Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information for

GC-TOF-MS based metabolomics of cucumber (*Cucumis sativus*) fruits reveal alteration of metabolites profile and biological pathway disruption induced by nano-Cu

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Table S1. Major properties of soils used in this study

Property	Grassland Soil
pH	5.90 ± 0.04
Electrical conductivity (μS cm^{-1})	18.9 ± 0.6
Cation exchange capacity (meq $100g^{-1}$)	25.8 ± 0.1
Loss-on-ignition organic matter (%)	3.11 ± 0.07
Bulk density (g cm^{-3})	0.981 ± 0.017
Sand / Silt / Clay (%)	54.0 / 29.0 / 17.0
Saturation percent (%)	43.0 ± 0.7
Water content of air-dry soil (wt. %)	10.54 ± 0.02
Exchangeable PO_4-P (μg g^{-1})	15.3 ± 0.6
Exchangeable NH_4-N (μg g^{-1})	1.28 ± 0.04
Exchangeable NO_3-N (μg g^{-1})	11.5 ± 0.5
Exchangeable K (μg g^{-1})	206 ± 1
Total Ce (μg g^{-1})	30.3 ± 0.8
Total Cu (μg g^{-1})	25.4 ± 0.3
Total Ti (μg g^{-1})	1864 ± 10

Table S2. A summary of analysis of variance to detect significant differences among nano-Cu treatments with respect to gas exchange, biomass, Cu and Fe content.

		p value
Gas exchange **(Figure 1)**	Potosynthesis rate	0.0066
	Transpiration rate	0.7136
	Stomatal conductance	0.373
	Water usage efficiency	0.0009
Biomass (**Figure S1**)	Root	0.139
	Stem	0.807
	Leaf	0.677
	Fruit	0.355
Cu content (**Figure 2**)	Root	0
	Stem	0.001
	Leaf	0.001
	Fruit	0.002
Fe content (**Figure 3**)	Root	0.009
	Stem	0.607
	Leaf	0.024
	Fruit	0.454

Table S3 Metabolites identified in cucumber fruit grown in soil with 0 (Control), 200 (Low), 400 (Medium) and 800 (High) mg/kg nano-Cu

Metabolites	Control			Low			Medium			High		
glucose	1730412	±	703980	2640435	±	713824	1579289	±	353966	388798	±	2117660
fructose	1128642	±	467504	1553420	±	402029	1335231	±	247984	259163	±	1297591
malic acid	161913	±	71267	215443	±	63268	122256	±	50585	26052	±	137638
glutamine	89085	±	23592	101060	±	12094	100556	±	60848	30510	±	92230
oxoproline	66168	±	11329	64219	±	8539	57989	±	25787	23725	±	67504
alanine	47320	±	7913	51570	±	25671	57118	±	12413	11713	±	46167
sucrose	52193	±	14570	47872	±	31858	37929	±	3022	24822	±	35916
myo-inositol	62903	±	57351	32160	±	19400	21766	±	4824	15120	±	38254
maleic acid	14961	±	7947	16873	±	9810	25633	±	23040	27632	±	40521
pipecolinic acid	9988	±	5782	9775	±	2370	8096	±	1319	3771	±	11255
glycine	17358	±	7950	26110	±	4651	29646	±	10917	6723	±	27688
glyceric acid	17248	±	4113	20748	±	4072	16208	±	2806	4682	±	19154
leucine	16086	±	3423	18255	±	4203	22719	±	7882	4277	±	18206
isoleucine	14684	±	2249	16588	±	3694	18816	±	5880	3328	±	16411
valine	13794	±	3067	16006	±	4157	18275	±	3458	2802	±	16944
serine	11898	±	2519	15214	±	3163	12479	±	3861	1959	±	11542
phosphate	12552	±	4421	14078	±	2545	12244	±	2259	2407	±	12450
tyrosine	12562	±	3043	13272	±	5960	16588	±	6267	3057	±	13319
gamma-aminobutyric acid	10668	±	6917	14113	±	7833	13477	±	1119	6481	±	14118
glutamic acid	11889	±	2696	13553	±	5673	13040	±	3351	4341	±	10756
aspartic acid	8625	±	4941	11217	±	5744	3867	±	254	5357	±	7543
shikimic acid	18760	±	18452	12428	±	10226	6425	±	4934	13146	±	13737
proline	8262	±	2199	11927	±	4737	13276	±	2173	3200	±	11424
citric acid	11668	±	1701	9655	±	2374	7297	±	1075	5537	±	9575
phenylalanine	7063	±	2399	6720	±	1554	7365	±	2568	1889	±	6558

stearic acid	6534 ± 2144	10186 ± 4258	7000 ± 167	815 ± 7050
glycerol	5013 ± 1491	4382 ± 692	4891 ± 1655	1386 ± 5505
xylose	5477 ± 2624	8316 ± 1284	6750 ± 328	1711 ± 6213
tryptophan	5688 ± 1738	5232 ± 1933	6670 ± 3020	1408 ± 5842
threonine	3912 ± 1130	4207 ± 1576	4599 ± 1523	1107 ± 4189
fumaric acid	2544 ± 1172	3156 ± 1106	4432 ± 3783	3509 ± 5762
glycerol-3-galactoside	3745 ± 704	4882 ± 2312	2964 ± 209	1761 ± 4513
ornithine	3090 ± 625	2298 ± 748	1832 ± 412	613 ± 2415
alpha-ketoglutarate	2668 ± 418	3691 ± 319	2427 ± 212	1623 ± 3128
alpha-aminoadipic acid	2058 ± 761	2705 ± 749	2121 ± 578	1130 ± 2391
histidine	3473 ± 1749	3557 ± 2201	4175 ± 2763	737 ± 3006
palmitic acid	2309 ± 911	2920 ± 725	2468 ± 398	480 ± 2332
galactinol	2038 ± 556	2444 ± 1028	1377 ± 344	916 ± 2248
methionine	2104 ± 635	1722 ± 552	1584 ± 1156	638 ± 1839
citrulline	16638 ± 25715	1354 ± 475	956 ± 287	18627 ± 9078
ribitol	1251 ± 594	1056 ± 271	963 ± 185	408 ± 1157
asparagine	1211 ± 633	1624 ± 737	1470 ± 338	357 ± 1555
pelargonic acid	1392 ± 610	2077 ± 564	1691 ± 171	301 ± 1640
nornicotine	738 ± 231	914 ± 343	675 ± 149	181 ± 848
xylonic acid	727 ± 224	820 ± 95	639 ± 119	268 ± 804
1-monoolein	526 ± 225	453 ± 180	314 ± 83	253 ± 428
glucose-6-phosphate	1051 ± 544	1552 ± 511	1030 ± 116	307 ± 968
3,6-anhydro-D-galactose	637 ± 245	820 ± 190	700 ± 131	97 ± 710
lysine	2142 ± 3283	843 ± 448	973 ± 345	257 ± 760
5-hydroxynorvaline	689 ± 364	751 ± 160	587 ± 50	162 ± 633
1,2-cyclohexanedione	434 ± 163	466 ± 218	355 ± 155	317 ± 568
methionine sulfoxide	827 ± 391	824 ± 146	871 ± 437	200 ± 800
putrescine	507 ± 315	656 ± 333	297 ± 22	284 ± 445
hexose-6-phosphate	565 ± 194	616 ± 237	502 ± 108	245 ± 451
benzoic acid	593 ± 264	867 ± 271	739 ± 164	165 ± 715

fructose-6-phosphate	575	±	214	705	±	299	461	±	57	249	±	522
succinic acid	412	±	84	545	±	81	544	±	121	133	±	474
linolenic acid	745	±	294	1062	±	296	1008	±	91	247	±	674
galactose-6-phosphate	357	±	252	360	±	610	55	±	5	331	±	255
threonic acid	445	±	90	492	±	37	386	±	51	84	±	431
butyrolactam NIST	400	±	200	506	±	225	470	±	126	329	±	579
pyruvic acid	293	±	210	367	±	216	427	±	27	237	±	334
mucic acid	335	±	134	327	±	178	220	±	69	101	±	248
lactic acid	443	±	244	625	±	134	519	±	66	81	±	387
lauric acid	748	±	477	841	±	161	707	±	99	576	±	887
citramalic acid	314	±	182	383	±	59	269	±	19	95	±	304
beta-alanine	235	±	118	237	±	71	213	±	38	52	±	179
N-acetylmannosamine	424	±	241	458	±	133	247	±	194	230	±	379
methanolphosphate	300	±	157	477	±	311	193	±	27	140	±	325
pentitol	286	±	96	269	±	74	184	±	29	112	±	283
caprylic acid	392	±	123	619	±	145	500	±	47	178	±	478
urea	235	±	90	376	±	144	151	±	97	49	±	265
glycolic acid	274	±	210	390	±	190	328	±	62	89	±	342
erythritol	238	±	128	286	±	76	167	±	56	72	±	226
beta-gentiobiose	237	±	118	277	±	113	179	±	21	104	±	230
isothreonic acid	213	±	37	222	±	54	169	±	22	72	±	219
adipic acid	172	±	77	195	±	28	168	±	62	51	±	191
nicotinic acid	190	±	71	226	±	62	165	±	24	56	±	176
glycerol-alpha-phosphate	212	±	99	250	±	45	189	±	30	105	±	208
arachidic acid	203	±	127	471	±	155	274	±	102	125	±	317
linoleic acid	214	±	74	290	±	74	273	±	30	118	±	265
digalacturonic acid	110	±	45	158	±	74	155	±	18	37	±	109
epsilon-caprolactam	136	±	56	195	±	49	170	±	35	31	±	146
1-monopalmitin	317	±	285	298	±	277	243	±	68	84	±	183
beta-sitosterol	223	±	107	283	±	26	288	±	140	91	±	250

levoglucosan	165 ± 95	246 ± 48	163 ± 33	64 ± 210
2,5-dihydroxypyrazine	160 ± 57	147 ± 46	87 ± 24	29 ± 91
isomaltose	144 ± 65	149 ± 30	120 ± 34	60 ± 160
lignoceric acid	124 ± 72	173 ± 83	113 ± 40	59 ± 153
myristic acid	167 ± 53	196 ± 94	144 ± 8	46 ± 153
propane-1,3-diol NIST	155 ± 130	259 ± 112	205 ± 13	52 ± 171
xylitol	167 ± 54	206 ± 56	139 ± 38	105 ± 191
1-kestose	228 ± 167	96 ± 64	99 ± 42	14 ± 90
uracil	70 ± 34	126 ± 90	161 ± 66	68 ± 120
3-hydroxypropionic acid	139 ± 66	166 ± 45	149 ± 51	39 ± 148
octanol NIST	91 ± 44	114 ± 45	104 ± 49	12 ± 94
xylulose NIST	126 ± 56	152 ± 67	138 ± 104	51 ± 166
O-acetylserine	116 ± 62	117 ± 29	103 ± 31	29 ± 100
ribulose-5-phosphate	101 ± 29	136 ± 50	96 ± 30	38 ± 99
2-hydroxyglutaric acid	68 ± 49	90 ± 20	80 ± 12	29 ± 79
5'-deoxy-5'-methylthioadenosine	87 ± 26	88 ± 49	77 ± 20	32 ± 96
homoserine	109 ± 49	110 ± 26	116 ± 14	39 ± 127
octadecanol	101 ± 44	184 ± 99	328 ± 402	30 ± 111
capric acid	125 ± 67	119 ± 49	100 ± 24	29 ± 110
glutaric acid	50 ± 28	79 ± 27	70 ± 17	17 ± 58
lactitol	97 ± 56	127 ± 59	74 ± 2	29 ± 63
4-aminobutyric acid	505 ± 934	160 ± 134	103 ± 32	72 ± 154

The data are the means of four replicates.

Table S4. T-test screened metabolites significantly changed compared to control in cucumber fruit

Compounds	**Trend**	**Group**	***p* value**
proline	↑	*Amino acid*	**0.028072458**
glycine	↑		**0.014595707**
valine	↑		0.054666177
asparagine	↑		0.081489374
ornithine	↓		**0.002351659**
citric acid	↓	*Carboxylic acid*	0.062459638
pelargonic acid	↑		0.052356472
arachidic acid	↑	*Fatty acid*	**0.021941532**
caprylic acid	↑		**0.047793091**
xylose	↑	*Sugar*	**0.038463045**
1-kestose	↓		**0.003937712**
myo-inositol	↓	*Others*	**0.040726072**
2,5-dihydroxypyrazine	↓		**0.032809484**

control to all (Low, Medium, High)

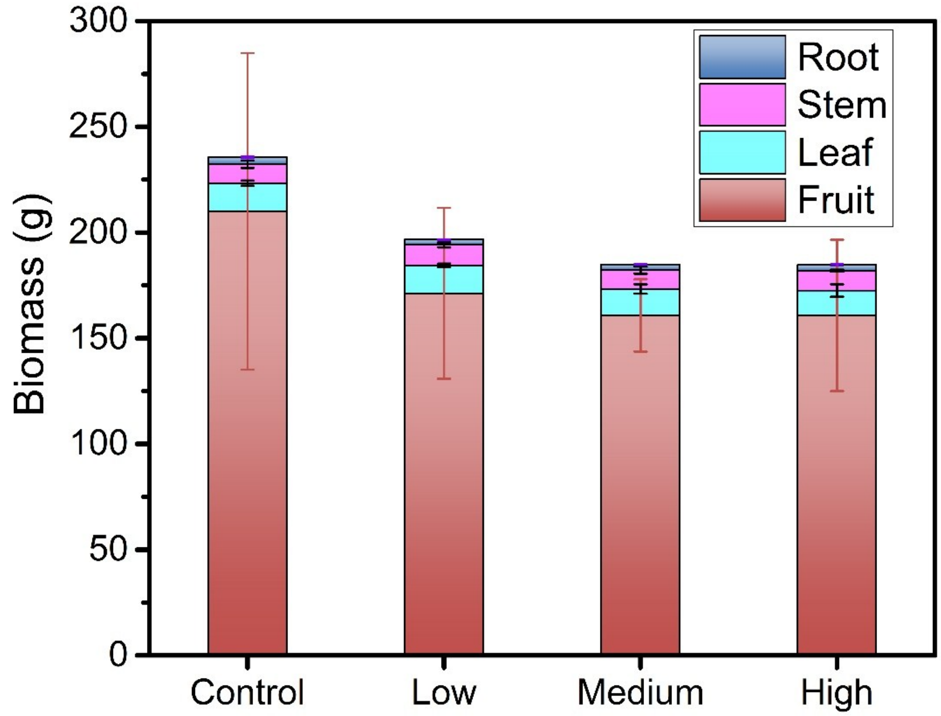

Figure S1. Effect of nano-Cu on cucumber biomass accumulation in root, stem, leaves and fruit. The data are the means of four replicates. For root, stem and leaves the data represent dry weight; and for fruit it is fresh weight. Error bars represent ± standard error. Statistical significance is presented in Table S1.

Experimental Design for additional experiment

In order to determine the dissolution of nano-Cu in comparison with $CuCl_2$, soil samples were spiked with 0, and 200 mg/kg nano-Cu or $CuCl_2$. Each treatment (growing pot) had 4 replicates. Cucumber seedlings were grown in the soils for 10 days. On day 10, cucumber seedlings were removed and the soils were collected for Cu ion analysis. To determine water-extractable Cu, 5 g of soil were weighed into 50 ml centrifuge tubes and 50 ml of nano pure water were added. The mixture was agitated for 24 hr. After filtration and dilution, the Cu concentration in the soil-derived solutions was analyzed by ICP-MS. Results are shown in Figure S2.

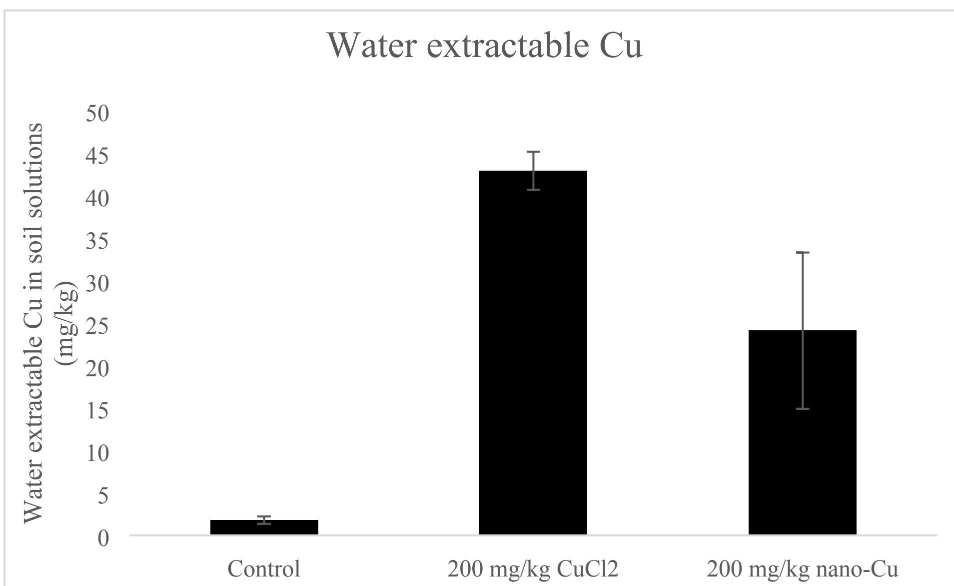

Figure S2. Water extractable Cu concentration in spiked soils: control, 200 mg/kg $CuCl_2$ and 200 mg/kg nano-Cu.

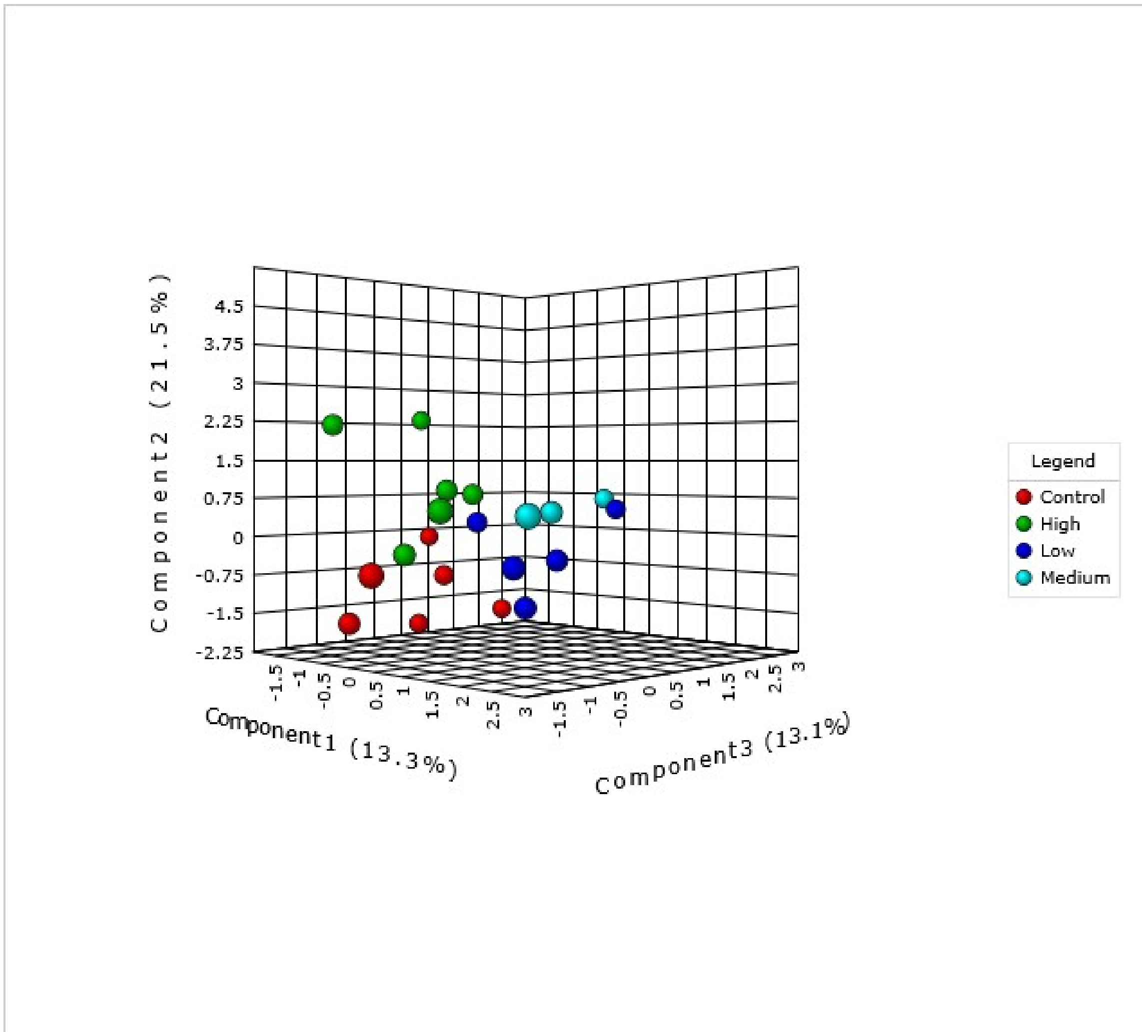

Figure S3. Score plot of partial least square (PLS) analysis of mineral elements in cucumber fruits affected by different concentrations of nano-Cu.

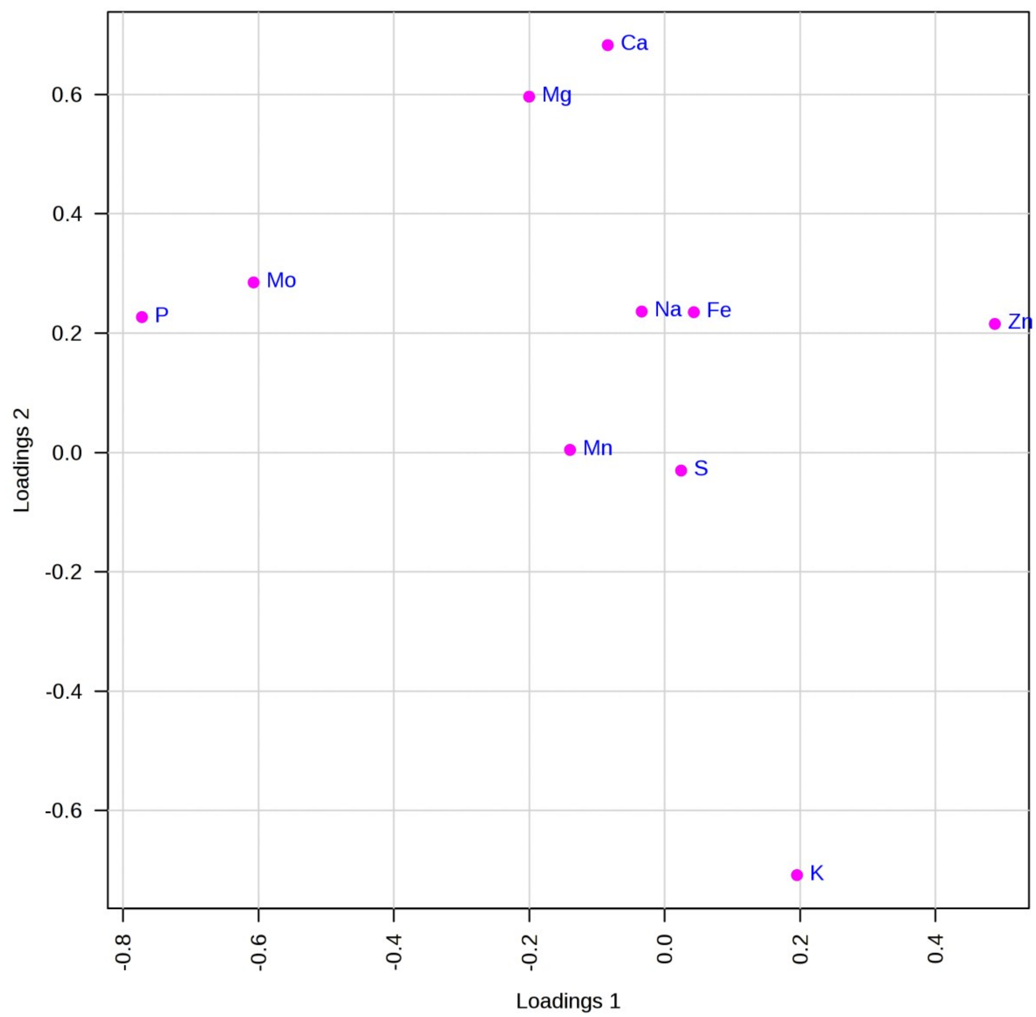

Figure S4. Loading plot of partial least square (PLS) analysis of mineral elements in cucumber fruits affected by different concentrations of nano-Cu.

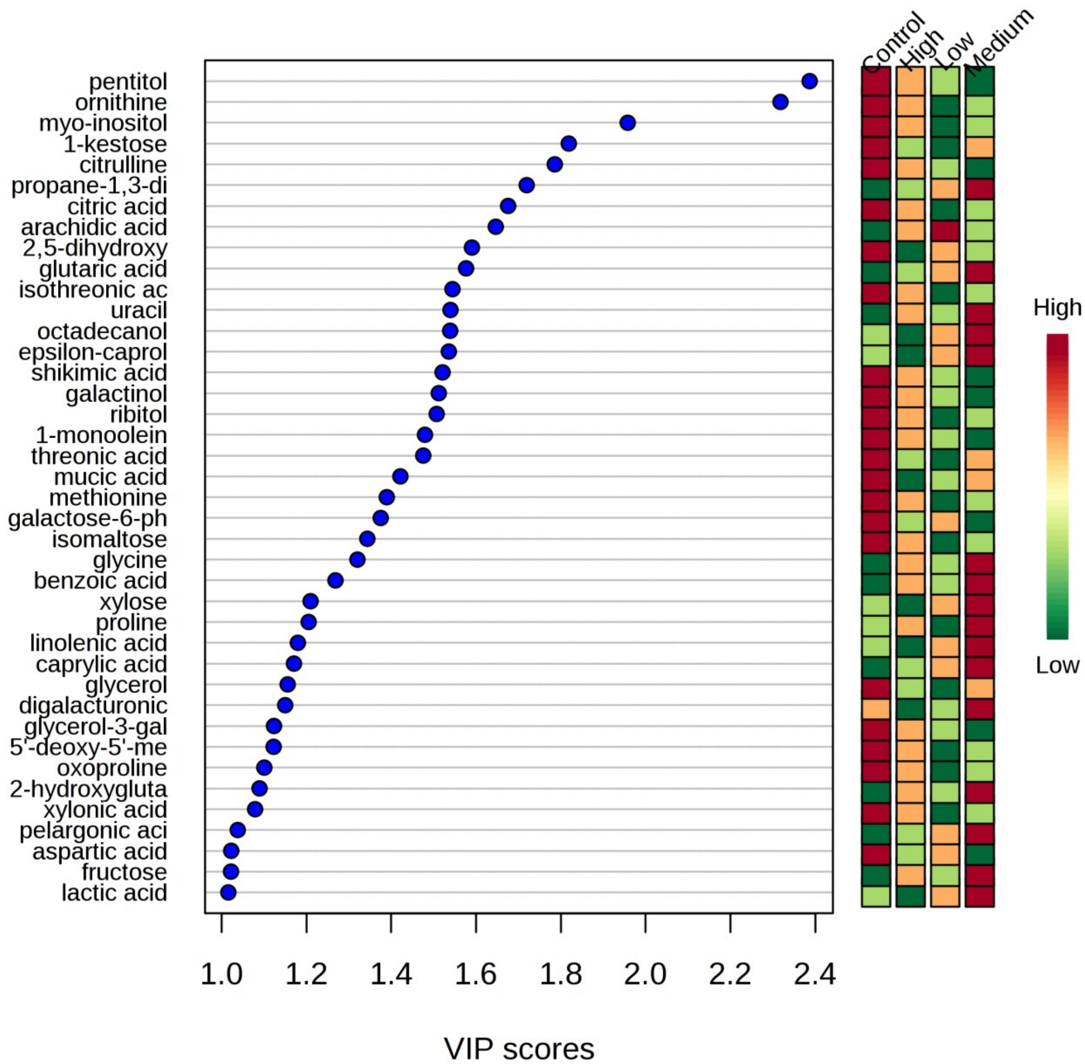

Figure S5. VIP scores from PLS-DA analysis showing how the discriminating metabolites induced the group separation.