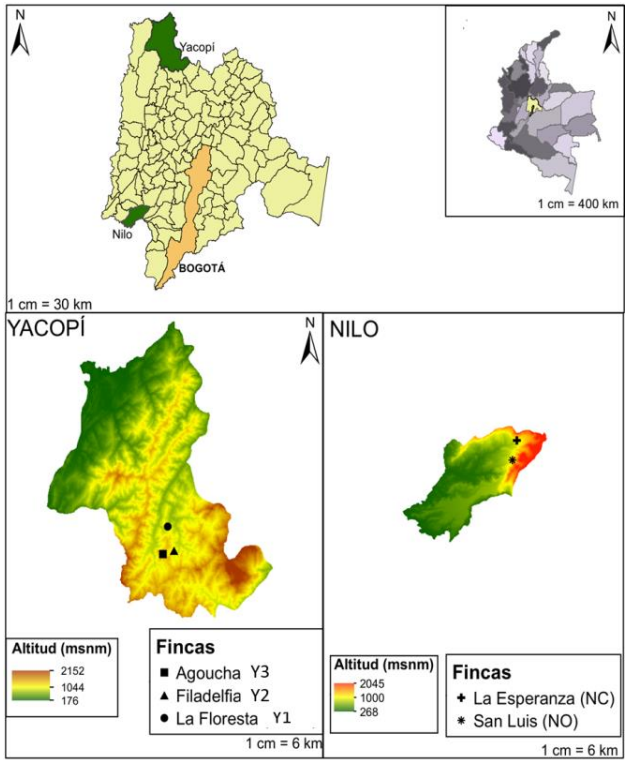




Objective

To characterize cacao soil microbial community tolerant to Cd as a contribution to soil biorremedation

MATERIALS AND METHODS



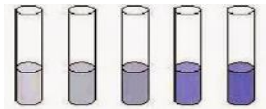
Cd levels (mg.kg^{-1}) present in the cocoa soils.

low <1.20; medium: 1.20 - 4.0; high > 4.0.

	Level of Cd	Total Cd (mg.kg^{-1})	Available Cd (mg.kg^{-1})
NO	Low	0.67	0.07
NC	Low	1.20	0.58
Y1	Low	0.7	<0.01
Y2	Medium	3.88	0.30
Y3	High	>4	>1

Soil sampling

Isolation of microorganisms



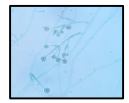
Dilutions 10^{-3}, 10^{-5} y 10^{-7}
selective medium
6 mg.kg^{-1} Cd.

Bact. → 37°C/2 days

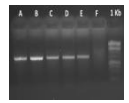
Fungi → 20°C/10 days

Characterization

Counting colony forming units (CFU)

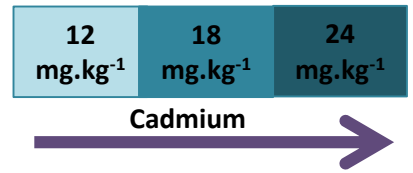


Characterization macro and micro



Molecular identification 16S/ITS1-4

Cadmium tolerance



Biogeochemical cycles

Preservation of microorganisms

RESULTS

Abundance of microorganisms

- The bacteria abundance was significantly higher than the fungi abundance for all localities.
- The abundance of cd tolerant microorganisms was lower than the microorganisms reported in agricultural soils without Cd. (logUFC10^8 - 10^9)
- It was not found correlation with the level of Cd present in the soils and the microorganisms abundance; neither with the clime and other edaphic characteristics.

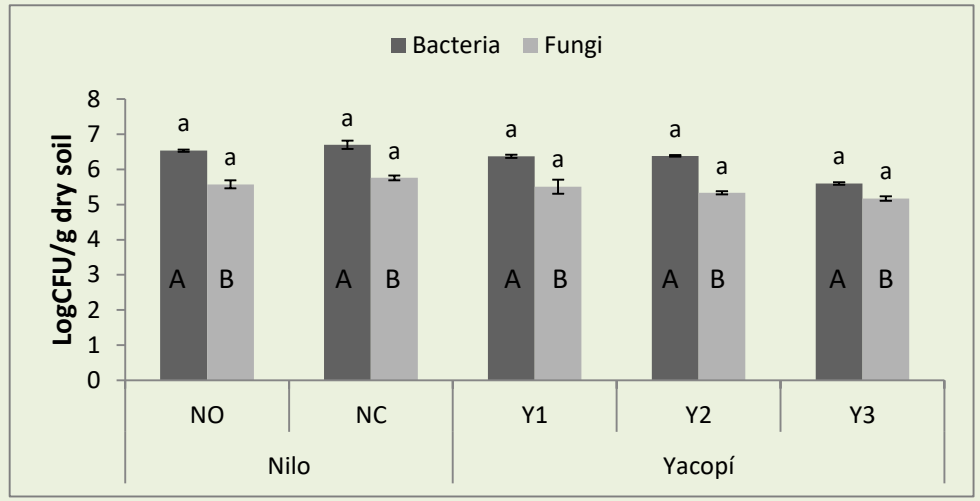


Figure 1. Count of logCFU in Mergeay medium with 6 mg.Kg^{-1} of Cd, isolate of soils of Nilo and Yacopí (Cundinamarca).

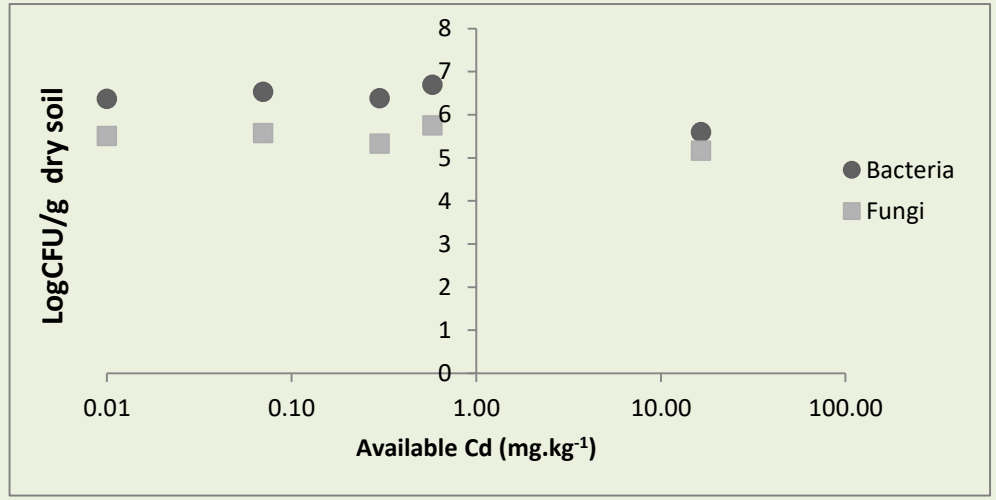


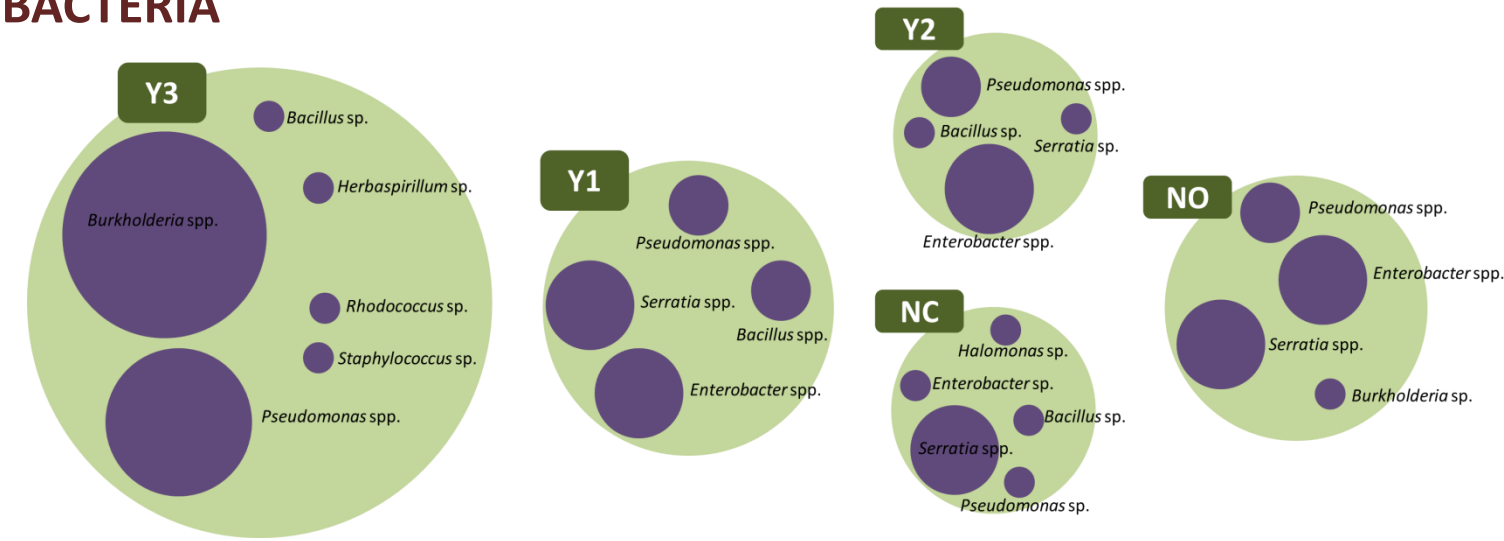
Figure 2. Relation of logCFU and cadmium in the selected soil, of microorganisms isolate in medium Mergeay with 6 mg.Kg^{-1} of Cd. This values are in logarithm scale (base 10) in the X axe.

Table 2. Alpha diversity analysis of the microorganisms cadmium tolerant isolates in Mergeay medium with 6 mg.kg^{-1} de Cd.

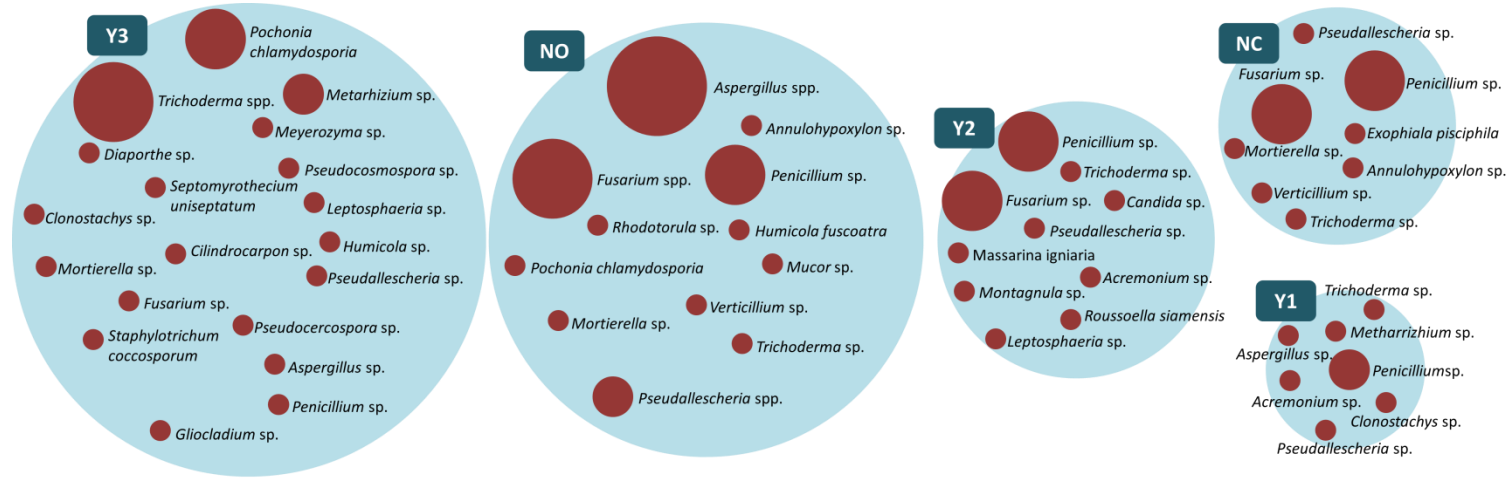
		Margalef	Simpson	Shanon
BACTERIA	NO	1,82	0,11	1,68
	NC	2,06	0,14	1,47
	Y1	2,60	0,09	1,83
	Y2	2,06	0,14	1,47
	Y3	2,82	0,12	1,95
FUNGI	NO	4,20	0,07	2,43
	NC	2,88	0,09	1,94
	Y1	2,88	0,04	1,90
	Y2	3,41	0,04	2,17
	Y3	5,66	0,03	2,83

Diversity of isolated microorganisms on medium with 6 mg.kg^{-1}

BACTERIA



FUNGI



Diversity of microorganisms

Table 3. Matrix of beta diversity (Jaccard Index) of microorganisms cadmium tolerant isolates in Mergeay medium with 6 mg.kg^{-1} Cd between localities.

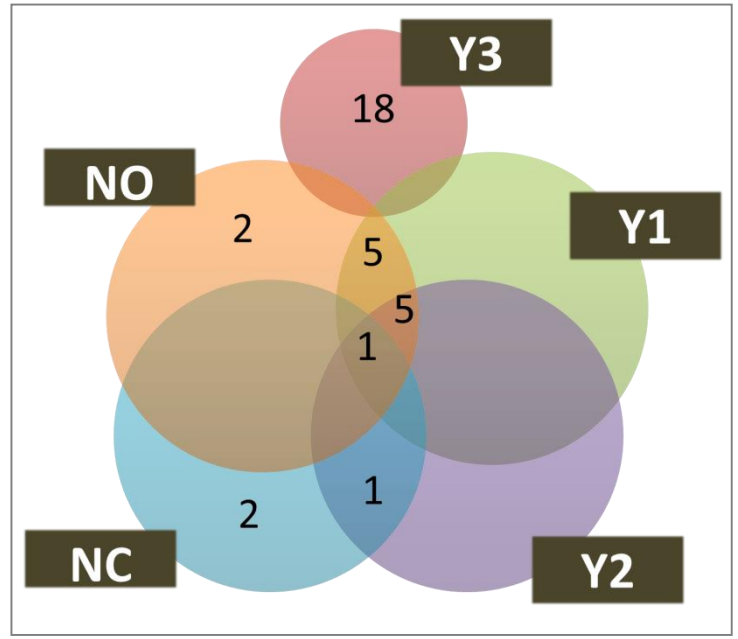
	NO	NC	Y1	Y2	Y3	GENERAL
NO		0,059	0,360	0,272	0,000	
NC	0,244		0,055	0,125	0,000	
Y1	0,090	0,130		0,260	0,000	0
Y2	0,077	0,103	0,185		0,000	
Y3	0,077	0,130	0,085	0,073		
GENERAL			0,036			

Bacteria

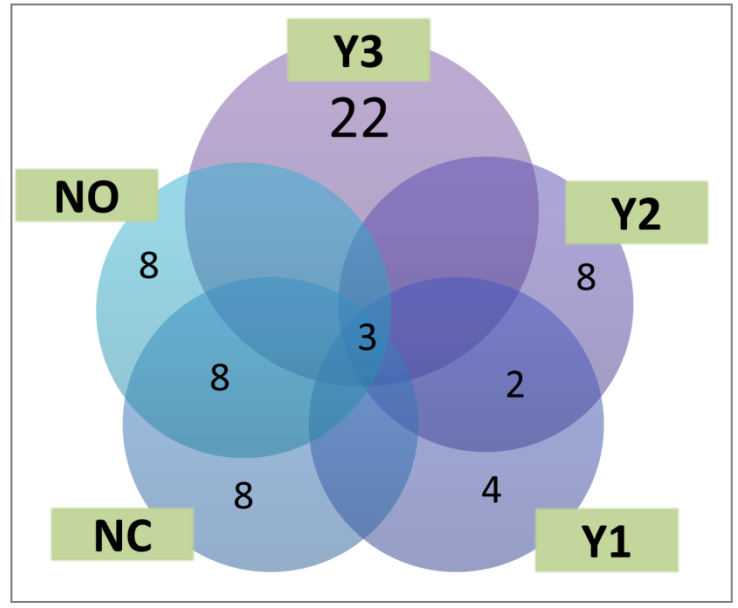
Fungi

- Y3 did not have similarity with other localities in bacteria, because the microorganisms isolated from this sample are unique for this locality.
- It was not found relation between the beta diversity and edaphic characteristics.

BACTERIA



FUNGI



Minimum inhibitory concentration: MIC

Table 4. Number of Cd tolerant morphotypes on Mergeay medium

		6 mg.kg^{-1}	12 mg.kg^{-1}	18 mg.kg^{-1}	24 mg.kg^{-1}
NO	**Bacteria**	9	9	8	7
	Fungi	19	18	18	18
NC	**Bacteria**	6	6	2	0
	Fungi	6	6	6	6
Y1	**Bacteria**	2	1	0	0
	Fungi	13	13	13	12
Y2	**Bacteria**	1	1	1	0
	Fungi	3	3	3	3
Y3	**Bacteria**	17	17	8	2
	Fungi	23	23	23	21

- The fungi showed greater tolerance to Cd, respect to bacteria

***Acremonium* sp.** | ***Aspergillus* sp.**

***Clonostachys* sp.** | ***Mortierella* sp.**

***Pochonia* sp.** | ***Pseudallescheria* sp.**

Trichoderma sp. | ***Meyerozyma* sp.**

Table 5. MIC results of bacteria isolates from Cundinamarca cocoa soils

STRAIN	MORFHOTYPE	Concentration of Cd			
		6 mg/kg	12 mg/kg	18 mg/kg	24 mg/kg
NB2	*Pseudomonas aeruginosa*	+	+	+	+
NB5	*Enterobacter* sp.	+	+	+	-
NB6	*Pseudomonas* sp.	+	+	-	-
NB10	*Burkholderia* sp.	+	+	+	-
NB11	*Enterobacter* sp.	+	+	+	-
NB19	No identificado	+	+	+	-
NB30	*Serratia marcescens*	+	+	+	-
NB40	*Halomonas* sp.	+	+	+	-
NB42	*Serratia marcescens*	+	+	+	-
NB48	*Enterobacter* sp.	+	+	+	-
NB58	*Enterobacter* sp.	+	+	-	-
NB59	*Serratia* sp.	+	+	-	-
NB61	*Serratia* sp.	+	+	-	-
NB64	*Bacillus oleronius*	+	+	+	-
NB80	*Serratia* sp.	+	+	-	-
YB13	*Pseudomonas* sp.	+	+	-	-
YB22	*Bacillus* sp.	+	-	-	-
YB43	*Serratia* sp.	+	+	+	-
GB13	*Burkholderia* sp.	+	+	-	-
GB16	*Burkholderia* sp.	+	+	-	-
GB17	*Rhodococcus* sp.	+	+	-	-
GB18	*Bacillus* sp.	+	+	-	-
GB58	*Burkholderia* sp.	+	+	-	-
GB66	*Burkholderia* sp.	+	+	-	-
GB67	*Burkholderia cepacia*	+	+	+	-
GB68	*Burkholderia cepacia*	+	+	+	+
GB71	*Pseudomonas* sp.	+	+	+	-
GB73	*Pseudomonas monteilli*	+	+	-	+
GB78	*Pseudomonas* sp.	+	+	-	-
GB82	*Burkholderia* sp.	+	+	+	-
GB84	*Staphylococcus epidermis*	+	+	+	-
GB85	No identificado	+	+	-	-
GB86	*Pseudomonas* sp.	+	+	+	-
GB88	*Pseudomonas* sp.	+	+	+	-
GB90	*Herbaspirillum* sp.	+	+	+	-

Functional assignment of cd tolerant microorganisms

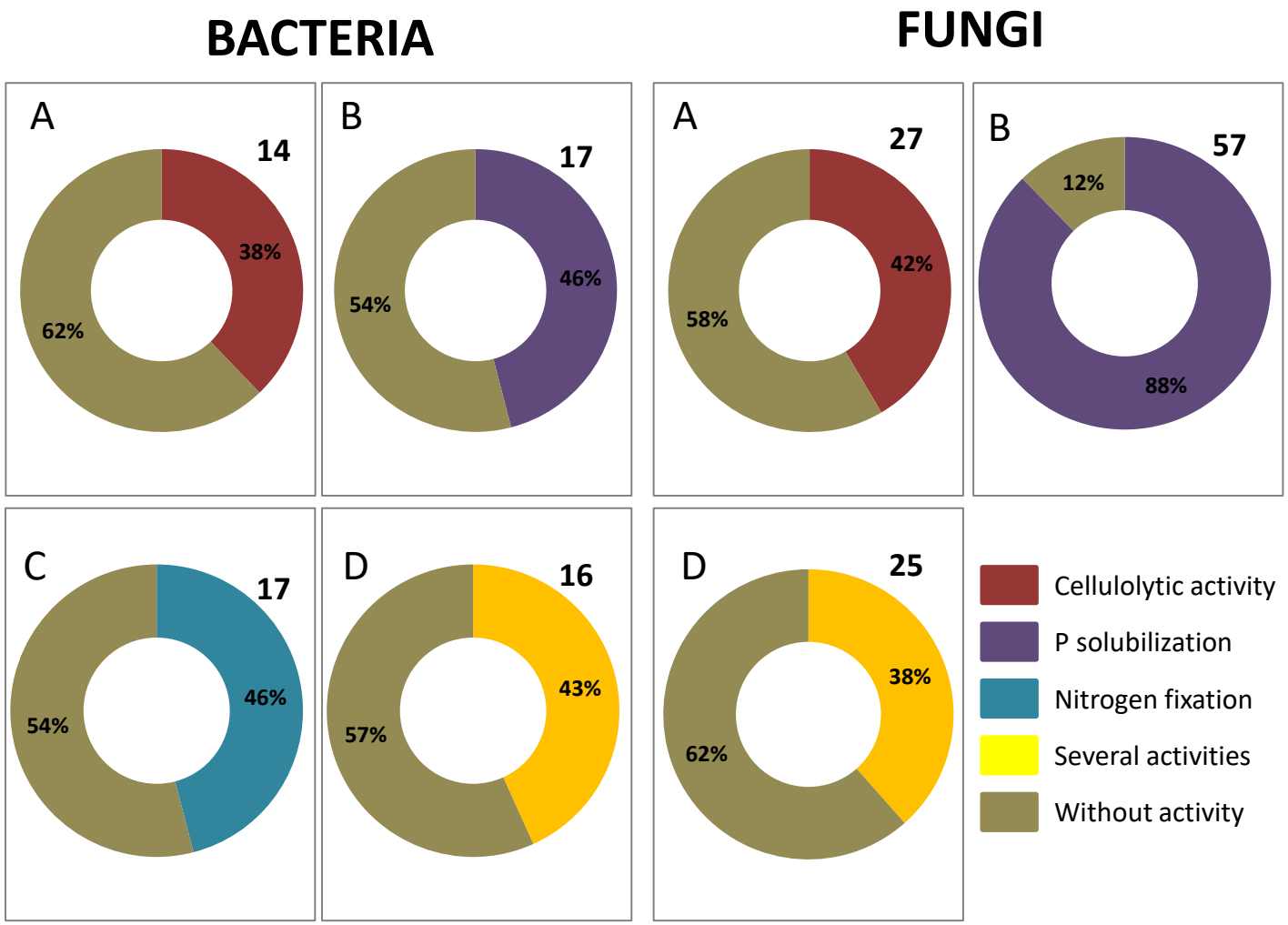


Figure 5. Assessment of cd tolerant microorganisms in cocoa soils. A. Cellulolytic activity; B. Phosphate solubilization; C. Nitrogen fixation; D. Microorganisms with more than one functional activity.

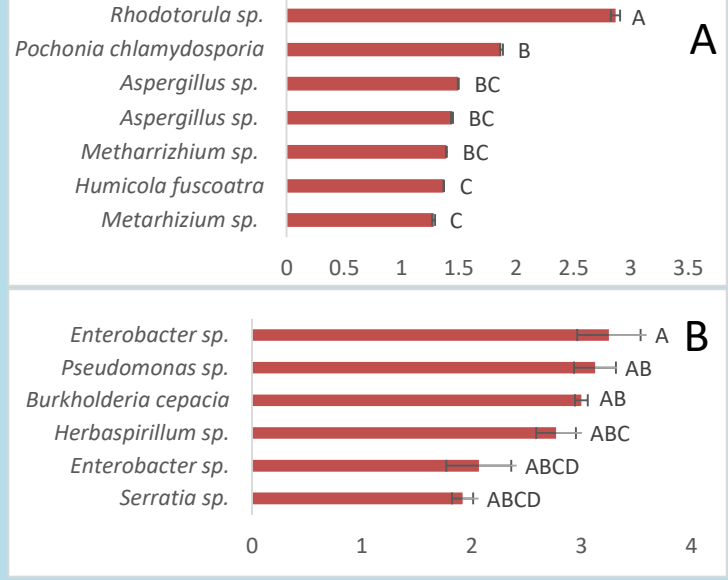


Figure 6. Microorganisms with the best cellulolytic activity. A. Fungi; B. Bacteria

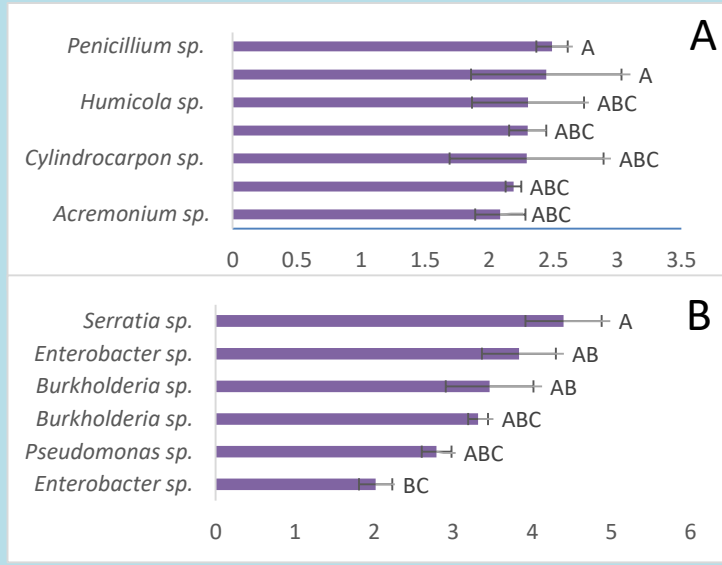


Figure 7. Microorganisms with the best phosphate solubilization activity. A. Fungi; B. Bacteria

CONCLUSIONS

- The diversity of cultivable microbial community is influence by the Cd concentration present in cacao soil.
- The locality with highest Cd concentration show low abundance and highest microorganism richness.
- The fungi showed higher tolerance to Cd (24mg.kg^{-1}) than bacteria, showing a better evolutionary adaptation to this abiotic stress.
- It was found Cd tolerant microorganisms with the potential to design crop management strategies that reduce the absorption of Cd by the root system of the cocoa plant.

Acknowledgment