

Supplementary material: Effects of *Lagarosiphon major* extracts on the metabolome and photosynthesis of *Microcystis aeruginosa*

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1. Material and methods

1.1. Sampling and samples preparation

Briefly, 10 mg of freeze-dried were added to a 2 mL microtube containing 150 mg of 0.5 mm silica beads. After the addition of 1 mL MeOH/MTBE (3:1; v:v) and 650 μ L of UPW:MeOH (3:1, v/v), the microtubes were homogenized with a Fast Prep 5G (3 homogenization cycles at 15 s at 6 m/s) and then centrifugated at 4 °C at 12000 RPM during 5 min in order to separate the two phases. Thus, 600 μ L of the lipophilic phase (upper phase) and 500 μ L of the hydrophilic phases were collected in amber glass vials. Then a second round of extraction was performed according the same conditions. In total, 1.1 mL of lipophilic

and 1.3 mL of hydrophilic were collected. In parallel of the sample, solvent alone (procedural blank) were also extracted. Following the extraction, 500 μ L of both the hydrophilic and lipophilic stock solutions were evaporated using rotary evaporator (Speed-Vac, ThermoScientific) and gentle nitrogen stream respectively. They were then resuspended in adequate solvent: 100 μ L of ACN/UW (50:50, v:v) for hydrophilic extract and 100 μ L of ACN/ISO (50:50, v:v) for lipophilic extract. For both extracts, a pool of 5 μ L of all the extracts were prepared as pool QC for further intensity and retention time drift correction (see part X).

In parallel to the cyanobacteria extracts, samples from LS extracts aging experiment were directly freeze-dried and resuspended in ACN/H20 in order to get an equivalent concentration to the exposure experiment.

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1.2. UPLC-TOF HRMS analyses

1.2.1. Chromatographic separation

For the chromatography, 2 different gradients were used:

For the hydrophilic gradient, the mobile phases consisted of ultrapure water supplemented with 0.1% formic acid (A), acetonitrile supplemented with 0.1% formic acid (B), and a mixture of acetonitrile and isopropanol (50:50, v:v) (Table S1a).

Supplementary Table S1. Detailed description of the gradient programs used in the chromatographic
method for the analysis of the hydrophilic (a) and the lipophilic fraction(b), respectively

(a) Hydrophilic gradient (A: UW with 0.1% formic acid,				
B: ACN an	d 0.1% formic acid; C:	ACN/IS	0, 50:50	, v:v)
Time (min)	Flow rate (mL/min)	A (%)	B (%)	C (%)
0.00	0.4	99	1	0
4.00	0.4	99	1	0
16.00	0.4	1	99	0
20.00	0.4	1	99	0
20.01	0.2	1	99	0
22.00	0.2	0	0	100
23.00	0.2	0	0	100
24.00	0.2	0	100	0
25.00	0.3	0	100	0
26.00	0.3	99	1	0
27.00	0.4	99	1	0
30.00	0.4	99	1	0

(b) Lipophilic gradient (A: ACN/UW with 0.1				
formic acid	l; B ACN/ISOP with 0.1	l formic	acid)	
Time (min)	Flow rate (mL/min)	A (%)	B (%)	
0.00	0.3	70	30	
5.0	0.3	55	45	
14.0	0.3	30	70	
21.0	0.3	1.0	99	
24.0	0.3	1.0	99	
24.10	0.3	70.0	30	
30.00	0.3	70.0	30	

1.2.2. HRMS acquisition

UPLC-TOF. Details on MSe acquisition parameters are provided in Table S2.

Supplementary Table S2. Sample and lockspray sources parameters

		Parameters	ESI+	ESI-
		Capillary (kV)	0.7 kV	0.5 kV
	Source	Sampling cone	30 V	30 V
Commission of the second se		Source offset	80 V	80 V
Sample source	Temperature	Source (°C) Desolvation (°C)	100 °C 550 °C	100 °С 550 °С
	Gas flow	Cone gas (L/h) Desolvation gaz (L/h)	50 L/h 1000 L/h	50 L/h 1000 L/h
Lockspray source		Lockspray capill	3 kV	2.1 kV
		Collision	20 V	29 V

UPLC-QExactive+. Since the match between MSe mass spectra and in house library was quite low, additional injections of sample fractions were per-

formed on UPLC-Qexactive+ instrument in order to get actual MS2 spectra. Details on source parameters are provided in Table S3.

Parameters	ES	[+	ESI-	
	Full scan	MS/MS	Full scan	MS/MS
Scan range	75–1500	-	75–1500	-
Resolution (FWHM)	70,000	17,500	70,000	17,500
Automated gain control (AGC)	500,000	500,000	500,000	500,000
Spray voltage (kV)	4	4	4	4
Sheath gas flow rate (μA)	40	40	40	40
Capillary temperature (°C)	350	350	350	350
NCE	-	50	-	50
Loop count	-	2	-	2

Supplementary Table S3. Orbitrap-Qexactive parameters (DDA top5 mode)

1.2.3. Data processing (preprocessing and filtration)

At a first step, all HRMS data acquired with the UPLC-TOF were processed by using W4M (Giacomoni *et al.* 2017), as previously reported in Creusot *et al.* 2021 with small modifications. Preprocessing step and parameters step are detailed below.

W4M. The FUNC003 (LockMass signal) was suppressed from the raw data prior to data conversion into mzML files by using MS-Convert (ProteoWizard) based on CWT algorithm in Peak-Picking filter. mzML files were pre-processed by using W4M (https://workflow4metabolomics.usegalaxy.fr/) based on XCMS scripts as described in Table S4.

	method	centWave			
	ppm	15			
	peakwidth	c(5, 25)			
	snthresh	10			
	mzdiff	0.01			
	integrate	2			
	prefilter	c(3, 1000)			
XcmsSet	noise	0			
	<i>a</i> .		CUDl		
	fitgauss	FALSE	тпреакs	method	Chrom
	scanrange	numeric()		mzabs	0.015
	sleep	0		ppm	5
				calceas	FALSE
	verbose.column	FALSE		calcuis	TRUE
	method	density		calciso	FALSE
	mzwid	0.1 then 0.05		cor_eic_th	0.75
	minfrac	0.5		fc_th	NULL
Group	bw	5 then 2		graphivietod	"ncs"
(2 times)	ignore sample			intval	"into"
	class			max_peaks	100
	minsamp	1	CANAEDA	maxcharge	3
	max	50	CAIVIERA	maxiso	4
	sleep	0	annotate	mintrac	0.5
	method	peakgroups		multiplier	3
	smooth	c("loess")		nsiaves	1
	extra	1		perrwnm	U.6
	missing	0.9		polarity	positive / negative
- .	family	c("gaussian", "symmetric")		psg_list	NULL
Retcor	span	0.2		pval	0.05
				pval_th	NULL
	col	NULL		quick	FALSE
	plottype	c("none", "deviation",		rules	NULL
		"mdevden")		sample	NA
	ty	NULL		sigma	6

Supplementary Table S4. W4M pre-processing parameters

The obtained peak list were further filtrated for missing signal (NA > 75%), corrected for missing values (NA replaced by the minimal value of the entire dataset divided per 5), corrected for signal drift using all loess function based on OC samples. Features with CV above 30% between QC samples and with an intensity in the sample lower than in QC samples were removed from the dataset. The features were then filtrated against the procedural blank signal and injection blanks (i.e. only features with Sample/Blanks > 10 were conserved). The intensity of the features was then normalized by using the cyanobacteria dry weigh. The peak lists were then filtrated against the correlated signals at similar retention times by using between-table correlation and analytical correlation filtration functions. Finally, for hydrophilic datasets of the endometabolome, only the features not found in the macrophyte extract were kept. The processing allowed to get four datasets according to the fraction (i.e. hydrophilic vs lipophilic) and ionization mode (i.e. ESI+ vs ESI-) that were used to implement chemometrics analyses.

DROMICs. DROMICS tool (Shiny version, https:// lbbe.univ-lyon1.fr/fr/dromics, Larras *et al.* 2018) was used in order to prioritize the metabolites annotation towards features that follow a quadratic trend (increase, decrease, U-shape, Bell) with increasing concentration of the macrophyte extract, according to five regression models (linear, hill, exponential, gauss-probit, log-Gauss-probit). This tool allows to encompass both monophasic (increase, decrease) and biphasic (U-shape, Bell) trends of each feature and further determination of benchmark dose (BMD) summarized as empirical cumulative distribution function (ECDF).

- **Step 1:** the data were normalized (median), transformed (cube root) and scaled (Pareto).
- **Step 2:** the relevant features (i.e. those displaying a significant change along the concentration gradient) were identified using a quadratic trend test with a False Discovery Rate (Benjamin-Hochberg test) of 0.001 because of the high number of metabolites, as suggested by Larras *et al.* (2018).

- **Step 3:** concentration response modelling was performed for each of the selected features based on nominal concentrations. All these curves were manually curated to avoid further miscalculation and false modelling. In particular for U- and bell-shaped curves, the features for which the modelled curves did not fit the experimental values were removed from the dataset before reimplementation of steps 1–3.
- **Step 4:** the model with the best fit was used to derive a benchmark dose (BMD-_{1SD}) for each

feature, as recommended by EFSA (Committee 2017). The BMD_{1SD} values are the concentration corresponding to a Benchmark Response (BMR-*z*SD) defined as follows: BMR-*z*SD = $y_0 + l - z *$ SD, where y_0 is the mean control response, and SD is the residual standard deviation of the considered concentration-response model and *z* is the SD factor (*z* fixed at 1 by default). These BMD values give an indication of the concentration leading to a significant level of change compared to the control (non-exposed in case of concentration-response).

MS-DIAL (v4.9.221218).

	TOF	Qex
Data collection	MS1: 0.015	MS1: 0.01
	MS2: 0.05	MS2: 0.05
	Advanced	Advanced
	Isotope:	Isotope:
	Max: 2	Max: 2
	Cl&Br: checked	Cl&Br: checked
Peak detection	Peak heigt: 5000	Peak heigt: 25000
	Mass slice width: 0.05	Mass slice width: 0.1
	Advanced	Advanced
	Linear weighted moving average	Linear weighted moving average
	Smoothing: 3	Smoothing: 3
	Minimum peak width: 5	Minimum peak width: 5
MS2Dec	Sigma 0.5	Sigma 0.5
	MS/MS 10	MS/MS 10
	Advanced	Advanced
	Exclude after: checked	Exclude after: checked
	Keep the isotopic: 3 Da	Keep the isotopic: 3 Da
	Keep the isotopic ions w/o: checked	Keep the isotopic ions w/o: checked
Identification	MSP file	MSP file
	Rt: 100 min	Rt: 100 min
	MS1 0.01 Da	MS1 0.01 Da
	MS2 0.05 Da	MS2 0.05 Da
	Cut off: 80%	Cut off: 80%
	Advanced	Advanced
	Rt: 2 min	Rt: 2 min
	Accurate mass: 0.015	Accurate mass: 0.015
	Score cut off: 85%	Score cut off: 85%
	Relative abundance: 0%	Relative abundance: 0%

Supplementary Table S5. MS-Dial parameters

(continued on next page)

	TOF	Qex
Adduct	[M+H]+, [M+Na+], [M+K]+, [M+Li]+,	[M+H]+, [M+Na+], [M+K]+, [M+Li]+,
	$[M+ACN+H]+, [M+H-H_20]+,$	$[M+ACN+H]+, [M+H-H_20]+,$
	$[M+H2H_20]+, [M+ACN+Na]+, [2M+H]+,$	$[M+H2H_20]+, [M+ACN+Na]+, [2M+H]+,$
	[2M+Na]+, [2M+K]+, [2M+ACN+H]+,	[2M+Na]+, [2M+K]+, [2M+ACN+H]+,
	[2M+CAN+Na]+	[2M+CAN+Na]+
Alignment	Rt: 0.1 mn	Rt: 0.1 mn
	MS1: 0.025	MS1: 0.025
	Advanced:	Advanced:
	Rt factor 0.1	Rt factor 0.1
	MS1 factor 0.5	MS1 factor 0.5
	Peak 0%	Peak 0%
	N%: 0%	N%: 0%
	Remove feature: checked	Remove feature: checked
	Sample max / blank: 10 fold change	Sample max / blank: 10 fold change
	Keep reference matched: checked	Keep reference matched: checked
	Keep suggested (w/o MS): checked	Keep suggested (w/o MS): checked
	Keep removable features: checked	Keep removable features: checked
	Gap filling: checked	Gap filling: checked

Supplementary Table S5. (continued)

MS-Finder (v3.52).

Method Basic Formula finder Structure finder Data source Retention time	e CCS	Method Basic Formula finder Struct	ture finder Data source Retentio	n time CCS	Method Basic Formula finder Struct	ture finder Data source Retention time CCS
Search option		Mass tolerance setting			Formula calculation setting	
		Mass tolerance type:	⊖ Da 💿 ppm		LEWIS and SENIOR check:	v
Spectral database search		Mass tolerance (MS1):		10 +-Da or ppm	Isotopic ratio tolerance:	20 %
\checkmark Formula prediction and structure elucidation by in silico fragmenter		Mass tolerance (MS2):		15 +-Da or ppm	Element ratio check:	Common range (99.7%) v covering
Spectral database option		Abundance setting			Element probability check:	v
Use internal experimental library (MassBank, GNPS, ReSpect)		Relative abundance cut off:		1 %	Element selection	
Use the theoretical MS/MS spectra of lipids: CH3COONH4 ~		Mass range setting			🖌 O 🗌 N 🗌 P 🛄 S	F CI Br I Si
User-defined DR: C:\Users\nicolas.creusot\Documents\Data\Libraries\m	ISD Browse	Mass range max:		1500 Da	TMS-MEOX derivative compound	f
	biotise	Mass range min:		50 Da	Minimum TMS count:	1
Precursor ion option					Minimum MEOX count:	0
Precursor oriented spectral search		Method Basic Formula finder Structu	re finder Data source Retention ti	ime CCS	Options	
		Local Databases			Maximum report number:	10 up to 100
Method Basic Formula finder Structure finder Data source Retention tim	e CCS	HMDB (Human) Urine (H	uman) 🔄 Saliva (Human) 🗌	Feces (Human)	Time out (-1 means infinite):	1 min
In silico MC/MS or EL MS fragmenter setting		Serum (Human) CSF (Hu	man) SMPDB (Human)	LipidMAPS (Lipids)	A drawer of contribution for AVE	
		YMDB (Yeast) ECMDB	(E.coli) BMDB (Bovine)	DrugBank (Drug)	Advanced settings for Air.	Setting
Tree depth:	[1-3]	C Can DB (Can d)	(Direct)	T2D8 (Taxia)		
Use the fragmentation library for electron ionization (EI)		Plantcyc	(Plant) (Chebi (biomolecules)	I I SUB (TOURI)		
		STOFF (Environment) BLEX	P (blood exposome) 🗹 NPA (Nat	ural Products Atlas)		
Use the fragmentation library for low energy CID		✓ NANPDB (Natural product) ✓ C	OCONUT (Natural product)			
Options		KNApSAcK (Natural product)	PubChem (Biomolecules) 🕑 UNPE	0 (Natural product)		
Maximum report number: 100	up to 100	User-defined DB C:\Users\nicola	s.creusot\Documents\Data\Libraries\	Sust Browse		
Time out (-1 means infinite): 1	min	MINEs (Metabolic In silico Network Expa	nsions) setting			
Cut off for structure elucidation: 0	0-10 (total score)	O Never use it. Only use when	there is no query in local DBs. O A	lways use it.		
Cut off for spectral match: 80	0-100 (%)	PubChem Online setting				
		○ Never use it. ④ Only use when	there is no query in local DBs. O A	lways use it.		

Supplementary Figure S1. MS-Finder parameters.

SIRIUS (v5.6.3).

🚮 Compute		×
SIRIUS - Molecular Form	ula Identification	
	General Use DB formulas only Possible forizations ILP Instrument O-TOF Ø Ø ID Database Ø Ø IM + H]+ Tree timeo Filter by isotope pattern Ø Ø COCONUT Ø IM + Naj+ Campound timeo Campound timeo MSMS isotope source SCORE Ø Occonvut Ø IM + Naj+ Use heuristic above m Min candidates sored 10 a Ø Occonvut all none all none	ut 0 2 ut 0 2 ut 0 2 uz 300 2 uz 650 2 uz
SIRIUS	Elements allowed in Molecular Formula H 0 @mmmmmmp inf C 0 @mmmmmmp inf N 0 @mmmmmmp inf	0 0 Opponyoupper inf
	P 0 Propagangang Inf B 0 Propagangang auto SI 0 Propagangang 0 .	S 0 Proprogramming auto
	CI 0 Yununununun auto Se 0 Yunununun auto Br 0 Yununununun auto S	F 0 minimum 0
	Select elements	
ZODIAC - Network-based	improvement of SIRIUS molecular formula ranking	
ZODIAC	General Edge Filters Gibbs Sampling Considered candidates 300m/z 10 Edge Threshold 0.95 er Considered candidates 800m/z 50 Min Local Connections 10 Burn-In 2.000 er Use 2-step approach Separate Runs 10 10 10 10 10	
C SI:FingerID - Fingerprint	Prediction CSt:FingerID - Structure Database Search	
Predict FPs	Fallback Adducts General Search DBs General Ø III+ HI+ Score threshold Ø MI+H20+HI+ Score threshold Ø MI+H20+HI+ Ø Bio Database MI+H20+HI+ Ø CHEBI MI+H20+HI+ Ø CHEBI MI+H20+HI+ Ø CHEBI MI+H20+HI+ Ø COONUT Ø Cooconut Ø Cooconut Ø Cooconut Ø Cooconut	neral TagʻLipids 🗹
CANOPUS - Compound C	lass Prediction	
CANOPUS	Parameter-Free! Nothing to set up here. =)	
Recompute already com	puted tasks?	Show Command Compute Cancel

Supplementary Figure S2. SIRIUS parameters.

2. Results



2.1. Metabolomic response of Microcystis aeruginosa exposed to Lagarosiphon major extracts

Supplementary Figure S3. Metabolomics fingerprint summarized as PCA score plot and HCA heatmap of HydroPOS (a), HydroNeg (b), LipoPOS (c) and LipoNEG (d).



Supplementary Figure S4. ECDF trends (bell, dec, inc, U) of hydrophilic (a, ESI+; b, ESI–) and lipophilic (c, ESI+; d, ESI–) fractions of the metabolome.

Supplementary Table S6. List of annotated metabolites, pathways and classes in the endometabolome (hydrophilic phase) (Tabular file)

Supplementary Table S7. List of annotated metabolites, pathways and classes in the endometabolome (lipophilic phase) (Tabular file)

2.2. Biomolecules in L. major extracts



Supplementary Figure S5. NPC_superclass of the up/down regulated endo-metabolome (both hydrophilic and lipophilic phase).



Supplementary Figure S6. Distribution of significant fold change (between control and 10 mg/L) among the NPC superclass of the annotated putative metabolites for both hydrophilic and lipophilic phases. The fold change values were from VolcanoPlot analyses based on T.test anova (p value < 0.01) following DROMICs processing.

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Supplementary Figure S7. NPC superclass of the aged macrophyte extracts from UPLC-HRMS-analyses. Probability > 0.5, n = 1518.



Supplementary Figure S8. NPC class of the aged macrophyte extracts from UPLC-HRMS-analyses. Probability > 0.5, n = 1054.



Supplementary Figure S9. Weight of evidence elements related to p-coumaric acid annotation, as experimental vs in house databasis (a), formula (b) and structural (c) elucidation from SIRIUS.



Supplementary Figure S10. Weight of evidence elements related to caffeic acid annotation, as experimental vs in house databasis (a), formula (b) and structural (c) elucidation from SIRIUS.



Supplementary Figure S11. Weight of evidence elements related to saikosaponin a annotation, as experimental vs in house databasis (a), formula (b) and structural (c) elucidation from SIRIUS.

Supplementary Table S8. List of annotated metabolites, pathways and classes in the *L. major* extract (Tabular file)