

Supplementary Materials:

Table S1. Total of metabolites detected by GC-MS in *G. caudata* listed according to the retention time (RT) and the calculated Kovats retention index (RI).

Number	ID	RI	RT	Classification
1	Lactic acid	1059	6.31	Hydroxy acid
2	Acetic acid	1075	6.55	Carboxylic Acid
3	Undecane	1110	6.96	Fatty Acid
4	Leucine	1155	7.75	Amino acid
5	Norleucine	1177	8.08	Amino acid
6	Valine	1208	8.58	Amino acid
7	Butanoic acid	1233	8.90	Carboxylic Acid
8	Serine	1250	9.21	Amino acid
9	Phosphate	1266	9.37	Ester Anion
10	Pentanoic acid	1274	9.52	Carboxylic Acid
11	Threonine	1289	9.72	Amino acid
12	Glycine	1299	9.87	Amino acid
13	Decanoic acid	1309	10.00	Carboxylic Acid
14	Dodecane	1323	10.18	Fatty Acid
15	2(3H)-Furanone	1369	10.81	Carbohydrates
16	Malonic acid	1400	11.25	Carboxylic Acid
17	Aminomalonic acid	1462	12.02	Carboxylic Acid
18	Malic acid	1481	12.27	Carboxylic Acid
19	Erythritol	1497	12.49	Monosaccharide
20	Proline	1516	12.72	Amino acid
21	Threonic acid	1534	12.93	Sugar Acid
22	2-Aminobenzoxazole	1537	12.97	Amine
23	Alanine	1551	13.14	Amino acid
24	Sulfurous acid	1599	13.74	Mineral Acid
25	Hexadecane	1700	14.90	Fatty Acid
26	Phosphoric acid	1750	15.44	Mineral Acid
27	Ribonic acid	1763	15.58	Sugar Acid
28	Arabinonic acid	1790	15.88	Sugar Acid
29	Carboxylic acid	1807	16.07	Carboxylic Acid
30	Citric acid	1826	16.26	Carboxylic Acid
31	Tetradecanoic acid	1846	16.47	Fatty Acid
32	Mannose	1881	16.84	Carbohydrates
33	Glucitol	1923	17.27	Polyol (alcohol)
34	Ascorbic acid	1937	17.40	Vitamin
35	Talose	1964	17.67	Monosaccharide
36	Galactofuranose	2030	18.33	Monosaccharide
37	Hexadecanoic acid	2043	18.44	Fatty Acid
38	Eicosane	2121	19.18	Fatty Acid
39	β -D-glucopyranose	2210	20.02	Monosaccharide
40	Octadecanoic acid	2240	20.25	Fatty Acid
41	2- α -D-galactopyranosyl Glycerol (Floridoside)	2257	20.40	Heteroside
42	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide	2568	22.94	Stilbenes
43	Guaiacol- β -D-glucopyranoside	2726	24.13	Heteroside

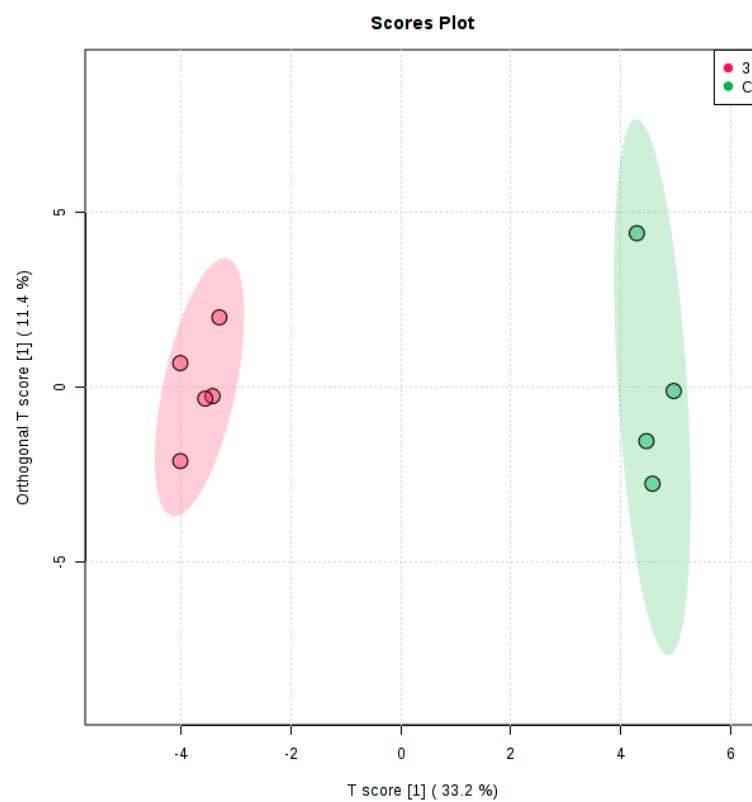


Figure S1. OPLS-DA in *G. caudata* exposed to Cd²⁺ (0 and 3 mg/L). Data converted to cubic root and Table 11. and T-score: 33,2%.

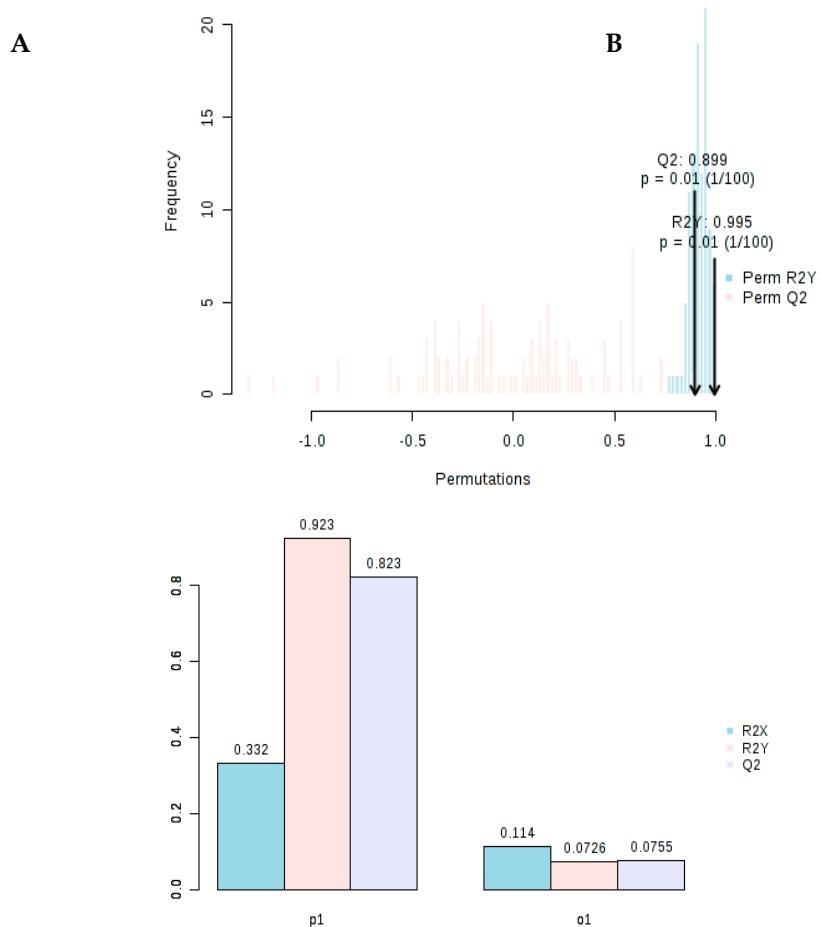


Figure S2. Validation tests for the OPLS-DA for the metabolic profile of *G. caudata* exposed to Cd²⁺ (0 and 3 mg/L). **(A)**. 100-permutation test that resulted in variables of Q2 de 0,899 ($p=0,01$) e R2Y 0,995 ($p=0,01$); **(B)**. graph bar for the calculated statistical variables of de Q2=0,823 e de R2Y= 0,923.

Table S2. Complete list of the metabolites detected in the analysis (represented by their respective retention time) and their correlation and covariance values assigned by the OPLS DA test.

Retention Time (RT)	Covariance	Correlation
6.31	-0,32092	-0,33708
6.55	-1,945	-0,99237
6.78	-0,713	-0,6173
6.96	0,12831	0,32994
7.75	-0,49521	-0,58547
7.85	0,34994	0,57297
8.08	-0,80895	-0,74084
8.43	-0,3419	-0,33112
8.58	-0,64211	-0,71186
8.90	0,081553	0,11086
9.21	-1,0741	-0,92262
9.37	-0,28467	-0,32801
9.41	0,70807	0,63154
9.52	0,19879	0,4501
9.72	-1,084	-0,86677
9.77	-0,64876	-0,93449
9.87	-0,86148	-0,86056
10.00	-0,65404	-0,77122

10.18	0,003942	0,0049752
10.81	-0,27026	-0,36146
11.09	-0,40794	-0,46676
11.25	-0,45375	-0,39488
12.02	-0,23714	-0,23053
12.27	-0,30431	-0,28472
12.37	0,24449	0,58726
12.49	-1,0223	-0,92648
12.72	-1,1561	-0,88113
12.93	-0,049981	-0,1076
12.97	0,15801	0,52723
13.14	-0,13175	-0,21195
13.74	-0,26113	-0,3969
14.90	0,26229	0,69538
15.44	-0,24707	-0,33194
15.58	-0,13681	-0,17204
15.88	-0,46129	-0,63174
15.97	-0,7982	-0,86817
16.07	-0,8532	-0,70374
16.26	-0,9269	-0,88942
16.47	-0,25492	-0,44246
16.84	0,41968	0,39102
17.27	-0,35726	-0,49679
17.40	-1,0816	-0,9126
17.67	0,48049	0,6829
18.33	0,32343	0,51997
18.44	0,072068	0,11463
19.18	0,13982	0,21117
20.02	1,5635	0,76734
20.13	0,27298	0,24042
20.25	-0,10179	-0,06081
20.27	-0,051087	-0,04968
20.35	0,020666	0,017632
20.40	-0,91115	-0,78662
20.47	0,29689	0,25707
21.10	-0,3912	-0,6268
21.6	-0,24058	-0,57604
22.94	0,62386	0,71181
24.13	-0,22203	-0,12466

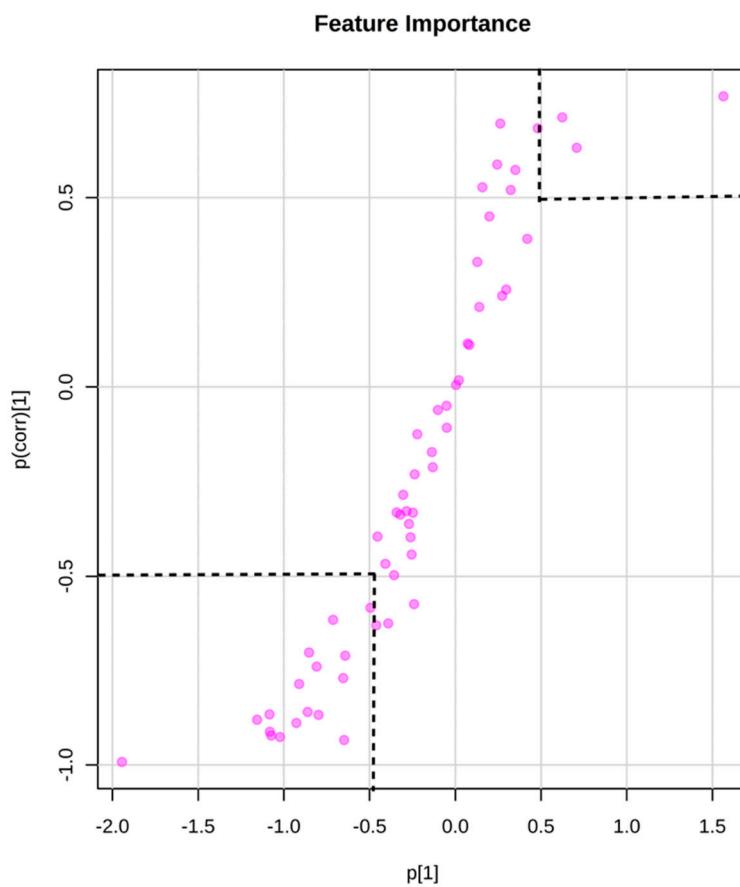


Figure S3. S-plot of the metabolites present in the OPLS-DA analysis in *G. caudata* exposed to Cd²⁺ (0 and 3 mg/L). The metabolites are plotted with their respective covariance (y-axis) and correlation (X-axis) values. The upper right and lower left ends (black dashed areas) stand out as the most important metabolites for the separation of the groups (correlation $> |0,05|$ and a covariance $> |0,5|$).

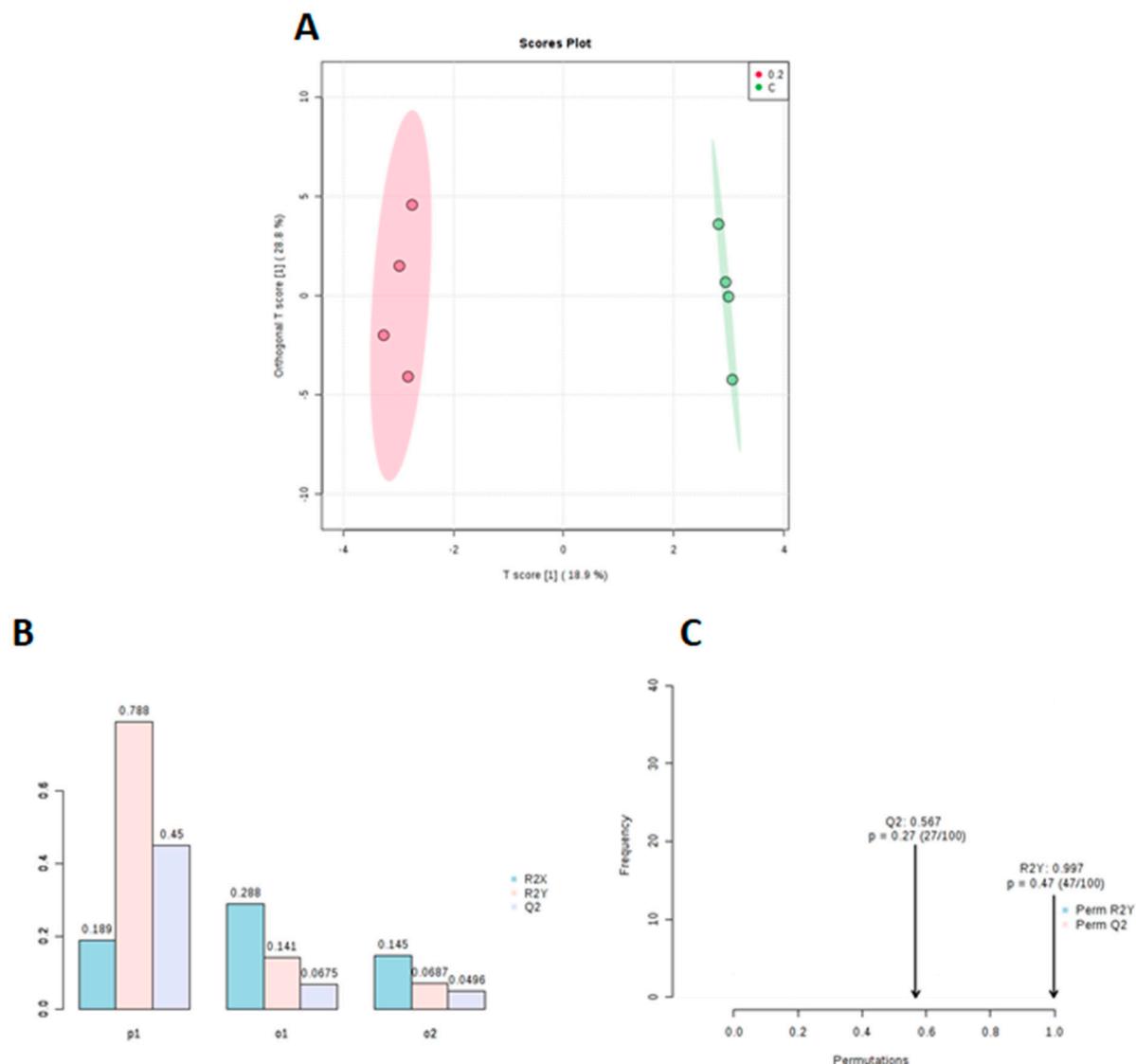


Figure S4. Invalid OPLS-DA and validation tests in *G. caudata* exposed to Cd²⁺ (0 and 0,2 mg/L). Data converted to cubic root and the Pareto scaling - (A) OPLS-DA Orthogonal T-score: 28,8% and T-score [1]: 18,9%; (B) 100-permutation test Q2: 0,567 ($p=0,27$) and R2Y: 0,997 ($p=0,47$); (C) Model validation Q2:0,45 and R2Y: 0,189.

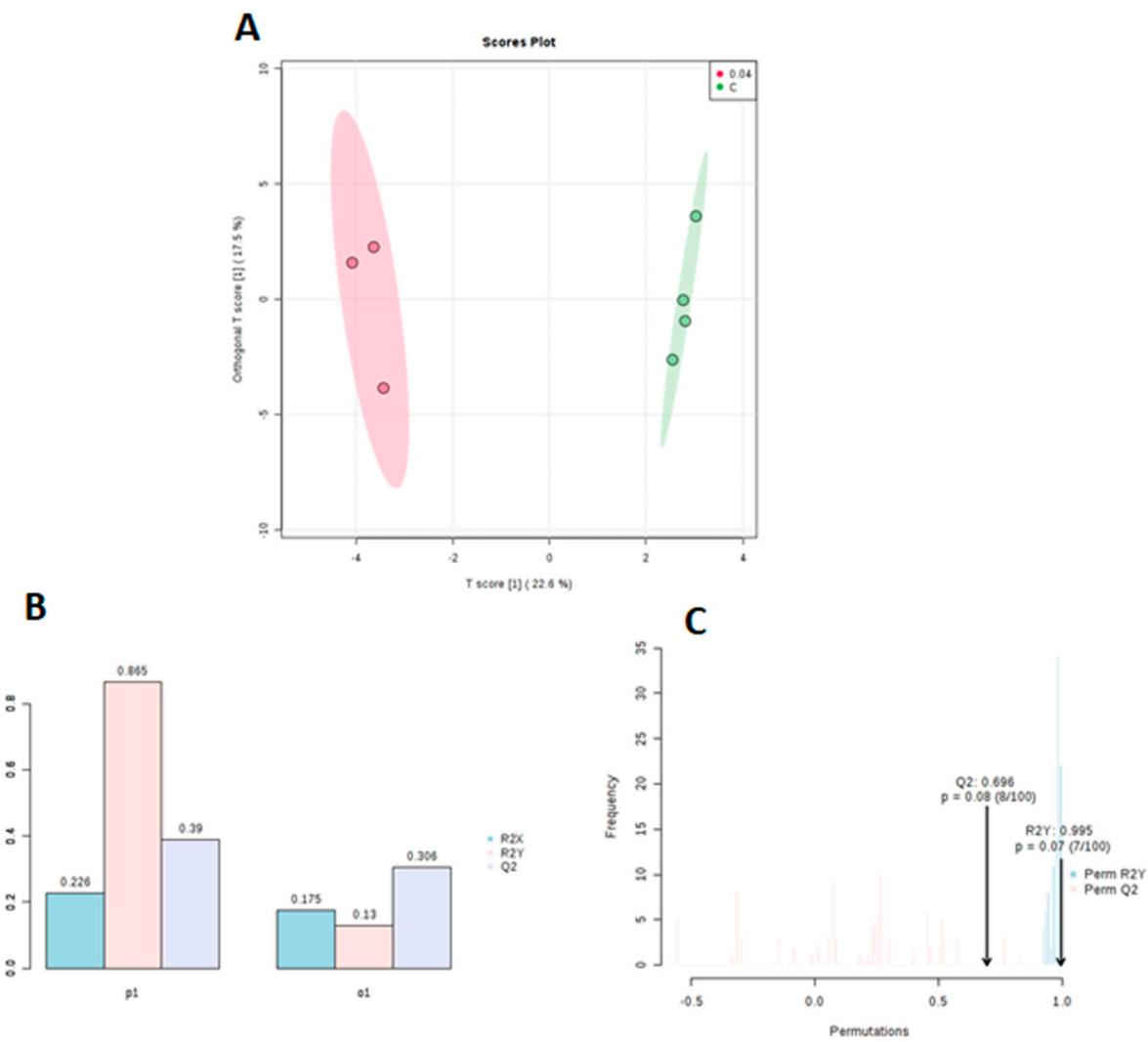


Figure S5. Invalid OPLS-DA and validation tests in *G. caudata* exposed to Cd²⁺ (0 and 0,04 mg/L). Data converted to cubic root and the Pareto scaling - **(A)** OPLS-DA Orthogonal T-score: 17,5% and T-score [1]: 22,6%; **(B)** 100-permutation test Q2: 0,696 ($p=0,08$) and R2Y: 0,995 ($p=0,07$); **(C)** Model validation Q2:0,39 and R2Y: 0,226.

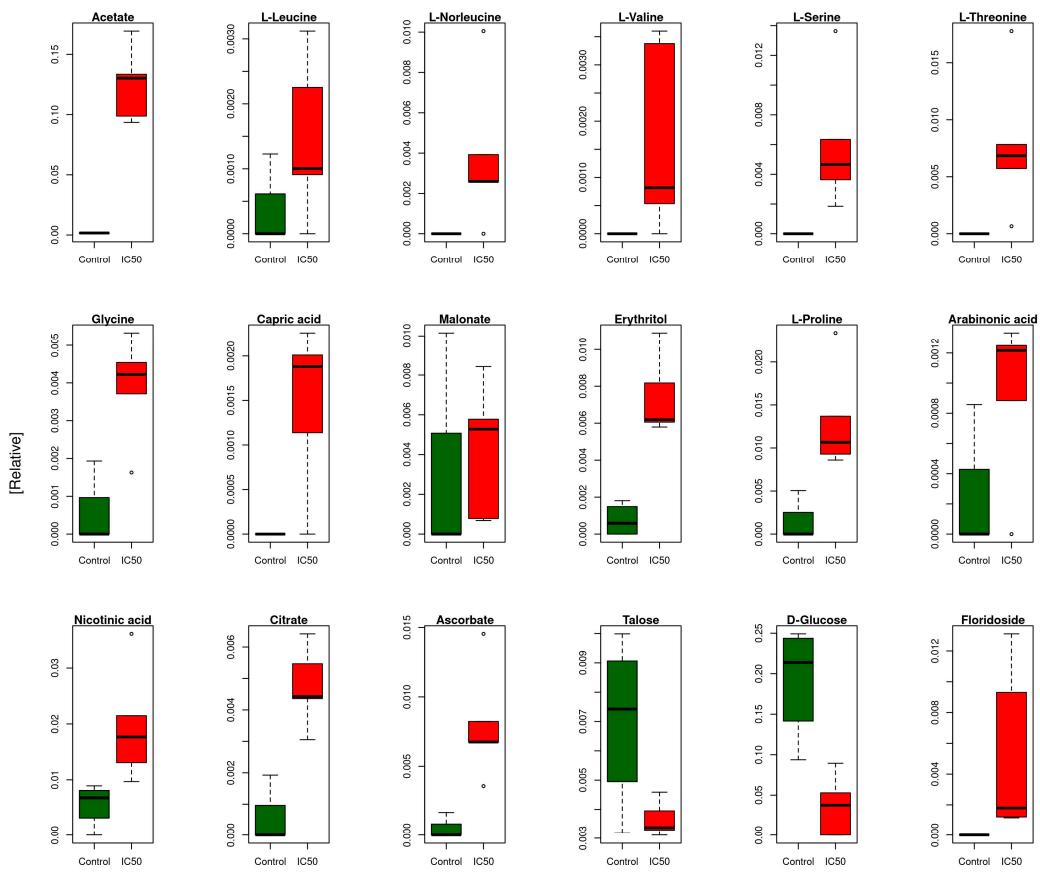


Figure S6. Comparison of relative concentrations of the 19 metabolites (Table 1) found alternated in the metabolome of *Gracilaria caudata* exposed to Cd²⁺ (3 mg/L).