ECOTOXICOLOGIA

AULA TP 7

Planeamento das Aulas

Aula 1 – Introdução aos Testes de Ecotoxicologia. Biomarcadores e Delineamento Experimental

Aula 2 – Efeitos do Glifosato no crescimento dos organismos teste (taxas de inibição e constantes de inibição IC50). Marcadores biofísicos de toxicidade I

Aula 3 – Marcadores biofísicos de toxicidade II

Aula 4 – Marcadores bioquímicos de toxicidade I – Pigmentos vegetais

Aula 5 – Marcadores bioquímicos de toxicidade II – Pigmentos vegetais II

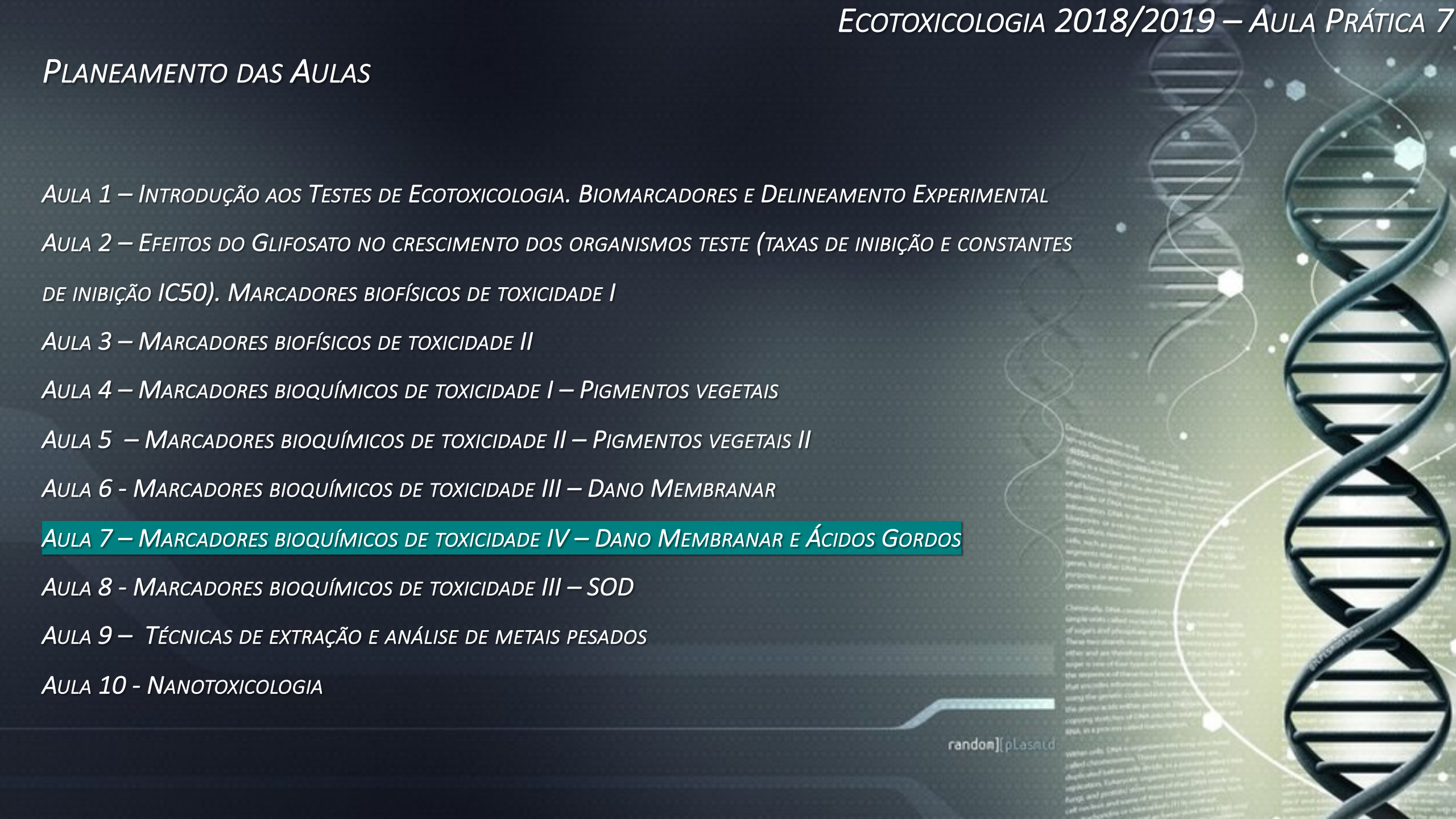
Aula 6 - Marcadores bioquímicos de toxicidade III – Dano Membranar

Aula 7 – Marcadores bioquímicos de toxicidade IV – Dano Membranar e Ácidos Gordos

Aula 8 - Marcadores bioquímicos de toxicidade III – SOD

Aula 9 – Técnicas de extração e análise de metais pesados

Aula 10 - Nanotoxicologia



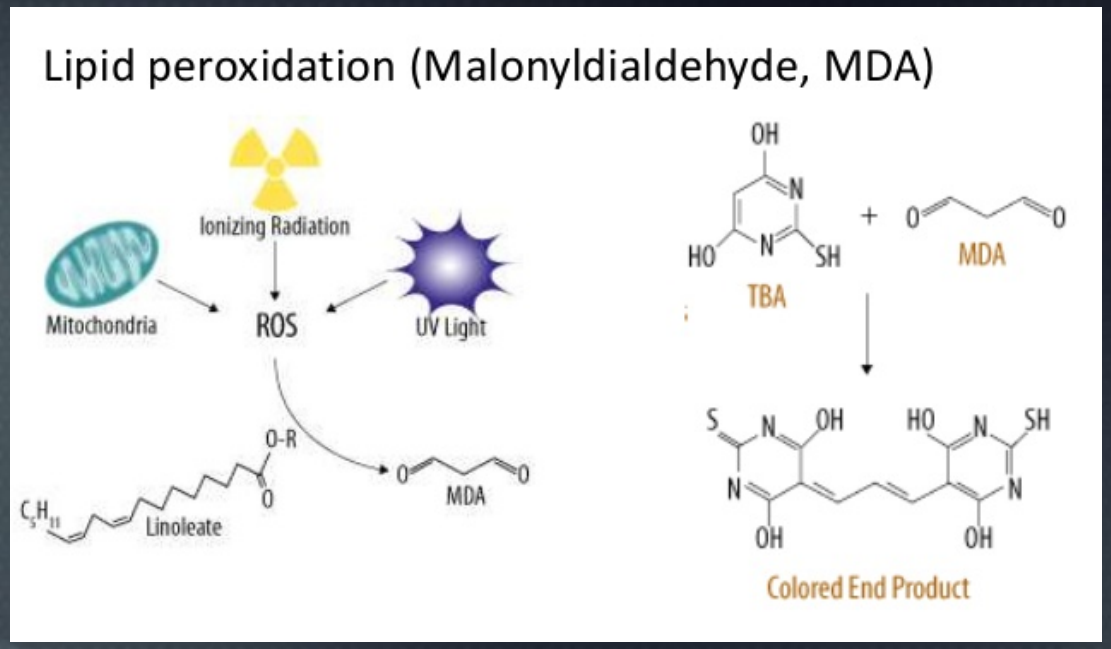
PEROXIDAÇÃO LÍPIDICA

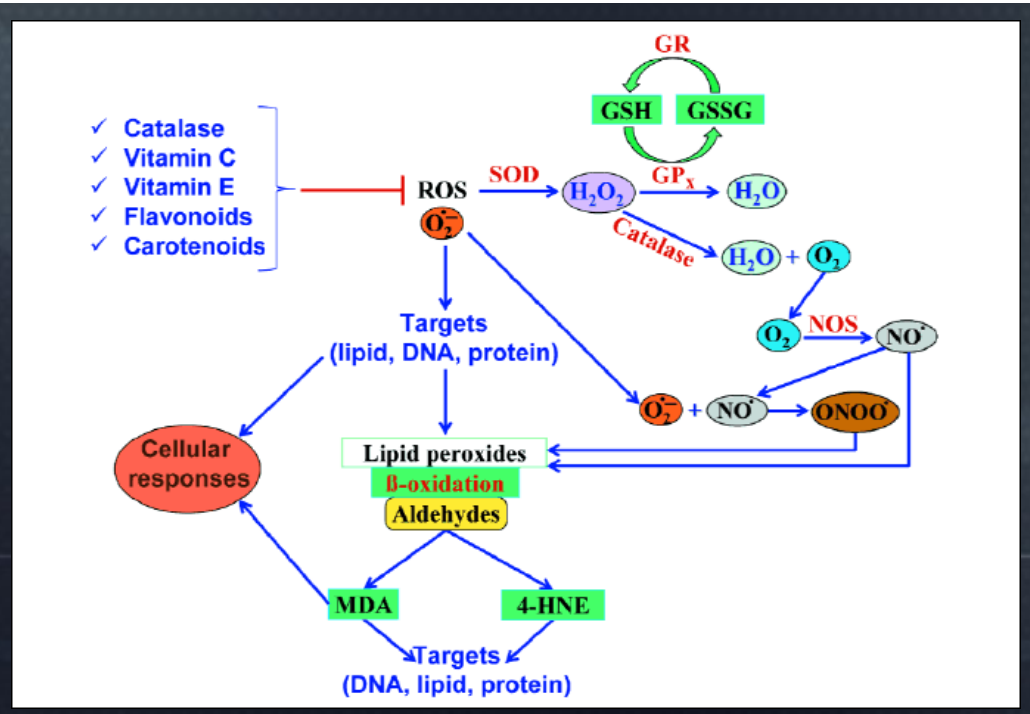
- Homogenizar o material vegetal numa proporção de 1 mL de solução TBA/TCA a cada amostra de *pellet*.
    - TBA 0.5 %
    - TCA 20%

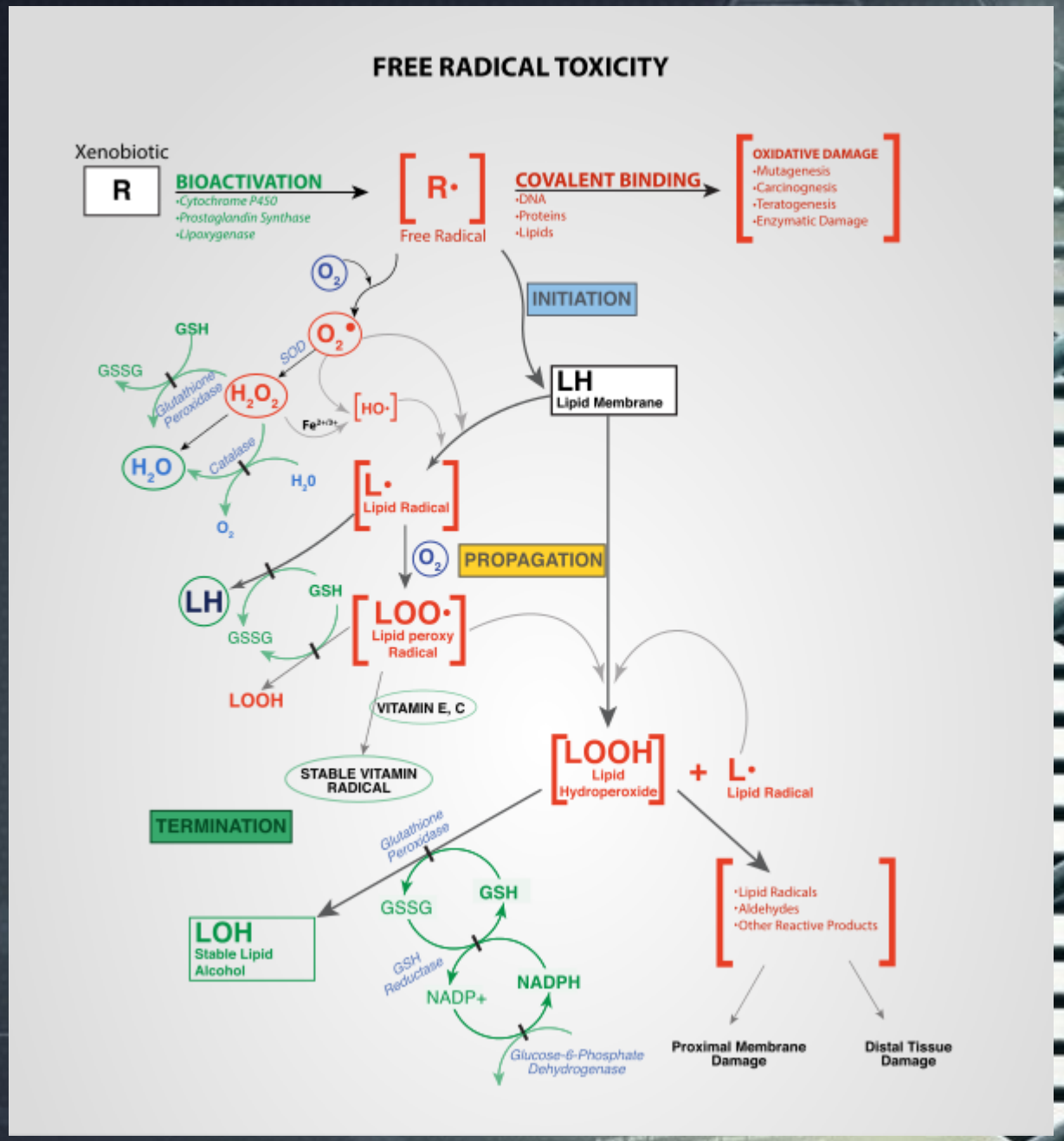
- Colocar o extrato a incubar durante 30 min a 95 ºC.

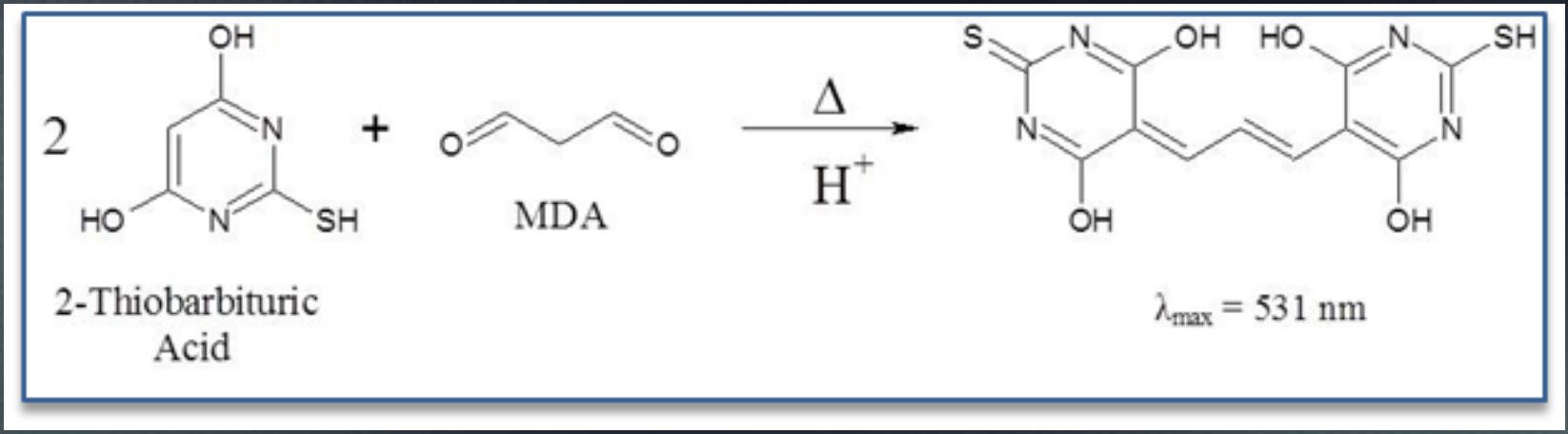
- Ler a absorvância a 532 nm e 600 nm e aplicar a equação (ε [MDA] = 155 mM^{-1} cm^{-1}):

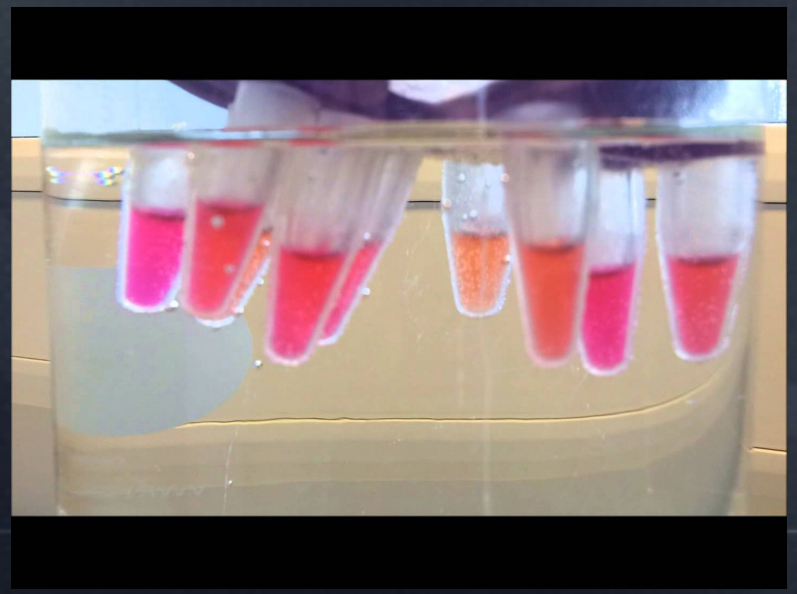
$$A_{532\,nm} - A_{600\,nm} = [MDA]mM \times \varepsilon MDA$$

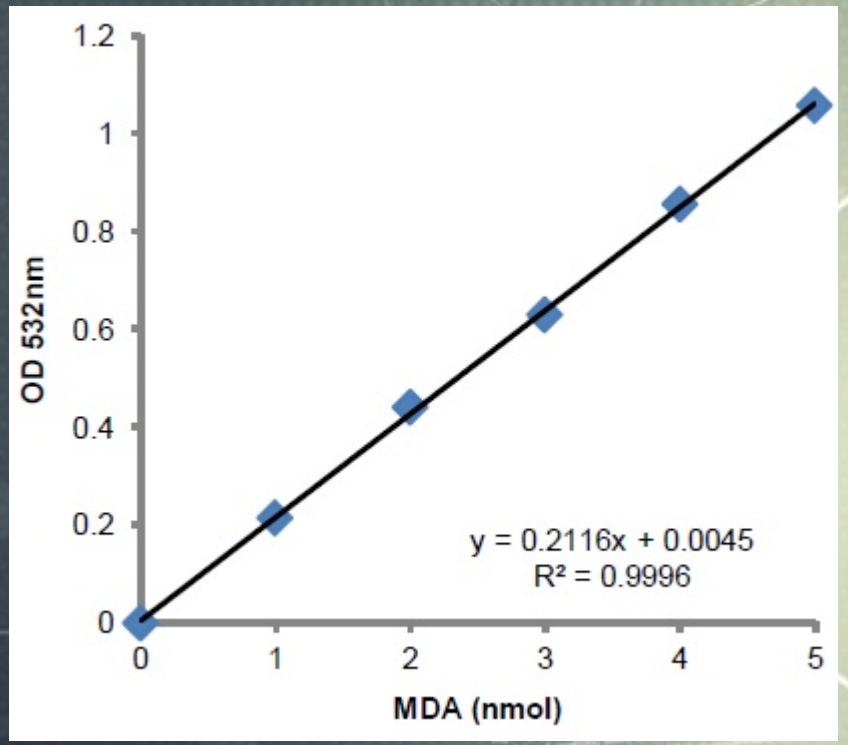

Lipid peroxidation (Malonyldialdehyde, MDA)
Ionizing Radiation
Mitochondria
ROS
UV Light
O-R
Linoleate
MDA
OH
HO
N
SH
TBA
+
MDA
Colored End Product


GR
GSH
GSSG
✓ Catalase
✓ Vitamin C
✓ Vitamin E
✓ Flavonoids
✓ Carotenoids
ROS
SOD
GPx
Catalase
NOS
Targets
(lipid, DNA, protein)
Cellular responses
Lipid peroxides
ß-oxidation
Aldehydes
MDA
4-HNE
Targets
(DNA, lipid, protein)

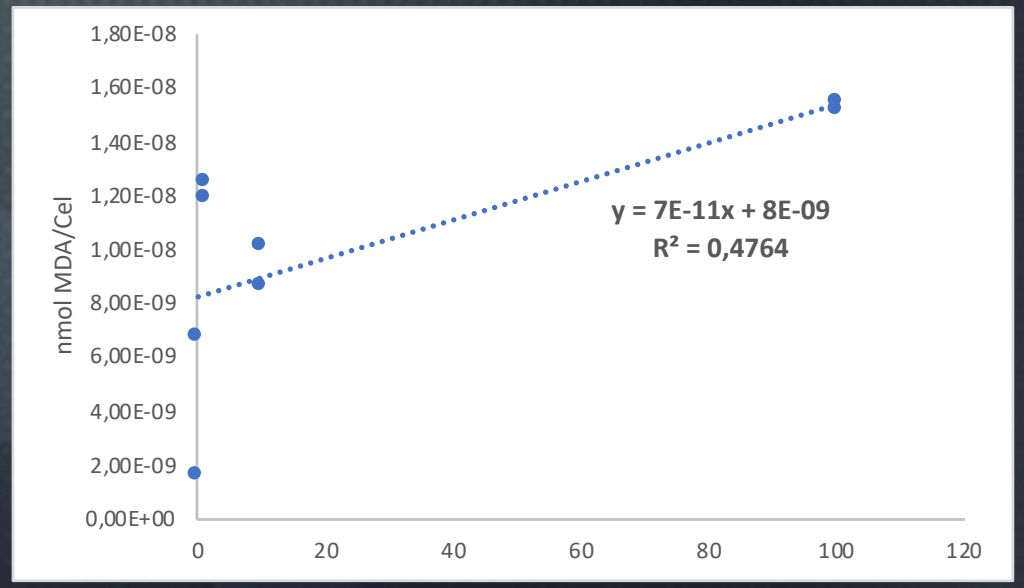

FREE RADICAL TOXICITY
Xenobiotic
R
BIOACTIVATION
•Cytochrome P450
•Prostaglandin Synthase
•Lipoxygenase
R•
Free Radical
COVALENT BINDING
•DNA
•Proteins
•Lipids
OXIDATIVE DAMAGE
•Mutagenesis
•Carcinogenesis
•Teratogenesis
•Enzymatic Damage
INITIATION
LH
Lipid Membrane
GSH
GSSG
Glutathione Peroxidase
SOD
Catalase
L•
Lipid Radical
PROPAGATION
LOO•
Lipid peroxy Radical
LOOH
VITAMIN E, C
STABLE VITAMIN RADICAL
TERMINATION
LOOH
Lipid Hydroperoxide
Lipid Radical
LOH
Stable Lipid Alcohol
GSH Reductase
NADP+
NADPH
Glucose-6-Phosphate Dehydrogenase
•Lipid Radicals
•Aldehydes
•Other Reactive Products
Proximal Membrane Damage
Distal Tissue Damage

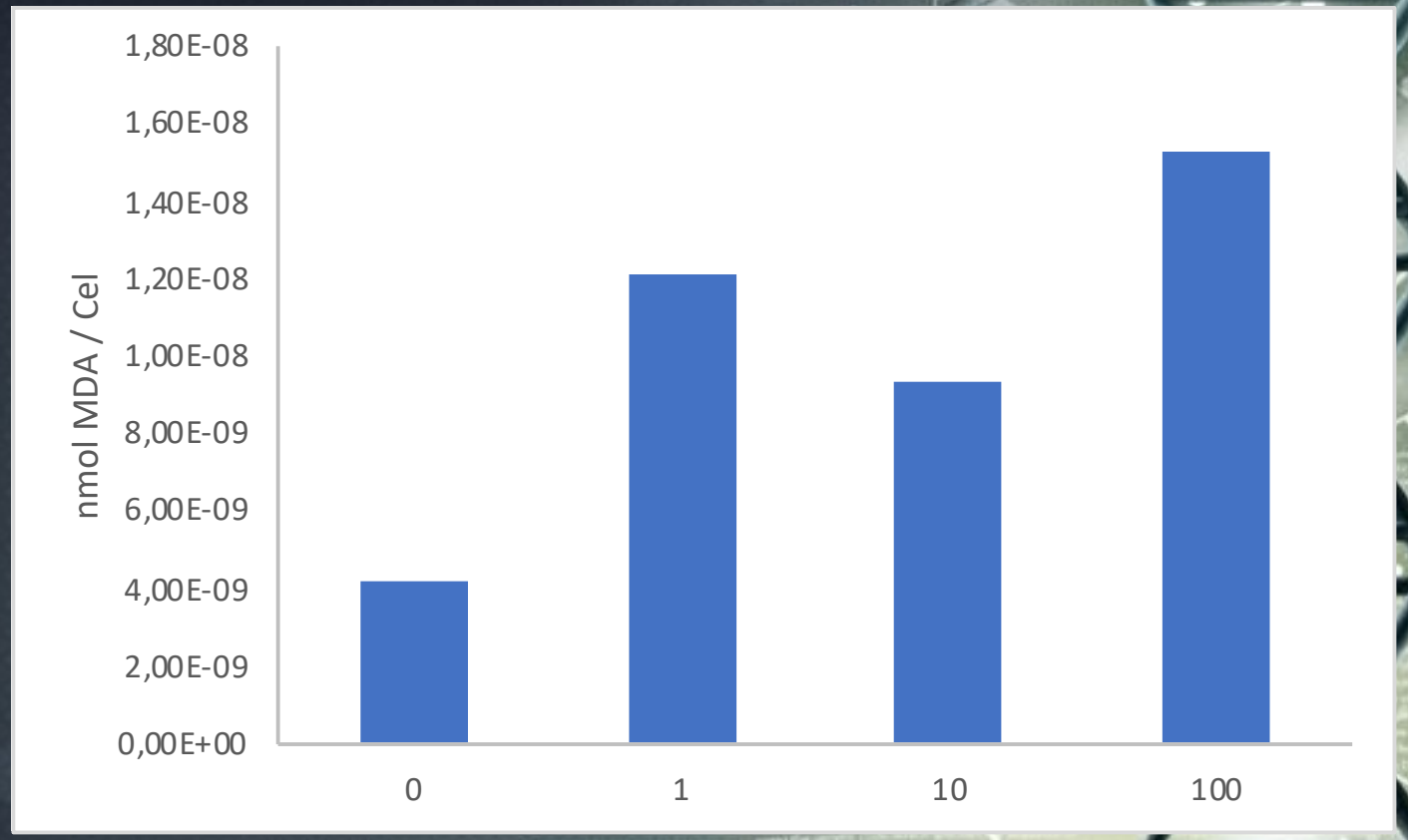

2
OH
N
HO
N
SH
+
O
O
MDA
Δ
H+
S
N
OH
N
OH
HO
N
SH
N
OH
OH
2-Thiobarbituric Acid
λmax = 531 nm



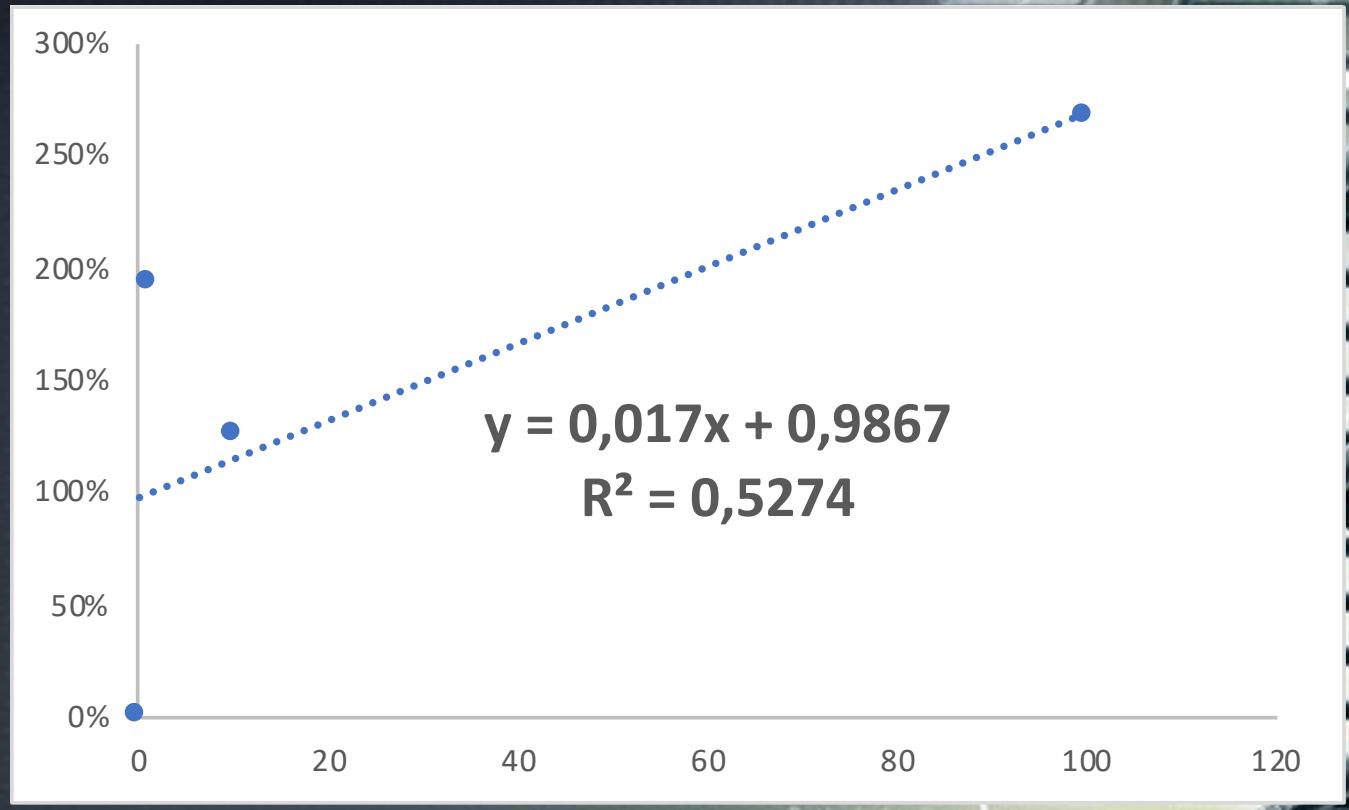

1.2
1
0.8
0.6
0.4
0.2
0
OD 532nm
0
1
2
3
4
5
MDA (nmol)
y = 0.2116x + 0.0045
R² = 0.9996

[Glif] ug/L	Nº Cel / mL	Volume Pellet (mL)	Cell Pellet	Abs 532nm	Abs 600 nm	532 - 600	V extrac (mL)	[MDA] mM	[MDA] uM	umol MDA	nmol MDA	nmol MDA/Cel	Average
0	4681650	30	140449500	0,41	0,27	0,15	1,00	9,42E-04	0,94	9,42E-04	0,94	6,71E-09	4,16E-09
0	4681650	30	140449500	0,29	0,26	0,04	1,00	2,26E-04	0,23	2,26E-04	0,23	1,61E-09	
1	3718101	30	111543030	0,49	0,28	0,22	1,00	1,39E-03	1,39	1,39E-03	1,39	1,24E-08	1,21E-08
1	3718101	30	111543030	0,51	0,30	0,21	1,00	1,32E-03	1,32	1,32E-03	1,32	1,19E-08	
10	4081575	30	122447250	0,42	0,26	0,16	1,00	1,05E-03	1,05	1,05E-03	1,05	8,59E-09	9,33E-09
10	4081575	30	122447250	0,48	0,29	0,19	1,00	1,23E-03	1,23	1,23E-03	1,23	1,01E-08	
100	2888283	30	86648490	0,49	0,29	0,20	1,00	1,31E-03	1,31	1,31E-03	1,31	1,51E-08	1,53E-08
100	2888283	30	86648490	0,50	0,29	0,21	1,00	1,34E-03	1,34	1,34E-03	1,34	1,54E-08	


1,80E-08
1,60E-08
1,40E-08
1,20E-08
1,00E-08
8,00E-09
6,00E-09
4,00E-09
2,00E-09
0,00E+00
nmol MDA/Cel
y = 7E-11x + 8E-09
R² = 0,4764
0
20
40
60
80
100
120


1,80E-08
1,60E-08
1,40E-08
1,20E-08
1,00E-08
8,00E-09
6,00E-09
4,00E-09
2,00E-09
0,00E+00
nmol MDA / Cel
0
1
10
100

[Glif] ug/L	nmol MDA/Cel	% Effect
0	4,15716E-09	0%
1	1,21463E-08	192%
10	9,32594E-09	124%
100	1,52637E-08	267%



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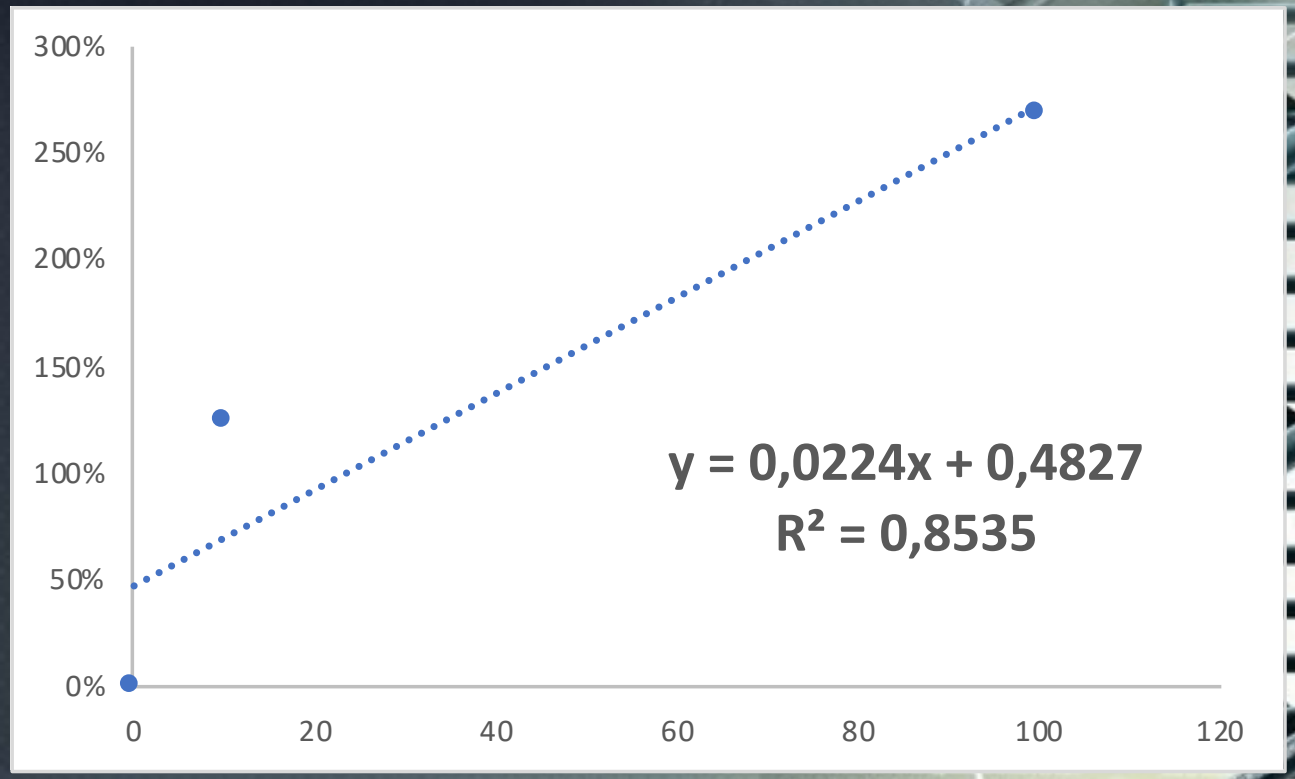

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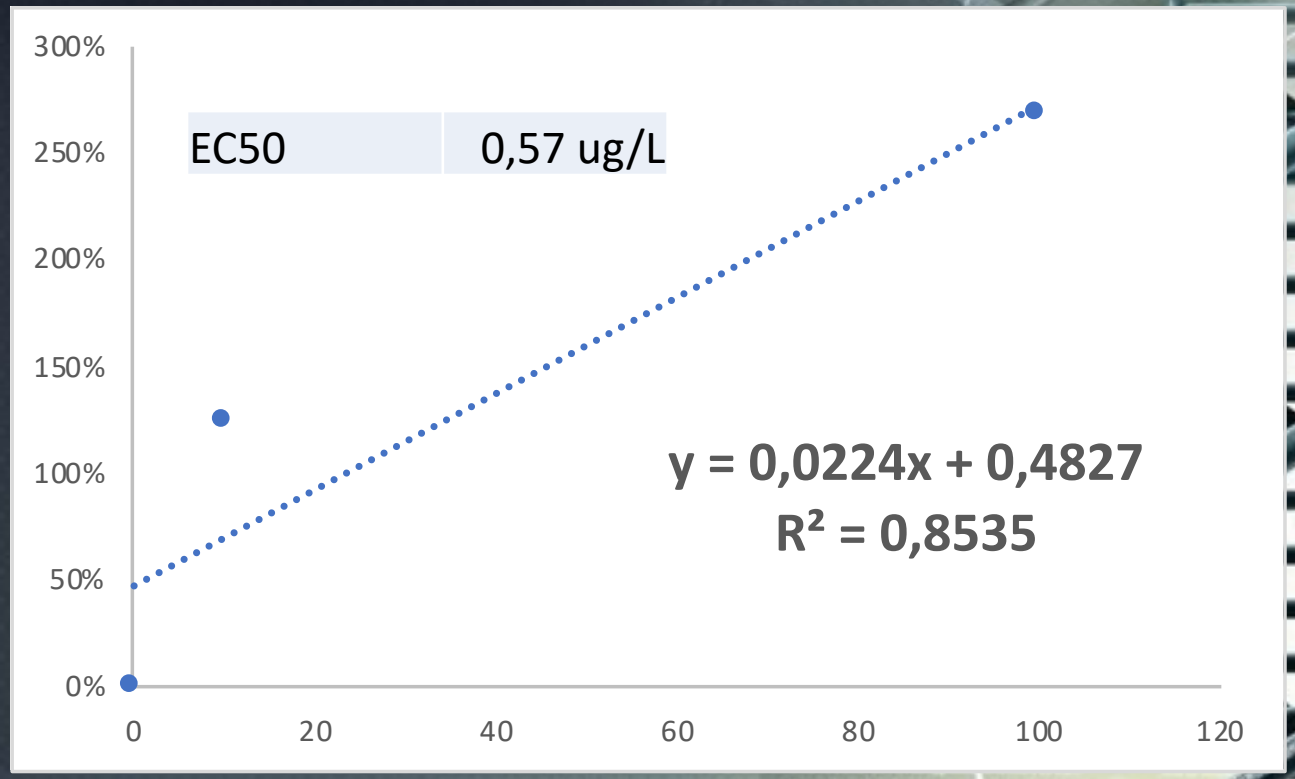
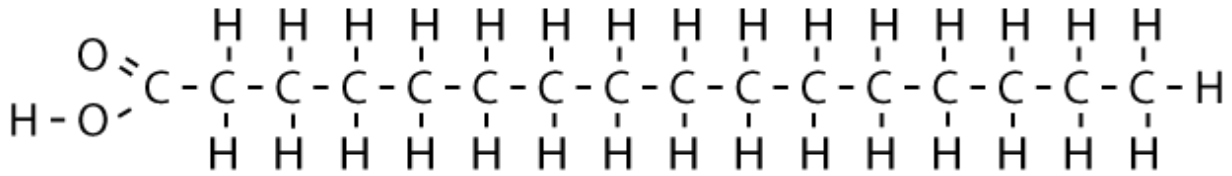
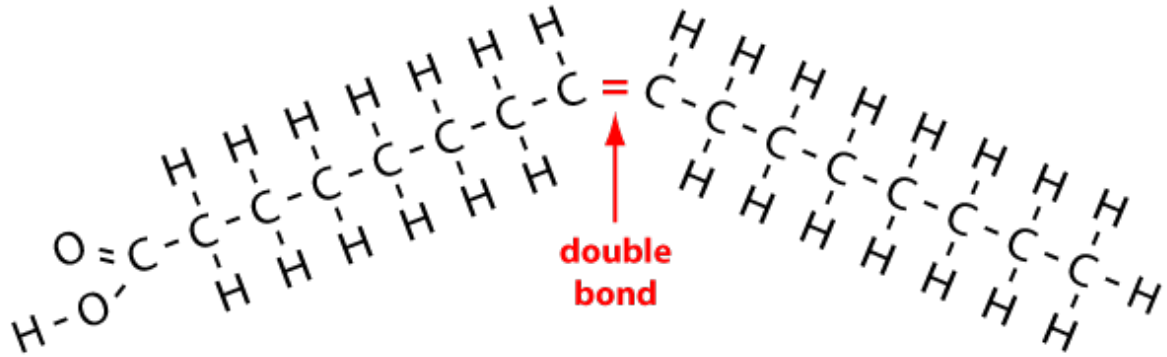


	IC50 (ug/L)
Crescimento	142,6
Phi_Pav	4945
Area	120,9
ABS	121,7
ET	117,782
TR	137,057
DI	127,5
Chl a	68,47
Diadinoxantina	121,71
Carotenoides	357,07
Chl a/c	62,42
TBARS (EC50)	0,57

saturated fatty acid



unsaturated fatty acid



random][plasmid

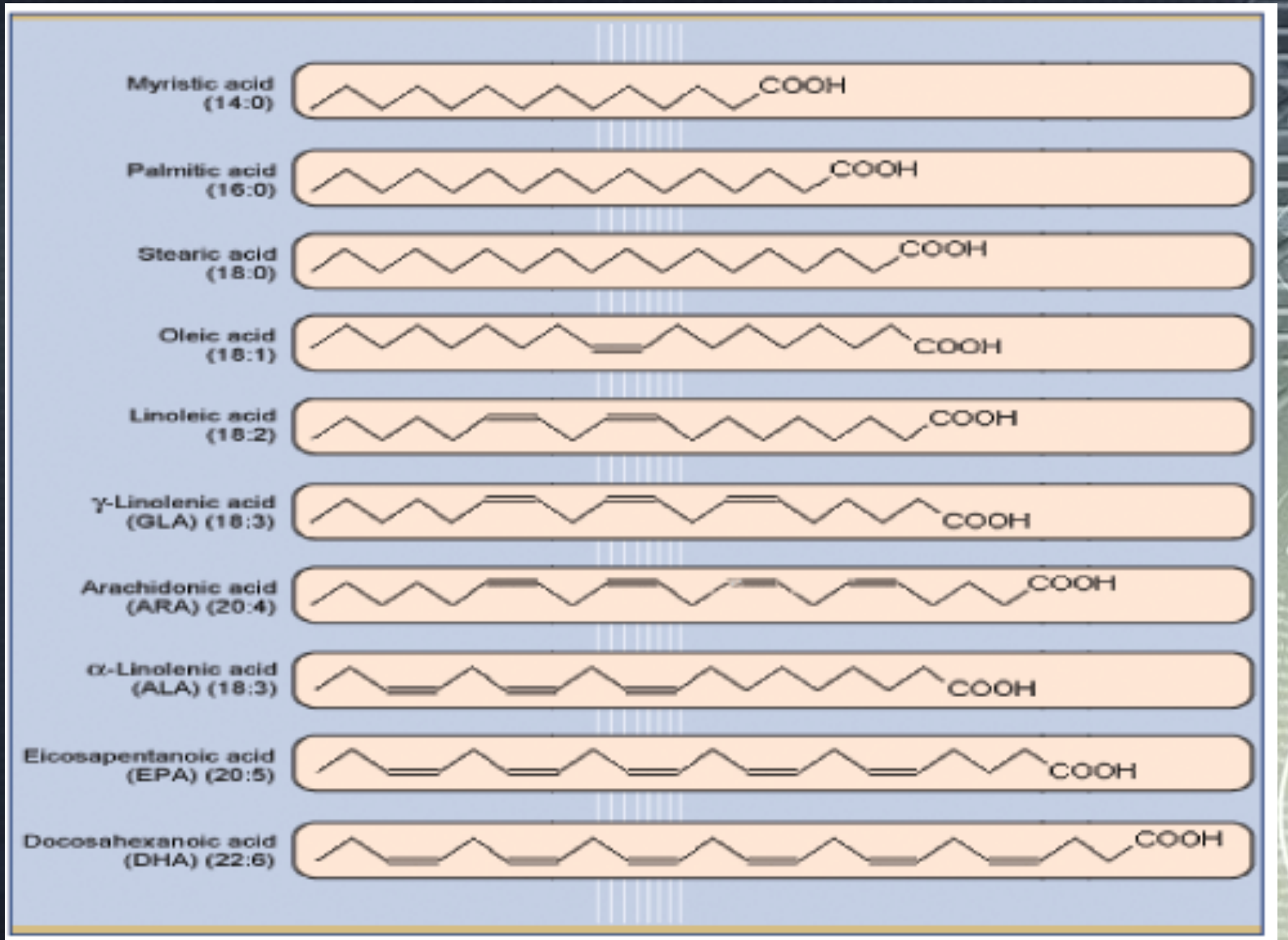
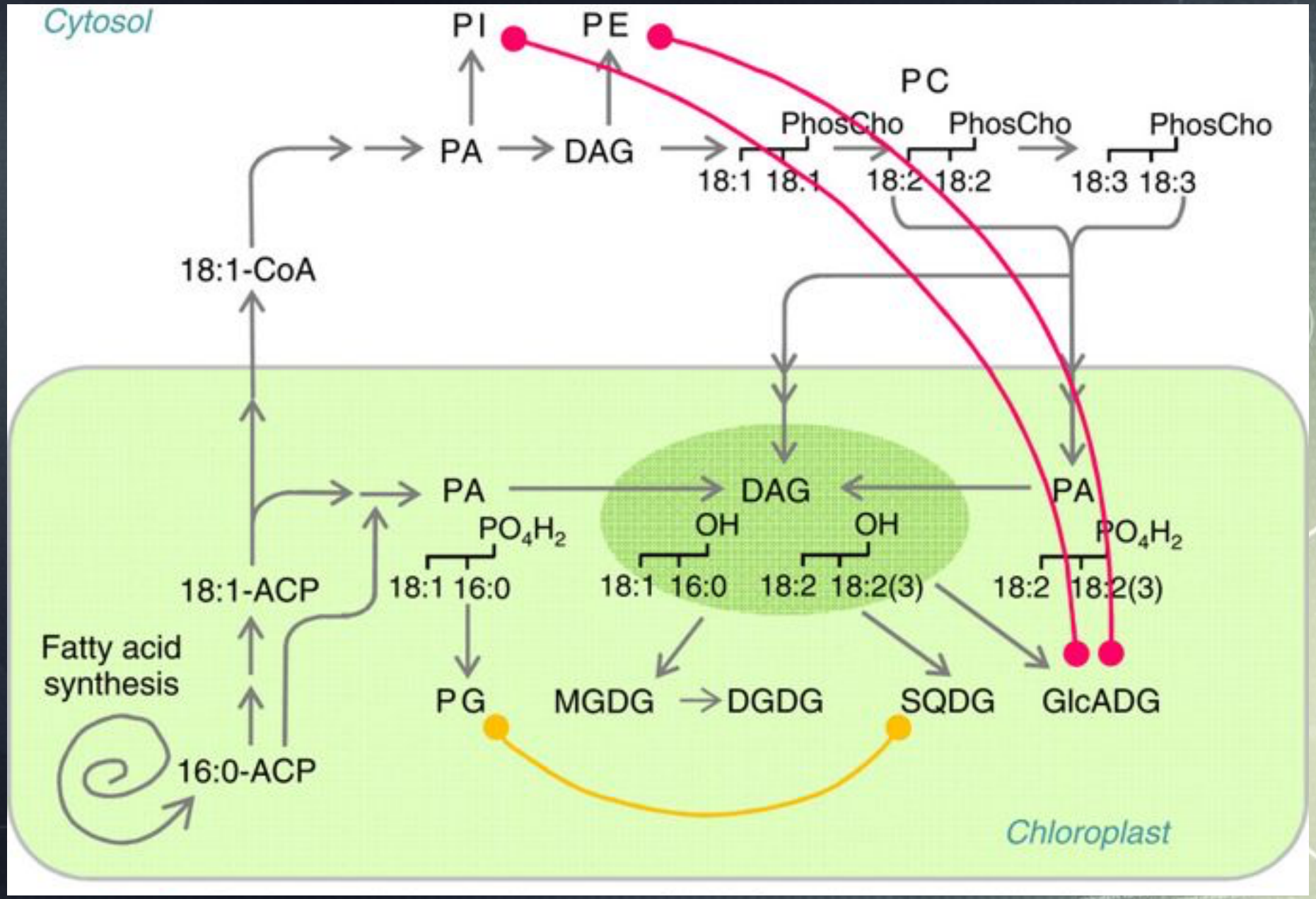
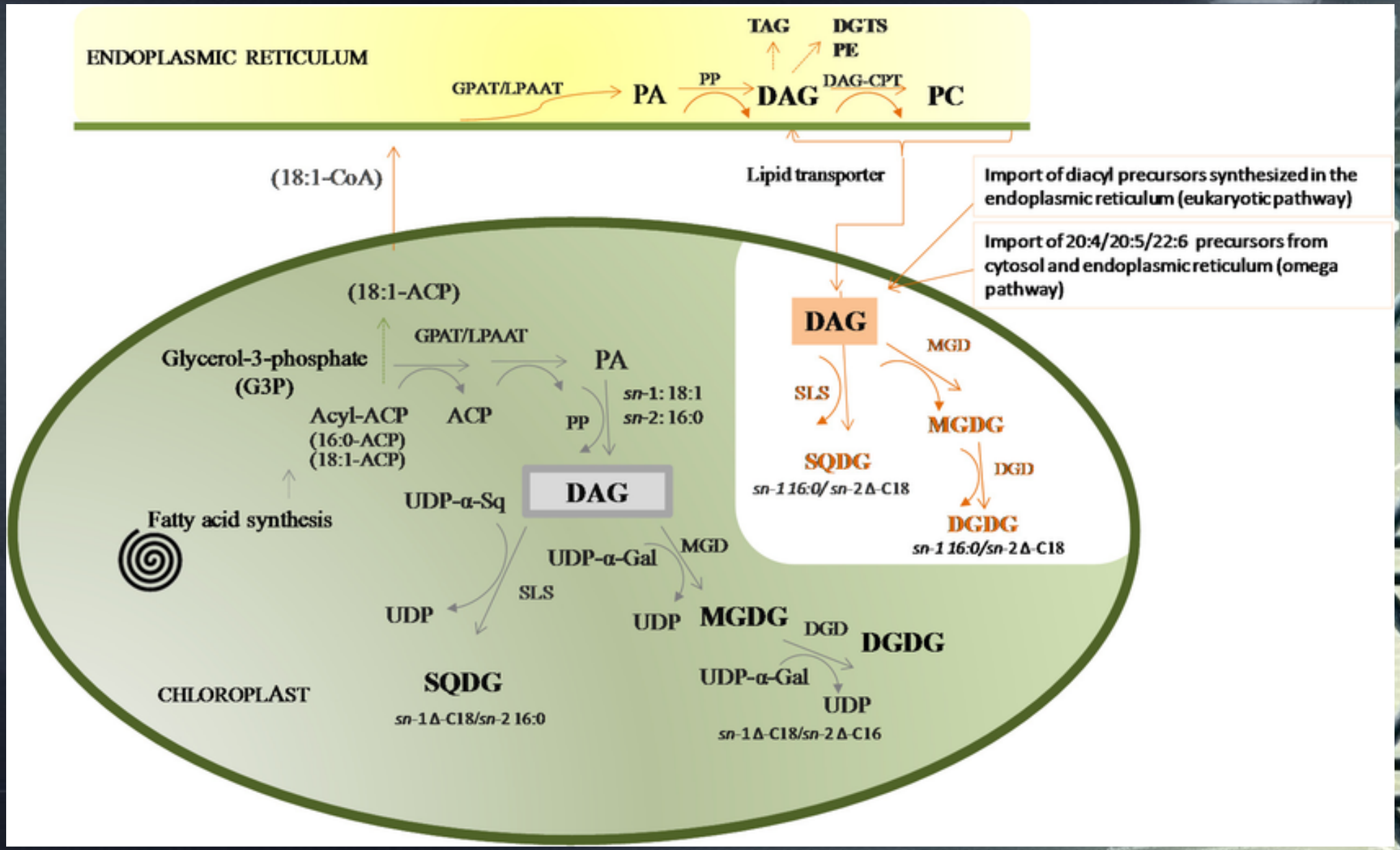
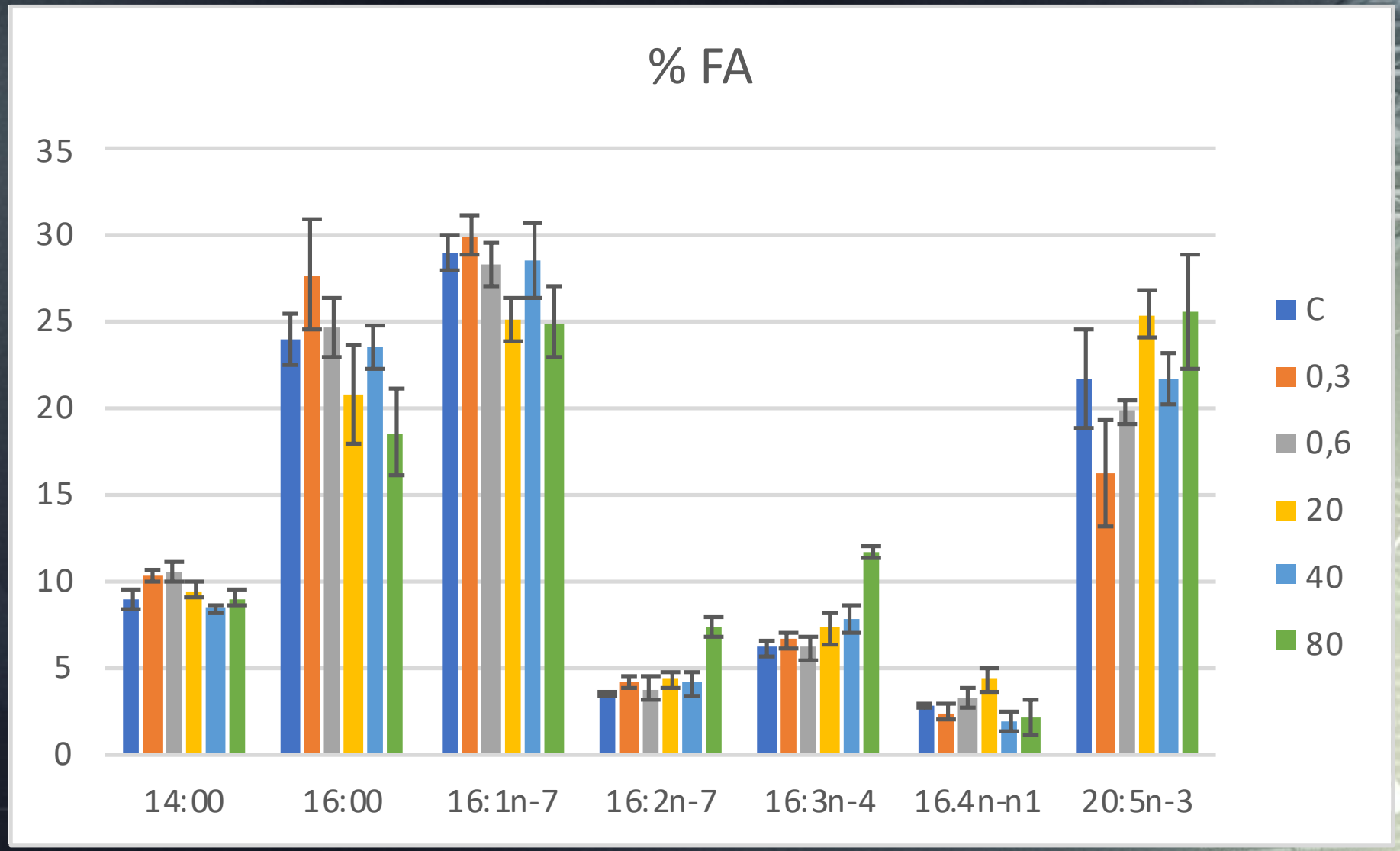
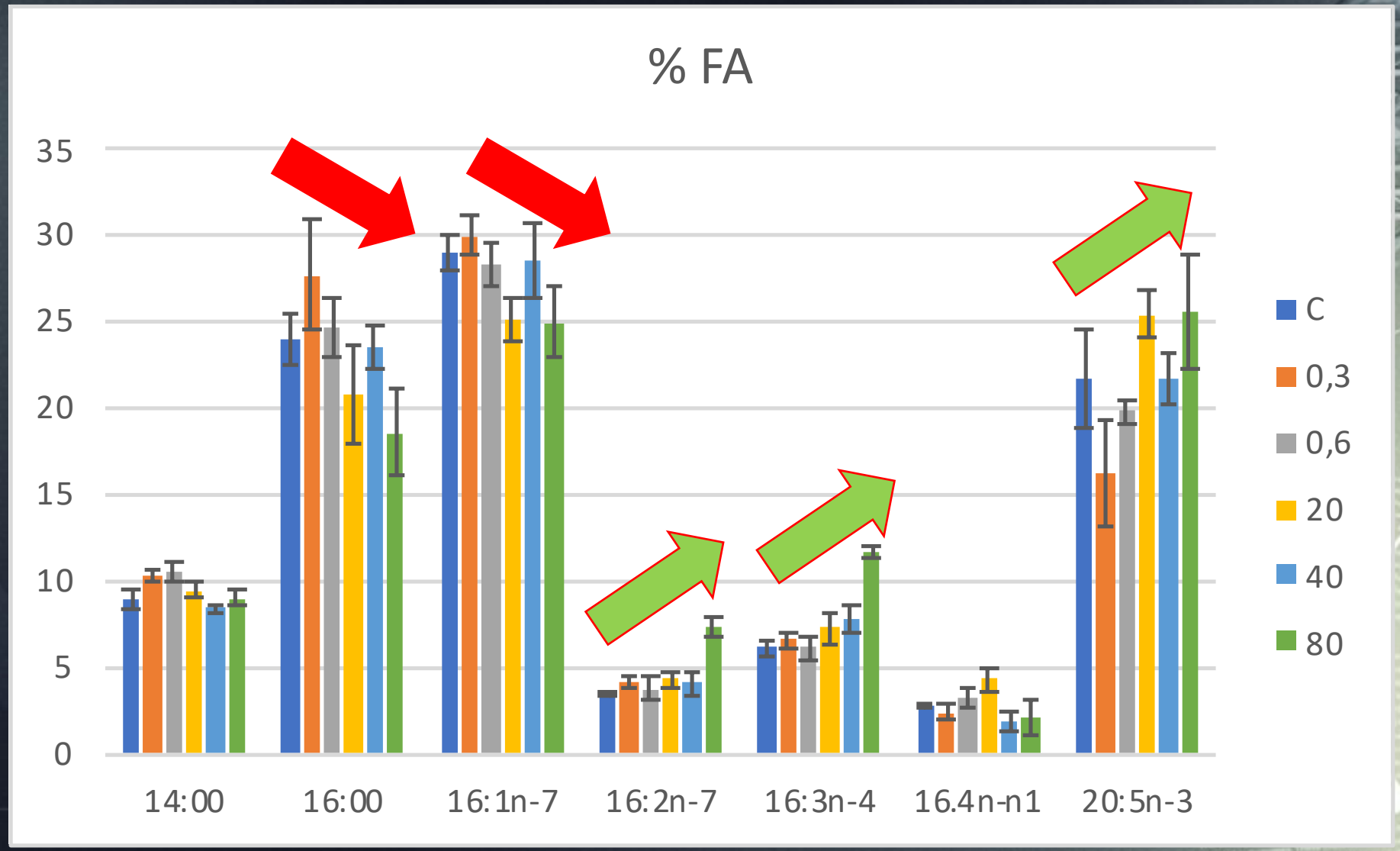


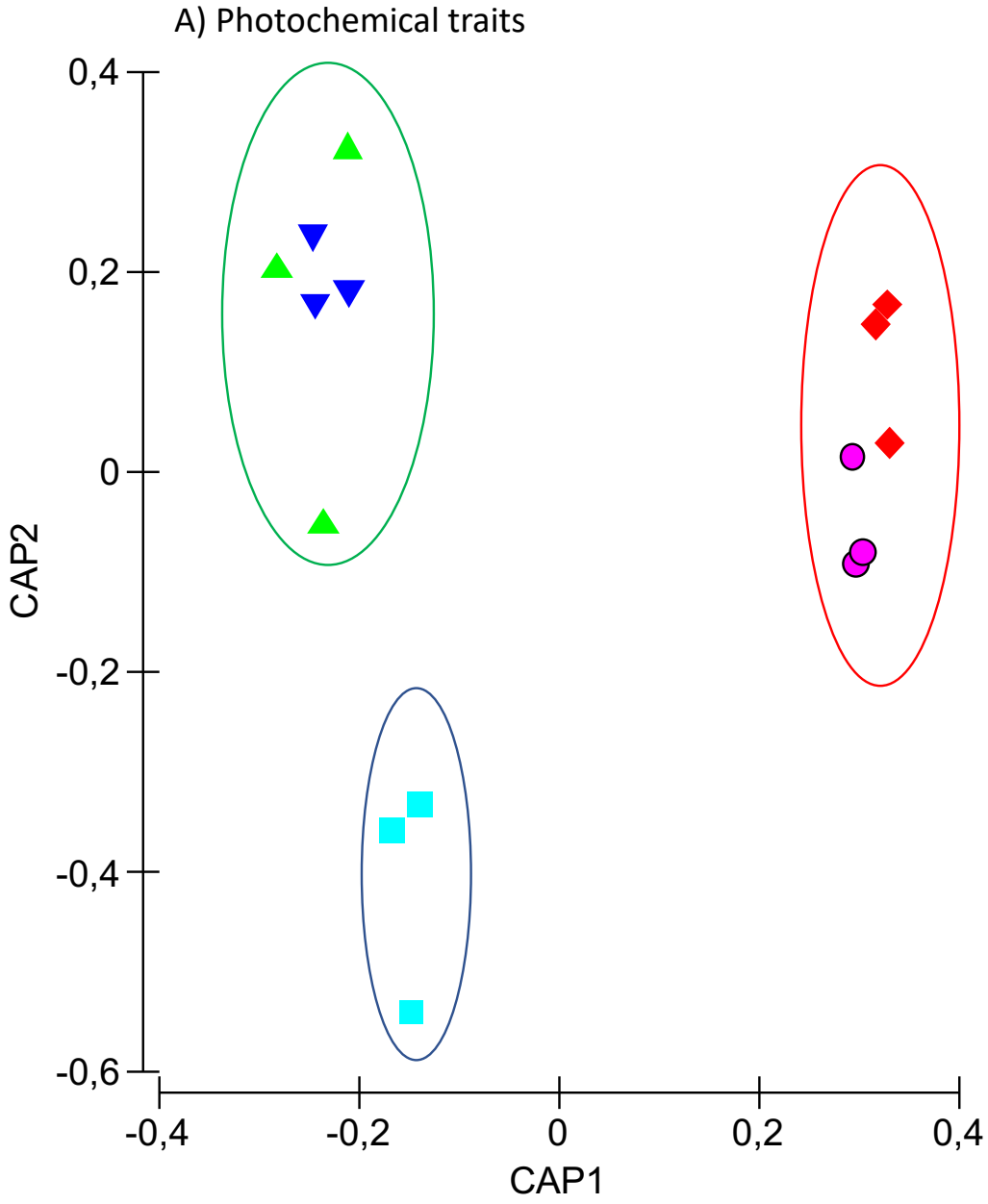
Figure 1 - Structural formulas of the saturated, monounsaturated, and polyunsaturated fatty acids also showing the methylene interrupted sequence of the polyunsaturated acids.

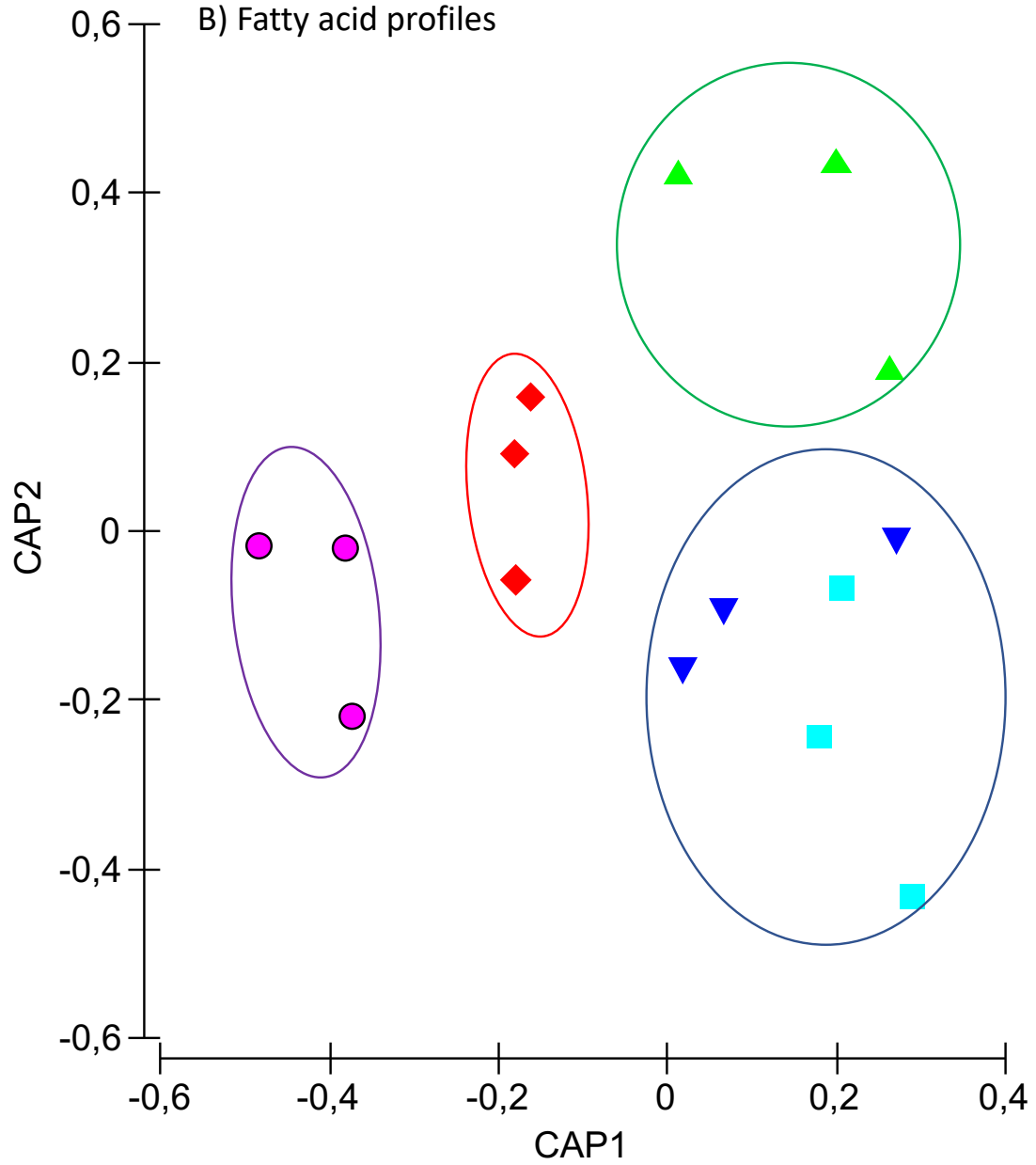

Cytosol
PI
PE
PC
PhosCho
PhosCho
PhosCho
PA
DAG
18:1 18:1
18:2 18:2
18:3 18:3
18:1-CoA
PA
DAG
PA
PO4H2
OH
OH
PO4H2
18:1-ACP
18:1 16:0
18:1 16:0
18:2 18:2(3)
18:2 18:2(3)
Fatty acid synthesis
PG
MGDG
DGDG
SQDG
GlcADG
16:0-ACP
Chloroplast


ENDOPLASMIC RETICULUM
TAG
DGTS
PE
GPAT/LPAAT
PA
PP
DAG
DAG-CPT
PC
(18:1-CoA)
Lipid transporter
Import of diacyl precursors synthesized in the endoplasmic reticulum (eukaryotic pathway)
Import of 20:4/20:5/22:6 precursors from cytosol and endoplasmic reticulum (omega pathway)
(18:1-ACP)
GPAT/LPAAT
Glycerol-3-phosphate (G3P)
PA
sn-1: 18:1
sn-2: 16:0
Acyl-ACP (16:0-ACP) (18:1-ACP)
ACP
PP
DAG
MGD
SLS
MGDG
SQDG
sn-1 16:0/ sn-2 Δ-C18
DGD
DGDG
sn-1 16:0/sn-2 Δ-C18
Fatty acid synthesis
UDP-α-Sq
DAG
UDP-α-Gal
MGD
SLS
UDP
UDP
MGDG
DGD
DGDG
UDP-α-Gal
UDP
CHLOROPLAST
SQDG
sn-1 Δ-C18/sn-2 16:0
sn-1 Δ-C18/sn-2 Δ-C16

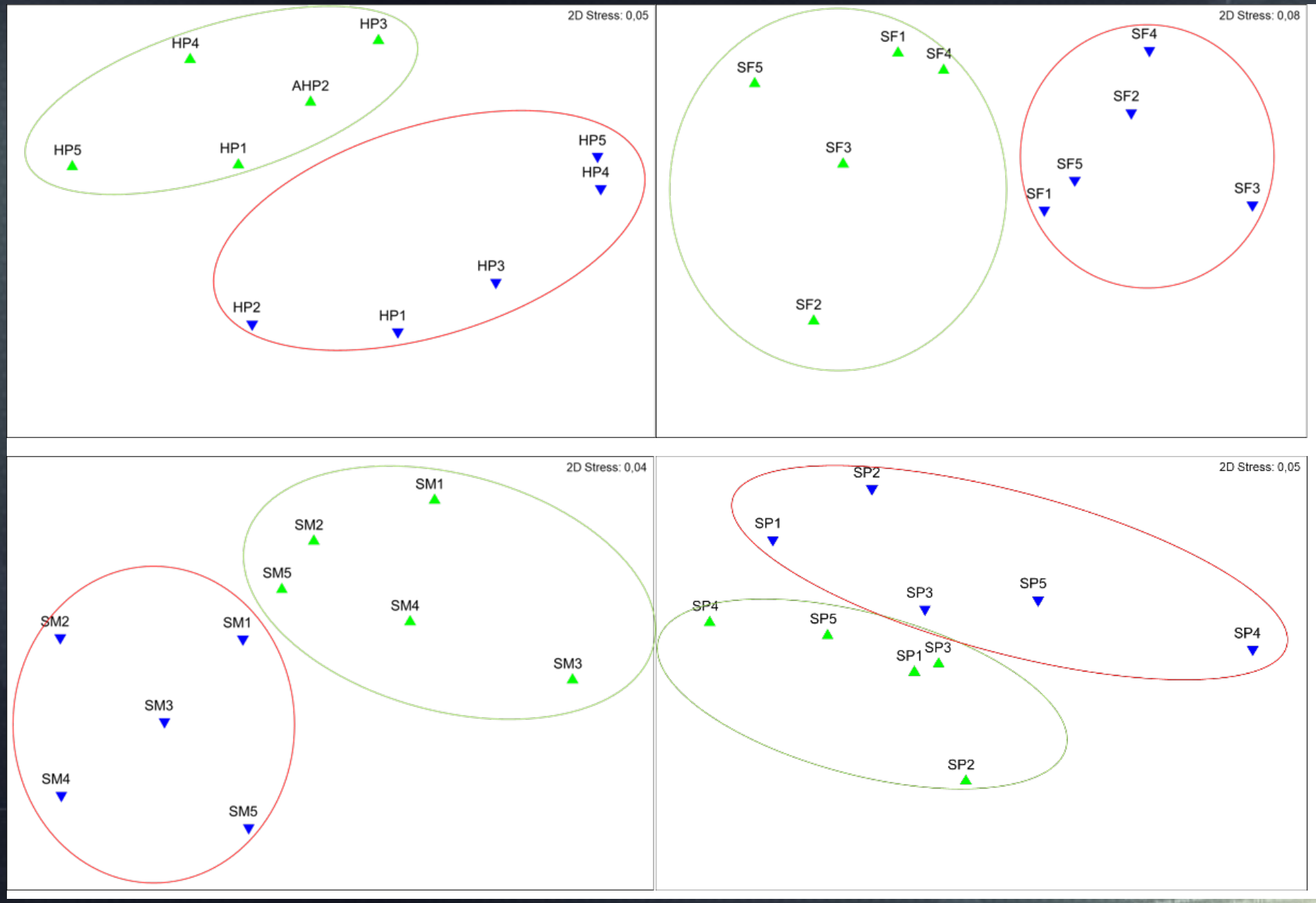

% FA
35
30
25
20
15
10
5
0
14:00
16:00
16:1n-7
16:2n-7
16:3n-4
16.4n-n1
20:5n-3
C
0,3
0,6
20
40
80

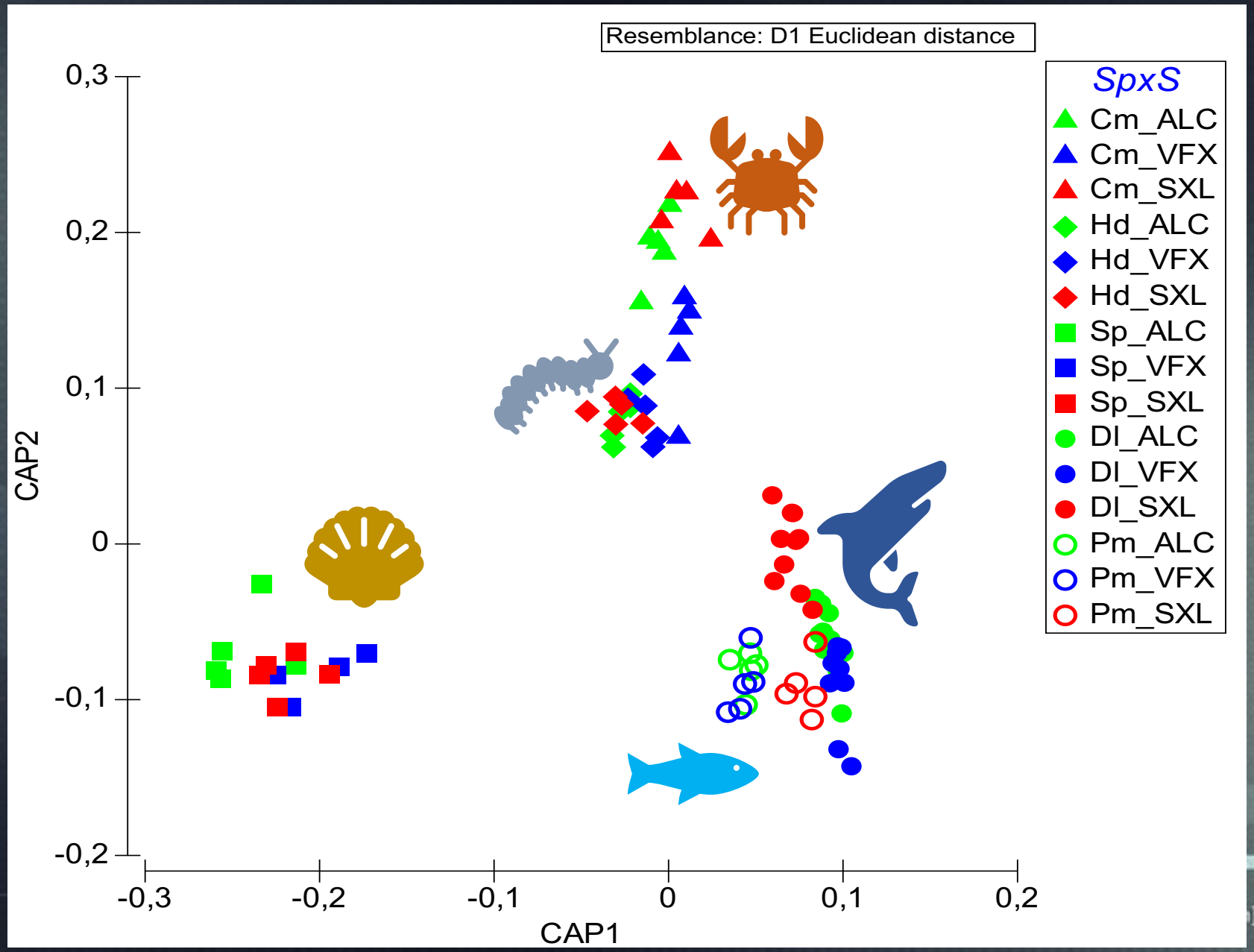

% FA
35
30
25
20
15
10
5
0
14:00
16:00
16:1n-7
16:2n-7
16:3n-4
16.4n-n1
20:5n-3
C
0,3
0,6
20
40
80

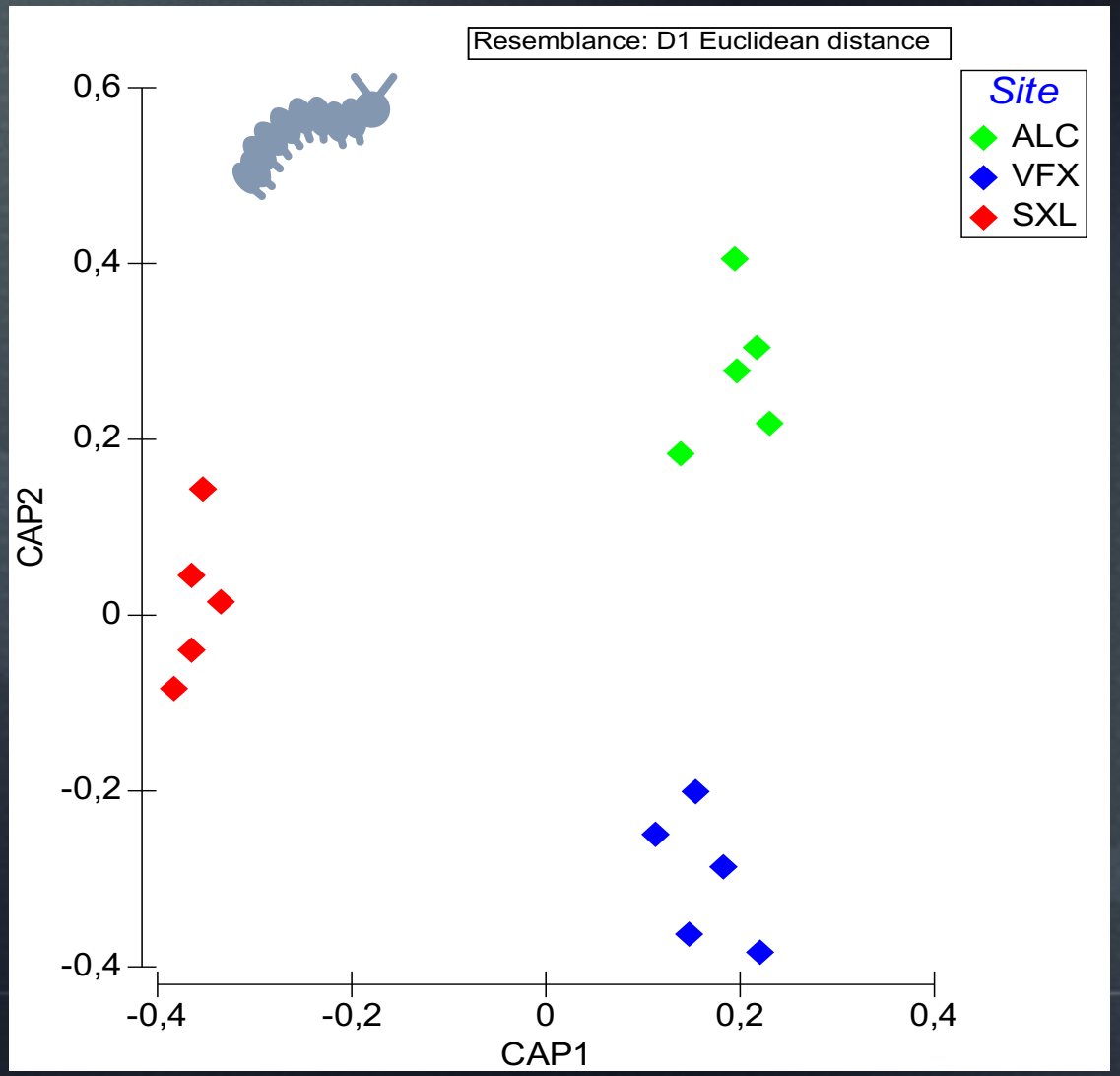

A) Photochemical traits
0,4
0,2
0
-0,2
-0,4
-0,6
CAP2
-0,4
-0,2
0
0,2
0,4
CAP1

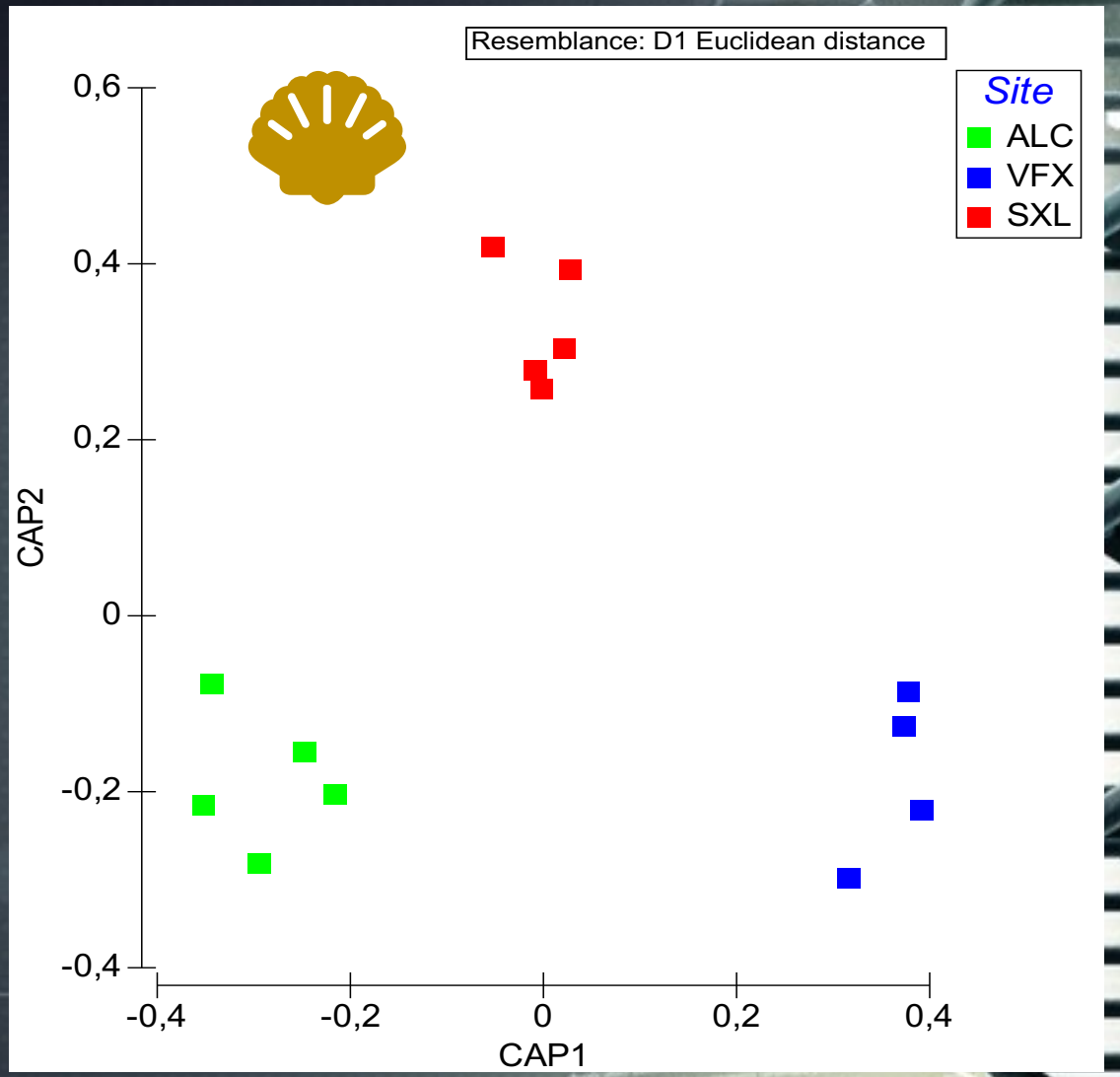

B) Fatty acid profiles
0,6
0,4
0,2
0
-0,2
-0,4
-0,6
CAP2
-0,6
-0,4
-0,2
0
0,2
0,4
CAP1

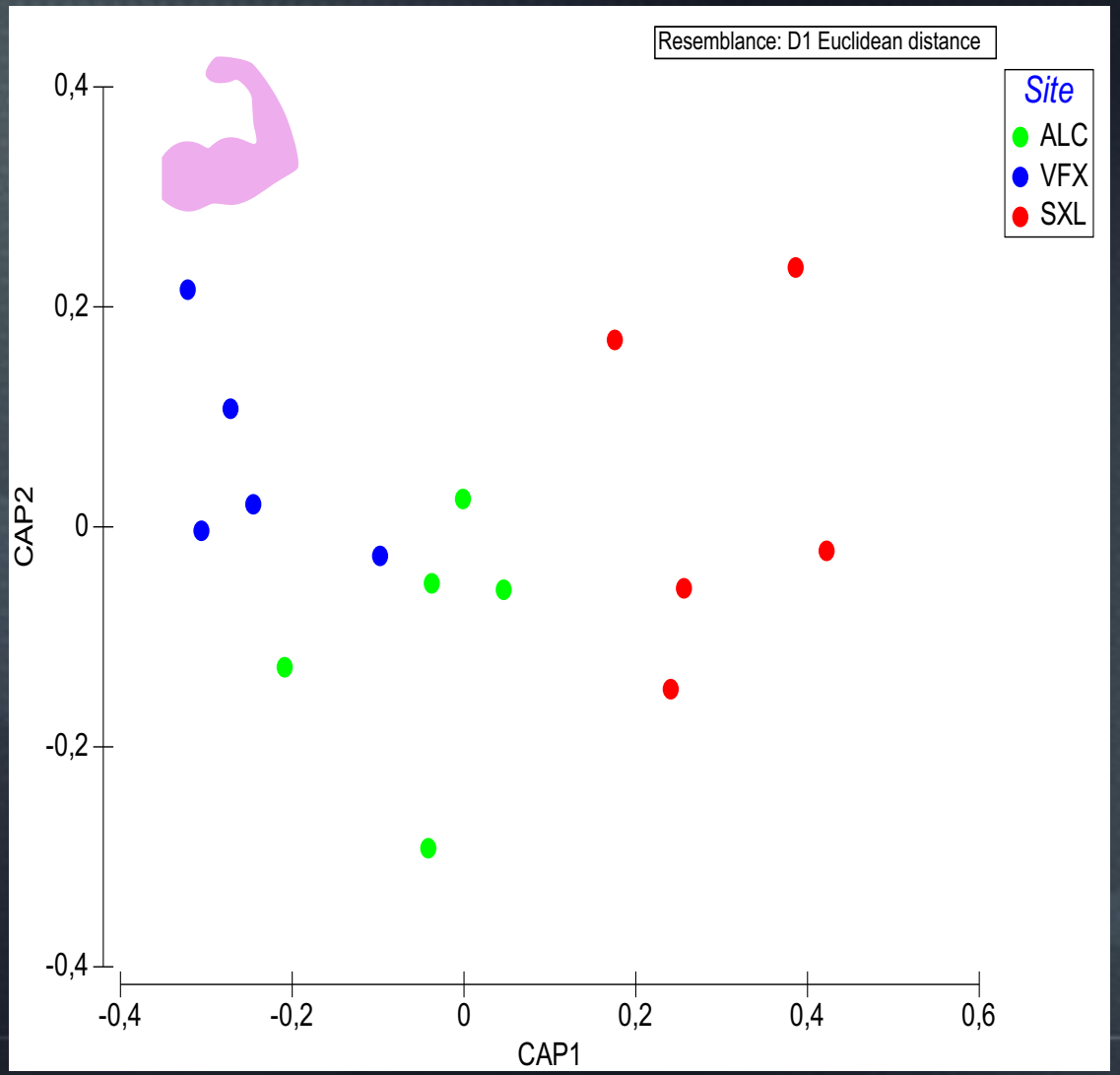

Bezafibrate
0 µg L-1
3 µg L-1
6 µg L-1
30 µg L-1
60 µg L-1

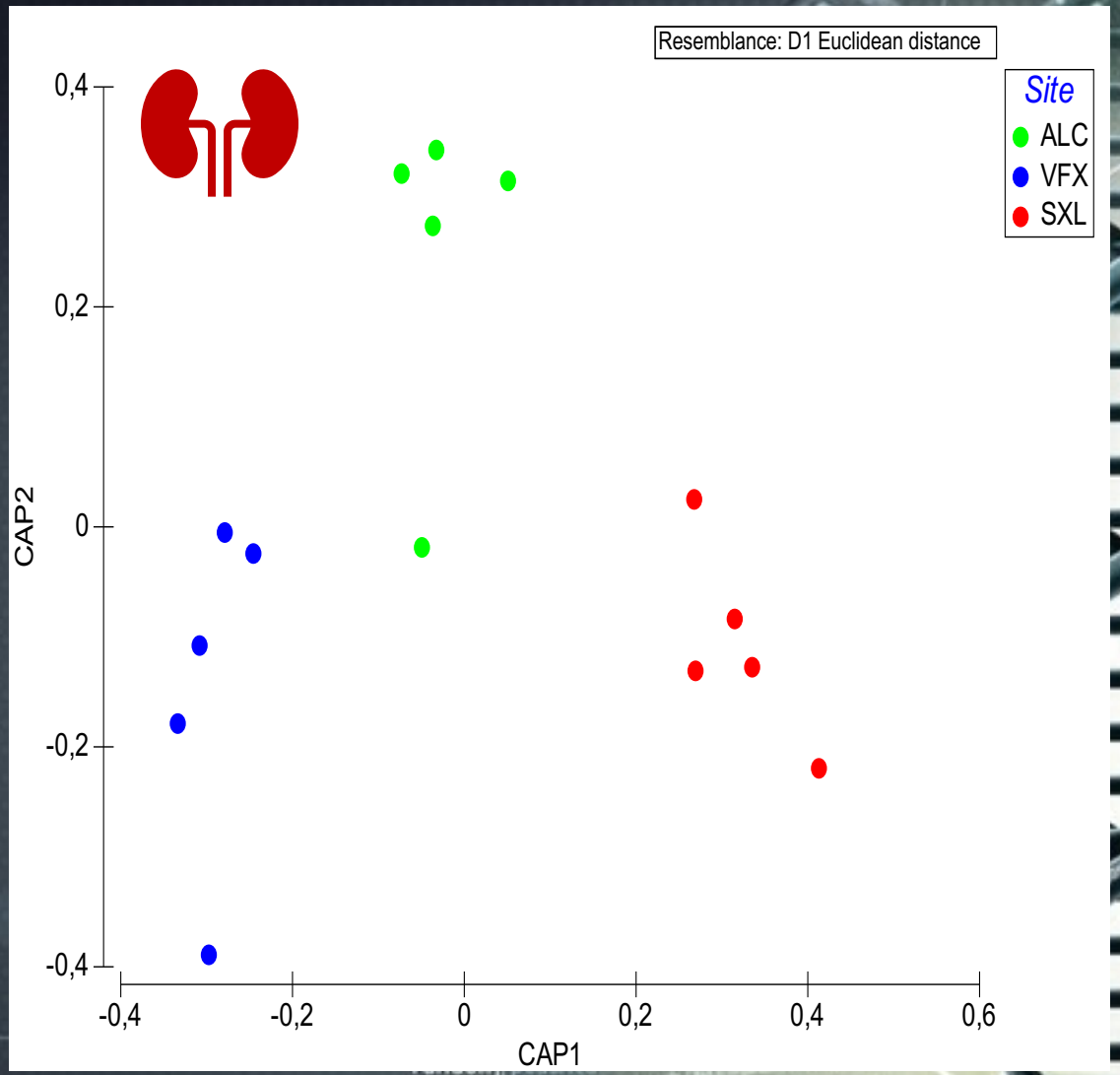

2D Stress: 0,05
HP3
HP4
AHP2
HP5
HP1
HP5
HP4
HP3
HP2
HP1
2D Stress: 0,08
SF1
SF4
SF5
SF3
SF2
SF4
SF2
SF5
SF1
SF3
2D Stress: 0,04
SM1
SM2
SM5
SM4
SM2
SM1
SM3
SM3
SM4
SM5
2D Stress: 0,05
SP2
SP1
SP3
SP5
SP4
SP4
SP5
SP1
SP3
SP2


Resemblance: D1 Euclidean distance
SpxS
Cm_ALC
Cm_VFX
Cm_SXL
Hd_ALC
Hd_VFX
Hd_SXL
Sp_ALC
Sp_VFX
Sp_SXL
Dl_ALC
Dl_VFX
Dl_SXL
Pm_ALC
Pm_VFX
Pm_SXL
0,3
0,2
0,1
0
-0,1
-0,2
CAP2
-0,3
-0,2
-0,1
0
0,1
0,2
CAP1


Resemblance: D1 Euclidean distance
Site
ALC
VFX
SXL
0,6
0,4
0,2
0
-0,2
-0,4
CAP2
-0,4
-0,2
0
0,2
0,4
CAP1


Resemblance: D1 Euclidean distance
Site
ALC
VFX
SXL
0,6
0,4
0,2
0
-0,2
-0,4
CAP2
-0,4
-0,2
0
0,2
0,4
CAP1


Resemblance: D1 Euclidean distance
Site
ALC
VFX
SXL
0,4
0,2
0
-0,2
-0,4
CAP2
-0,4
-0,2
0
0,2
0,4
0,6
CAP1


Resemblance: D1 Euclidean distance
Site
ALC
VFX
SXL
0,4
0,2
0
-0,2
-0,4
CAP2
-0,4
-0,2
0
0,2
0,4
0,6
CAP1