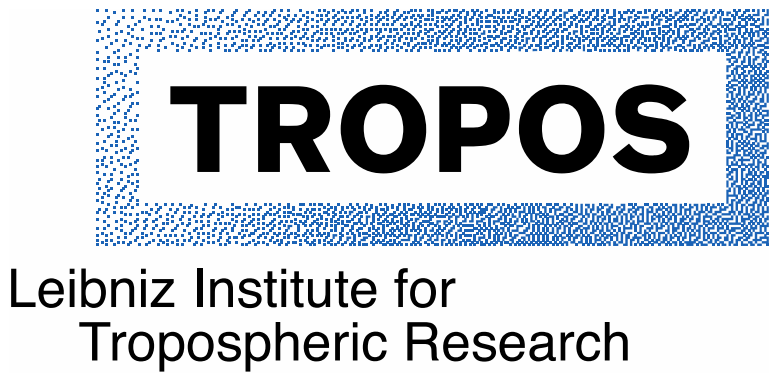


# Evaluating Synergisms and Antagonisms of Ascorbic Acid (AA) Redox Mechanism in Oxidative Potential (OP) Measurements

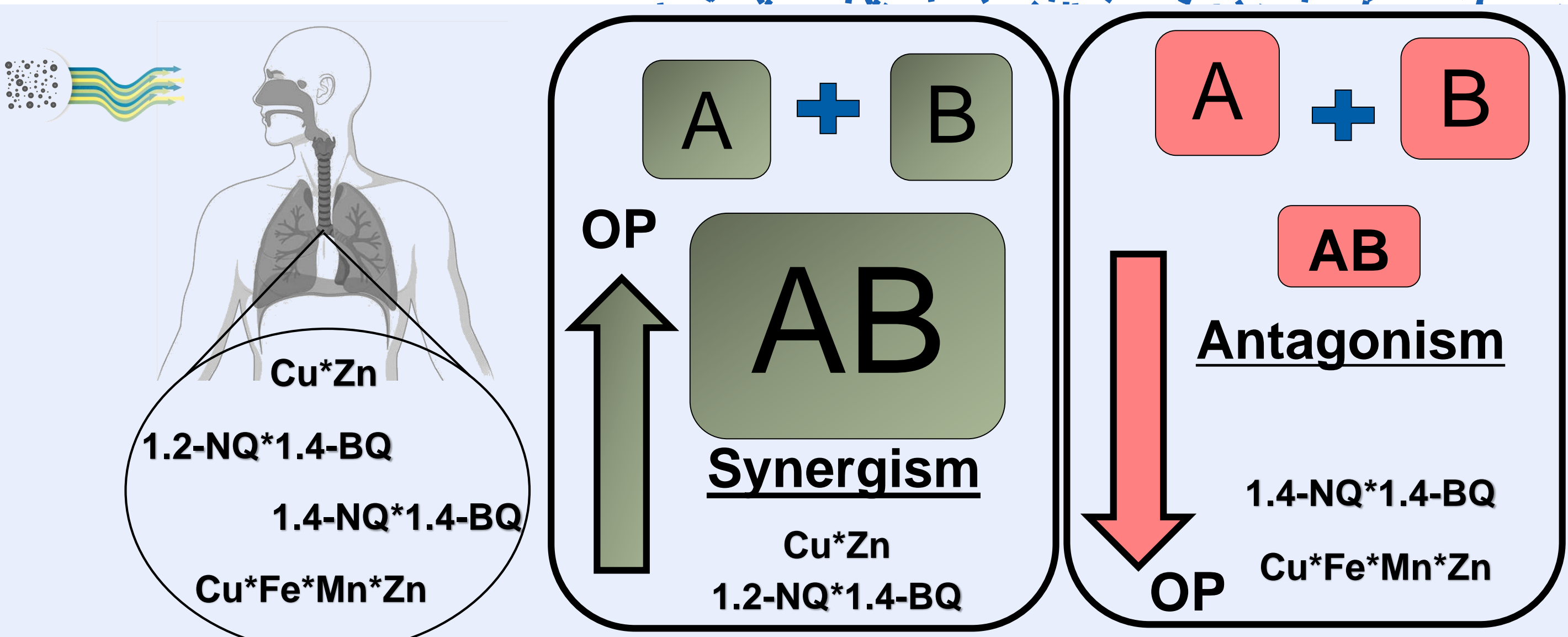
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## Introduction

**Synergistic** and **antagonistic effects** have the interesting ability to explain over or underestimated values of specific outcomes that is not fully understood by applying a simple sum of individual values. The oxidation of **ascorbic acid (AA)** - one of the most abundant antioxidants present in lung fluids - can be investigated through the dehydroascorbic acid formation when redox-active species in PM are reduced (Visentin, 2016). This study presents a critical evaluation of chemical interactions of two **oxidative potential (OP)** assays of the AA mechanism: simple chemical assay (**OP<sup>AA</sup>**) and the related redox potential (RP) in a simulated epithelial lining fluid (**SELF** and hence **OP<sup>AA-SELF</sup>**). A statistical model evaluation was carried out using **factorial design (FD)** - 2<sup>4</sup> (three central points) with 19 experiments - and **interaction factor (IF)** analysis to understand divergences between available OP assays since chemical interactions have shown to present different redox mechanisms which may influence the toxicity prediction of aerosol samples.



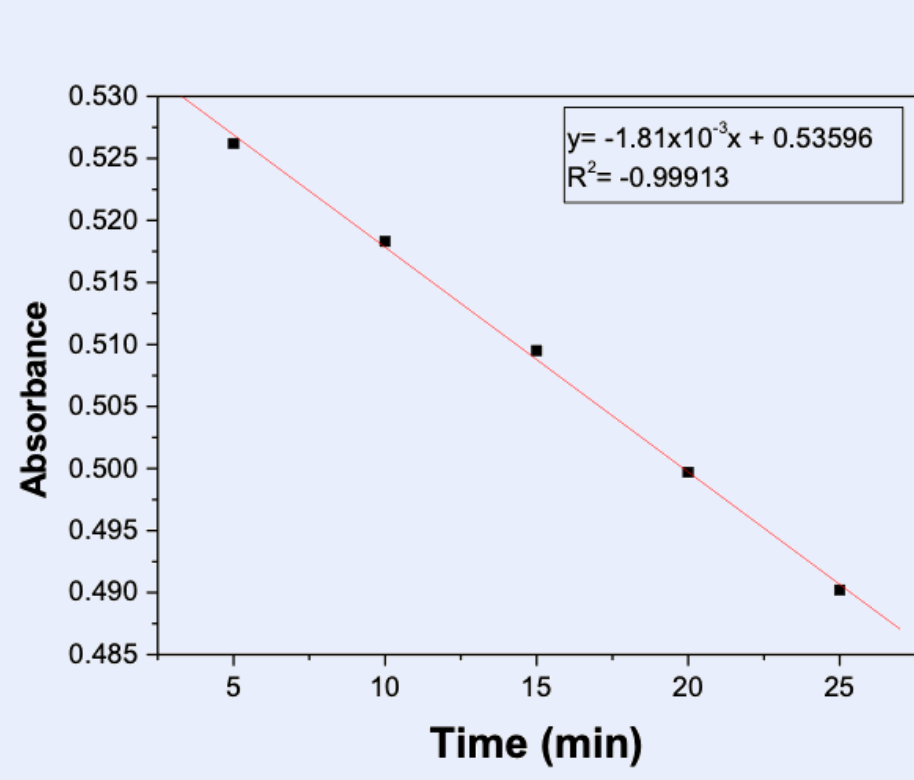
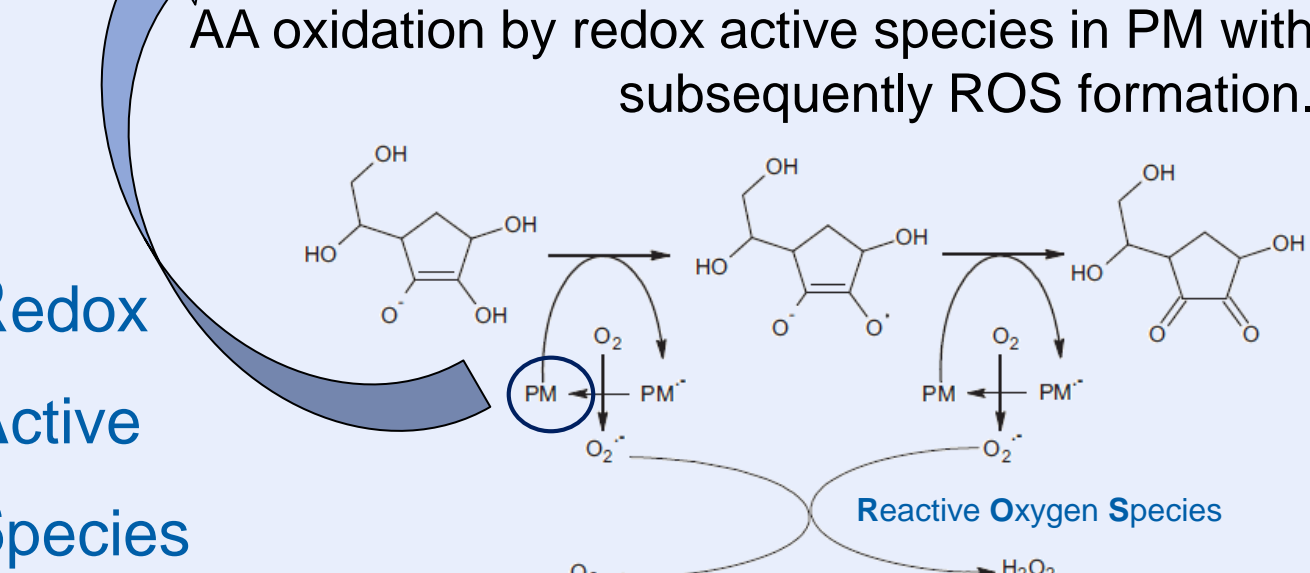
## Methods

### Oxidative Potential Assay

- ✓ OP<sup>AA</sup> and OP<sup>AA-SELF</sup> assays were investigated under 25 min of incubation.
- ✓ 30 µL of 10 mM AA - which contains 300 nmol of AA – into PM extract solutions and standard solutions were investigated.
- ✓ The AA depletion was investigated directly in the spectrophotometric cuvette due to the AA ion formation at 265 nm under 37 °C (ε=14500M<sup>-1</sup>cm<sup>-1</sup> at pH 7.4).
- ✓ The SELF in this work was made of phosphate-buffer saline solution, which was composed of a mixture of glutathione (GSH), citric acid (CA), and uric acid (UA) (200 µM each).

### Factorial Design Investigation

Chemical species	Low (-) (nM)	Central point (0) (nM)	High (+) (nM)
1,2-naphthoquinone (1.2-NQ)	0	40	80
1,4-naphthoquinone (1.4-NQ)	0	40	80
9,10-phenanthroquinone (9.10-PQ)	0	40	80
1,4-benzoquinone (1.4-BQ)	0	40	80
Cu	0	50	100
Fe	0	500	1000
Mn	0	500	1000
Zn	0	500	1000



$\sigma_{AA} = \sigma_{Abs} \cdot (N / Abs \cdot)$

**Abs**: slope  
**Abs·**: intercept  
**N**: Initial mol of AA  
**V<sub>a</sub>**: Volume incubated with AA  
**V<sub>e</sub>**: Extraction volume  
**V**: Total air volume sampled  
**A**: Area taken for AA assay  
**A<sub>Total</sub>**: Total area of the sample  
**M**: PM mass concentration (µg m<sup>-3</sup>)

### OP Calculation

**AA<sub>V</sub>**  
(nmol min<sup>-1</sup> m<sup>-3</sup>)

$$\frac{\sigma_{Sample} - \sigma_{Blank}}{\frac{V_a}{V_e} \times V \times \frac{A}{A_{Total}}}$$

**AA<sub>M</sub>**  
(nmol min<sup>-1</sup> µg<sup>-1</sup>)

$$\frac{AA_V}{M}$$

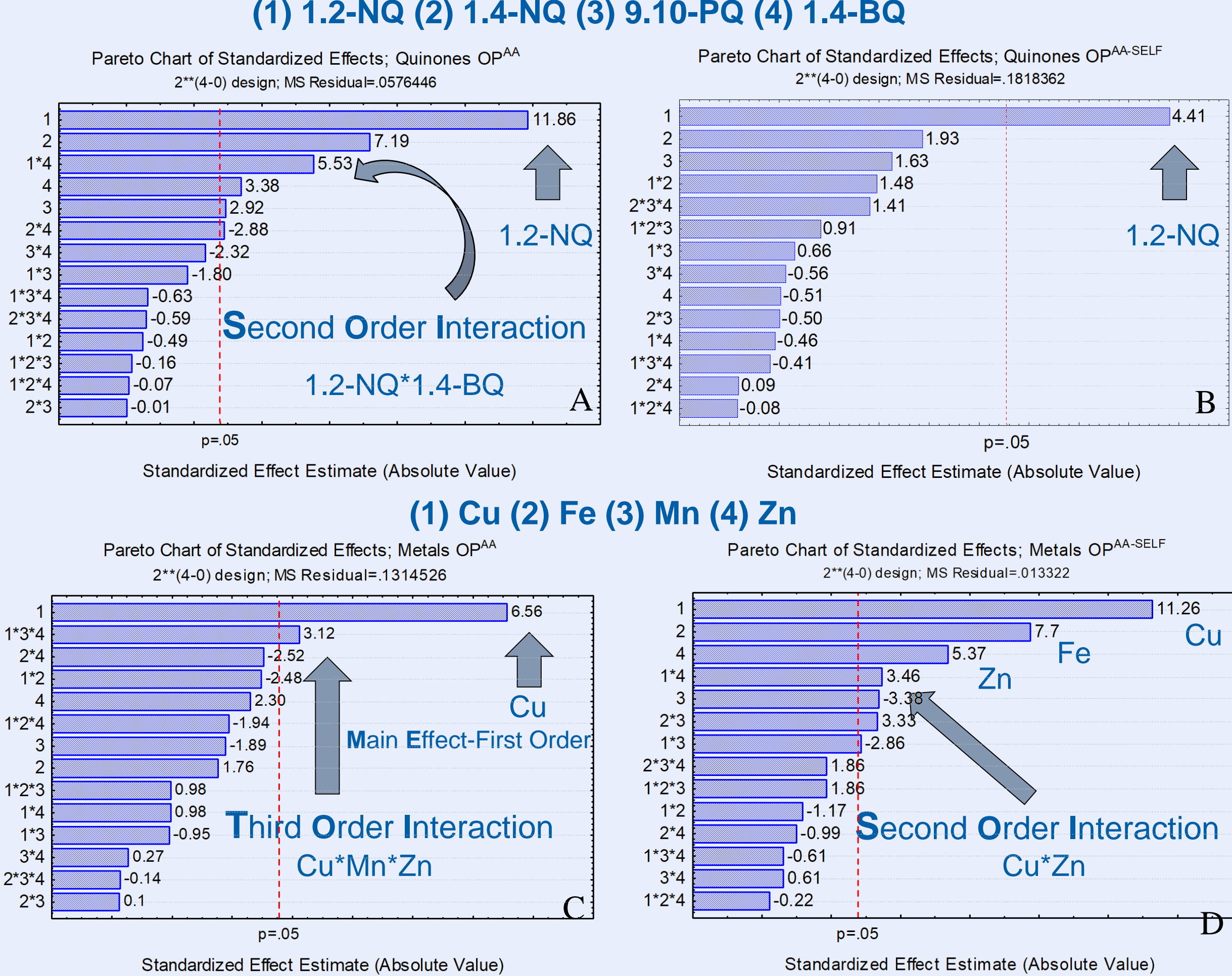
### IF Calculation

$$IF_{A,B,...,k} = \frac{OP_{AB...k}}{OP_A + OP_B + \dots + OP_k}$$

**IF < 1 Antagonism**  
**IF > 1 Synergism**  
**IF = 1 ± 0.2 Additive**

## Results

### Pareto Diagrams



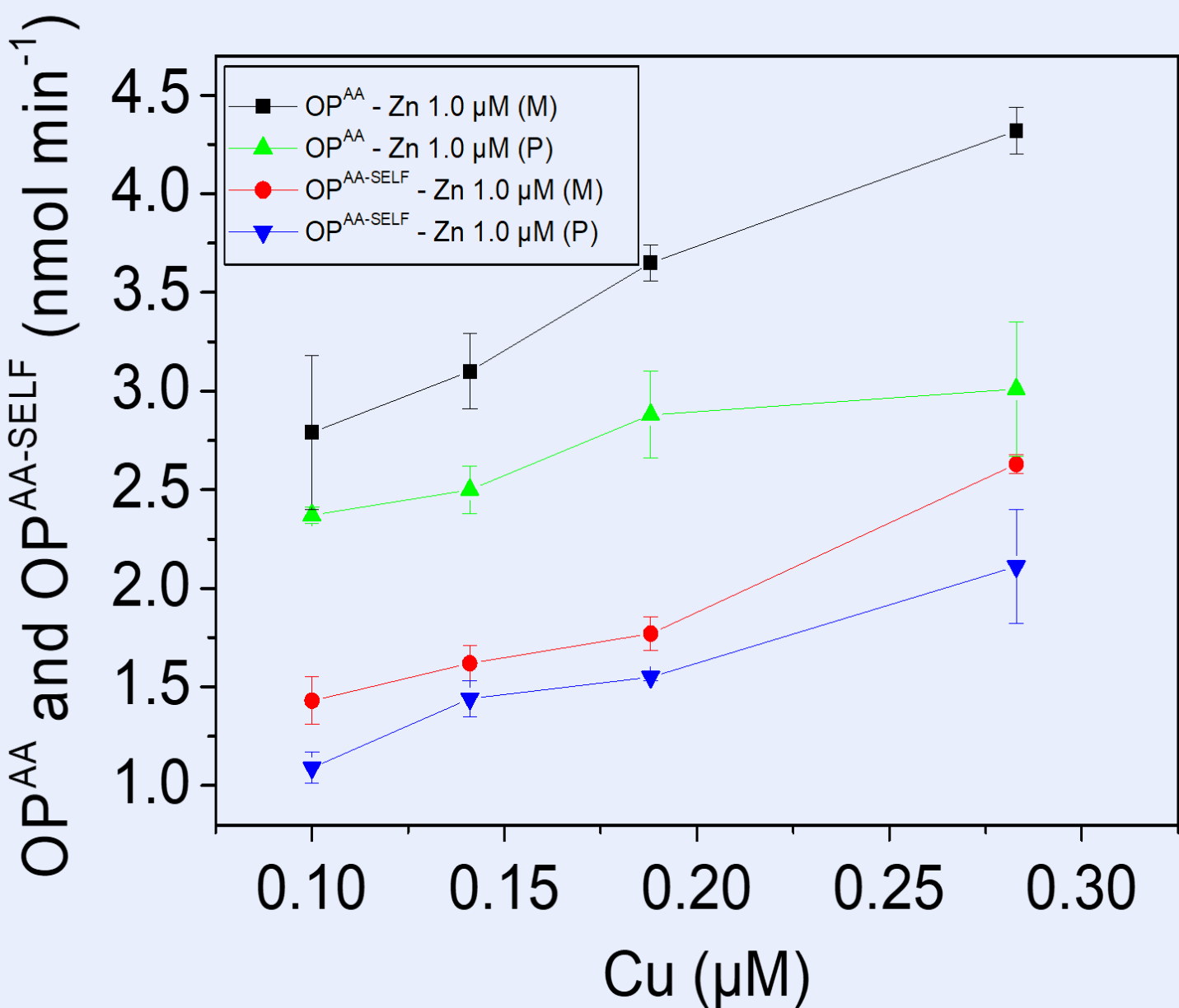
In total, 55 different mixtures were investigated

38 Antagonisms  
15 Synergisms  
2 Additives

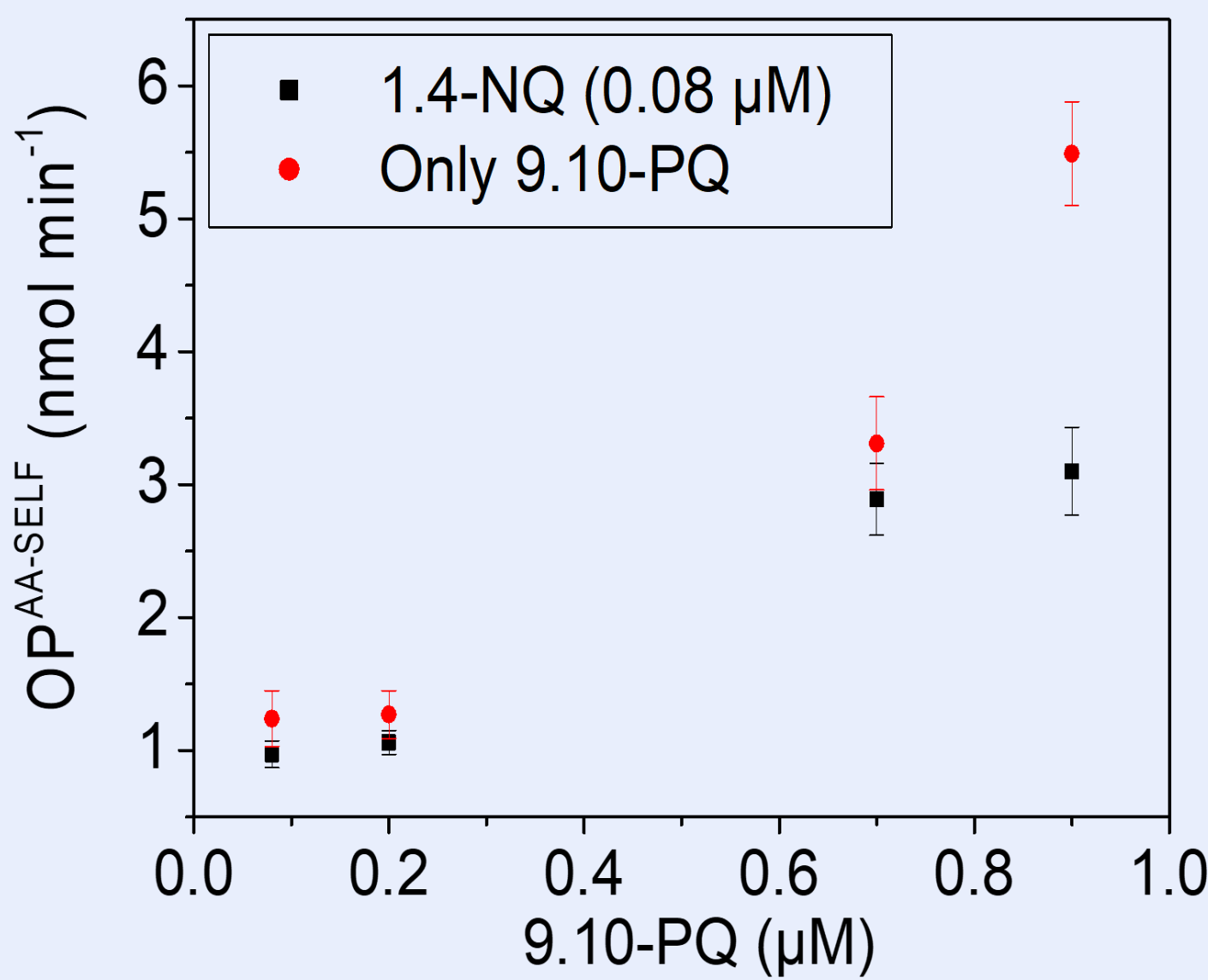
### IF for binary, ternary and quaternary mixtures

Chemical Interaction	Interaction Factor	
	OP <sup>AA</sup>	OP <sup>AA-SELF</sup>
Cu*Zn	1,18	1,31
Cu*Fe*Zn	0,46	0,81
1.2-NQ*1.4-BQ	2.64	1.33
1.4-NQ*9.10-PQ*1.4-BQ	0.59	0.33
Cu*9.10-PQ*Zn*1.4-NQ	-	0.36

- ✓ 1.2-NQ is a significant parameter for both assays (Fig. 1 A&B), but second-order interactions involving 1.2-NQ is only representative for OP<sup>AA</sup>
- ✓ 1.4-NQ, 9.10-PQ and 1.4-BQ are also significant parameters to modify OP<sup>AA</sup> but in a smaller order of magnitude
- ✓ Cu is a statistically significant factor in both assays achieving higher effects in OP<sup>AA-SELF</sup>, which showed a higher estimated factor compared to Fe.



Evaluating the OP<sup>AA-SELF</sup> assay under variation on 9.10-PQ concentration in the presence of 0.08 µM of 1.4-NQ



Measured and predicted OP values for the binary mixture of Cu and Zn in OP<sup>AA</sup> and OP<sup>AA-SELF</sup> assays.

## Conclusion

- AA assays present **more antagonistic effects** compared with **synergistic**.
- The redox system involving the **depletion of AA** by redox active species **poorly depends on additive effects**.
- Cu and Zn have demonstrated **strong synergism** for both OP assays.
- 1.4-NQ combined with 9.10-PQ cause **antagonisms** for **binary, ternary and quaternary mixtures**.

## References

Visentin et al. Env Pol 2020  
Pietrogrande et al. Atm Env 2019  
Bates et al. Env Sci Tec 2019