



# Ecotoxicology TP Course

Concepts, Tests & Biomarkers

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MARE – Marine and Environmental Sciences Centre



# TP3 Photobiology

Activities

**Control****10 ug/L Glifosato****250 ug/L Glifosato****500 ug/L Glifosato**

## PROTOCOL

- Add 5 mL acetone to the pellet
- Sonicate the samples;
- Store at -20 °C
- Centrifuge;
- Read the absorbance

$$\text{Chl a} = -2,6839 \times A_{632 \text{ nm}} + 13,2654 \times A_{665 \text{ nm}}$$

$$\text{Chl c} = 28,8191 \times A_{632 \text{ nm}} - 6,0138 \times A_{665 \text{ nm}}$$

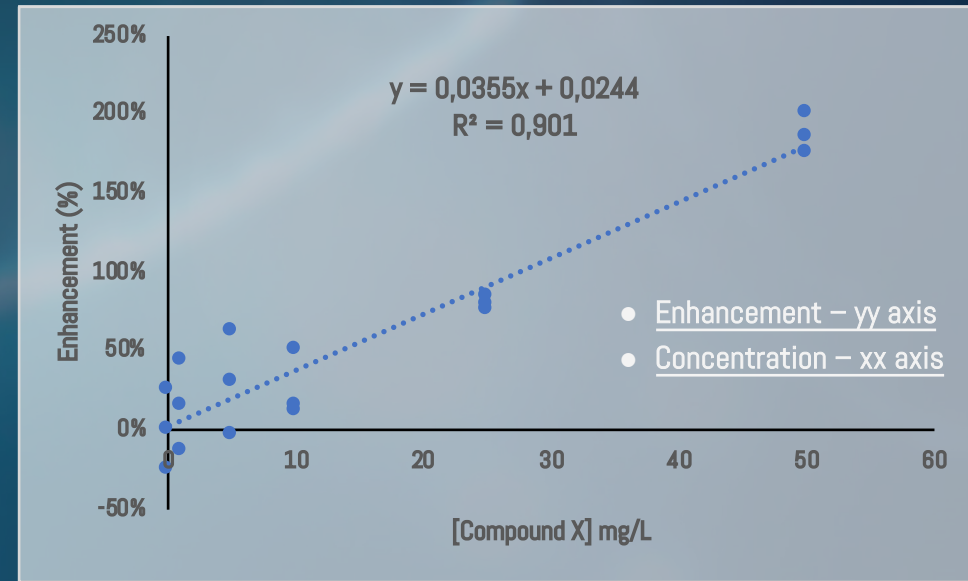
$$C + X = (1000 \times A_{470 \text{ nm}} - 2,13 \times \text{Clorofila a} - 97,63 \times \text{Clorofila c}) / 209$$

# O2 PARAMETERIZATION : Effective Concentration

[Compound X] mg/L	Variable A (a.u.)	Enhancement (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
50	0,055	175%



$$\text{Enhancement (\%)} = \frac{\text{Test} - \overline{\text{Control}}}{\overline{\text{Control}}}$$



Using the linear regression equation calculate the concentration at which the enhancement is 50% ( $EC_{50}$ )

$$50\% = 0.0355x + 0.0244 \Leftrightarrow 0.5 = 0.0355x + 0.0244 \Leftrightarrow 0.5 - 0.0244 = 0.0355x$$

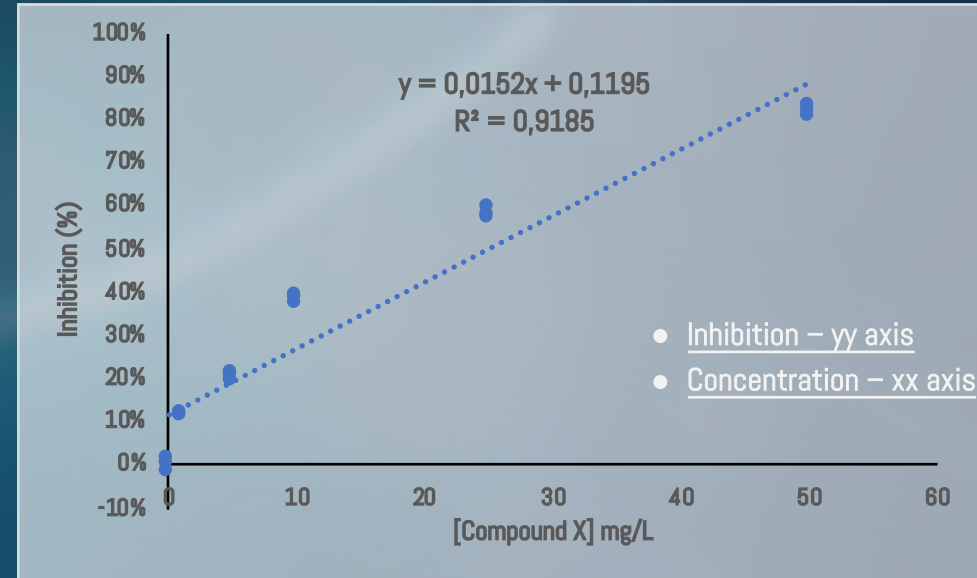
$$x = 13.40 \text{ mg/L} = EC_{50}$$

Upon the application of 13.40 mg/L the variable A suffers a 50% increase relative to the control.

[Compound X] mg/L	Variable A (a.u.)	Inhibition (%)
0	0,02	0%
0	0,015	-25%
0	0,025	25%
1	0,023	15%
1	0,01725	-14%
1	0,02875	44%
5	0,026	30%
5	0,0195	-3%
5	0,0325	63%
10	0,03	50%
10	0,0225	13%
10	0,023	15%
25	0,035	75%
25	0,037	85%
25	0,036	80%
50	0,06	200%
50	0,057	185%
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$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \text{Test}}{\overline{\text{Control}}}$$

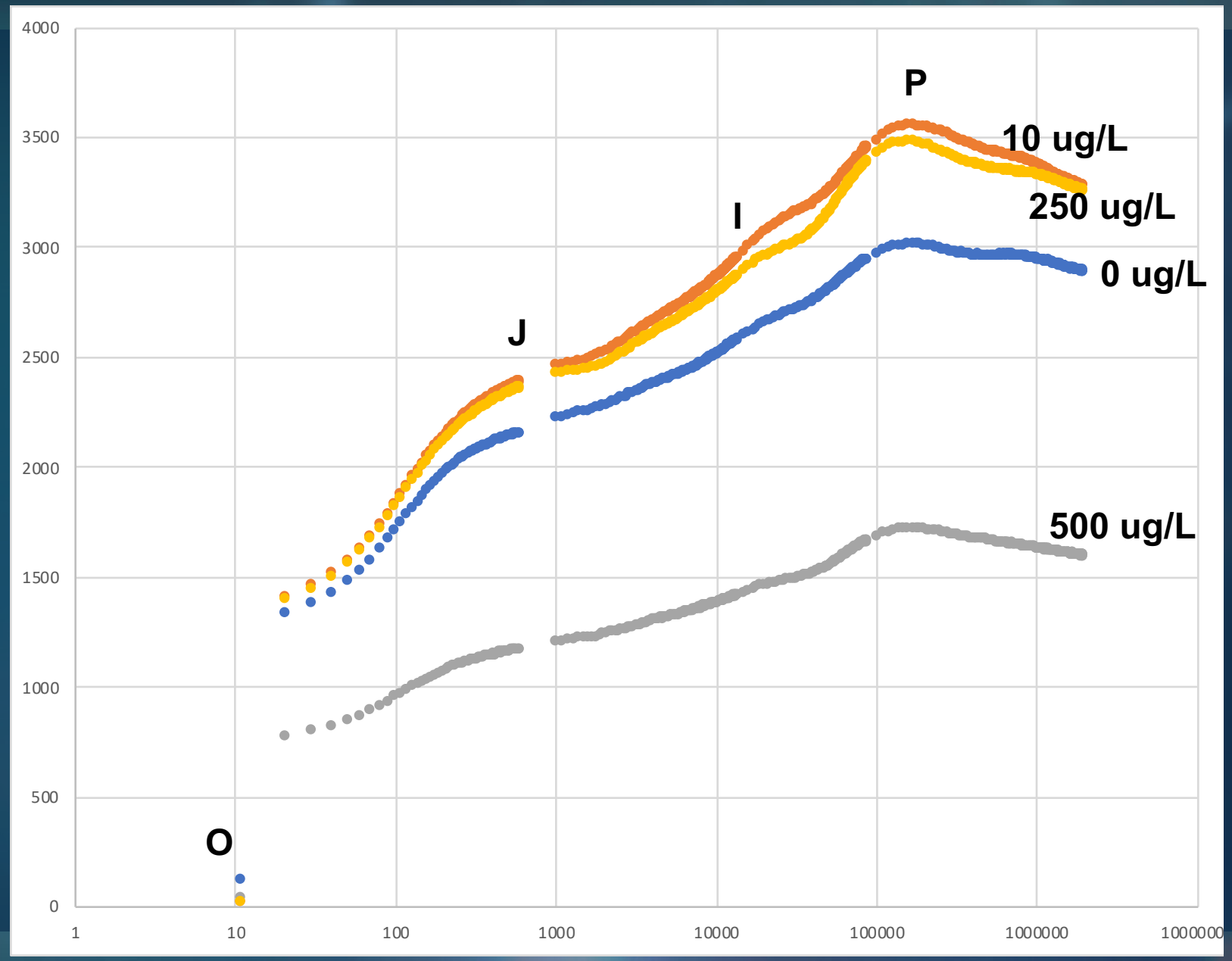


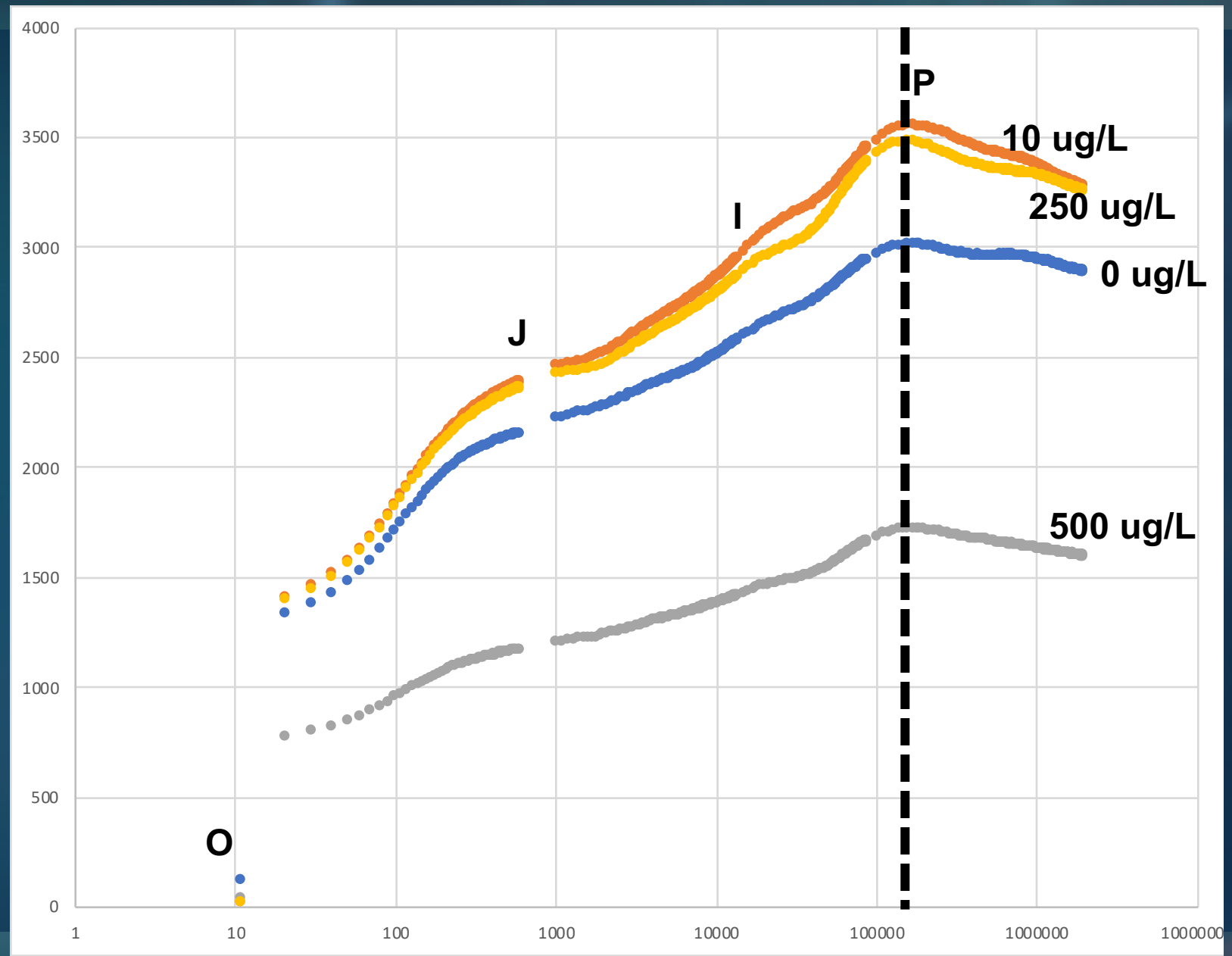
Using the linear regression equation calculate the concentration at which the inhibition was 50% ( $IC_{50}$ )

$$50\% = 0.0152x + 0.1195 \Leftrightarrow 0.5 = 0.0152x + 0.1195 \Leftrightarrow 0.5 - 0.1195 = 0.0152x$$

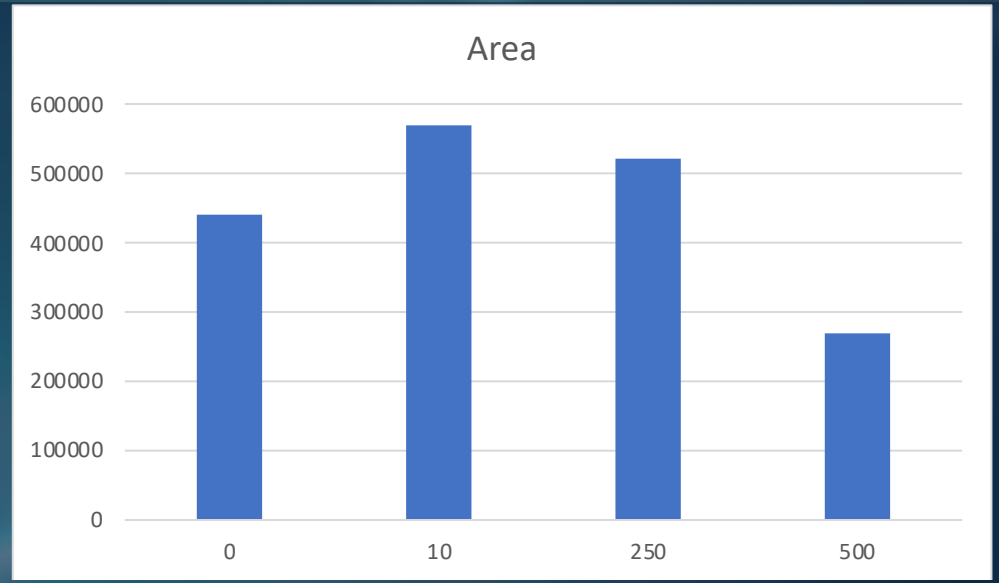
$$x = 13.40 \text{ mg/L} = IC_{50}$$

Upon the application of 13.40 mg/L the variable A suffers a 50% inhibition relative to the control.

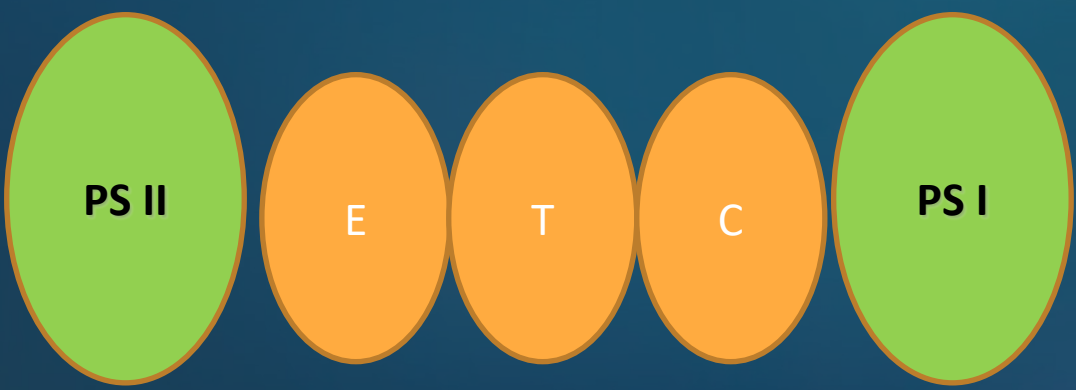




[Glif] ug/L	Area
0	440619
10	569439
250	521764
500	268274



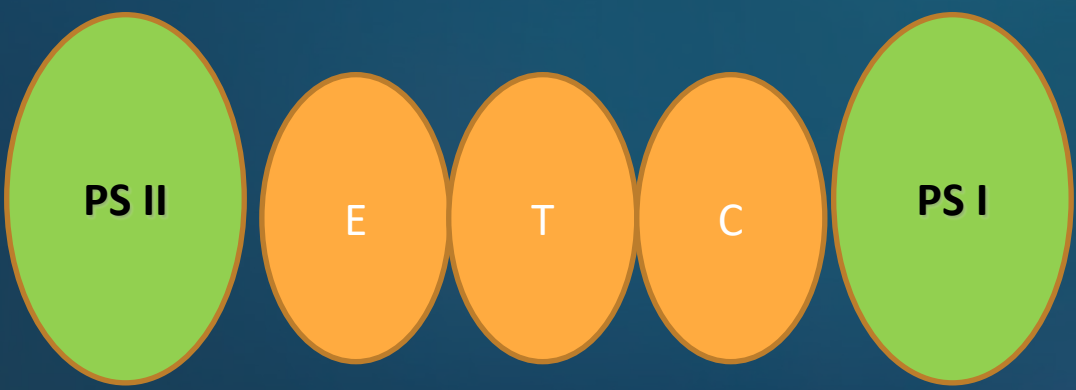
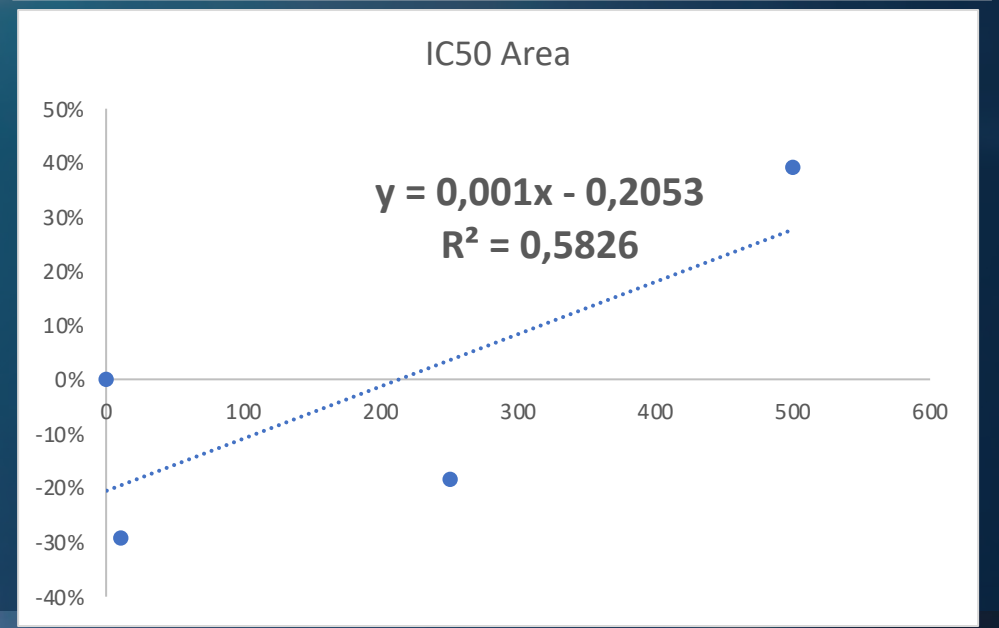
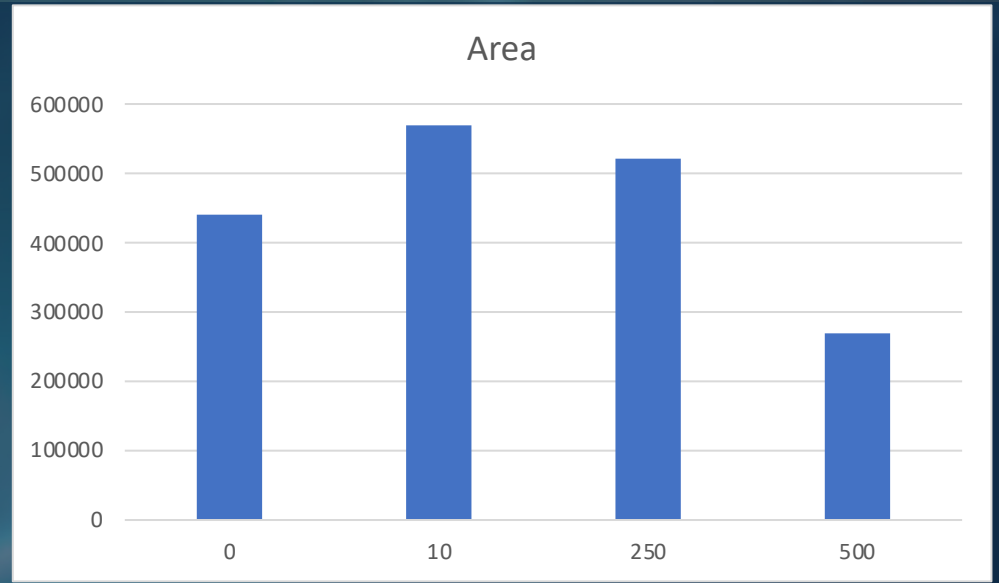
$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \overline{\text{Test}}}{\overline{\text{Control}}}$$





[Glif] ug/L	Area	%inib
0	440619	0%
10	569439	-29%
250	521764	-18%
500	268274	39%

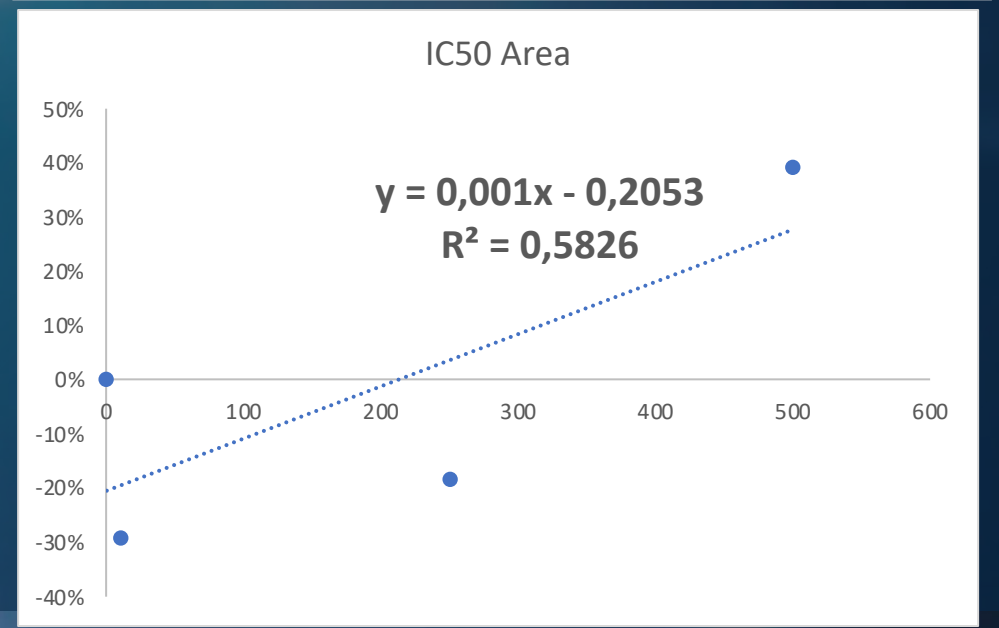
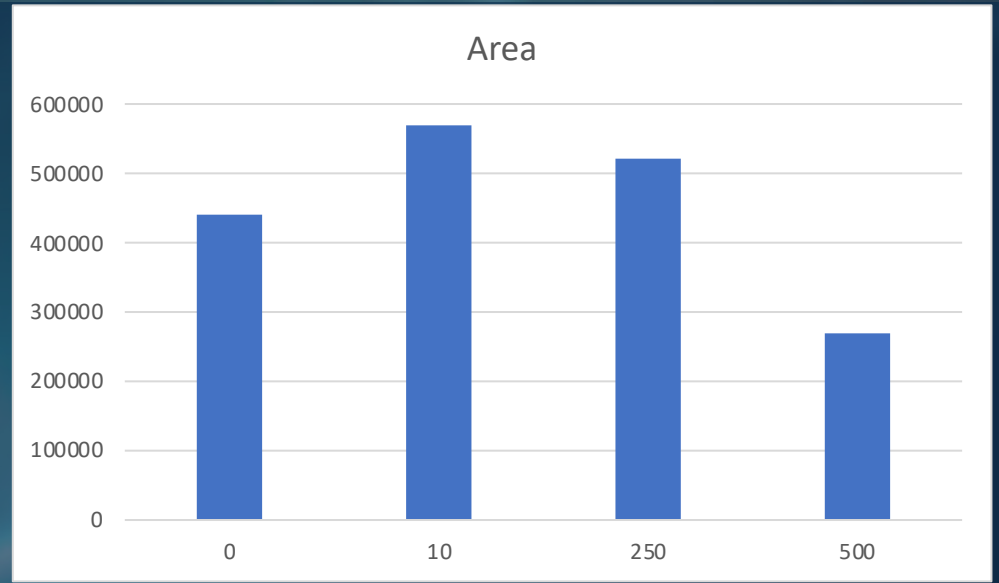
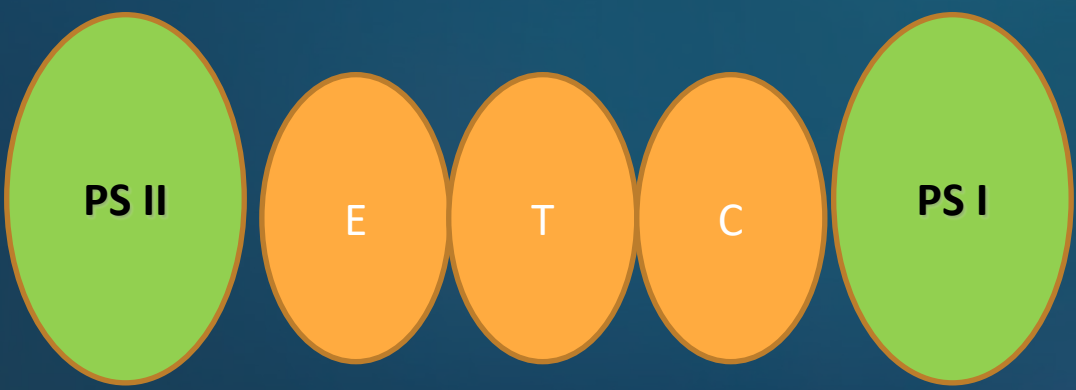
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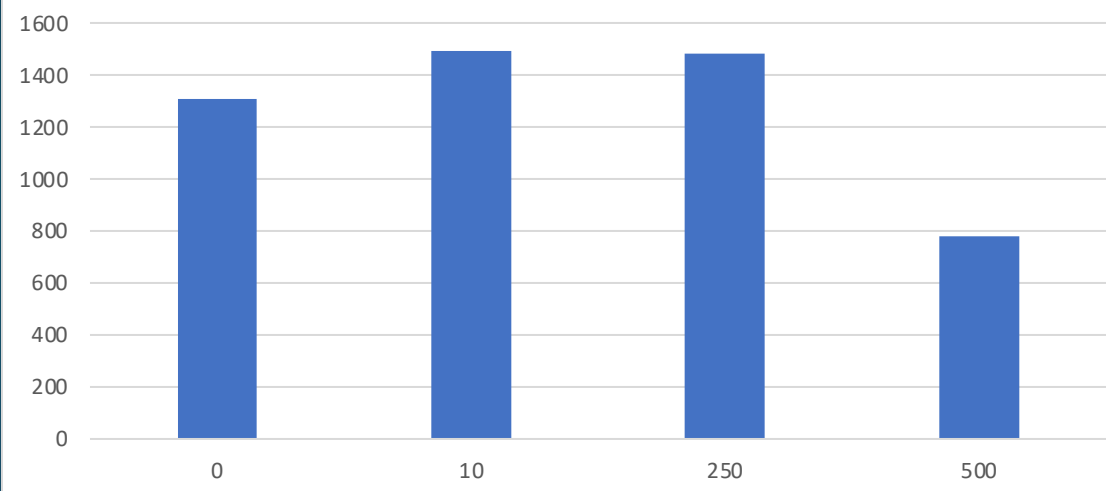
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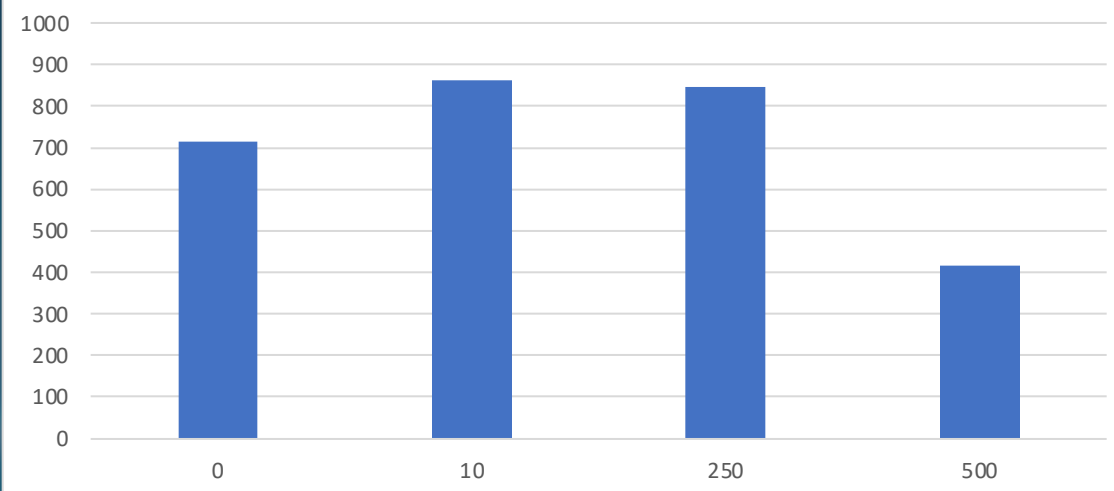
IC<sub>50</sub> = 705,3 ug/L



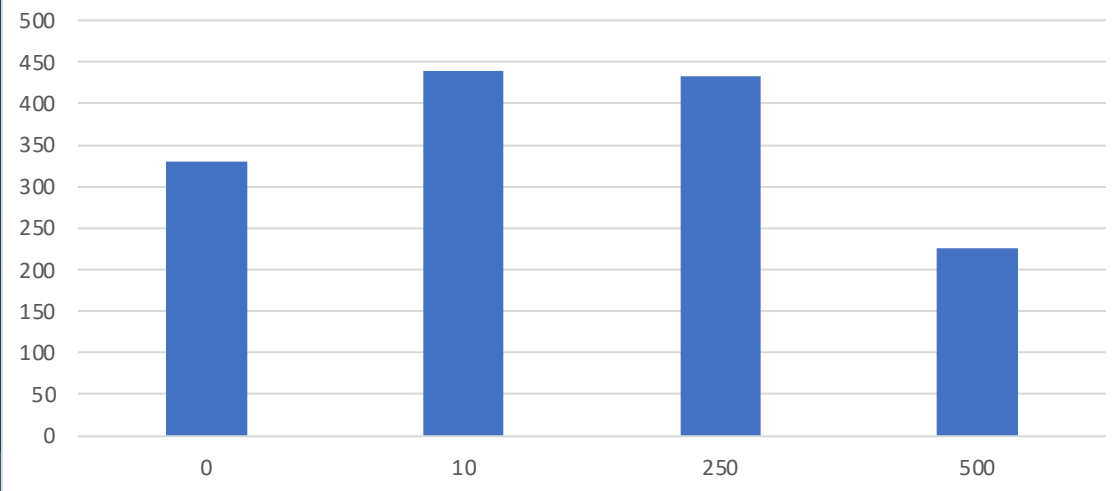
### ABS/CS



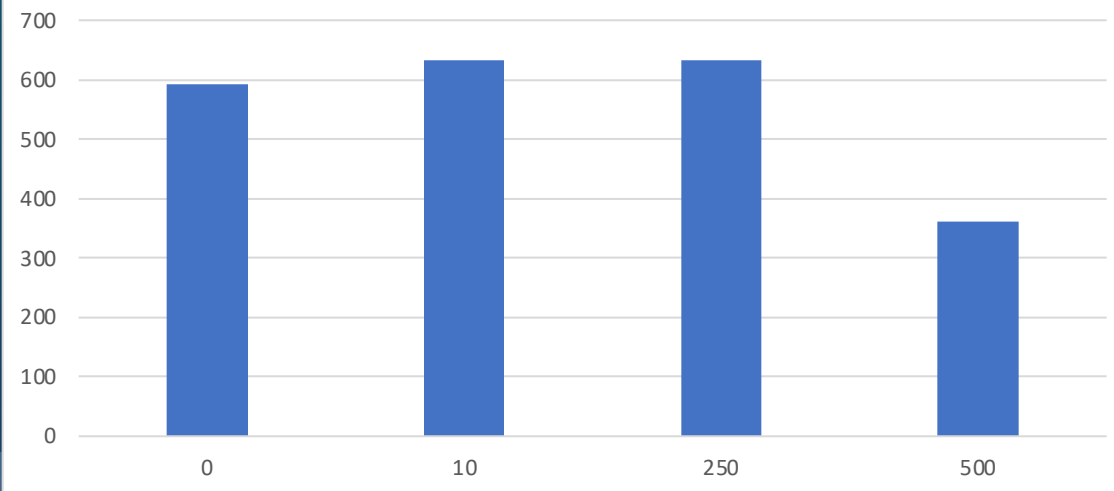
### TR/CS



### ET/CS



### DI/CS



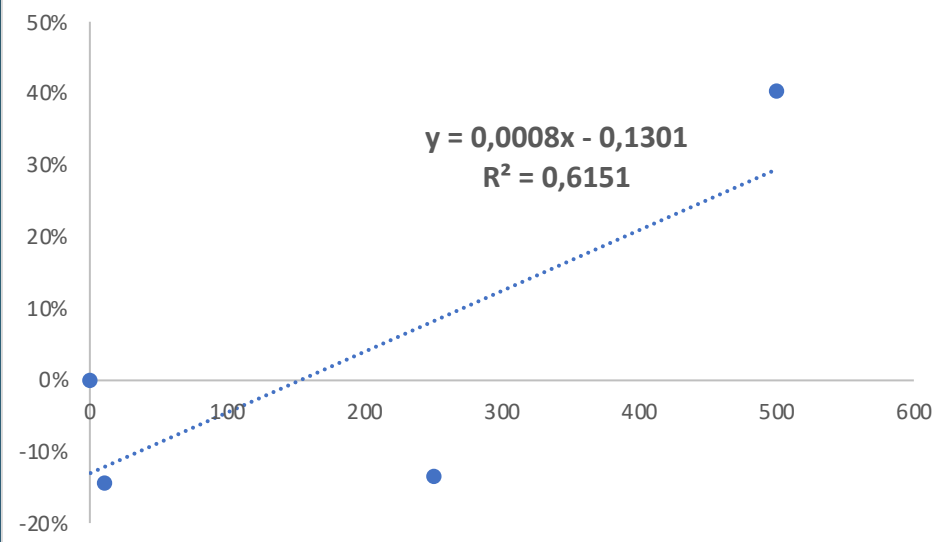
Glif (ug/L)	ABS/CS	TR/CS	ET/CS	DI/CS
0	1 306,00	714,38	330,76	591,62
10	1 494,00	862,04	438,78	631,96
250	1 481,00	847,13	432,88	633,87
500	779,00	417,54	225,06	361,46

$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \overline{\text{Test}}}{\overline{\text{Control}}}$$

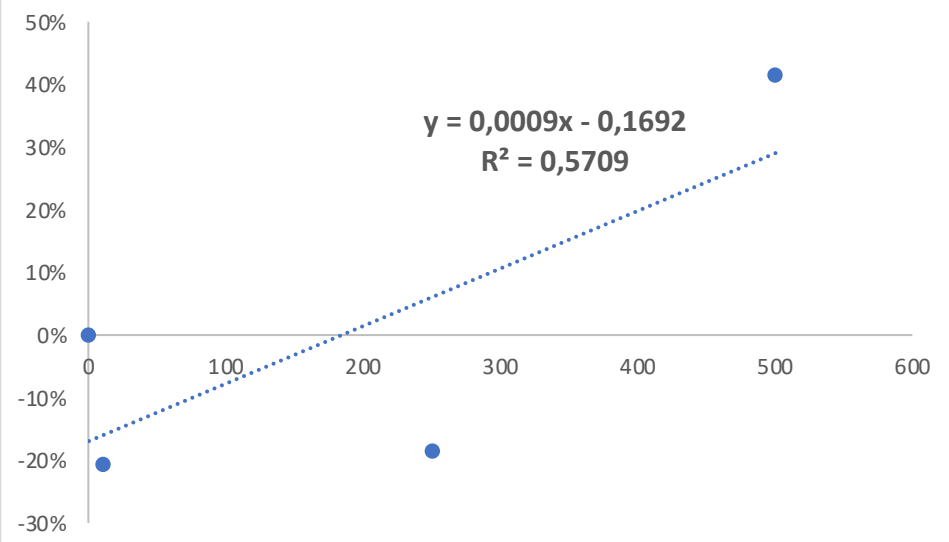
<b>Glif (ug/L)</b>	<b>ABS/CS</b>	<b>%inib ABS</b>	<b>TR/CS</b>	<b>%inib TR</b>	<b>ET/CS</b>	<b>%inib ET</b>	<b>DI/CS</b>	<b>%inib DI</b>
0	1 306,00	0%	714,38	0%	330,76	0%	591,62	0%
10	1 494,00	-14%	862,04	-21%	438,78	-33%	631,96	-7%
250	1 481,00	-13%	847,13	-19%	432,88	-31%	633,87	-7%
500	779,00	40%	417,54	42%	225,06	32%	361,46	39%

$$\text{Inhibition (\%)} = \frac{\overline{\text{Control}} - \overline{\text{Test}}}{\overline{\text{Control}}}$$

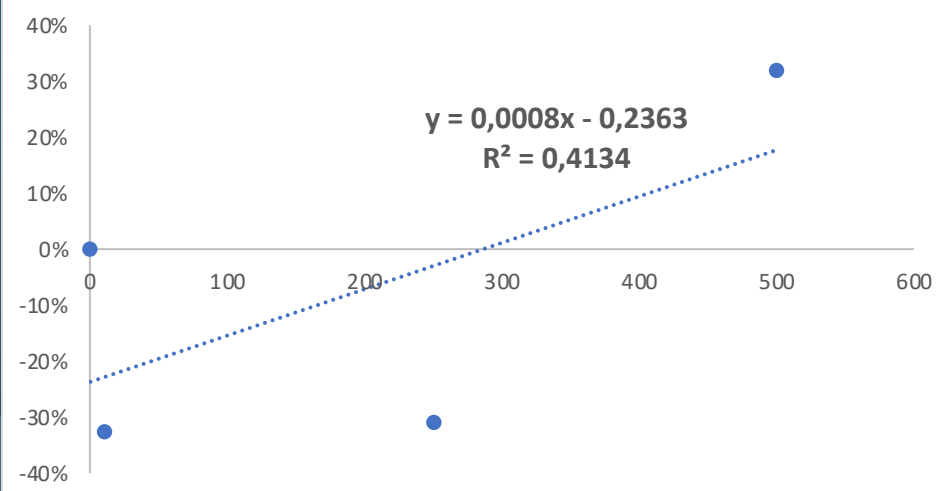
### IC50 Abs



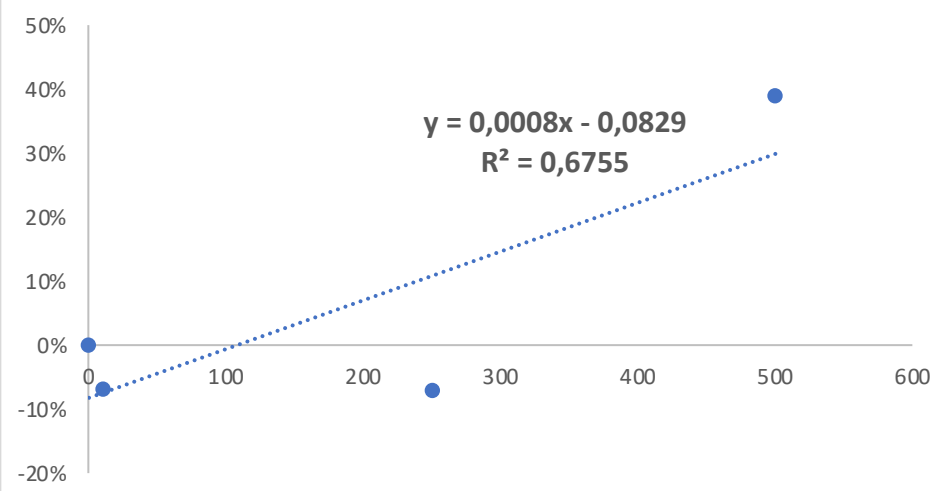
### IC50 TR

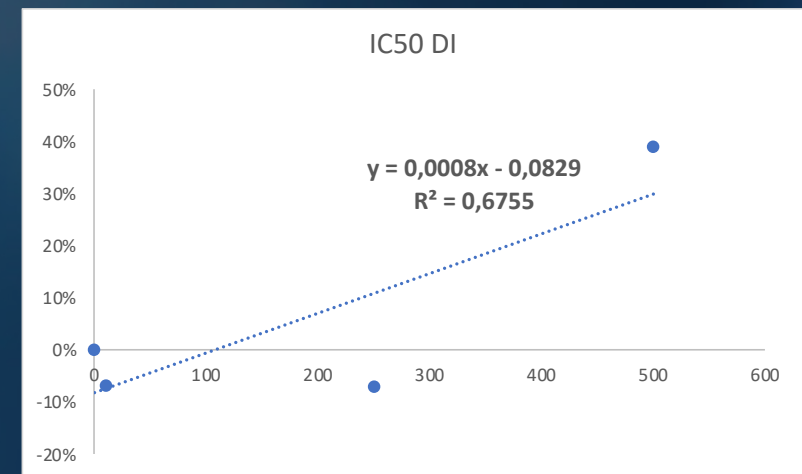
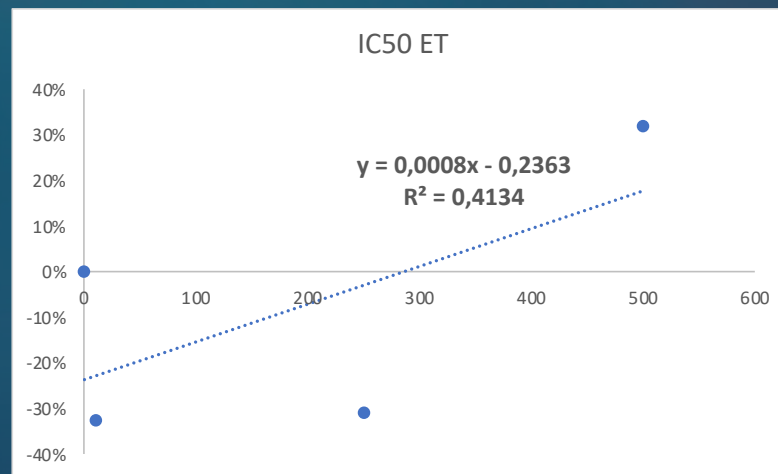
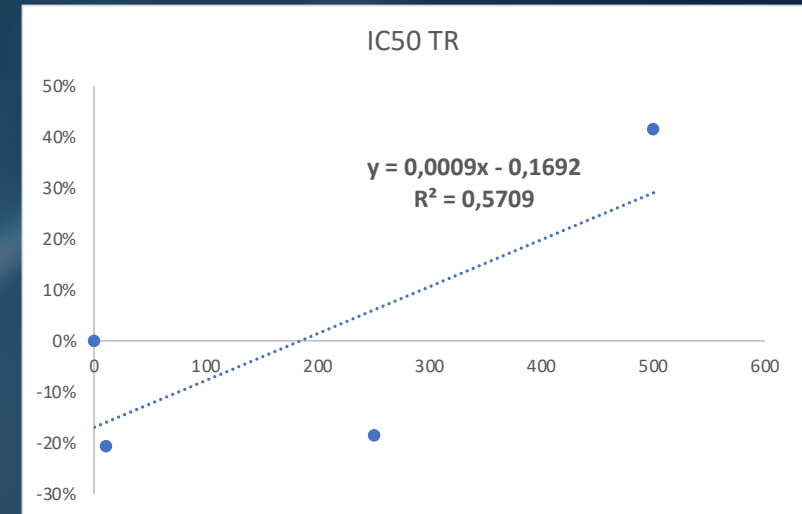
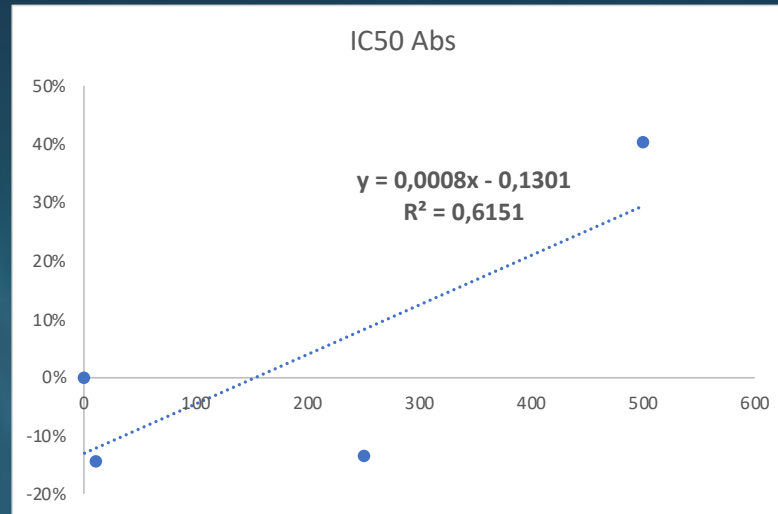


### IC50 ET

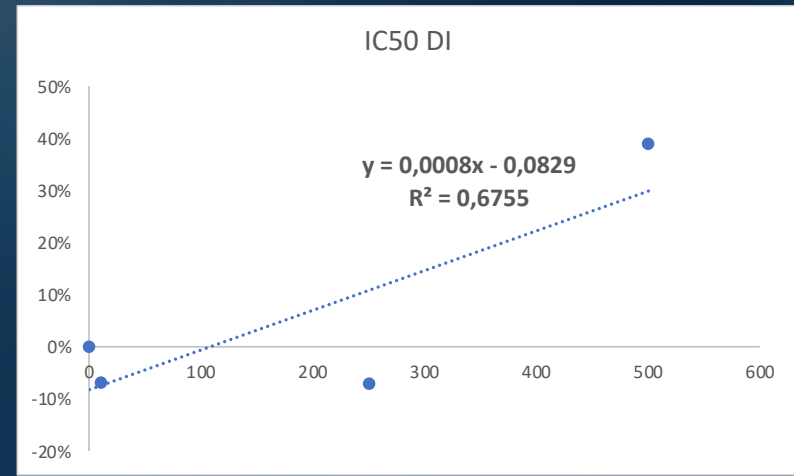
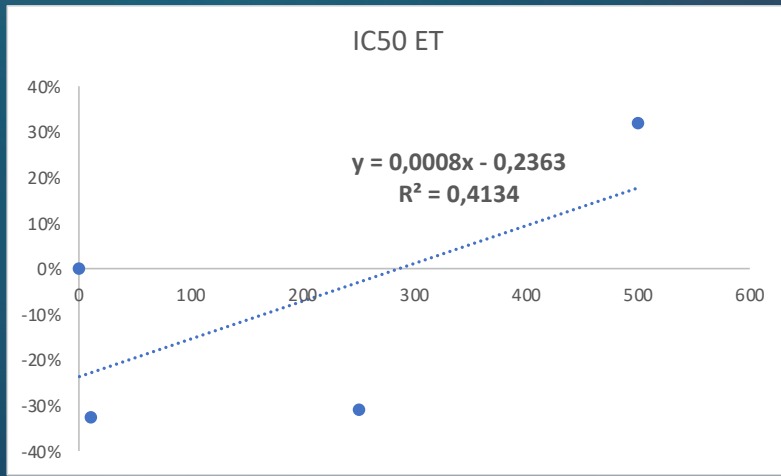
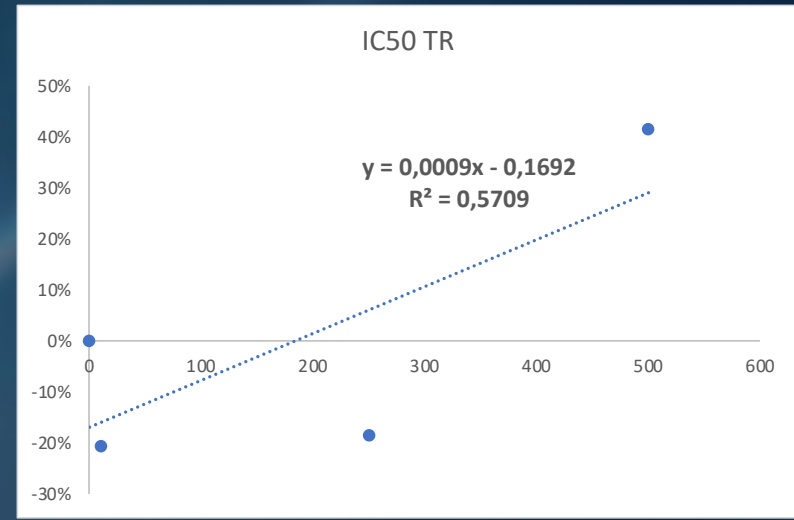
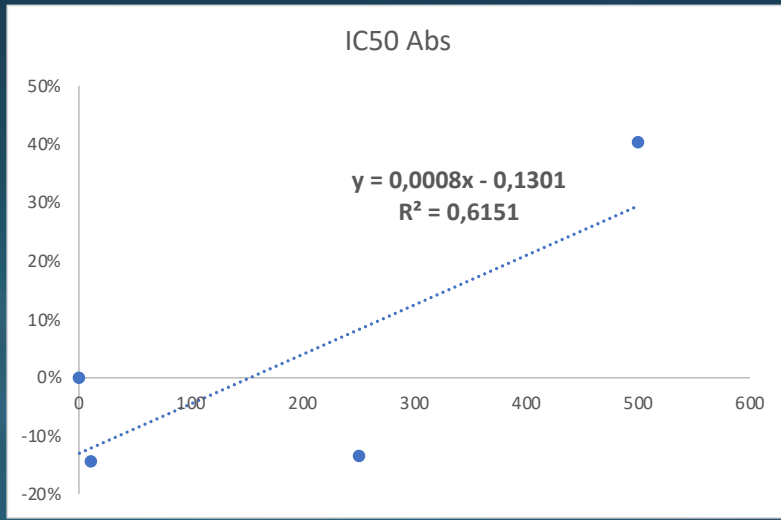


### IC50 DI



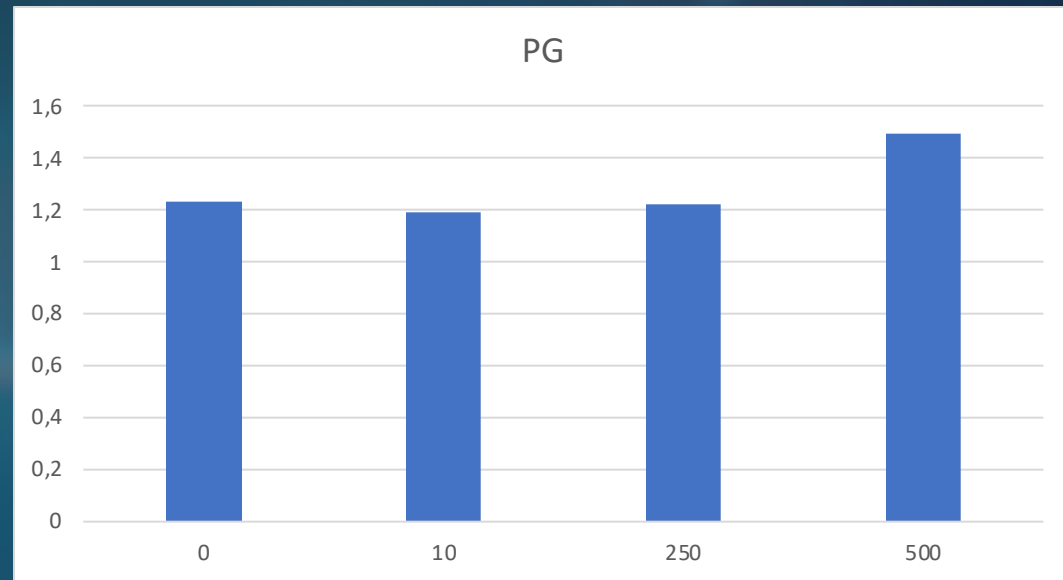
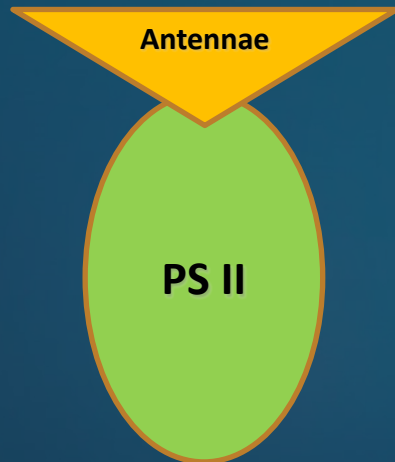


	IC <sub>50</sub> (ug/L)
<b>ABS</b>	787,625
<b>TR</b>	743,556
<b>ET</b>	920,375
<b>DI</b>	728,625



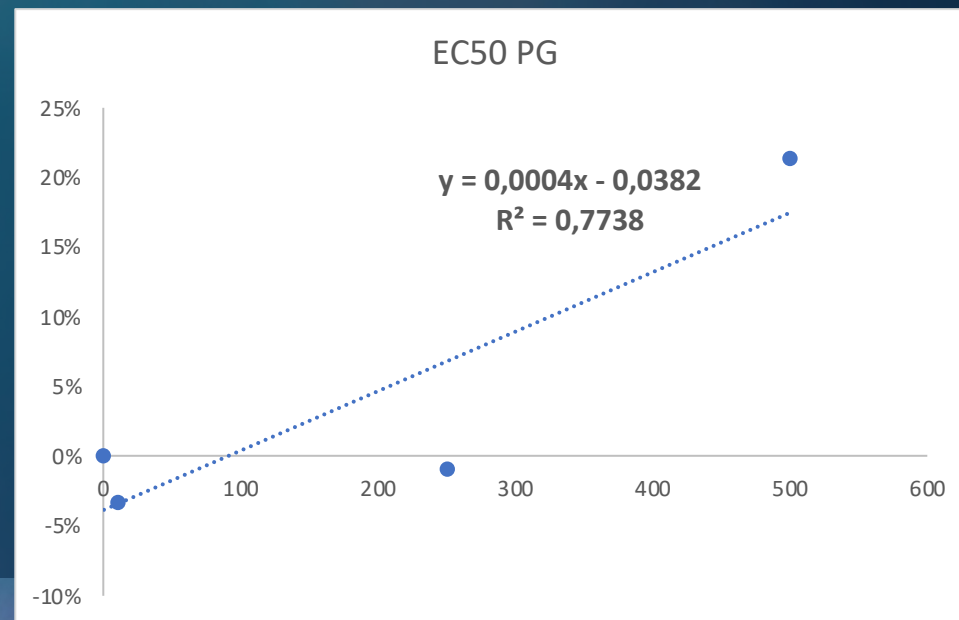
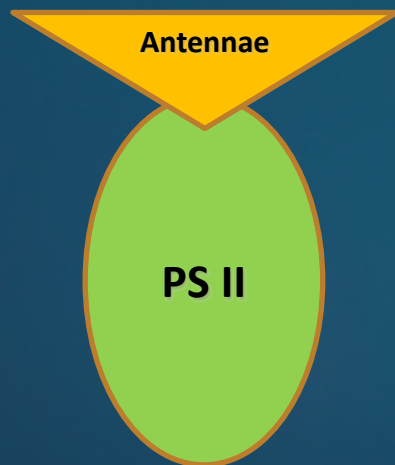
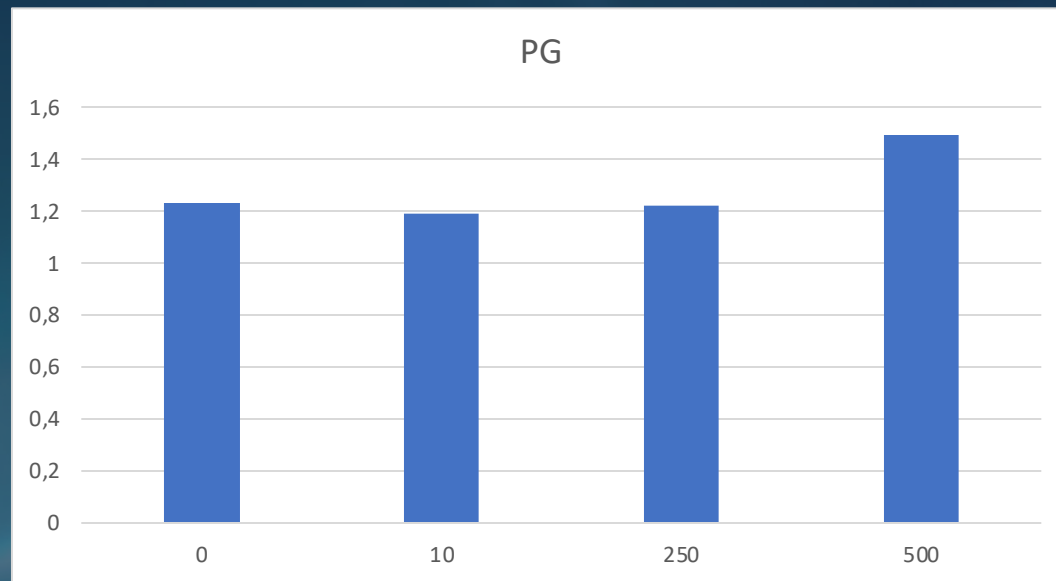


[Glif] ug/L	PG
0	1,23174396
10	1,19064228
250	1,22072997
500	1,49509889



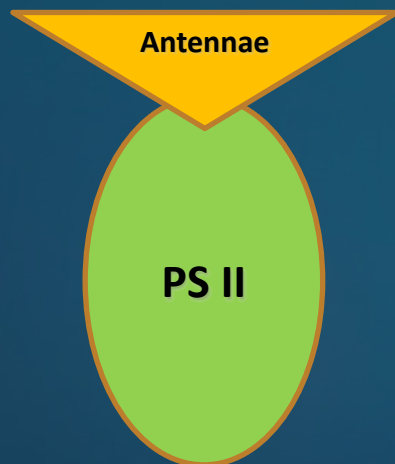
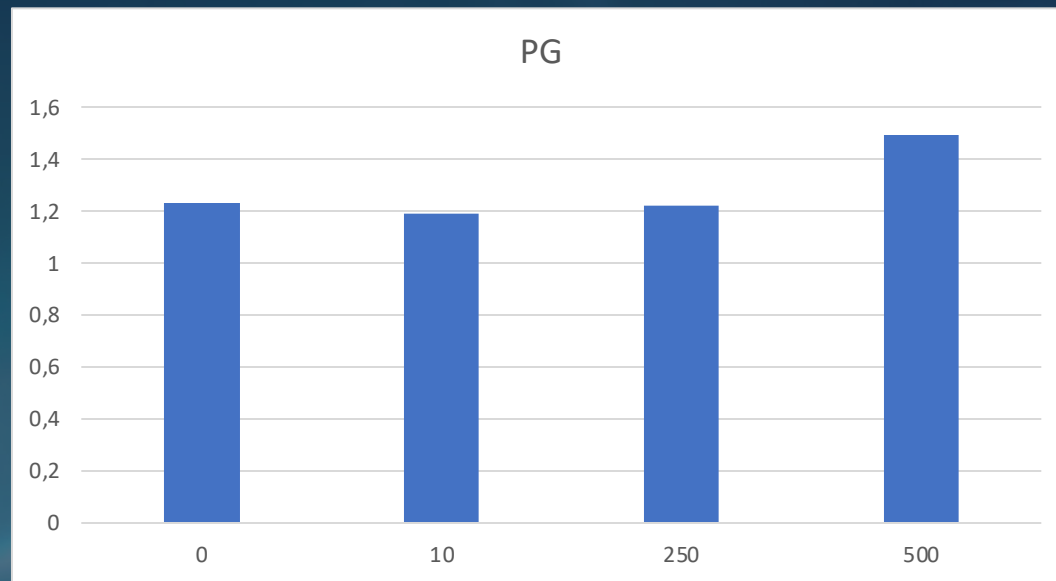
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[Glif] ug/L	PG	%Enhanc
0	1,23174396	0%
10	1,19064228	-3%
250	1,22072997	-1%
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$EC_{50} = 1154,5 \text{ ug/L}$

