

Article

**In vitro removal of Cd and Pb through the bacterial strain *Burkholderia zhejiangensis* CEIB S4-3, efficiency and cellular mechanisms implicated in the process**

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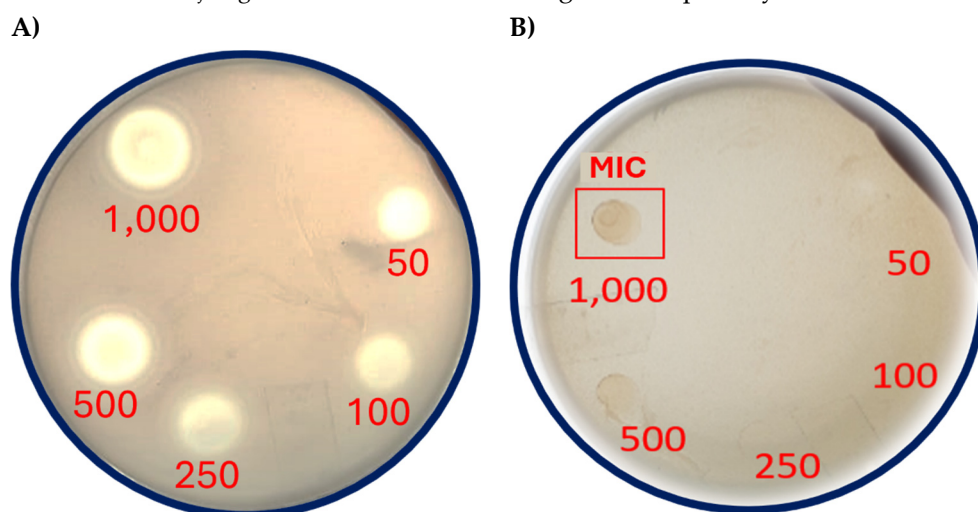
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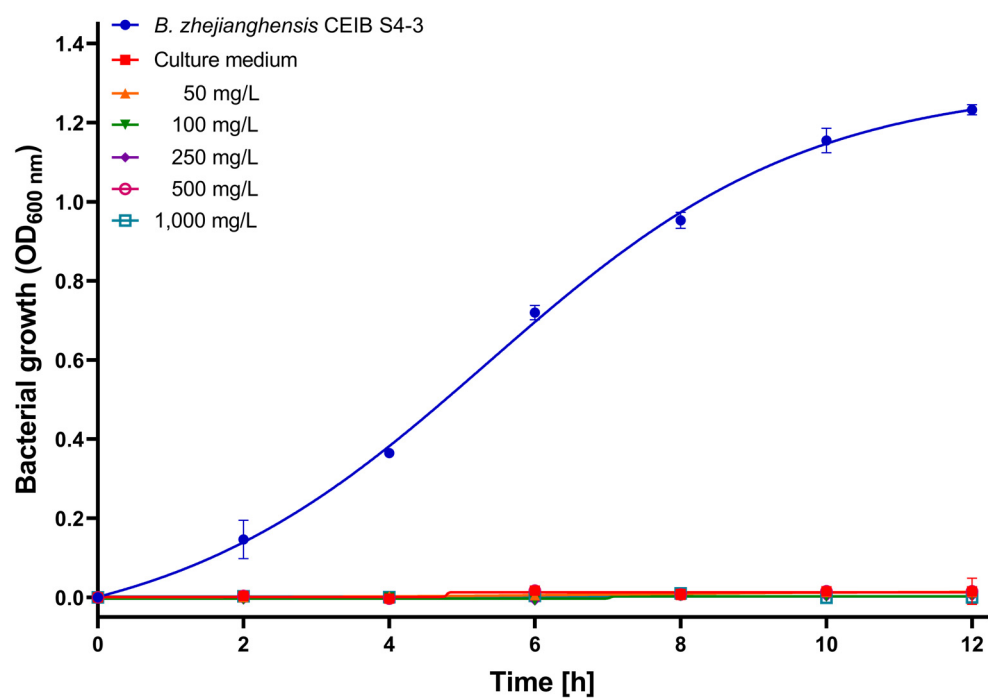
**Supplementary Data S1: Results of the Cd<sup>+2</sup> and Pb<sup>+2</sup> inhibition assays on *B. zhejiangensis* CEIB S4-3**

To evaluate the susceptibility of the bacterial strain to the two evaluated heavy metals, Cadmium (Cd<sup>+2</sup>) and lead (Pb<sup>+2</sup>), inhibition tests were conducted in the solid phase, Trip-suitcase Soy Agar (TSA) plates and liquid cultures, Trypticase Soy Broth, (TSB). Increasing concentrations (50, 100, 250, 500, and 1,000 mg/L) of both heavy metals were supplemented for inhibitory assays. For agar plate assays, the minimum inhibitory metal concentration (MIC) was defined as the minimum concentration that inhibits visible bacterial growth (clear halos) after incubation, while in the inhibition assay in liquid cultures, the minimum inhibitory metal concentration (MIC) was defined as the minimum concentration that inhibits bacterial growth (DO<sub>600 nm</sub>). As is shown in Fig. S1A and S2A, all Cd<sup>+2</sup> concentrations evaluated inhibited the bacterial growth; due to these results, the MIC of Cd<sup>+2</sup> for *B. zhejiangensis* CEIB S4-3 were below 50 mg/L in both inhibition assays. In the Pb<sup>+2</sup> inhibition assays on agar plates, it was observed that just the concentration of 1,000 mg/L showed a slight inhibitory effect over the group of the bacterial strain (Fig. S1B). In contrast, in the liquid culture inhibition assays, all concentrations evaluated just showed a bacteriostatic effect on the growth of *B. zhejiangensis* CEIB S4-3 (Fig. S2B). Overall, the bacterial strain *B. zhejiangensis* CEIB S4-3 showed higher susceptibility to Cd<sup>+2</sup> than Pb<sup>+2</sup>.



**Figure S1.** Heavy metal inhibition assays. A) Cd<sup>+2</sup> inhibition in TSA plates; B) Pb<sup>+2</sup> inhibition in TSA plates.

A)



B)

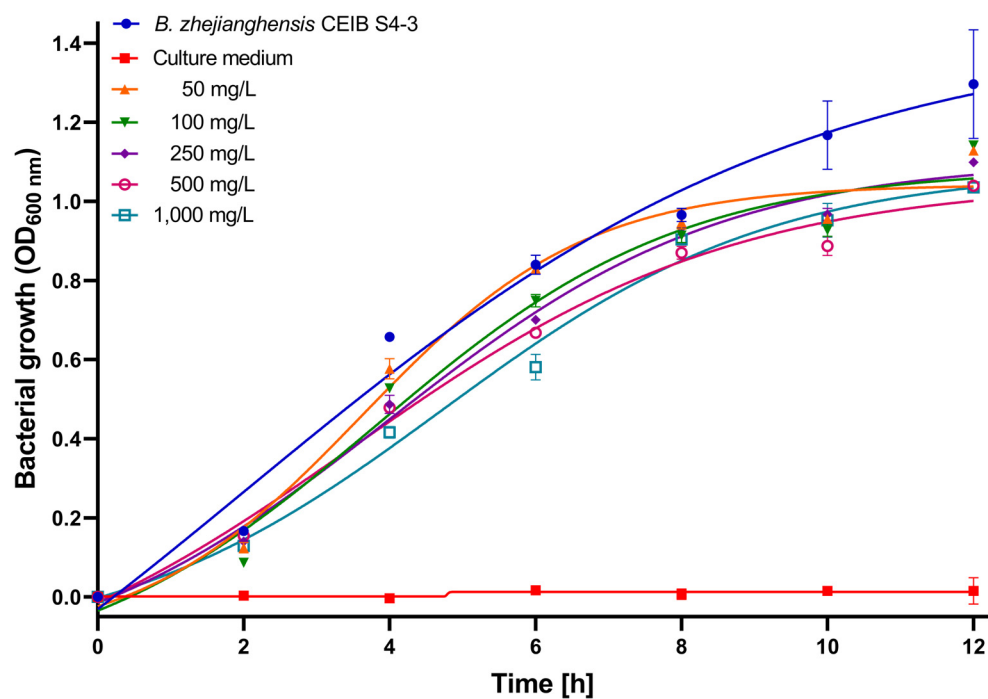


Figure S2. Heavy metal inhibition assays. A) Cd<sup>+2</sup> inhibition in TSB liquid culture; B) Pb<sup>+2</sup> inhibition in TDB liquid culture.

## Supplementary Data S2: Results of the statistical analyses

### Pb<sup>+2</sup> removal kinetics by *B. zhejiangensis* CEIB S4-3

#### Two-way ANOVA Results

Analysis	Statistic F	p value
Removal mechanisms (RM)	F (1, 64) = 254.1	P<0.0001
Time (t)	F (3, 64) = 1.01	P=0.3943
Interaction RM x t	F (3, 64) = 54.84	P<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0:Biosorption vs. 0:Bioaccumulation	-20.40	-27.48 to -13.32	Yes	****	<0.0001
0:Biosorption vs. 2:Biosorption	-2.239	-9.322 to 4.845	No	ns	0.9742
0:Biosorption vs. 2:Bioaccumulation	-19.06	-26.14 to -11.97	Yes	****	<0.0001
0:Biosorption vs. 4:Biosorption	7.867	0.7831 to 14.95	Yes	*	0.0193
0:Biosorption vs. 4:Bioaccumulation	-29.97	-37.05 to -22.88	Yes	****	<0.0001
0:Biosorption vs. 6:Biosorption	-9.961	-17.04 to -2.878	Yes	**	0.0010
0:Biosorption vs. 6:Bioaccumulation	-6.978	-14.06 to 0.1058	No	ns	0.0564
0:Bioaccumulation vs. 2:Biosorption	18.16	11.08 to 25.24	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Bioaccumulation	1.344	-5.739 to 8.428	No	ns	0.9988
0:Bioaccumulation vs. 4:Biosorption	28.27	21.18 to 35.35	Yes	****	<0.0001
0:Bioaccumulation vs. 4:Bioaccumulation	-9.567	-16.65 to -2.483	Yes	**	0.0018
0:Bioaccumulation vs. 6:Biosorption	10.44	3.355 to 17.52	Yes	***	0.0005
0:Bioaccumulation vs. 6:Bioaccumulation	13.42	6.339 to 20.51	Yes	****	<0.0001
2:Biosorption vs. 2:Bioaccumulation	-16.82	-23.90 to -9.733	Yes	****	<0.0001
2:Biosorption vs. 4:Biosorption	10.11	3.022 to 17.19	Yes	***	0.0008
2:Biosorption vs. 4:Bioaccumulation	-27.73	-34.81 to -20.64	Yes	****	<0.0001
2:Biosorption vs. 6:Biosorption	-7.722	-14.81 to -0.6386	Yes	*	0.0232
2:Biosorption vs. 6:Bioaccumulation	-4.739	-11.82 to 2.345	No	ns	0.4284
2:Bioaccumulation vs. 4:Biosorption	26.92	19.84 to 34.01	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Bioaccumulation	-10.91	-17.99 to -3.828	Yes	***	0.0002
2:Bioaccumulation vs. 6:Biosorption	9.094	2.011 to 16.18	Yes	**	0.0037
2:Bioaccumulation vs. 6:Bioaccumulation	12.08	4.994 to 19.16	Yes	****	<0.0001
4:Biosorption vs. 4:Bioaccumulation	-37.83	-44.92 to -30.75	Yes	****	<0.0001
4:Biosorption vs. 6:Biosorption	-17.83	-24.91 to -10.74	Yes	****	<0.0001
4:Biosorption vs. 6:Bioaccumulation	-14.84	-21.93 to -7.761	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Biosorption	20.01	12.92 to 27.09	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Bioaccumulation	22.99	15.91 to 30.07	Yes	****	<0.0001
6:Biosorption vs. 6:Bioaccumulation	2.983	-4.100 to 10.07	No	ns	0.8880

## Cd<sup>2+</sup> removal kinetics by *B. zhejiangensis* CEIB S4-3

### Two-way ANOVA Results

Analysis	Statistic F	p value
Removal mechanisms (RM)	F (1, 64) = 141.3	P<0.0001
Time (t)	F (3, 64) = 0.1024	P=0.3943
Interaction RM x t	F (3, 64) = 19.66	P<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0:Biosorption vs. 0:Bioaccumulation	15.85	10.54 to 21.16	Yes	****	<0.0001
0:Biosorption vs. 2:Biosorption	-0.8556	-6.163 to 4.452	No	ns	0.9996
0:Biosorption vs. 2:Bioaccumulation	16.06	10.75 to 21.36	Yes	****	<0.0001
0:Biosorption vs. 4:Biosorption	7.044	1.737 to 12.35	Yes	**	0.0023
0:Biosorption vs. 4:Bioaccumulation	8.639	3.331 to 13.95	Yes	****	<0.0001
0:Biosorption vs. 6:Biosorption	5.294	-0.01343 to 10.60	No	ns	0.0510
0:Biosorption vs. 6:Bioaccumulation	11.22	5.909 to 16.52	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Biosorption	-16.71	-22.01 to -11.40	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Bioaccumulation	0.2056	-5.102 to 5.513	No	ns	>0.9999
0:Bioaccumulation vs. 4:Biosorption	-8.806	-14.11 to -3.498	Yes	****	<0.0001
0:Bioaccumulation vs. 4:Bioaccumulation	-7.211	-12.52 to -1.903	Yes	**	0.0017
0:Bioaccumulation vs. 6:Biosorption	-10.56	-15.86 to -5.248	Yes	****	<0.0001
0:Bioaccumulation vs. 6:Bioaccumulation	-4.633	-9.941 to 0.6745	No	ns	0.1309
2:Biosorption vs. 2:Bioaccumulation	16.91	11.60 to 22.22	Yes	****	<0.0001
2:Biosorption vs. 4:Biosorption	7.900	2.592 to 13.21	Yes	***	0.0004
2:Biosorption vs. 4:Bioaccumulation	9.494	4.187 to 14.80	Yes	****	<0.0001
2:Biosorption vs. 6:Biosorption	6.150	0.8421 to 11.46	Yes	*	0.0124
2:Biosorption vs. 6:Bioaccumulation	12.07	6.764 to 17.38	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Biosorption	-9.011	-14.32 to -3.703	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Bioaccumulation	-7.417	-12.72 to -2.109	Yes	**	0.0011
2:Bioaccumulation vs. 6:Biosorption	-10.76	-16.07 to -5.453	Yes	****	<0.0001
2:Bioaccumulation vs. 6:Bioaccumulation	-4.839	-10.15 to 0.4690	No	ns	0.0993
4:Biosorption vs. 4:Bioaccumulation	1.594	-3.713 to 6.902	No	ns	0.9806
4:Biosorption vs. 6:Biosorption	-1.750	-7.058 to 3.558	No	ns	0.9675
4:Biosorption vs. 6:Bioaccumulation	4.172	-1.136 to 9.480	No	ns	0.2302
4:Bioaccumulation vs. 6:Biosorption	-3.344	-8.652 to 1.963	No	ns	0.5068
4:Bioaccumulation vs. 6:Bioaccumulation	2.578	-2.730 to 7.886	No	ns	0.7930
6:Biosorption vs. 6:Bioaccumulation	5.922	0.6143 to 11.23	Yes	*	0.0184

**Pb<sup>+2</sup> removal in mixture with Cd<sup>+2</sup> kinetics by *B. zhejiangensis* CEIB S4-3****Two-way ANOVA Results**

Analysis	Statistic F	<i>p</i> value
Removal mechanisms (RM)	F (1, 64) = 1358	P<0.0001
Time (t)	F (3, 64) = 1.187	P=0.3217
Interaction RM x t	F (3, 64) = 11.13	P<0.0001

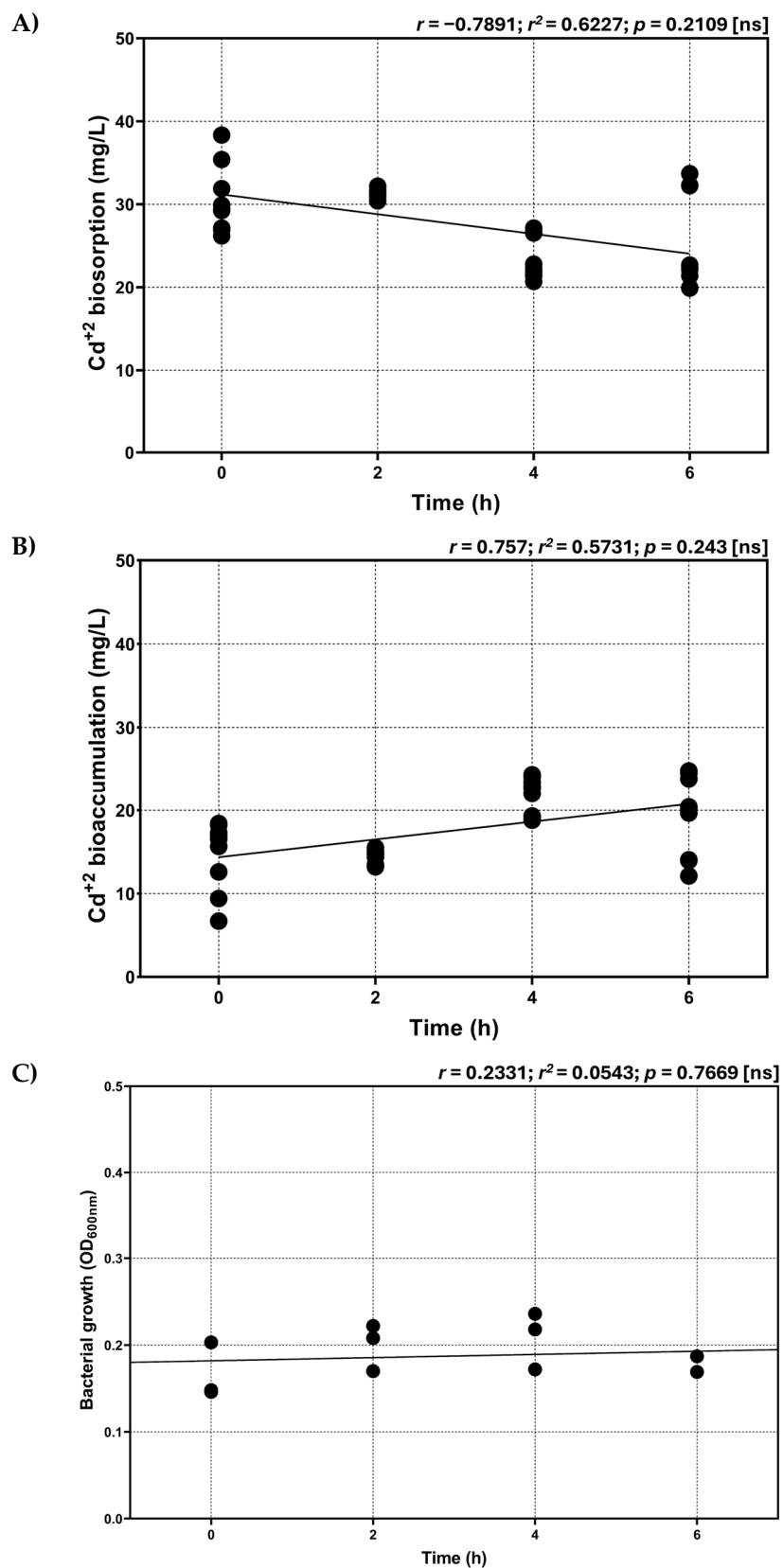
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0:Biosorption vs. 0:Bioaccumulation	27.61	23.47 to 31.76	Yes	****	<0.0001
0:Biosorption vs. 2:Biosorption	0.6111	-3.533 to 4.756	No	ns	0.9998
0:Biosorption vs. 2:Bioaccumulation	28.29	24.15 to 32.44	Yes	****	<0.0001
0:Biosorption vs. 4:Biosorption	3.122	-1.022 to 7.267	No	ns	0.2785
0:Biosorption vs. 4:Bioaccumulation	27.01	22.87 to 31.16	Yes	****	<0.0001
0:Biosorption vs. 6:Biosorption	6.294	2.150 to 10.44	Yes	***	0.0003
0:Biosorption vs. 6:Bioaccumulation	24.61	20.46 to 28.75	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Biosorption	-27.00	-31.14 to -22.86	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Bioaccumulation	0.6833	-3.461 to 4.828	No	ns	0.9995
0:Bioaccumulation vs. 4:Biosorption	-24.49	-28.63 to -20.34	Yes	****	<0.0001
0:Bioaccumulation vs. 4:Bioaccumulation	-0.6000	-4.745 to 3.545	No	ns	0.9998
0:Bioaccumulation vs. 6:Biosorption	-21.32	-25.46 to -17.17	Yes	****	<0.0001
0:Bioaccumulation vs. 6:Bioaccumulation	-3.006	-7.150 to 1.139	No	ns	0.3247
2:Biosorption vs. 2:Bioaccumulation	27.68	23.54 to 31.83	Yes	****	<0.0001
2:Biosorption vs. 4:Biosorption	2.511	-1.633 to 6.656	No	ns	0.5570
2:Biosorption vs. 4:Bioaccumulation	26.40	22.26 to 30.54	Yes	****	<0.0001
2:Biosorption vs. 6:Biosorption	5.683	1.539 to 9.828	Yes	**	0.0015
2:Biosorption vs. 6:Bioaccumulation	23.99	19.85 to 28.14	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Biosorption	-25.17	-29.32 to -21.03	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Bioaccumulation	-1.283	-5.428 to 2.861	No	ns	0.9770
2:Bioaccumulation vs. 6:Biosorption	-22.00	-26.14 to -17.86	Yes	****	<0.0001
2:Bioaccumulation vs. 6:Bioaccumulation	-3.689	-7.833 to 0.4556	No	ns	0.1160
4:Biosorption vs. 4:Bioaccumulation	23.89	19.74 to 28.03	Yes	****	<0.0001
4:Biosorption vs. 6:Biosorption	3.172	-0.9723 to 7.317	No	ns	0.2600
4:Biosorption vs. 6:Bioaccumulation	21.48	17.34 to 25.63	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Biosorption	-20.72	-24.86 to -16.57	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Bioaccumulation	-2.406	-6.550 to 1.739	No	ns	0.6100
6:Biosorption vs. 6:Bioaccumulation	18.31	14.17 to 22.46	Yes	****	<0.0001

**Cd<sup>+2</sup> removal in mixture with Pb<sup>+2</sup> kinetics by *B. zhejiangensis* CEIB S4-3****Two-way ANOVA Results**

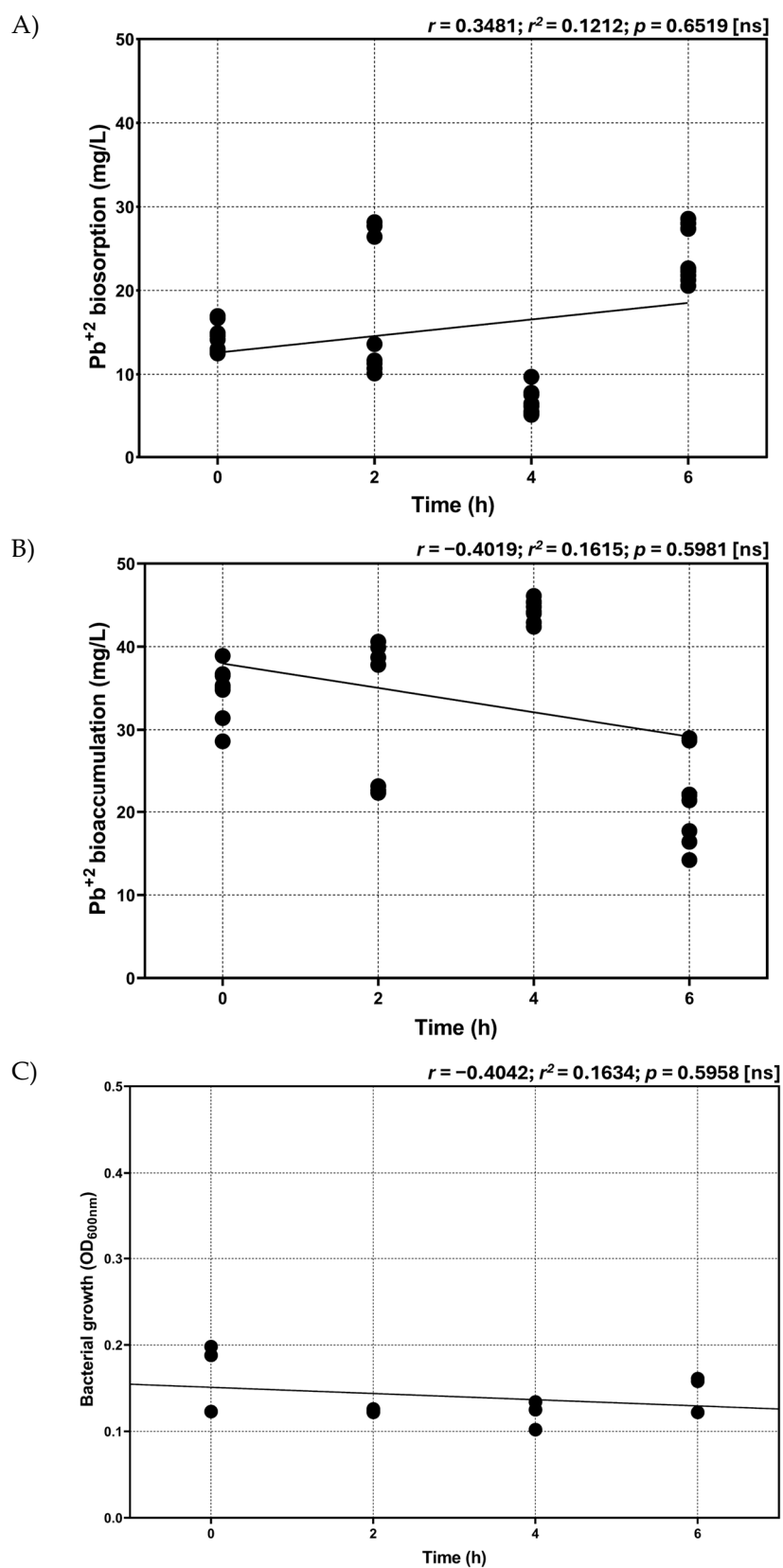
Analysis	Statistic F	<i>p</i> value
Removal mechanisms (RM)	F (1, 64) = 6805	P<0.0001
Time (t)	F (3, 64) = 8.496	P<0.0001
Interaction RM x t	F (3, 64) = 8.496	P<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0:Biosorption vs. 0:Bioaccumulation	28.91	26.67 to 31.15	Yes	****	<0.0001
0:Biosorption vs. 2:Biosorption	0.4222	-1.817 to 2.662	No	ns	0.9989
0:Biosorption vs. 2:Bioaccumulation	28.91	26.67 to 31.15	Yes	****	<0.0001
0:Biosorption vs. 4:Biosorption	0.9222	-1.317 to 3.162	No	ns	0.8992
0:Biosorption vs. 4:Bioaccumulation	28.91	26.67 to 31.15	Yes	****	<0.0001
0:Biosorption vs. 6:Biosorption	-3.650	-5.890 to -1.410	Yes	****	<0.0001
0:Biosorption vs. 6:Bioaccumulation	28.91	26.67 to 31.15	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Biosorption	-28.48	-30.72 to -26.24	Yes	****	<0.0001
0:Bioaccumulation vs. 2:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
0:Bioaccumulation vs. 4:Biosorption	-27.98	-30.22 to -25.74	Yes	****	<0.0001
0:Bioaccumulation vs. 4:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
0:Bioaccumulation vs. 6:Biosorption	-32.56	-34.80 to -30.32	Yes	****	<0.0001
0:Bioaccumulation vs. 6:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
2:Biosorption vs. 2:Bioaccumulation	28.48	26.24 to 30.72	Yes	****	<0.0001
2:Biosorption vs. 4:Biosorption	0.5000	-1.740 to 2.740	No	ns	0.9967
2:Biosorption vs. 4:Bioaccumulation	28.48	26.24 to 30.72	Yes	****	<0.0001
2:Biosorption vs. 6:Biosorption	-4.072	-6.312 to -1.833	Yes	****	<0.0001
2:Biosorption vs. 6:Bioaccumulation	28.48	26.24 to 30.72	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Biosorption	-27.98	-30.22 to -25.74	Yes	****	<0.0001
2:Bioaccumulation vs. 4:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
2:Bioaccumulation vs. 6:Biosorption	-32.56	-34.80 to -30.32	Yes	****	<0.0001
2:Bioaccumulation vs. 6:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
4:Biosorption vs. 4:Bioaccumulation	27.98	25.74 to 30.22	Yes	****	<0.0001
4:Biosorption vs. 6:Biosorption	-4.572	-6.812 to -2.333	Yes	****	<0.0001
4:Biosorption vs. 6:Bioaccumulation	27.98	25.74 to 30.22	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Biosorption	-32.56	-34.80 to -30.32	Yes	****	<0.0001
4:Bioaccumulation vs. 6:Bioaccumulation	0.000	-2.240 to 2.240	No	ns	>0.9999
6:Biosorption vs. 6:Bioaccumulation	32.56	30.32 to 34.80	Yes	****	<0.0001

Supplementary Data S3: Correlation analyses

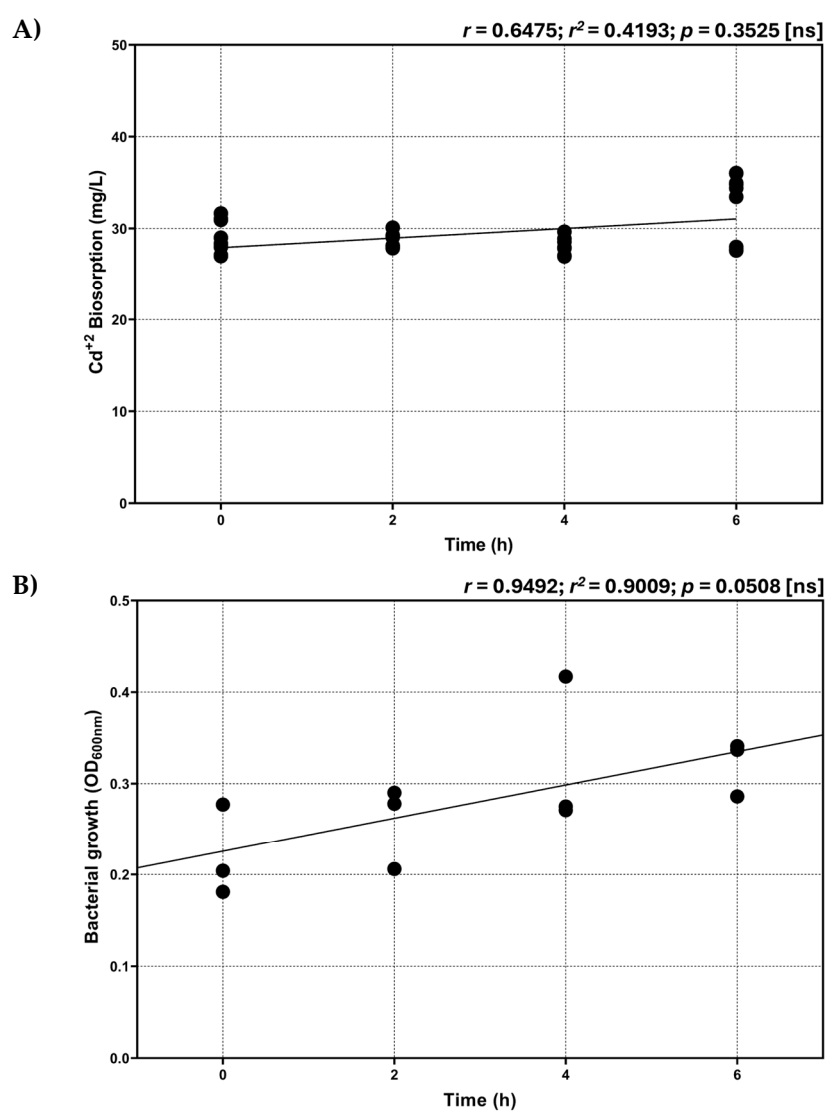


**Figure S3.** Correlation analyses  $\text{Cd}^{+2}$  removal. A) Biosorption of  $\text{Cd}^{+2}$  and time; B) Bioaccumulation of  $\text{Cd}^{+2}$  and time; C) Bacterial growth  $\text{OD}_{600\text{nm}}$  and time.



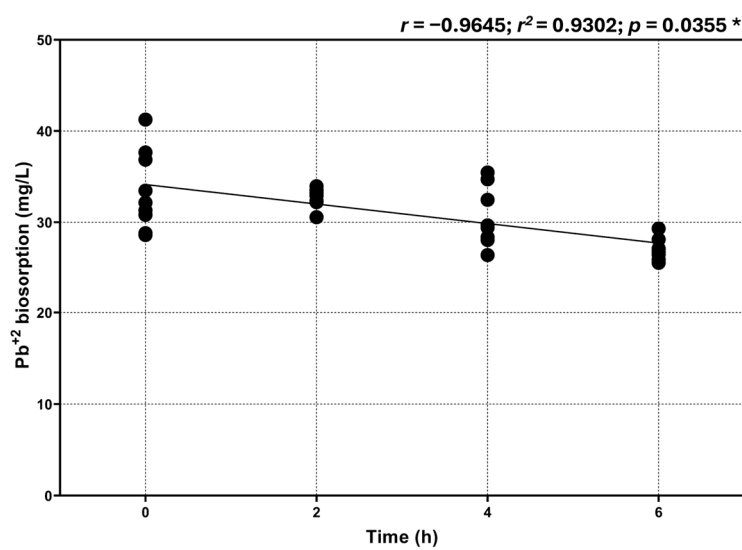
**Figure S4.** Correlation analyses Pb<sup>+2</sup> removal. A) Biosorption of Pb<sup>+2</sup> and time; B) Bioaccumulation of Pb<sup>+2</sup> and time; C) Bacterial growth OD<sub>600nm</sub> and time.



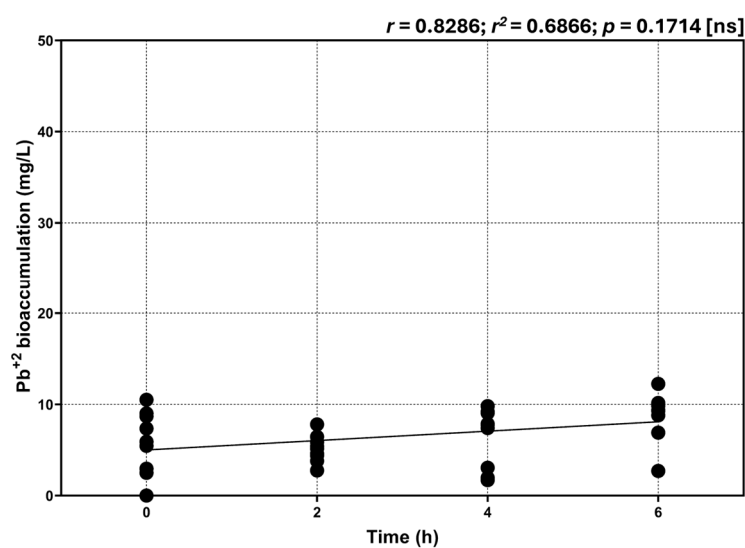


**Figure S5.** Correlation analyses Cd<sup>2+</sup> removal in mixture. A) Biosorption of Pb<sup>2+</sup> and time; B) Bacterial growth OD<sub>600nm</sub> and time.

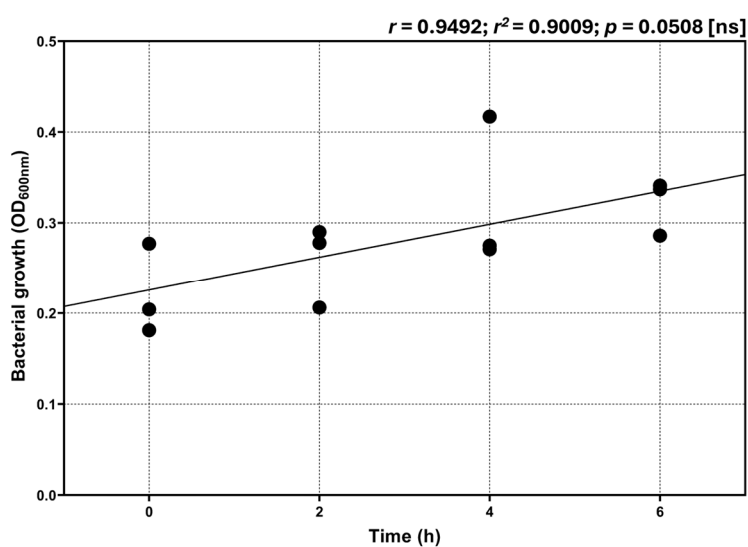
A)



B)



C)



**Figure S6.** Correlation analyses  $Pb^{+2}$  removal in mixture. A) Biosorption of  $Pb^{+2}$  and time; B) Bioaccumulation of  $Pb^{+2}$  and time; C) Bacterial growth  $OD_{600nm}$  and time.

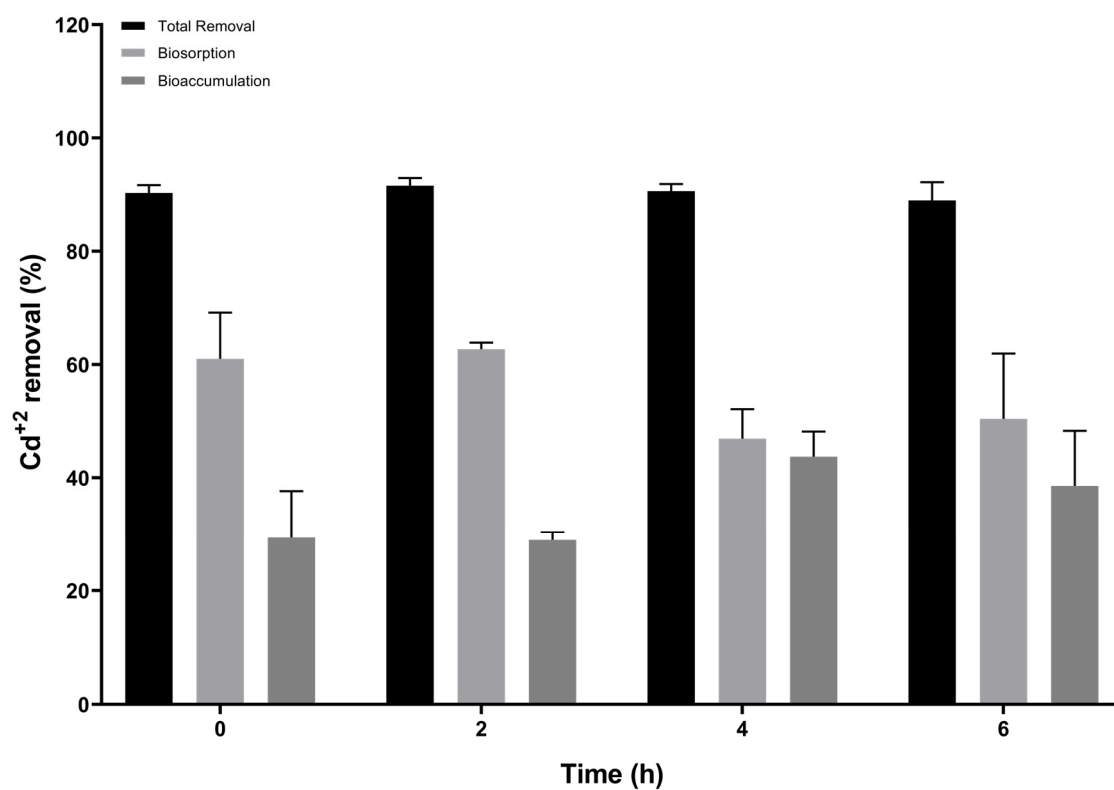
## Supplementary Data S4: Heavy metals removal percentages

Table S1. Heavy metals removal percentages

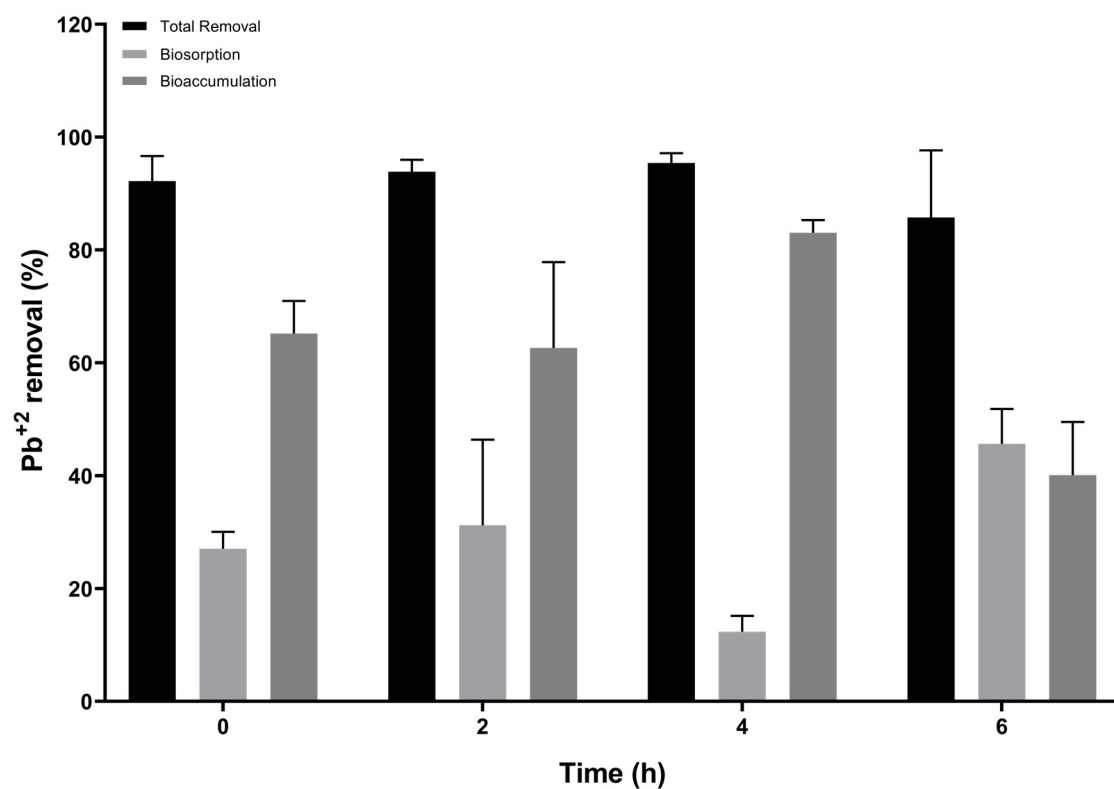
Heavy metal*	Time (h)	Biosorption (%)	Bioaccumulation (%)	Total Removal (%)
Cd <sup>+2</sup>	0	60.99±8.17	29.29±8.33	90.28±1.38
	2	<u>62.7±1.15</u>	28.88±1.45	<u>91.58±1.35</u>
	4	46.9±5.19	<u>43.71±4.43</u>	90.61±1.23
	6	50.4±11.52	38.56±9.71	88.96±3.21
Pb <sup>+2</sup>	0	27.04±2.99	65.16±5.79	92.2±4.45
	2	31.23±15.15	63.63±15.21	93.85±2.1
	4	12.34±2.81	<u>83.05±2.23</u>	<u>95.39±1.76</u>
	6	<u>45.66±6.16</u>	40.08±9.41	85.74±11.91
Cd <sup>+2</sup> in mixture	0	57.81±3.73	0	57.81±3.73
	2	56.97±1.59	0	56.97±1.59
	4	55.97±1.85	0	55.97±1.85
	6	<u>65.11±7.33</u>	0	<u>65.11±7.33</u>
Pb <sup>+2</sup> in mixture	0	<u>66.86±8.62</u>	11.63±6.93	<u>78.49±15.54</u>
	2	65.63±2.04	10.27±2.98	75.9±5.01
	4	60.61±6.32	12.83±6.50	73.44±12.82
	6	54.27±2.37	<u>17.54±5.38</u>	71.91±7.75

\*Concentrations of heavy metals: 50 mg/L Cd<sup>+2</sup> or Pb<sup>+2</sup>; heavy metals in mixture 50 mg/L of each heavy metal [Cd<sup>+2</sup> + Pb<sup>+2</sup>]

A)

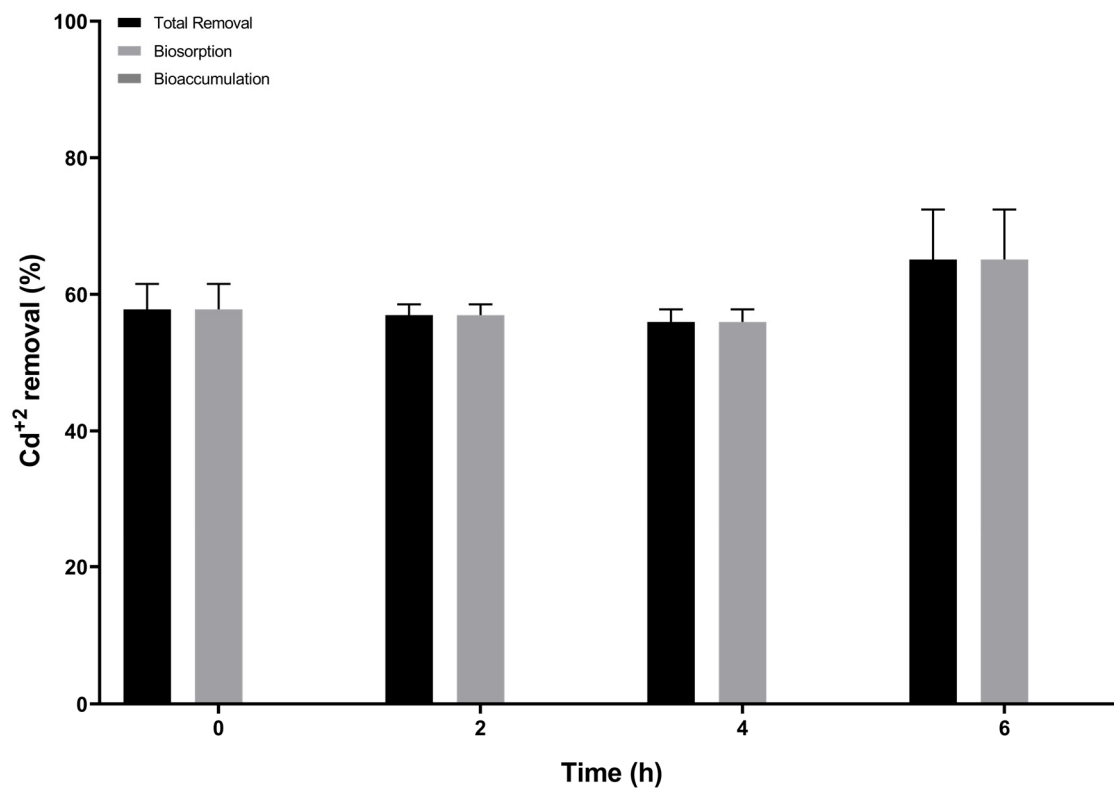


B)



**Figure S7.** Removal percentages of  $\text{Pb}^{+2}$  and  $\text{Cd}^{+2}$  by *B. zhejiangensis* CEIB S4-3. A)  $\text{Cd}^{+2}$  and B)  $\text{Pb}^{+2}$ .

A)



B)

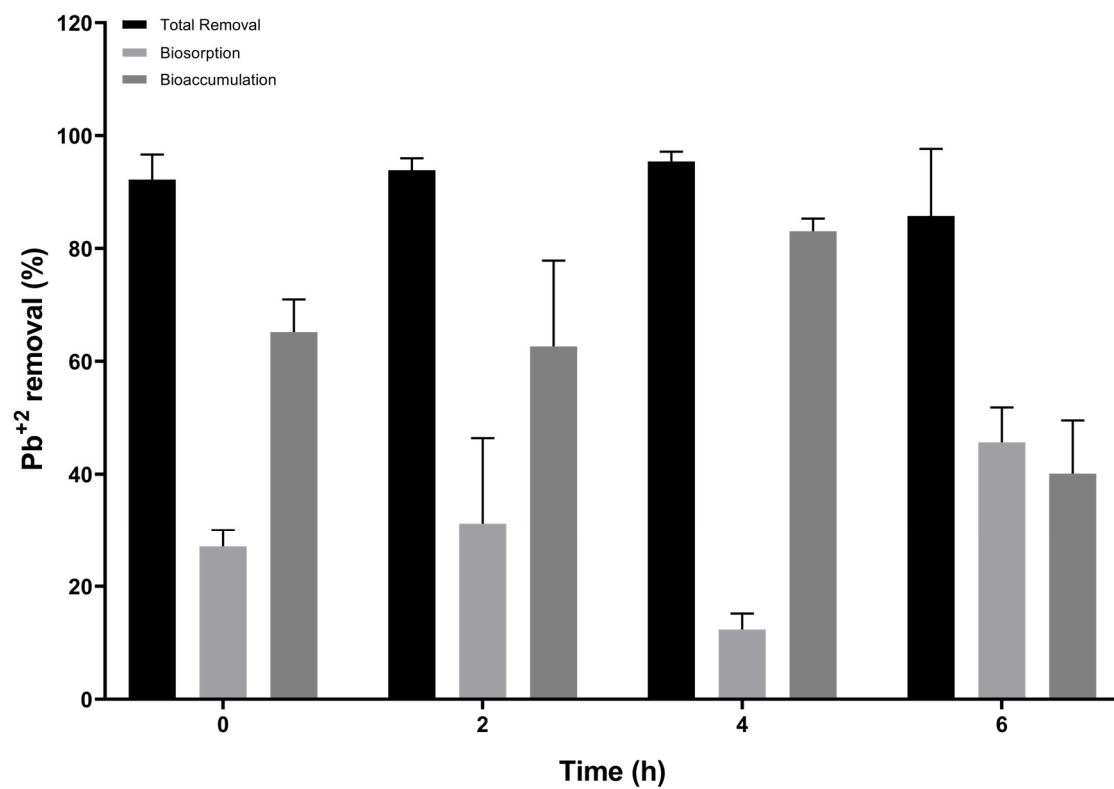


Figure S8. Removal percentages of mixtures of  $\text{Pb}^{+2}$  and  $\text{Cd}^{+2}$  by *B. zhejiangensis* CEIB S4-3. A)  $\text{Cd}^{+2}$  and B)  $\text{Pb}^{+2}$ .