Comparison between five acellular oxidative potential measurement assays performed with detailed chemistry on PM_{10} samples from the city of Chamonix (France)

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Section 1 Details on the chemical compounds analyzed

Briefly, the instrumental techniques used for PM_{10} chemical characterization are:

- A Sunset instrument and the EUSAAR2 protocol for elemental and organic carbon (OC, EC).
- An AE33 Aethalometer for BC and the distinction between wood burning and fossil fuel BC.
- Ionic chromatography for soluble anions and cations (NO_3^-, SO_4^{2-}, Cl^- and NH_4^+, Mg^{2+}, Na^+, Ca^{2+}, K^+) after water extraction.
- ICP-MS for inorganic elements (Al, Fe, Ti, As, Ba Cd, Ce, Cr, Cu, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sn, Sr, V, Zn and Zr) after acidic digestion.
- HPLC-PAD method for sugar alcohols (arabitol, sorbitol, and mannitol, also called polyols) and monosaccharide anhydrides (levoglucosan, mannosan and galactosan) after a water extraction.
- A combination of separation on a DEAE resin and quantification with a DOC analyzer for humic like substances (HULIS°) after a Milli-Q water.
- GC-MS for polar and apolar organics tracers (alkanes, hopanes, methoxyphenols, and substituted derivatives (methyl-PAHs) and polycyclic aromatic sulfur heterocycles (PASHs) after an ASE extraction
- HPLC-fluorescence for polycyclic aromatic hydrocarbons (PAH) after ASE extraction

Details on the organic compounds analyzed are given below:

- ∑ **alkanes** correspond to the sum of C11-C40 alkanes plus pristane and phytane.
- ∑ **hopanes** correspond to the sum of 10 analyzed hopanes.

- ∑ **methoxyphenols** correspond to the sum of the following analyzed methoxyphenols**:** vanillin, acetovanillone, guaiacyl acetone, coniferylaldehyde, vanillic acid, homovanillic acid, syringol, 4-methylsyringol, 4-propenylsyringol, acetosyringone, syringyl acetone, sinapyl aldehyde, syringic acid.
- ∑ **PAHs** correspond to the sum of the following analyzed PAH: phenanthrene, anthracene, fluoranthene, pyrene, triphenylene, retene, benzo[a]anthracene, chrysene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k]fluoanthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene, indeni[1,2,3-cd]pyrene, coronene.
- ∑ **methyl-PAHs** correspond to the following compounds: 2-methylnaphtalène, 1-methylfluorene, methylphenanthrenes, methylanthracenes, methylpyrenes, methylchrysene/benzoanthracene, benzo[a]anthracene-d12.
- ∑ **PASHs** correspond to the following compounds: dibenzothiophene, phenanthro[4,5-bcd]thiophene, benzo[b]naphthothiophenes, dinaphto(2,1-b:1'2'-d]thiophene, benzo[b]phenanthro(2,1-d)thiophene.

Section 2 DTT and AA assays

Linear response to PM concentrations : DTT and AA assays:

In both cases, 1 to 6 punches from 2 samples (different year period) were extracted using Gamble + DPPC solution and in order to investigate assay response pattern towards PM concentration. Non-linear response was observed in the case of the DTT assays (Figure S1 A) whereas for the AA assays linear pattern was observed (Figure S1 B).

Field vs lab blank filters : DTT assay:

Field blanks equally distributed over the sampling year were analysed. Sampling dates of these blanks are: 23/12/2013, 17/02/2014, 12/05/2014, 23/06/2014, 25/08/2014 and 06/10/2014. DTT depletion by field blanks was higher than DTT depletion by lab blanks, possibly highlighting some contamination (Figure S2). However, the fieldblank depletion was stable over the sampling year (CV 9%) and no back corrections were realized.

Field vs lab blank filters :AA assays:

Field blank sampling dates were identical to those employed in the DTT assay. For the AA assay, no difference in AA depletion was found between field and lab blanks (Figure S3).

Section 3 The ESR assay

For ESR assay, used parameters were: Microwave power, 1.7 mW; Modulation amplitude, 0.18 mT; Modulation frequency, 100 KHz. The spectra exhibit the 4-line pattern with ratio of intensity 1:2:2:1 that is reminiscent of the $HO^{\bullet}$ adduct of DMPO. Before quantification we ensured that the spectra were recorded under non-saturating conditions. For the measurement of intensity two methods were tested: Double integration of the whole spectra or measurement of the height of the central lines (the linewidth was constant within the series). The second method was preferred since more accurate and reproducible.

We investigate the influence of the extraction time on the intensity of the ESR signal. Results are depicted in the Figure S4, a plateau was reached around 40 min. After approximately one hour the signal slightly decreases, presumably due to the decomposition of the OH-DMPO adduct.

The compatibility between the assay and the use of Gamble + DPPC solution was tested as follows: filter punches from the same sample (03/12/2013) were placed in 1 mL tubes to which were added 125 µL of H_2O_2 (0.5 M) and 250 µL of DMPO (0.05 M). Then, 125µL of either Milli-Q water or a Gamble + DPPC solution was added to the tubes. Tubes were vortexed during 15 seconds before being placed in an agitator plate for incubation at 37° C for 40 min. After incubation, the suspension was vortexed again for 15 s. 35 µL of the suspension was then transferred into a capillary tube to measure hydroxyl radical ($HO^{\bullet}$) formation catalyzed by PM_{10} by ESR spectroscopy. The results showed much weaker signal intensity for the samples prepared in the Gamble + DPPC medium in comparison to Milli-Q water (Figure S5).

Calibration curve was obtained as follows: Half, 1 and 2 filter punches deriving from the same sample (03/12/2013) were placed in 1 ml tubes, 125 µL Milli-Q water, 125 µL H_2O_2 (0.5 M) and 250 µl of DMPO (0.05 M) were added. The 3 sub-samples were vortexed for 15s, placed in incubation at 37°C for 40 min, vortexed again and finally, 35 µl were transferred to capillary tubes for analyses. This relationship shows that the intercept = 0 was not reached ($y= 2484.4x + 32\,700$). We made the supposition that the relationship between 0 µg and the 1st measuring point (2.5 µg) was linear. For samples overpassing 2.5 µg of PM in the capillary tube, the signal was corrected using the linear curve. Thirty-seven samples were corrected out of the 75 analyzed samples.

Section 4 Cumulative score of correlations

Spearman correlations were employed to relate the chemical composition of PM_{10} (48 species/ compounds) with OPv measurements. A score of 1 was attributed when the correlation was >0.45 (i.e. moderate). A cumulative score of correlations was then calculated by adding the number of correlation > 0.45.

As an example, 5/5 cumulative score indicates that the 5 OPv measurements exhibit moderate to strong correlations with the chemical compounds of interest. A 37/48 cumulative score indicates that 37 over 48 chemical species show moderate to strong correlations with OPv.

Section 5 Multivariable linear regression model

An hypothesis on the additive effect of the chemical species on the OP measurement has been assumed (Charrier et al., 2015).

Data set preparation:

Extreme values of chemistry have been removed by performing monthly boxplots for each chemical compound over the sampling year. Samples (sampling days) showing values that lie an abnormal distance (Q75th +1.5 IQR, with IQR Q75th-Q25th) from other values (i.e. outliers) were manually discarded.

Variable transformations were then made to obtain as close as possible, normal distribution. Table S2 summarizes information on the observations used for the models, the variable transformation used, as well as the p value from the Shapiro-Wilk test to test the null hypothesis that OP data or transformed OP data come from a normally distributed population.

For the ESR assay, no transformation was necessary, since the initial distribution is already quasi normal. For DTT, AA and ASC assay log transformations were done. It has to be noted that for the AA assay (single compound assay), two distinct models were performed since the overall data set is distributed among 2 normal distributions, one in a colder period (19 observations from November to mid-March), and other in a warmer period (40 observations from mid-March to October). Finally, for the GSH assay, square root transformations were done, but normal distribution was not really reached. However, analysis was pursued for this assay (GSH assay) as a first rough investigation.

Model realizations and validations:

BIC number allows selecting variables that are significant to explain observed OP values. For models evaluation, residues and collinearity between predictors (chemical species) were taken into account (Table S3). All residue models are quite well randomly and normally distributed around zero. In the case of OP GSHv, the normal distribution was, once again, not fully reached (p-value = 0.05). The residual plot for log (AAv) for the cold period shows that residues are not evenly distributed. Because of the small dataset for this period (n=19), it is difficult to conclude whether it is the effect of the very small dataset

or the presence of two different groups than cannot be modeled similarly. This second assumption for OP AAv cannot be excluded since two groups have already been identified for the two seasons.

To evaluate collinearity, VIF (variance inflation factors) were calculated and were under 10 indicating no collinearity between chemical species used in the model.

Application to the overall data set:

For some observations, the models, when applied to the general data set, overestimated OP values (more than 2 times higher than observed OPv). These values represent only between 6 % (OP AAv) to 13% (OP GSHv) of the number of observations and are mostly found during the cold period, indicating that PM of very different nature can happen at this time. The smaller number of overestimated observations in the case of the AA assay is due to the two models used, with one centered on the cold period, such combination of 2 models leading to the best overall model explaining OP variance.

Contribution of each predictor:

The daily contribution of predictors was calculated by using coefficients obtained in the different model and daily atmospheric concentration of predictors. Log(OPv) and √OPv were back-transformed in order to obtain predicted OPv and daily contribution of predictors to the predicted OPv. Cold and warm period means were calculated, without taking overestimated values.

References

Charrier, J. G., Richards-Henderson, N. K., Bein, K. J., Mcfall, A. S., Wexler, A. S. and Anastasio, C.: Oxidant production from source-oriented particulate matter – Part 1 : Oxidative potential using the dithiothreitol (DTT) assay, Atmos. Chem. Phys., 15, 2327–2340, doi:10.5194/acp-15-2327-2015, 2015.

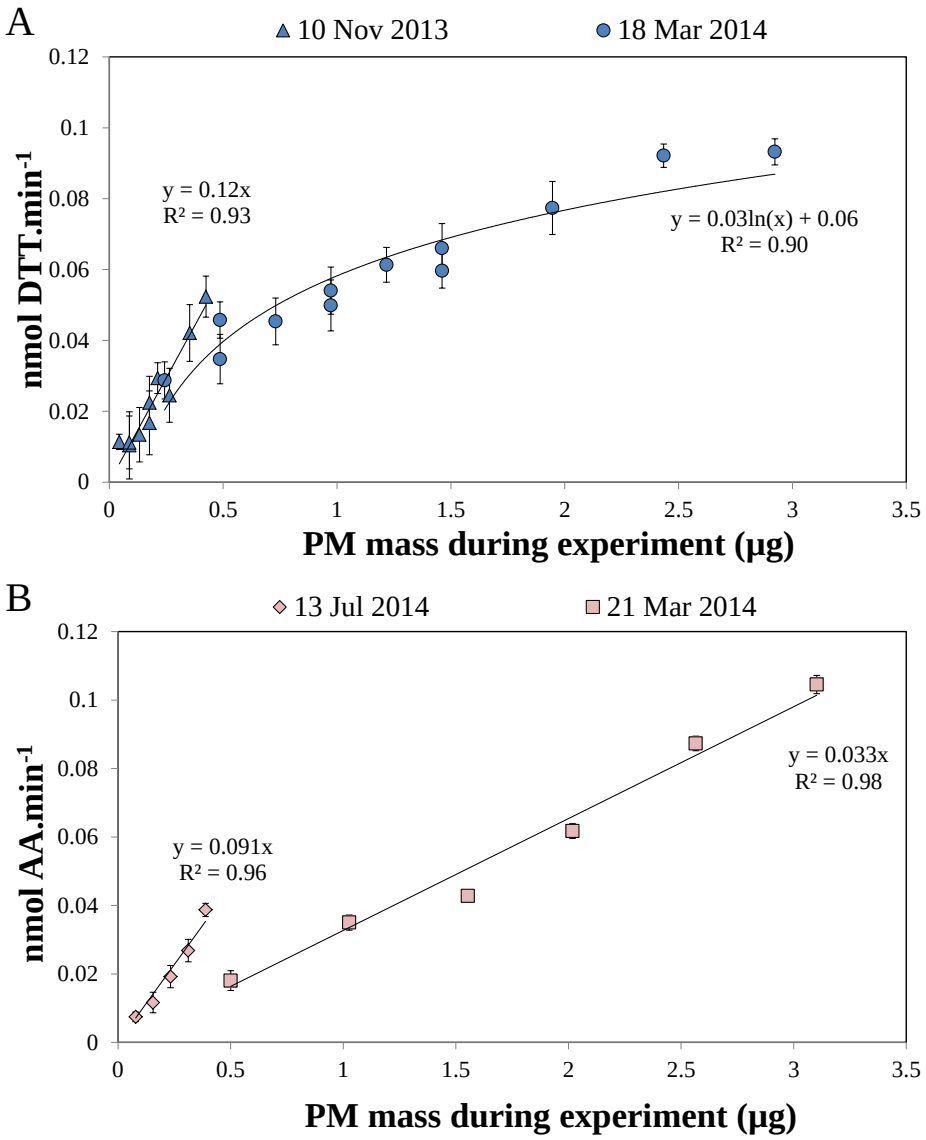


Figure S1: Linear response of OP assays towards PM mass added during the experiments (A: DTT assays, B: AA assays)

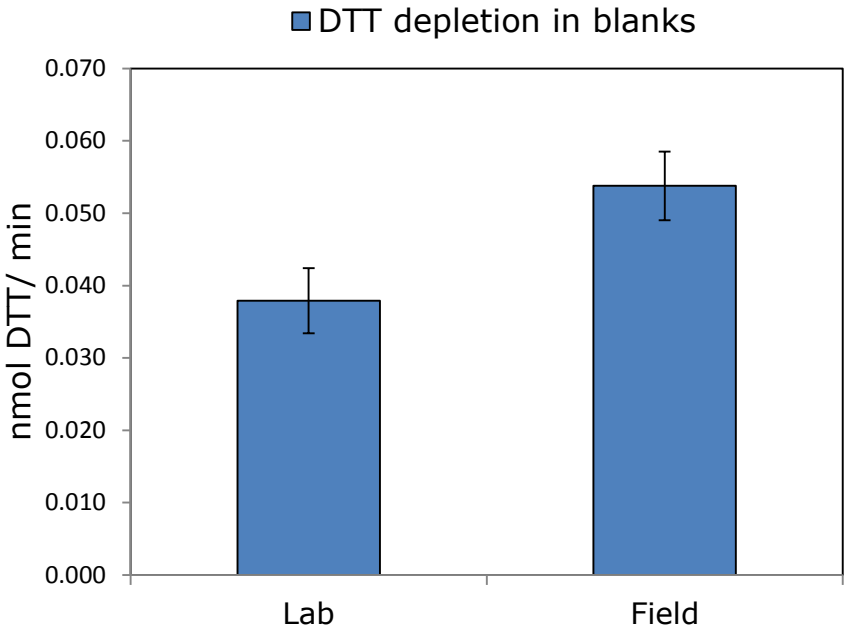


Figure S2 : DTT depletion in laboratory (n=13) and field (n=6) blank filters analyzed in triplicates.

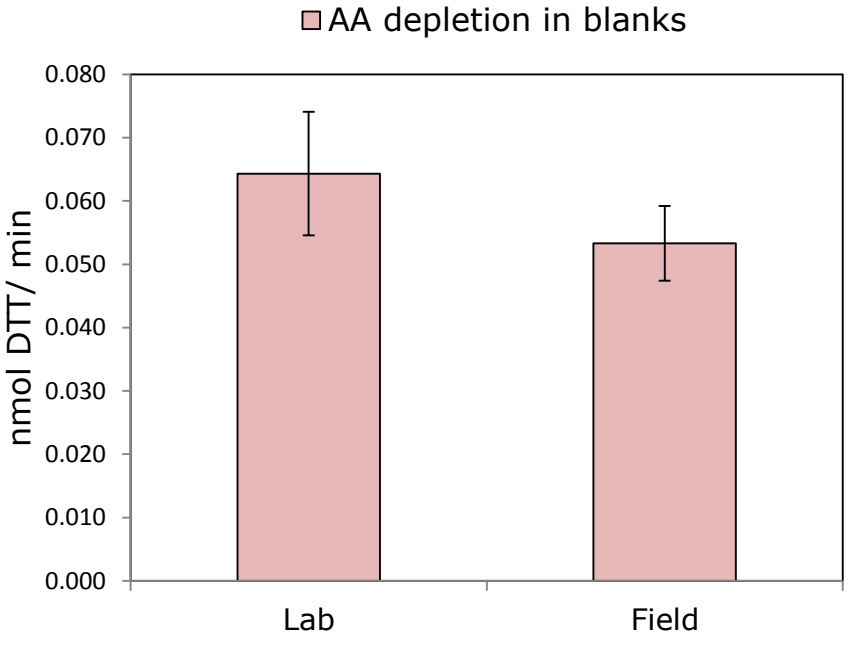


Figure S3 : AA depletion in laboratory (n=7) and field (n=6) blank filters analyzed in triplicates.

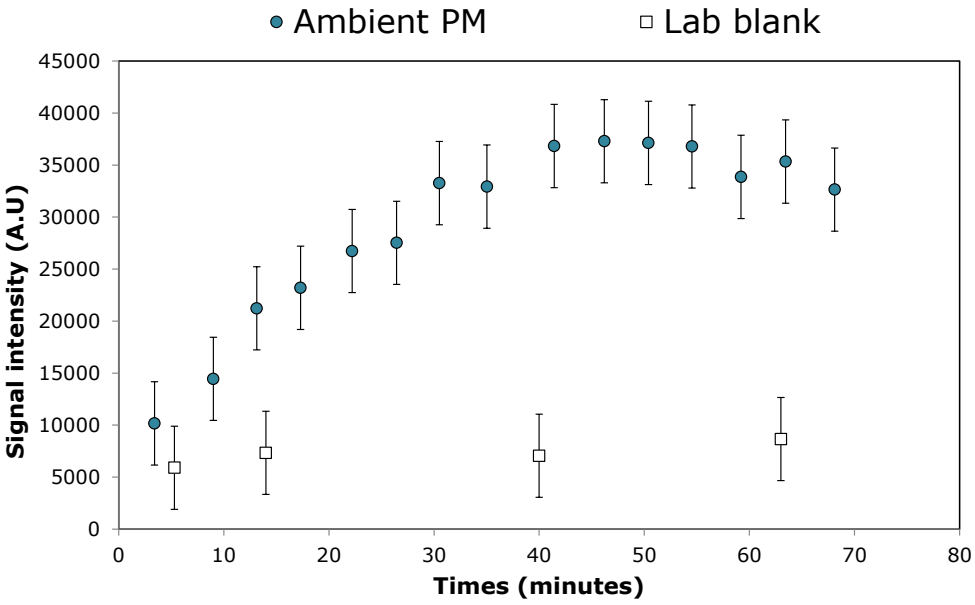


Figure S4 : Extraction kinetics in the ESR assay.

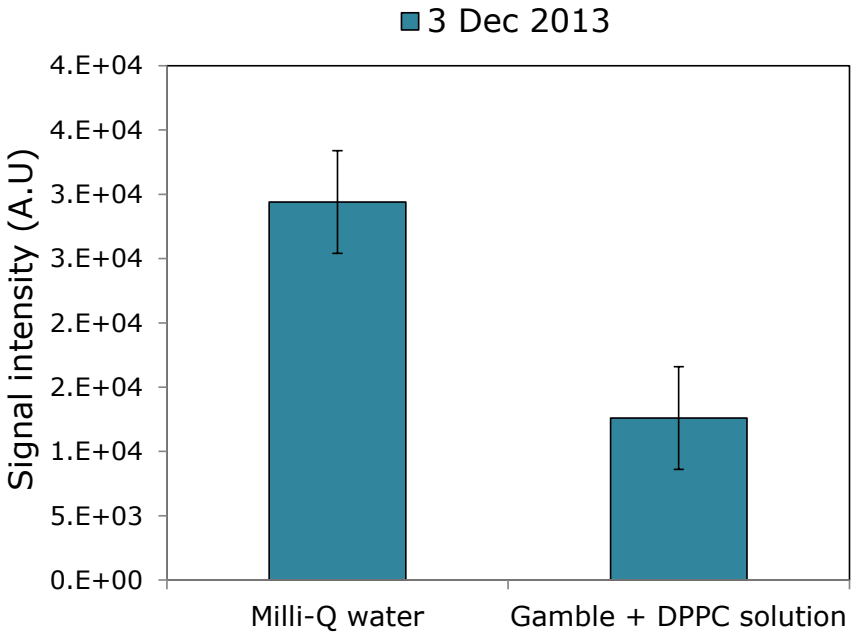


Figure S5 : ESR signal intensity modifications among extraction filter solutions.

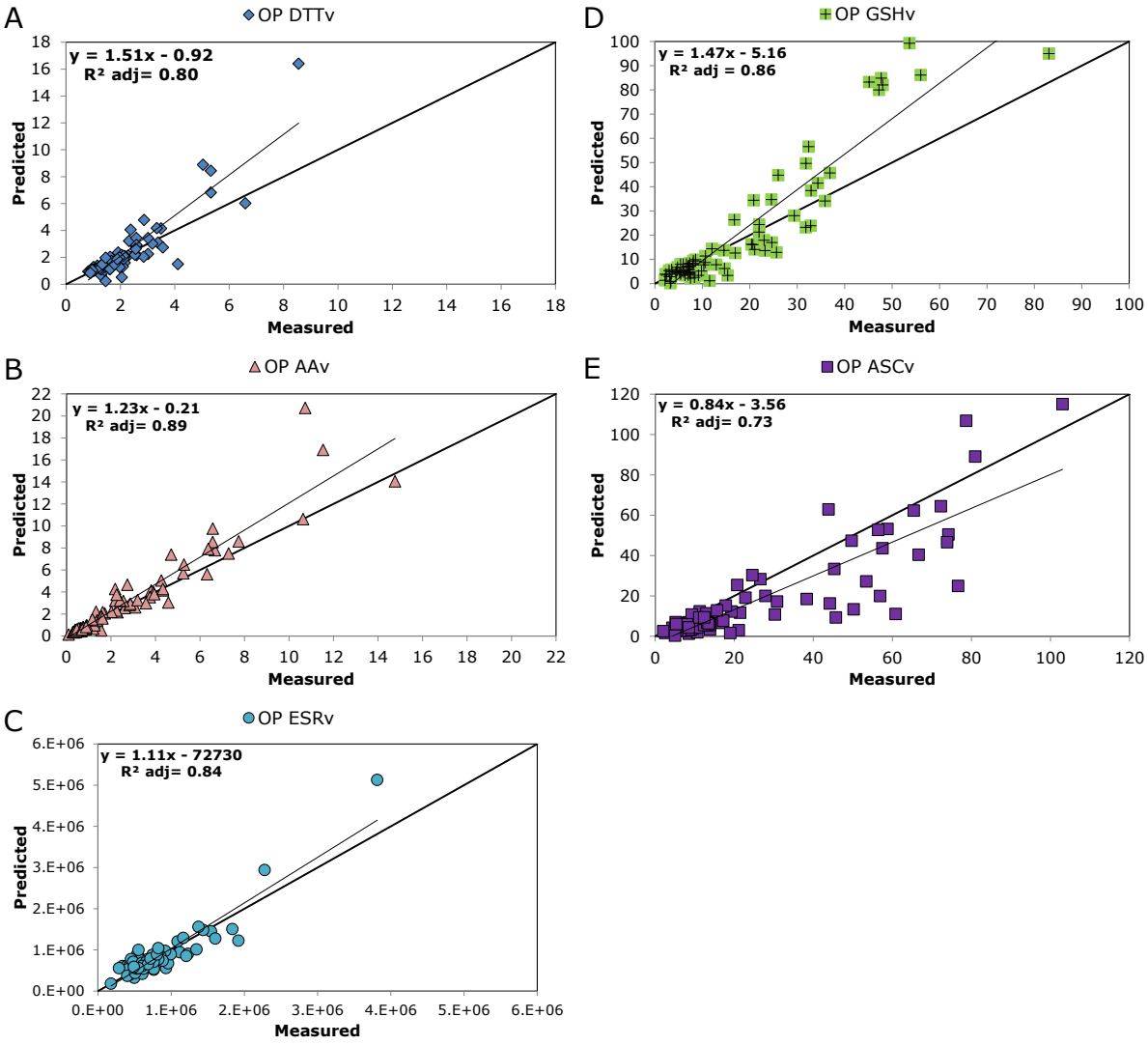


Figure S6 : Predicted OPv as a function of measured OPv, application of the models on the overall available data. (A: OP DTTv, B: OP AAv, C: OP ESRv, D: OP GSHv, E: OP ASCv).

Table S1 : Total number of samples (n: number of samples)

Months	n
Nov	7
Dec	10
Jan	11
Feb	7
Mar	8
Apr	9
May	5
Jun	8
Jul	5
Aug	8
Sep	9
Oct	11
Total	**98**

Table S2 : Information about the observations used for the models (number of observations, variable values and p value of the normality test.).

OP measurement	Observations used in model	Variable transformation	p value (Shapiro-Wilk test)
OP DTTv	51	log	0.26
OP AAv	40 and 19	log	0.27 and 0.34
OP ESRv	40	no transformation	0.046
OP GSHv	51	square root	0.017
OP ASCv	53	log	0.061

Table S3 : Parameters for the models evaluation: Variance inflation factor (VIF), residues vs endogenous variables and distribution of residues (p-value from Shapiro.Wilk normality test).

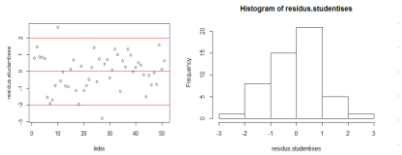
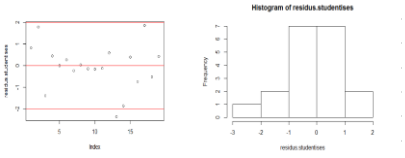
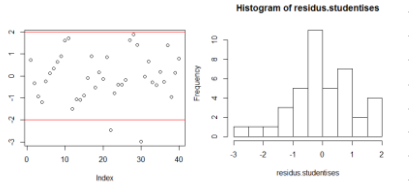
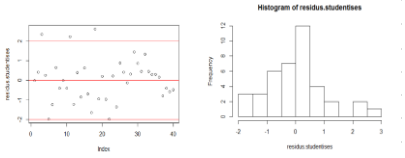
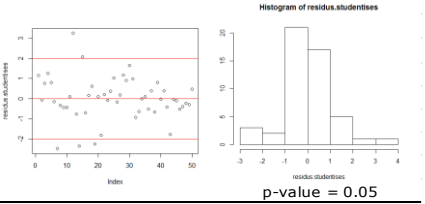
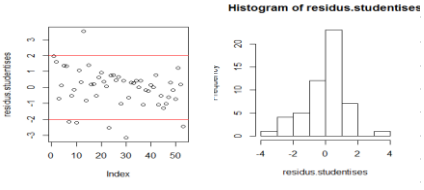
OP	Predictors (species)	VIF	Residue distributions
log(DTTv)	Cu	2.9	 p-value = 0.85
	∑PAH	2.6	
	Mg2+	3.6	
	As	1.4	
	Mo	2	
	MSA	1.9	
	∑Polyols	2.4	
	Na+	3.3	
log(AAv) cold period (19 obs)	∑Monosaccharides	1.3	 p-value = 0.39
	Ni	4.1	
	Mo	3.7	
	Fe	2.9	
log(AAv)	∑Monosaccharides	1.4	 p-value = 0.32
	Ca2+	2.7	
	Sb	4.5	
	Cu	7.1	
	Na+	1.7	
	Fe	2.3	
	Zn	4.8	
ESRv	Cu	3.7	 p-value = 0.30
	∑Alkanes	2.6	
	Cd	1.1	
	Ti	1.9	
√GSHv	∑Monosaccharides	1.5	 p-value = 0.05
	Cu	1.8	
	Mg	2	
	MSA	1.5	
log(ASCv)	NO3	5.8	 p-value = 0.10
	NH4	2.7	
	Fe	3.4	
	OC*	7.5	
	Cu	5	

Table S4 : Multivariate regression analyses results. * < 0.001 level, ** p<0.01 level, * p<0.05 level, . p<0.10 level. [a]51 observations used in model. [b]19 observations used in model. [c]40 observations used in model. [e]51 observations used in model. [f]53 observations used in model.**

OP	predictors (species)	coefficients	SD	p values	adjusted R^2
log(DTTv)[a]	(intercept)	-0.44	0.0695	***	0.81 (***)
	Cu	0.101	0.0181	***	
	∑PAH	0.0259	0.00571	***	
	Mg2+	-0.0247	0.00737	**	
	As	0.273	0.0997	**	
	Mo	0.318	0.133	*	
	MSA	0.00512	0.00215	*	
	∑Polyols	0.002	0.000870	*	
	Na+	0.00135	0.000567	*	
log(AAv)19[b]	(intercept)	0.433	0.0632	***	0.93 (***)
	∑Monosaccharides	0.000387	0.0000353	***	
	Ni	0.756	0.211	**	
	Mo	-0.372	0.130	**	
	Fe	0.000538	0.000166	*	
log(AAv)40[c]	(intercept)	-1.43	0.0827	***	0.87 (***)
	∑Monosaccharides	0.00163	0.000202	***	
	Ca2+	-0.00171	0.000394	***	
	Sb	1.69	0.554	**	
	Cu	0.117	0.041	**	
	Na+	0.00208	0.000589	**	
	Fe	0.00119	0.0003810	**	
	Zn	0.0435	0.0181	*	
ESRvd	(intercept)	339 953	63 950	*	0.62 (***)
	Cu	73 037	15 009	***	
	∑Alkanes	-6 725	1 860	***	
	Cd	-441 351	190 579	*	
	Ti	20 795	8 839	.	
√GSHv[e]	(intercept)	1.21	0.299	***	0.48 (***)
	∑Monosaccharides	0.00161	0.000302	***	
	Cu	0.210	0.0680	**	
	Mg	-0.0642	0.0294	*	
	MSA	0.0268	0.0117	*	
log(ASCv)[f]	(intercept)	1.79	0.266	***	0.73 (***)
	NO3	0.000659	0.0000919	**	
	NH4	-0.000901	0.000564	*	
	Fe	-0.000843	0.000771	*	
	OC*	0.000247	0.0000919	*	
	Cu	0.103	0.0587	.	