Supporting information for

Time-dependent Responses of Earthworms to Soil Contaminated with Low Levels of Lead as Detected using ^{1}H NMR Metabolomics

Ronggui Tang[a, d], Changfeng Ding[a], Yibing Ma[b], Junsong Wang[c], Taolin Zhang[a], XingxiangWang[a*]

a. Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, People's Republic of China

b. Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, People's Republic of China

c. Center for Molecular Metabolism, School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, 210014, People's Republic of China

d. University of the Chinese Academy of Sciences, Beijing, 100049, People's Republic of China

* Corresponding author. Prof. Xingxiang Wang. E-mail address: xxwang@issas.ac.cn, Tel: +86-02586881200.

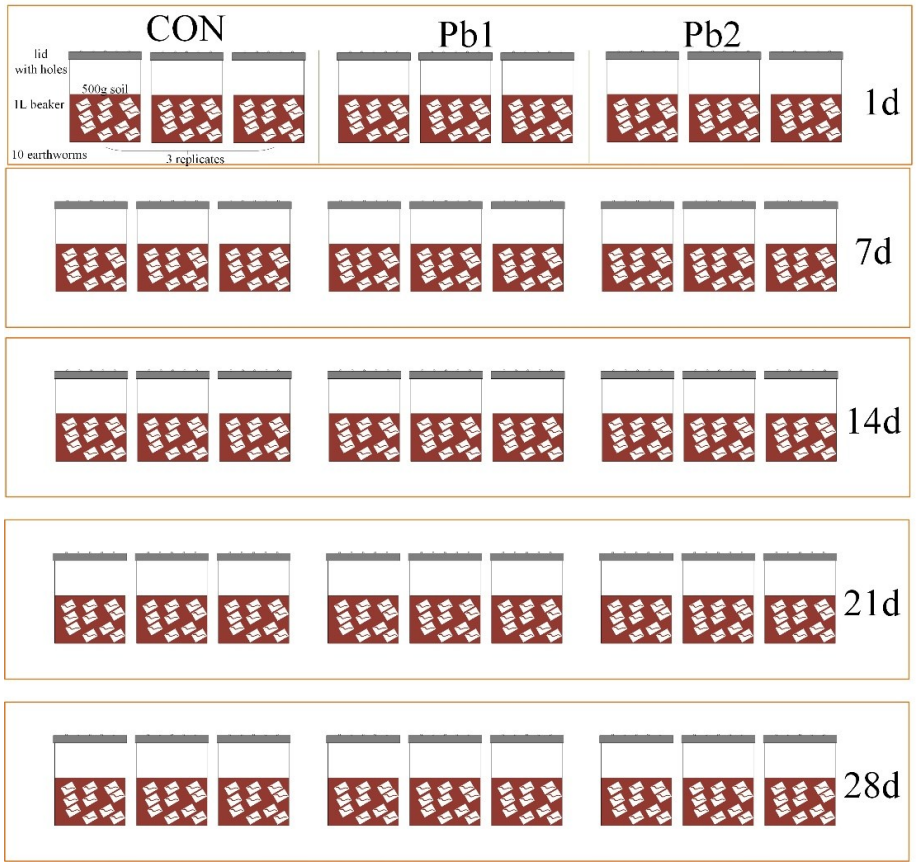


Figure S1. The detailed experiment design of this work

Table S1. Basic physical and chemical properties of the exposed soil

Soil type	pH	Pb concentrations ($mg.kg^{-1}$)	OC ($g.kg^{-1}$)	CEC ($cmol.kg^{-1}$)	Particles(SI)		
					Sand	Silt	Clay
Ferrosols	4.84	23.2±0.5	5.43	9.31	32.3	21.9	45.8

Table S2. The univariate analysis of groups and time to weight of earthworms

Tests of Between-Subjects Effects

Dependent Variable: Weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.093[a]	17	.005	54.441	.000
Intercept	12.355	1	12.355	123553.500	.000
Time	.035	5	.007	69.500	.000
Groups	.047	2	.024	235.500	.000
Time * Groups	.011	10	.001	10.700	.000
Error	.004	36	.000		
Total	12.452	54			
Corrected Total	.096	53			

a. R Squared = .963 (Adjusted R Squared = .945)

Table S3. Metabolites identified from the polar tissue extracts of earthworms.

NO.	Metabolites	Assignments	Chemical shift (ppm)
1	HEFS		0.85(t), 1.19(t),1.28(m), 1.30(m),1.64(q),2.60(m),2.83(t),6.19(s)
2	Isoleucine	9’CH_3	0.99(d)
3	Leucine	8’CH_3, 9’ CH_3	0.95(t)
4	Valine	7’ CH_3	1.03(d)
5	Lactate	3’CH_3	1.32(d)
6	Alanine	6’ CH_3	1.48(d)
7	Acetate	4’ CH_3	1.91(s)
8	Acetylcholine	8’CH_3	2.15(s)
9	Glutamate	6’CH_2,7’CH_2	2.04(m),2.34(m)
10	Asparagine	6’CH_2	2.94(m)
11	Pyruvate	6’CH_3	2.46(s)
12	Glutamine	6’CH_2,7’CH_2	2.13(m),2.45(m)
13	Succinate	4’CH_2,5’CH_2	2.39(s)
14	Malate	5’CH_2	2.66(dd)
15	Dimethylamine	2’CH_3,3’CH_3	2.72(s)
16	Dimethylglycine	6’CH_3,7’CH_3	2.91(s)
17	Lysine	8’CH_2, 9’ CH_2	1.71(m), 3.02(t)
18	GABA-betaine	4’CH_2	3.12(s)
19	Choline	5’CH_3,6’CH_3,7’CH_3	3.19(s)
20	Glycerophosphocholine	5’CH_3,6’CH_3,13’CH_3	3.20(s)
21	Betaine	3’CH_2, 5’CH_3, 7’CH_3, 8’CH_3	3.25(s),3.89 (s)
22	Myo-Inositol	2’CH, 6’CH, 3’CH, 5’CH, 1’CH	3.52(dd),3.61(t),4.05(t)
23	Scyllo-Inositol		3.35(s)

24	Glycine	4’CH_2	3.54(s)
25	Lombricine		3.48(t), 4.26(dd)
26	Glucose	2’CH	5.22(d)
27	Maltose	2’CH	5.40(d)
28	Uridine	2’CH,10’CH	5.88(d),5.90(d)
29	Inosine	2’CH	6.06(d)
30	ADP/AMP	2’CH,12’CH,7’CH	6.13(d),8.24(s),8.53(s)
31	Fumarate	4’CH,5’CH	6.51(s)
32	Tyrosine	2’CH, 6’CH, 3’CH, 5’CH	6.88(m),7.17(m)
33	Adenosine	2’CH, 7’CH	6.02(d),8.28(s)
34	N,N-dimethylhistidine		7.08(s)
35	Phenylalanine	(1-6)’CH	7.32(d),7.36(m),7.42(m)
36	Niacinamide	5’CH, 6’CH, 2’CH	7.58(dd),8.70(dd),8.92(s)
37	τ-Methylhistidine	2’CH	7.67(s)
38	Xanthine	7’CH	7.87(s)
39	Histidine	5’CH, 2’CH	7.09(d),7.90(d)
40	UDP-glucose	32’CH	7.95(d)
41	NAD+/ NADP+		8.21(m),8.41(s),8.84(d),9.15(d),9.33(s)
42	ATP	2’CH,12’CH, 7’CH	6.10(d), 8.23(s), 8.58(s)
43	NADH /NADPH		8.24(s), 8.46(s)

Multiplicity: (s) singlet, (d) doublet, (t) triplet, (q) quartets, (m) multiplets, (dd) doublet of doublets.

1 2-hexyl-5-ethyl-3-furansulfonate (HEFS); 2 Isoleucine(Ile); 3 Leucine (Leu); 4 valine (Val); 5 Lactate(Lac); 6 Alanine(Ala) 7 Acetate (Ace); 8 O-Acetylcholine (OAce); 9 Glutamate (Gln); 10 Asparagine (Asp); 11 Pyruvate (Pyr) 12 Glutamine (Glu);13 Succinate (Suc); 14 Malate (Mal); 15 Dimethylamine (DMA); 16 N,N-Dimethylglycine (DMG);17 Lysine (Lys); 18 GABA-betaine; 19 Choline(Cho); 20 Glycerophosphocholine (GPC); 21 Betaine (Bet) = glycine-betaine; 22 Myo-Inositol

(MI); 23 Scyllo-Inositol (SIno); 24 Glycine (Gly); 25 Lombricine (Lom); 26 Glucose；27 Maltose；28 Uridine(Uri); 29 Inosine (Ino); 30 Adenosine Diphosphate (ADP)/ Adenosine monophosphate (AMP); 31 Fumarate (Fum); 32 Tyrosine (Tyr) ; 33 Adenosine (Ade), 34 N,N-dimethylhistidine (DTH)；35 Phenylalanie (Phe); 36 Niacinamide (Nia); 37τ-Methlhistidine (MHis); 38 Xanthine (Xan); 39 Histidine (His); 40 UDP-glucose (UDG-glu); 41 Nicotinamide Adenine Dinucleotide (NAD^+)/ Nicotinamide adenine dinucleotide phosphate ($NADP^+$); 42 Adenosine Triphosphate (ATP); 43 Nicotinamide adenine dinucleotide (NADH)/Nicotinamide adenine dinucleotide phosphate (NADPH).

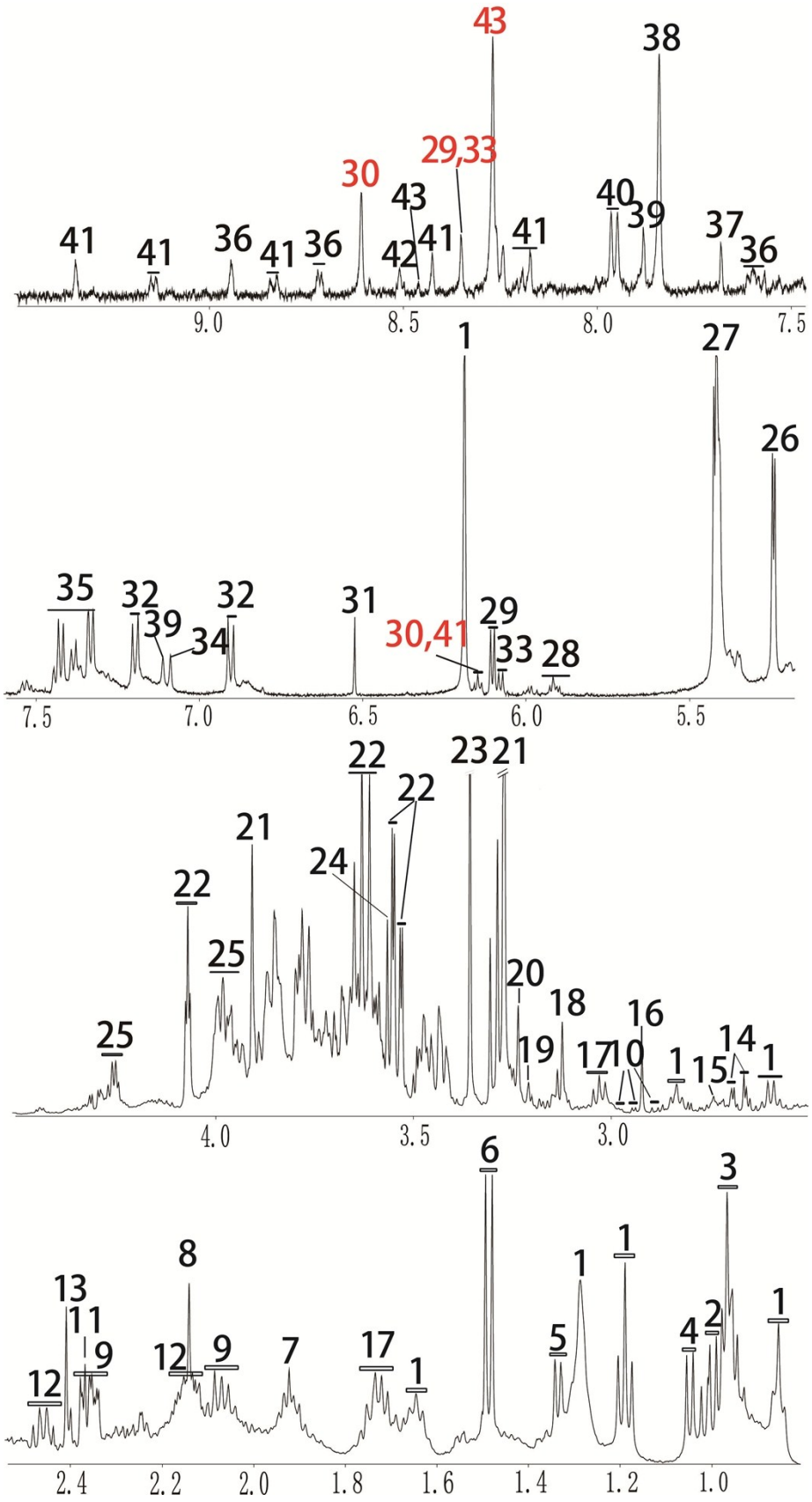
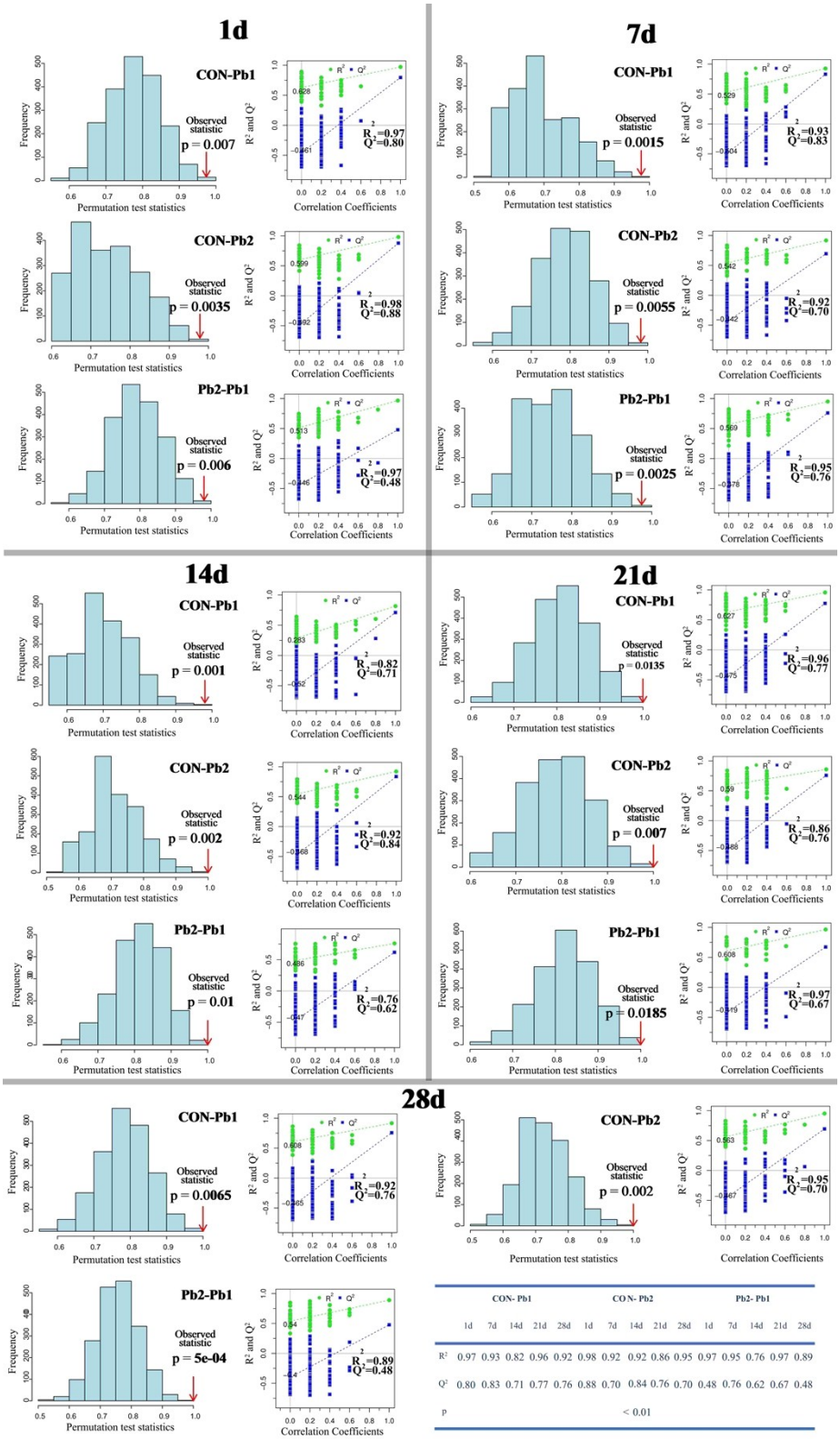


Figure S2. The identification of earthworm metabolites. The red numbers stand for multiple metabolites.



	CON- Pb1					CON- Pb2					Pb2- Pb1				
	1d	7d	14d	21d	28d	1d	7d	14d	21d	28d	1d	7d	14d	21d	28d
R^2	0.97	0.93	0.82	0.96	0.92	0.98	0.92	0.92	0.86	0.95	0.97	0.95	0.76	0.97	0.89
Q^2	0.80	0.83	0.71	0.77	0.76	0.88	0.70	0.84	0.76	0.70	0.48	0.76	0.62	0.67	0.48
p	< 0.01														

Figure S3. The Histograms for 2000 times permutation tests and the R^2 and Q^2 parameters of a repeated twofold cross-validation of OSC-PLS-DA models in different groups. The red arrows

indicated the performance based on the original labels, significant for a p-value less than 0.05. R^2 and Q^2 values were in vertical axis, whereas the correlation coefficients between the permuted and true class in the horizontal axis.

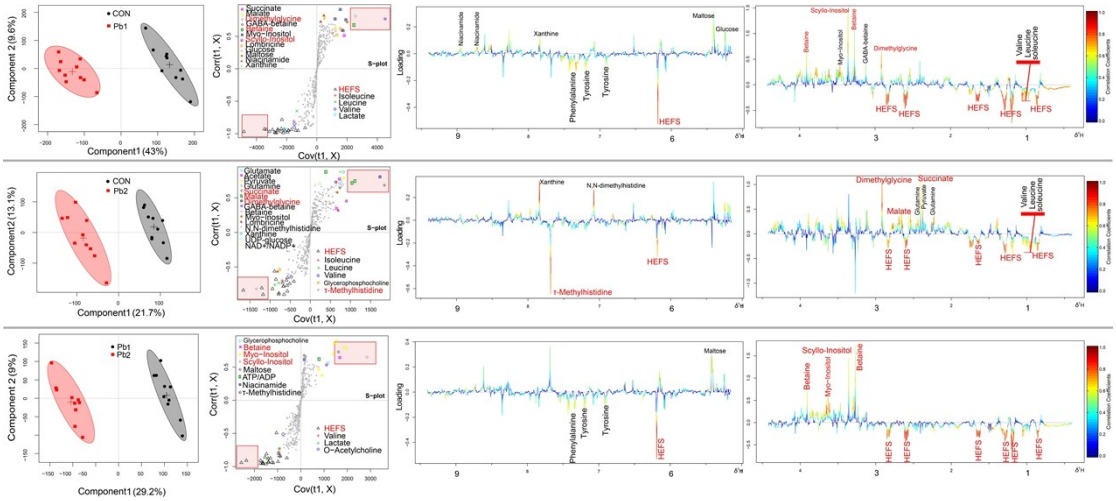


Figure S4. The score plots, s-plot and loading plots in different groups (CON-Pb1, CON-Pb2 and Pb1-Pb2) on the 7th day. Score plot where one point represented one sample and one ellipse corresponded to a confidence interval of 95% stood for a grouping. S-plot where points represented different variables (metabolites). Loading plots (0.4-4.42and 5.15-9.4 ppm) color coded according to the correlation coefficients from blue to red. The color bar corresponds to the weight of the corresponding variable in the discrimination of statistically significant (Red) or no significant (Blue). Positive and negative peaks indicated a relatively decreased and increased metabolite level in the Pb (Pb1 or Pb2) exposed groups.

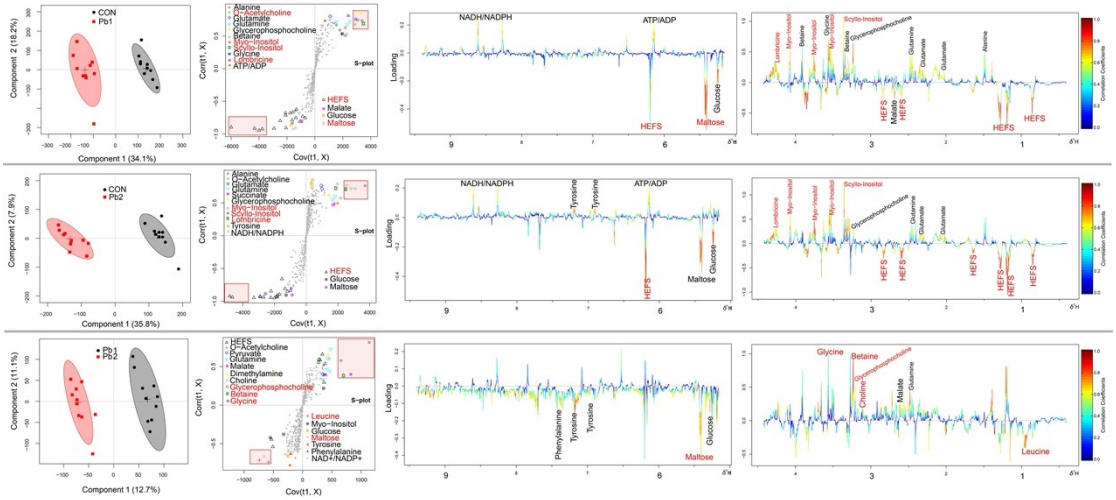


Figure S5. The score plots, s-plot and loading plots in different groups (CON-Pb1, CON-Pb2 and Pb1-Pb2) on the 14th day. Score plot where one point represented one sample and one ellipse corresponded to a confidence interval of 95% stood for a grouping. S-plot where points represented different variables (metabolites). Loading plots (0.4-4.42and 5.15-9.4 ppm) color coded according to the correlation coefficients from blue to red. The color bar corresponds to the weight of the corresponding variable in the discrimination of statistically significant (Red) or no significant (Blue). Positive and negative peaks indicated a relatively decreased and increased metabolite level

in the Pb (Pb1 or Pb2) exposed groups.

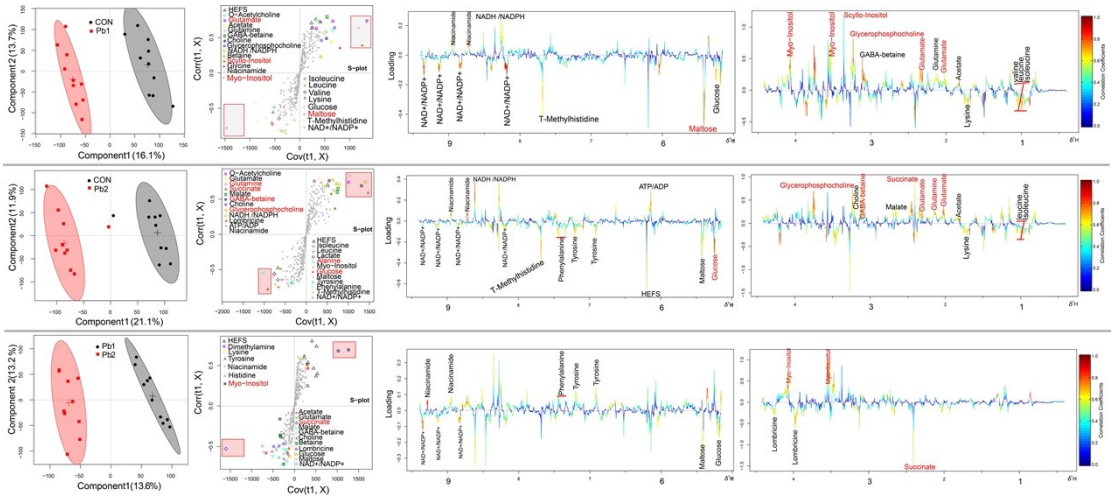


Figure S6. The score plots, s-plot and loading plots in different groups (CON-Pb1, CON-Pb2 and Pb1-Pb2) on the 21st day. Score plot where one point represented one sample and one ellipse corresponded to a confidence interval of 95% stood for a grouping. S-plot where points represented different variables (metabolites). Loading plots (0.4-4.42and 5.15-9.4 ppm) color coded according to the correlation coefficients from blue to red. The color bar corresponds to the weight of the corresponding variable in the discrimination of statistically significant (Red) or no significant (Blue). Positive and negative peaks indicated a relatively decreased and increased metabolite level in the Pb (Pb1 or Pb2) exposed groups.

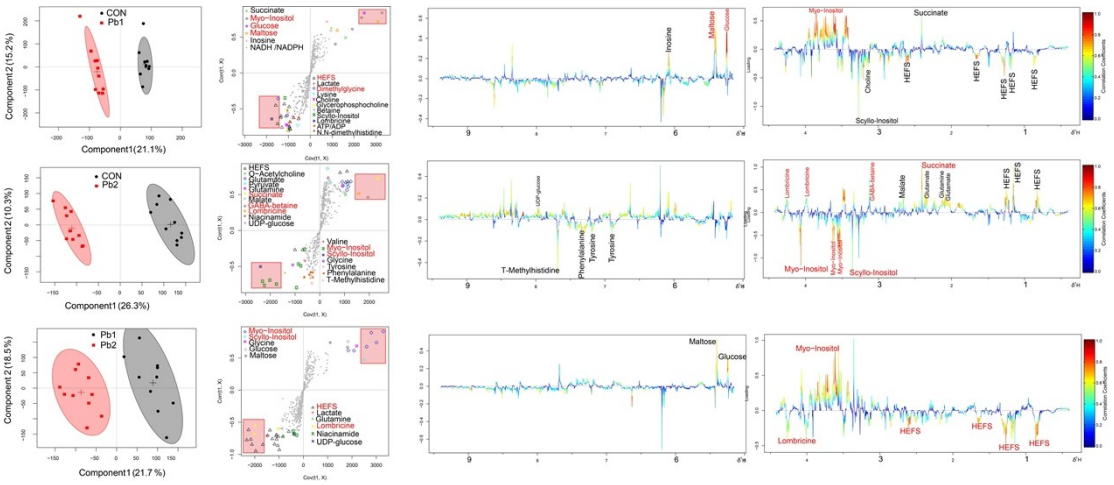


Figure S7. The score plots, s-plot and loading plots in different groups (CON-Pb1, CON-Pb2 and Pb1-Pb2) on the 28th day. Score plot where one point represented one sample and one ellipse corresponded to a confidence interval of 95% stood for a grouping. S-plot where points represented different variables (metabolites). Loading plots (0.4-4.42and 5.15-9.4 ppm) color coded according to the correlation coefficients from blue to red. The color bar corresponds to the weight of the corresponding variable in the discrimination of statistically significant (Red) or no significant (Blue). Positive and negative peaks indicated a relatively decreased and increased metabolite level in the Pb (Pb1 or Pb2) exposed groups.

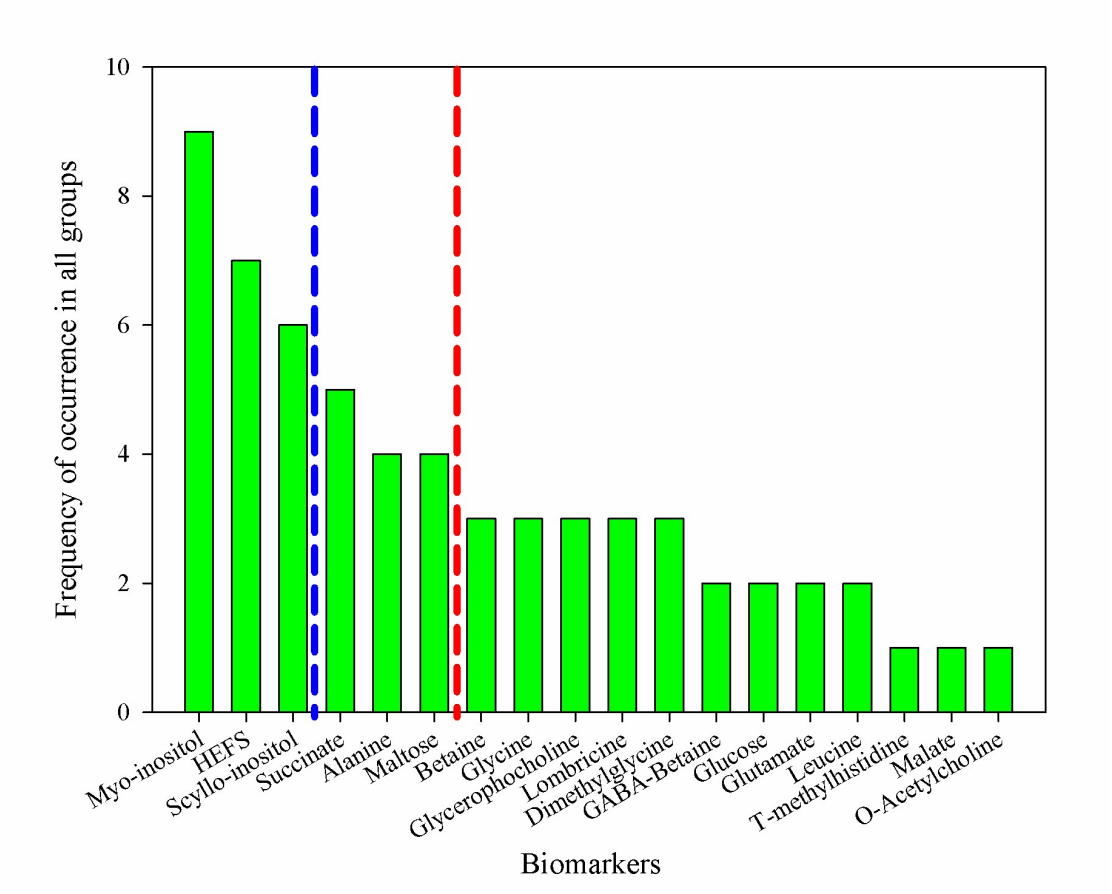


Figure S8. The frequency of occurrence of the biomarkers in all groups.

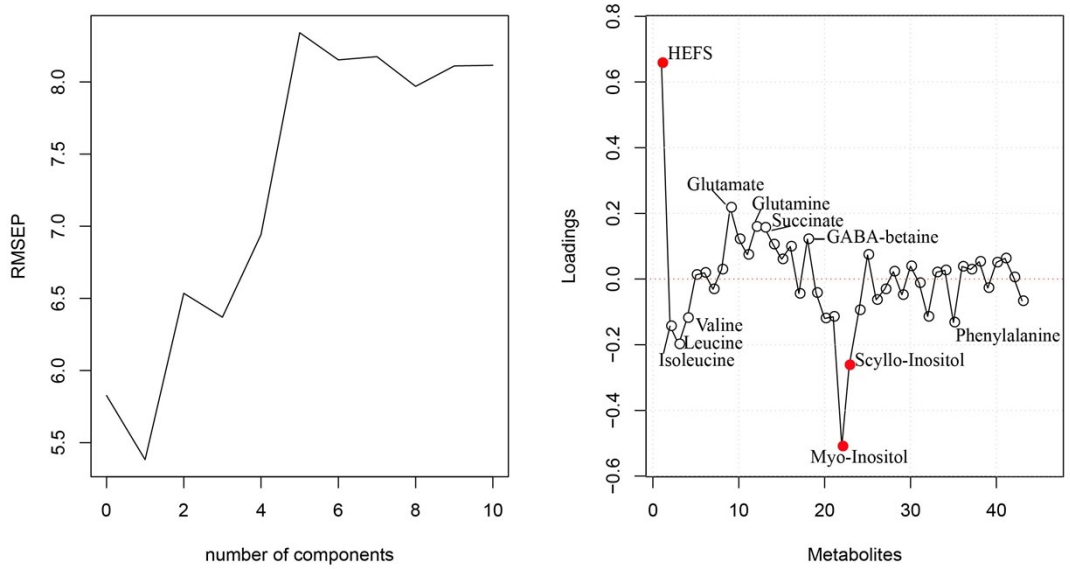


Figure S9. Validation plot for PLS regression with metabolites as X variables and Pb concentrations as Y variable (Left); PLS loadings for the first latent variables (right). The red circles stand for strong correlation between Pb concentration and metabolites of earthworms.