

Supplementary material: Heavy Metal Tolerance of Microorganisms isolated from Coastal Marine Sediments
and Their Lead Removal Potential

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Supplementary material Table S1. Culture media for microbial isolation

Culture media	Components (g.L ⁻¹)		pH	Processing method
Starch casein agar (SCA)	Soluble starch	10	7.2 ± 0.2	Direct seeding of no-pre-treatment sediments, phenol pre-treatment and selective pressure method (HgCl ₂)
	Casein	0.3		
	Sodium nitrate	2.0		
	Sodium chloride	2.0		
	Magnesium sulphate	0.05		
	Calcium carbonate	0.02		
	Iron sulphate	0.01		
	Seawater: distilled water	60:40		
	Agar	18		
Either Boyd and Kohlmeyer (B&K) medium	Yeast extract	1.0	7.0 ± 0.2	Direct seeding of no-pre-treatment sediments and selective pressure method (HgCl ₂)
	Peptone	2.0		
	Glucose	10		
	Seawater: distilled water	1:1		
	Streptomycin antibiotic	0.1		
	Agar	18		
Malt Extract agar	Malt extract	30.0	5.4 ± 0.2	Direct seeding of no-pre-treatment

Culture media	Components (g.L ⁻¹)	pH	Processing method	
	Peptone	5.0	sediments and selective pressure method (HgCl ₂)	
	Streptomycin antibiotic	0.1		
	Agar	15		
International <i>Streptomyces</i> project 2 (ISP2) medium	Yeast extract	4.0	Direct seeding of no-pre-treatment sediments and selective pressure	
	Malt extract	10		
	Glucose	4.0		
	Sodium chloride	10		
	Agar	18		
International <i>Streptomyces</i> project 4 (ISP4) medium	Soluble starch	10	Phenol pre-treatment and selective pressure method (HgCl ₂)	
	Dibasic potassium phosphate	1.0		
	Magnesium sulphate heptahydrate	1.0		
	Sodium chloride	10		
	Ammonium sulphate	2.0		
	Calcium carbonate	2.0		
	Iron sulphate heptahydrate	0.001		
	Manganese (II) chloride tetrahydrate	0.001		
	Zinc sulphate heptahydrate	0.001		
Agar	18			
International <i>Streptomyces</i> project 3 (ISP3) medium	White Oats	20	7.3 ± 0.2	Selective pressure method (HgCl ₂)
	Iron sulphate heptahydrate	0.001		
	Manganese (II) chloride tetrahydrate	0.001		
	Zinc sulphate heptahydrate	0.001		
	Agar	18		
Marine agar Difco™ (MA)	Commercial preparation		Direct seeding of no-pre-treatment sediments, phenol pre-treatment and selective pressure method (HgCl ₂)	
Rose Bengal Chloramphenicol agar (RBC) Merck®	Commercial preparation		Direct seeding of no-pre-treatment sediments and selective pressure method (HgCl ₂)	
Tryptic soy agar (TSA) Scharlau Scharlab, S.L	Commercial preparation		Direct seeding of no-pre-treatment sediments	

Sampling site	Consecutive number MHNMC	Type of microorganisms	Tolerance screening on Luria Bertani agar (LB)											
			Cadmium (Cd) mg.L ⁻¹				Lead (Pb) mg.L ⁻¹				Zinc (Zn) mg.L ⁻¹			
			50	150	250	350	50	150	250	350	50	150	250	350
MARM	76870	Bacteria	+	+	+	±	+	+	+	+	+	+	+	±
MARM	76871	Bacteria	±	-	-	-	+	+	+	+	+	+	±	-
MARM	76872	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76873	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76874	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76875	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
MARM	76876	Bacteria	±	-	-	-	+	±	-	-	+	+	±	±
MARM	76877	Bacteria	+	+	+	+	+	+	-	-	-	-	-	-
MARM	76878	Bacteria	+	+	+	±	+	+	+	+	+	+	+	+
MARM	76879	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76880	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76881	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76882	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76883	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76884	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
MARM	76885	Bacteria	-	-	-	-	+	+	+	+	+	-	-	-
MARM	76886	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76887	Bacteria	-	-	-	-	+	+	+	+	±	±	±	±
MARM	76888	Bacteria	-	-	-	-	+	+	+	+	±	-	-	-
MARM	77109	yeast	+	+	+	+	+	+	+	+	+	+	+	+
MARM	77111	yeast	+	+	±	±	+	+	+	+	+	+	+	+
MRAR	77115	yeast	+	-	-	-	+	+	+	+	+	+	+	+
MRAR	77116	yeast	+	-	-	-	+	+	+	+	+	+	+	+
MRAR	77118	yeast	+	-	-	-	+	+	+	+	+	+	+	+
MPAR	77124	yeast	-	-	-	-	+	+	+	+	+	+	+	+
MRAR	77127	yeast	-	-	-	-	+	+	+	+	+	+	+	-
MRAR	77129	yeast	+	-	-	-	+	+	+	+	+	+	+	+
MRAR	76891	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MRAR	76892	Bacteria	±	±	-	-	+	+	±	±	+	+	-	-
MRAR	76893	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MRAR	76894	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MPAR	76895	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MPAR	76896	Bacteria	+	±	-	-	+	+	+	+	+	+	+	+
MPAR	76897	Bacteria	+	±	±	-	+	+	+	+	+	+	+	+
MPAR	76898	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MPAR	76899	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MPAR	76900	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+

Sampling site	Consecutive number MHNMC	Type of microorganisms	Tolerance screening on Luria Bertani agar (LB)											
			Cadmium (Cd) mg.L ⁻¹				Lead (Pb) mg.L ⁻¹				Zinc (Zn) mg.L ⁻¹			
			50	150	250	350	50	150	250	350	50	150	250	350
MPAR	76901	Bacteria	-	-	-	-	-	-	-	-	±	-	-	-
MPAR	76902	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MPAR	76903	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76905	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76906	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76907	Bacteria	-	-	-	-	+	+	+	+	+	+	+	-
MRAR	76908	Bacteria	-	-	-	-	+	+	+	+	+	±	-	-
MARM	76909	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76910	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76911	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	76912	Bacteria	±	-	-	-	+	+	+	+	+	+	±	±
MARM	76913	Bacteria	±	±	±	±	+	+	+	+	+	±	±	±
MARM	76914	Bacteria	-	-	-	-	+	+	+	+	+	+	±	±
MRAR	76916	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	76917	Bacteria	-	-	-	-	+	±	±	±	+	+	-	-
OBR	76918	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	76919	Bacteria	-	-	-	-	+	+	+	+	+	+	±	±
OBR	76920	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
OBR	76921	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	76922	Bacteria	±	±	±	±	+	+	+	+	+	±	±	±
OBR	76923	Bacteria	-	-	-	-	+	+	+	+	+	+	-	-
OBR	76924	Bacteria	+	-	-	-	+	+	+	+	+	+	+	+
OBR	76925	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	76926	Bacteria	+	±	-	-	+	+	+	+	+	+	+	+
OBR	76927	Bacteria	-	-	-	-	+	+	+	+	+	-	-	-
OBR	76928	Bacteria	±	-	-	-	+	+	+	+	+	+	+	±
OBR	76929	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	76930	Bacteria	+	±	±	-	+	+	+	+	+	+	+	+
MVUBR	76931	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MVUBR	76932	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MVUBR	76933	Bacteria	-	-	-	-	+	+	+	+	-	-	-	-
MVUBR	76934	Bacteria	+	±	±	±	+	+	+	+	+	+	+	+
MVUBR	76935	Bacteria	+	±	-	-	+	+	+	+	+	+	+	-
MVUBR	76936	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MVUBR	76937	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MVUBR	76938	Bacteria	+	-	-	-	+	+	+	+	+	+	+	+
MVUBR	76939	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+

[illegible]

Sampling site	Consecutive number MHNMC	Type of microorganisms	Tolerance screening on Luria Bertani agar (LB)											
			Cadmium (Cd) mg.L ⁻¹				Lead (Pb) mg.L ⁻¹				Zinc (Zn) mg.L ⁻¹			
			50	150	250	350	50	150	250	350	50	150	250	350
MCHSJ2	76975	Bacteria	-	-	-	-	-	-	-	-	-	-	-	-
MCHSJ2	76976	Bacteria	±	±	±	±	+	+	+	+	+	+	+	±
MCHSJ2	76977	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MCHSJ2	76978	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MCHSJ2	76979	Bacteria	±	±	±	±	±	±	±	±	+	+	+	+
MCHSJ2	76980	Bacteria	+	+	+	+	+	+	+	+	+	-	-	-
MSJ2	76981	yeast	±	±	-	-	+	+	+	+	+	+	+	+
MSJ2	76982	Bacteria	+	±	±	-	+	+	+	+	+	+	+	+
MSJ2	76983	Bacteria	+	-	-	-	+	+	+	+	+	+	+	+
MSJ2	76984	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MSJ2	76985	Bacteria	-	-	-	-	+	+	+	+	+	+	+	-
MSJ2	76986	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MSJ2	76987	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MSJ2	76988	Bacteria	+	+	±	±	+	+	±	±	+	+	+	+
MSJ2	76989	Bacteria	-	-	-	-	+	+	+	+	+	+	+	-
MSJ2	76991	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MSJ2	76992	Bacteria	+	±	-	-	+	+	+	+	+	+	+	+
MSJ2	76993	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
MSJ2	76994	Bacteria	±	-	-	-	+	+	+	+	+	+	+	-
MSJ2	76995	Bacteria	-	-	-	-	+	+	+	+	+	+	+	+
BSJM2	76996	Bacteria	-	-	-	-	+	+	+	+	-	-	-	-
BSJM2	76997	Bacteria	-	-	-	-	-	-	-	-	-	-	-	-
BSJM2	76998	Bacteria	-	-	-	-	+	+	+	±	+	+	±	±
BSJM2	76999	Bacteria	+	+	±	±	+	+	+	+	+	+	+	±
BSJM2	77000	Bacteria	±	±	±	±	+	+	-	-	-	-	-	-
BSJM2	INV PRT211*	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
BSJM2	77002	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
BSJM2	77003	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
BSJM2	77004	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
BSJM2	77005	Bacteria	-	-	-	-	+	+	+	+	+	+	+	±
MCHSJ2	77144	yeast	-	-	-	-	+	+	+	+	+	+	+	+
MCHSJ2	77145	yeast	-	-	-	-	+	+	+	+	+	+	+	+
MCHSJ2	77146	yeast	±	-	-	-	+	+	+	+	+	+	±	-
MCHSJ2	77149	yeast	-	-	-	-	-	-	-	-	+	+	+	+
MCHSJ2	77152	Bacteria	±	-	-	-	+	+	+	+	±	±	±	±
BSJM2	77153	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
BSJM2	77154	yeast	+	±	±	±	+	+	+	+	+	+	+	+

[illegible]

[illegible]

Sampling site	Consecutive number MHNMC	Type of microorganisms	Tolerance screening on Luria Bertani agar (LB)											
			Cadmium (Cd) mg.L ⁻¹				Lead (Pb) mg.L ⁻¹				Zinc (Zn) mg.L ⁻¹			
			50	150	250	350	50	150	250	350	50	150	250	350
OBR	77077	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	77078	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
OBR	77079	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
MCHSJ2	77080	Bacteria	±	±	±	±	+	+	+	+	+	+	+	+
MCHSJ2	77081	Bacteria	±	±	±	±	+	+	+	+	+	+	+	+
MCHSJ2	77082	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MRAR	77083	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	77084	Bacteria	+	-	-	-	+	+	+	+	+	+	+	+
MARM	77085	Bacteria	-	-	-	-	-	-	-	-	-	-	-	-
MARM	77086	Bacteria	+	+	+	-	+	+	+	+	+	+	+	-
MVUBR	77087	Bacteria	±	±	±	±	+	+	+	+	+	+	+	+
MVUBR	77088	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MARM	77089	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	77090	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	77091	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MARM	77092	Bacteria	+	+	+	-	+	+	+	+	+	+	+	+
MARM	77093	Bacteria	+	+	+	+	-	-	-	-	+	+	+	+
BSJM2	77094	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MVUBR	77096	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
MVUBR	77097	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MVUBR	77098	Bacteria	+	+	-	-	+	+	+	+	+	+	+	+
MCHSJ2	77099	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MCHSJ2	77100	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MCHSJ2	77101	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
BSJM2	77103	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
BSJM2	77104	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MPAR	77105	Bacteria	±	-	-	-	+	+	+	+	+	+	+	+
MPAR	77106	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+
MPAR	77107	Bacteria	+	+	+	+	+	+	+	+	+	+	+	+

* Catalog number of the Marine Museum of Natural History of Colombia–Makuriwa located in INVEMAR

Supplementary material Table S3. Cadmium (Cd²⁺), Lead (Pb²⁺), and Zinc (Zn²⁺) tolerance screening trials at increased concentrations of Pb²⁺, Cd²⁺ and Zn²⁺ ions of eleven microorganisms. A microorganism was catalogued as tolerant (+) if growth was observed after 24-48 h of culture; not tolerant (-) if growth was inhibited entirely around wells, or sensitive (±) if some colonies were formed (36).

Sampling site	Consecutive numbers MHNMC	Type of microorganisms	Tolerance screening on Luria Bertani agar (LB)									
			Cadmium (Cd) mg.L ⁻¹		Lead (Pb) mg.L ⁻¹				Zinc (Zn) mg.L ⁻¹			
			550	750	450	550	650	750	450	550	650	750
Atrato river mouth	76869	Bacteria	-	-	+	+	+	+	+	+	+	+
	77030	Bacteria	+	+	+	+	+	+	+	+	+	+
	INV PRT213*	Bacteria	+	+	+	+	+	+	+	+	+	+
San Juan river mouth	76956	Bacteria	-	-	+	+	+	-	+	+	-	-
	77001	Bacteria	+	+	+	+	+	+	+	+	+	+
	77050	Bacteria	+	+	+	+	+	+	+	+	+	+
	INV PRT215*	Bacteria	+	+	+	+	+	+	+	+	+	+
	77103	Bacteria	-	-	+	+	+	+	+	+	+	+
Baudó river mouth	76952	Bacteria	+	-	+	+	+	-	+	+	-	-
	INV PRT216*	Bacteria	+	+	+	+	+	+	+	+	+	+
	77058	Bacteria	-	-	+	-	-	-	+	+	+	+

* Catalog number of the Marine Museum of Natural History of Colombia–Makuriwa located in INVEMAR

Supplementary material Table S4. Results obtained by comparing the 16S rRNA sequences to those in the Reference RNA Sequences database of the NCBI.

Catalog number MMNHC*	Description Genera	Accession number GenBank	Query cover	Ident	E-value
INV PRT211	<i>Stenotrophomonas panacihumi</i>	NR_117406.1	99%	97.88%	0.0
	<i>Xanthomonas hortensias</i>	NR_181958.1	99%	97.53%	0.0
	<i>Xanthomonas campestris</i>	NR_074936.1	99%	97.53%	0.0
	<i>Xanthomonas arboricola pv. juglandis</i>	NR_113167.1	99%	97.53%	0.0

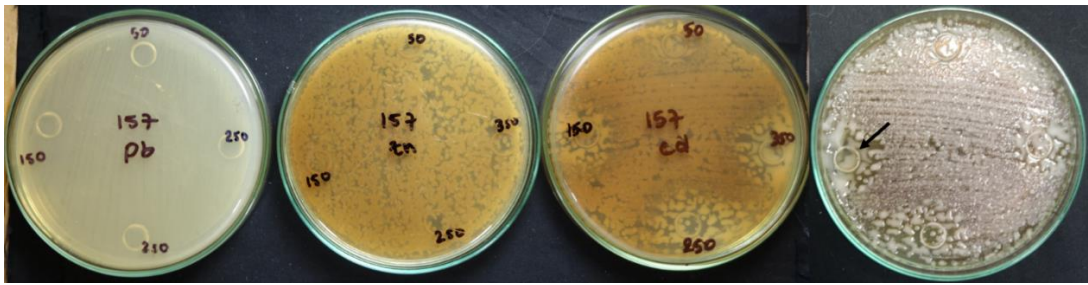
Catalog number MMNHC*	Description Genera	Accession number GenBank	Query cover	Ident	E-value
INV PRT212	<i>Xanthomonas hortorum</i> pv. <i>gardneri</i>	NR_104793.1	99%	97.53%	0,0
	<i>Enterobacter sichuanensis</i>	NR_179946.1	100%	99.68%	0,0
	<i>Enterobacter chengduensis</i>	NR_179167.1	100%	99.68%	0,0
	<i>Enterobacter quasiroggenkampii</i>	NR_179166.1	100%	99.68%	0,0
	<i>Enterobacter bugandensis</i>	NR_148649.1	100%	99.68%	0,0
	<i>Enterobacter mori</i>	NR_146667.2	100%	99.68%	0,0
	<i>Enterobacter soli</i>	NR_117547.1	100%	99.15%	0,0
	<i>Huaxiibacter chinensis</i>	NR_184601.1	100%	98.58%	0,0
INV PRT213	<i>Enterobacter asburiae</i>	NR_024640.1	99%	98.28%	0.0
	<i>Klebsiella aerogenes</i>	NR_113614.1	100%	98.15%	0.0
	<i>Klebsiella aerogenes</i>	NR_102493.2	100%	98.15%	0.0
	<i>Pseudomonas protegens</i>	NR_114749.1	100%	99.56%	0.0
INV PRT215	<i>Pseudomonas sesami</i>	NR_149822.1	100%	99.34%	0.0
	<i>Pseudomonas saponiphila</i>	NR_116905.1	99%	98.75%	0.0
	<i>Pseudomonas meliae</i>	NR_178423.1	100%	98.67%	0.0

Catalog number MMNHC*	Description Genera	Accession number GenBank	Query cover	Ident	E-value
	<i>Pseudomonas tremae</i>	NR_025549.1	100%	98.67%	0.0
	<i>Stenotrophomonas maltophilia</i>	NR_041577.1	100%	99.43%	0.0
	<i>Stenotrophomonas maltophilia</i>	NR_113648.1	100%	99.36%	0.0
INV PRT216	<i>Stenotrophomonas maltophilia</i>	NR_119220.1	100%	99.36%	0.0
	<i>Stenotrophomonas maltophilia</i>	NR_112030.1	100%	99.36%	0.0
	<i>Stenotrophomonas pavanii</i>	NR_116793.1	100%	99.14%	0.0

* Catalog number of the Marine Museum of Natural History of Colombia–Makuriwa located in INVEMAR

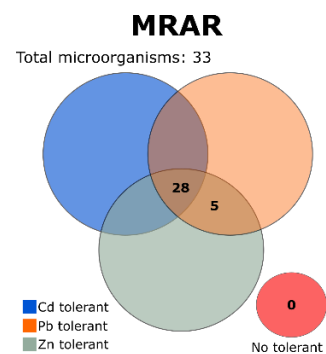
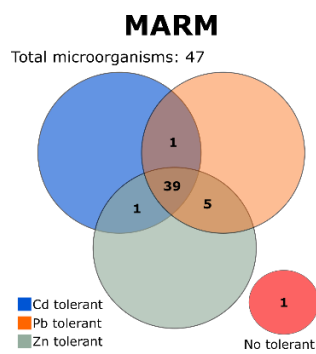
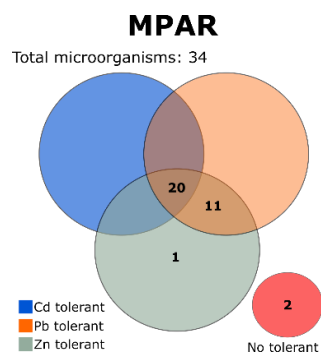


Supplementary material Figure S1. Phenotypic characteristics of some isolated colonies.

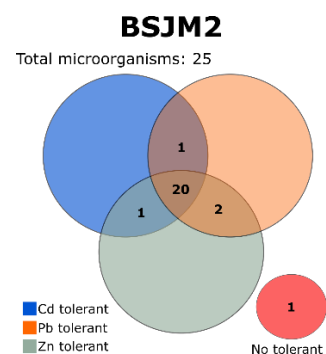
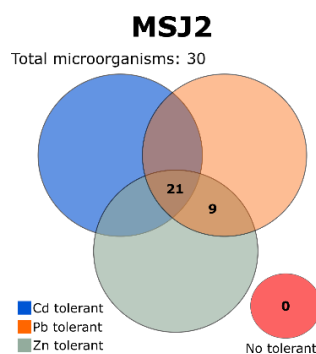
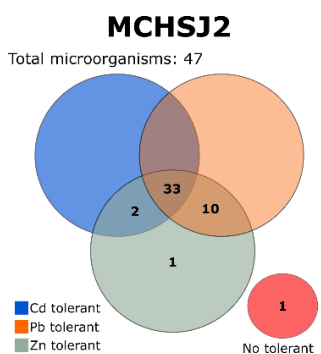


Supplementary material Figure S2. Photographic record of pigments and substances excreted by bacterium 76955 under Cd^{2+} and Zn^{2+} stress conditions.

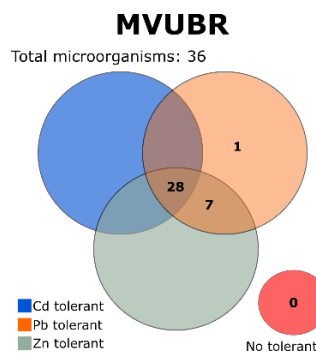
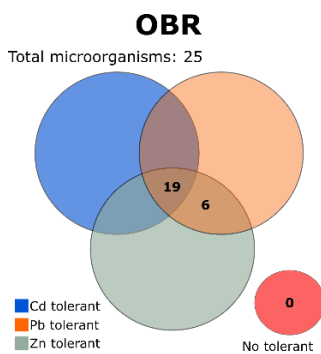
Atrato



San Juan



Baudó



Supplementary material Figure S3. Number of strains tolerant to Cd^{2+} , Pb^{2+} , and Zn^{2+} ions by sampling site. Sampling sites: Atrato river mouth (MPAR, MARM, MRAR), Baudó river mouth (OBR, MVUBR), and San Juan river mouth (MCHSJ2, MSJ2, BSJM2)