



The use of cyclohexane as new solvent for airborne pollen sampling

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Received: 13 December 2018 / Accepted: 7 February 2019
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Abstract Almost all the aerobiological networks used the non-volatile silicone fluid as a capture medium adhesive on the Melinex tape of Hirst-type volumetric samplers. This adhesive was prepared with carbon tetrachloride, considered as a carcinogenic agent. Since that, there is a spreading interest to find an alternative solvent for continuing the aerobiological sampling. In this study, we examined the impaction efficiency of the cyclohexane and carbon tetrachloride solvent, widely used in aerobiological studies. The results revealed that there is no significant difference between the two solvents and that cyclohexane can be used as a new solvent of the adhesive substance for airborne pollen sampling.

Keywords Solvent · Airborne pollen · Aerobiology sampling

1 Introduction

Carbon tetrachloride (CCl₄), used as a solvent for the non-volatile silicone fluid in airborne pollen sampling advised by the Spanish Aerobiological Network (Galán et al. 2007), has been recently interdicted by

the Registration Evaluation Authorization of Chemicals (REACH) protocol due to its dangerous effects on environment and human health (Manibusan et al. 2007), and it will continue to be used for restricted purposes. Therefore, the need to find a new solvent for the silicon-coating solution is mandatory in order to continue the aerobiological research. In a previous study, Thibaudon et al. (2015) concluded that there were no significant differences between diethyl ether and carbon tetrachloride and the first can be used as a solvent for capturing airborne pollen, but some chemical properties of this product make it inadvisable because it is a very volatile, highly flammable liquid that is mildly irritating to skin and mucous membranes (NCBI 2015). Many adhesives have been proposed to be used in Hirst pollen traps as an alternative to non-volatile silicone fluid, such as petroleum jelly (Comtois and Mandrioli 1997; Galán and Domínguez-Vilches 1997; Hasnain et al. 2007; Galán et al. 2014; MayaManzano et al. 2018) and double-sided self-adhesive tape (3 M) (Alcázar et al. 2003). The importance of this topic led us to investigate the ability of another solvent to be used alternatively for the adhesive coating solution. The aim of this preliminary study is to compare the impaction efficiency of airborne pollen grain on non-volatile silicone fluid by using cyclohexane solvent as an alternative to carbon tetrachloride, the common solvent used by the Spanish Aerobiology Network (REA).

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2 Materials and methods

The study was carried out in Malaga (Southern Spain), from February 16, 2015, to April 26, 2015 (10 weeks), with the aid of a 7-day Hirst-type volumetric trap (Hirst 1952), located on the roof of the Faculty of Science, 15 m above ground level. A double-blind comparison of carbon tetrachloride and cyclohexane was done. For this, the drum was covered by a Melinex tape, coated with non-volatile silicone fluid containing two different solvents: one half of the tape with carbon tetrachloride and the other half with cyclohexane, following the methodology previously described by Thibaudon et al. (2015). The position of the two sampling adhesives was reversed from right to left every week (Fig. 1). The counting method consisted of 2 continuous horizontal sweeps per each half of the

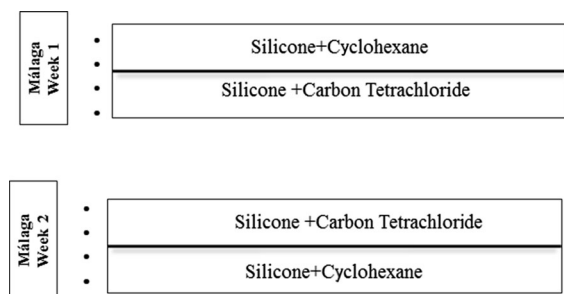


Fig. 1 Reading microscope slides methodology during the studied period

Fig. 2 Comparison of total pollen counts using the carbon tetrachloride and cyclohexane solvents with non-volatile silicone fluid during the studied periods of 10 weeks

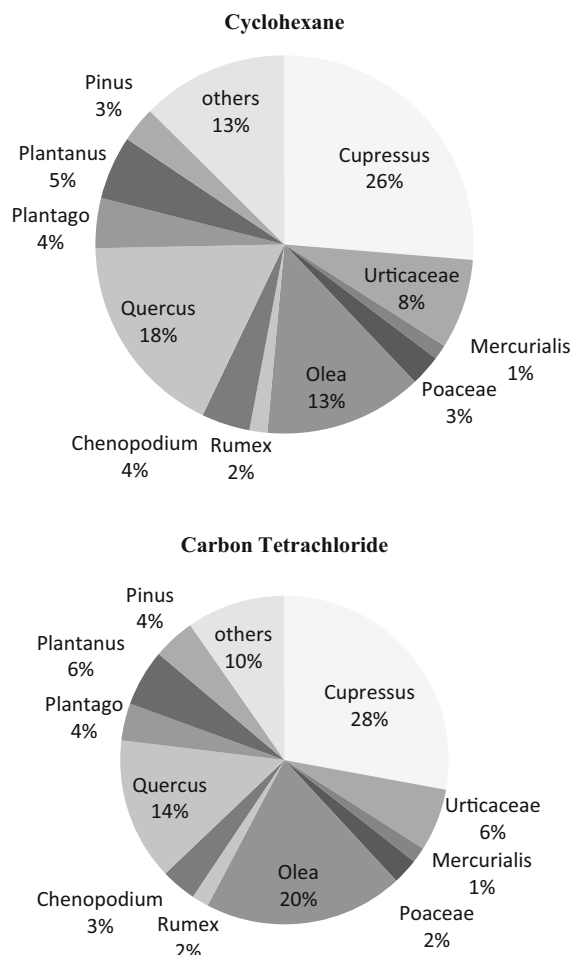
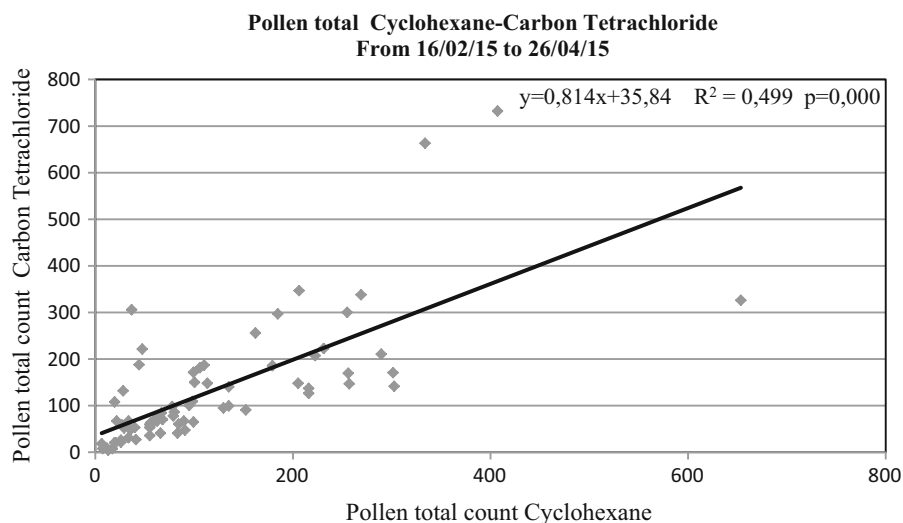


Fig. 3 Pollen percentages of each taxon compared to the total pollen count with each solvent

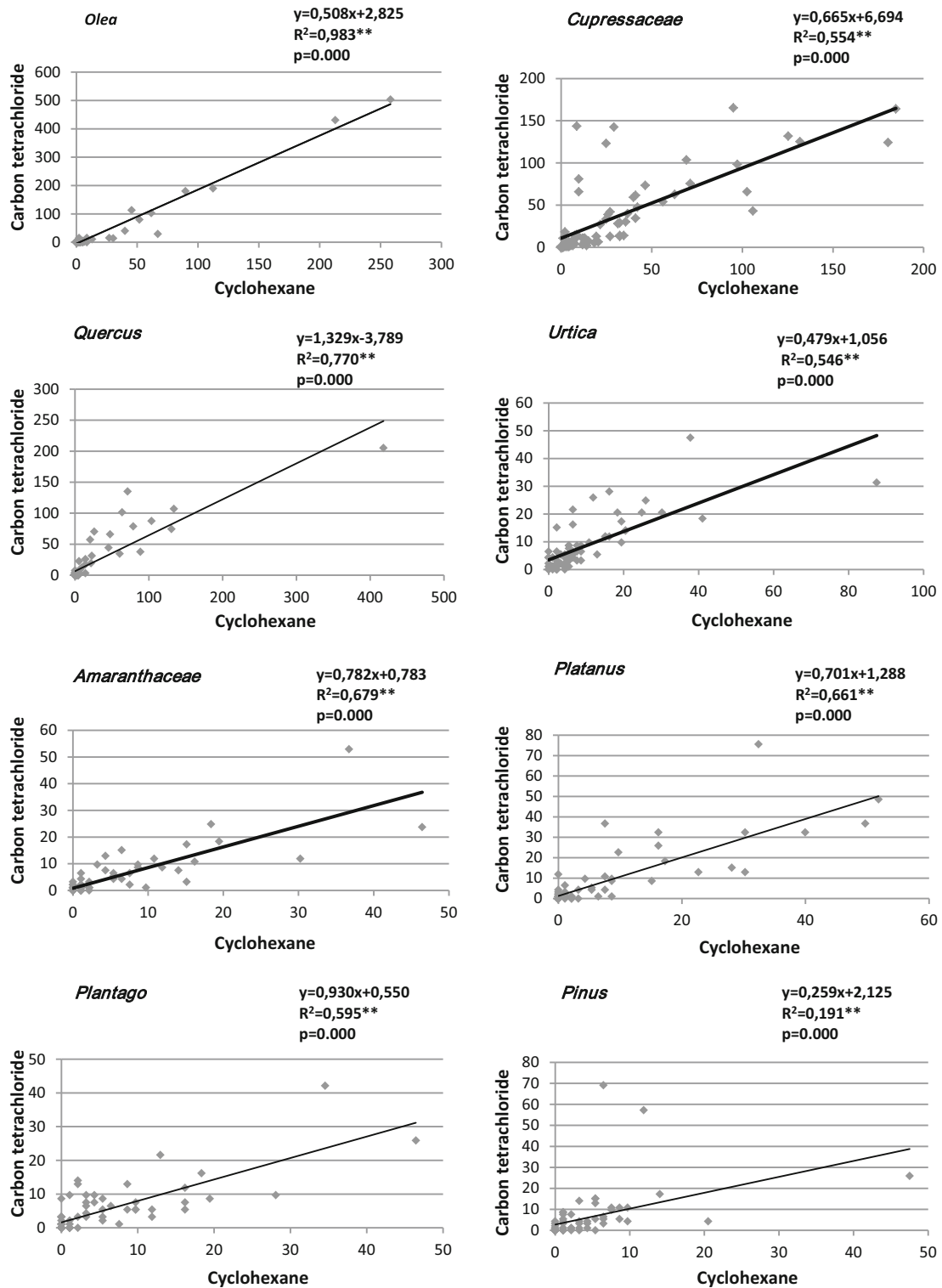


Fig. 4 Comparison of the daily mean concentrations of the pollen types recorded in the days of study using non-volatile silicone fluid with the tested solvents

tape. Only pollen types observed with daily mean pollen concentrations ≥ 1 grain/m³ would be considered. For the statistical analysis, daily mean pollen concentrations of each taxon were used, as well as the total pollen counts for the entire period (10 weeks). Data normality was evaluated by the Shapiro–Wilk test (W). Since the data did not adjust to normal distributions, nonparametric statistic tests were applied. To evaluate the relationships between the results obtained by the two solvent, Spearman's correlation coefficients were calculated, while the Wilcoxon test was used in order to search whether or not differences observed were significant. Additionally, regression analyses were also carried out. The statistical software package used was IBM SPSS 21.0.

3 Results and discussion

The use of silicone with carbon tetrachloride has been proposed as an adhesive by Hogan (1971) and recommended by the Spanish Aerobiological Network (Galán et al. 2007). The main advantage is the uniform behavior for a range of temperatures, between -20 and $+150$ °C (Galán and Domínguez-Vilches 1997). Its disadvantage is the carcinogenic properties of the initial solution. The relative efficiency of airborne

pollen grain capture on cyclohexane and carbon tetrachloride as solvents used with the non-volatile silicone fluid is shown in Fig. 2. We observed a positive linear correlation between the total pollen count with both solvents, showing a significant coefficient of determination ($R^2 = 0.499$; $p < 0.05$). When considering the most abundant pollen types levels found in the atmosphere of Malaga for the days' studies, results showed a similar percentage for each taxon using the tested solvents. The Cupressaceae pollen type was the most abundant genus captured, accounting for 28% of total pollen detected with carbon tetrachloride compared to 26% in the case of cyclohexane. The percentage of the other airborne pollen types captured is detailed in Fig. 3.

In addition, the scatter plots provide visual representations of the relationship between the pollen counts produced using the two solvent. The results have shown a positive linear correlation between the two variables for all pollen types, with a statistical significance of coefficients of determination (Fig. 4).

In addition, statistical analysis was carried out by Spearman's rank correlation, with the pollen concentrations recorded with tested adhesives, for all the case taxa and the total pollen. Results are shown in Table 1. It can be seen that for all paired comparisons for the pollen types separately or the pollen total, the

Table 1 Results of statistical analysis carried out between the series obtained for the different pollen types, as well as the total period using the non-volatile silicone fluid with the corresponding solvents

	Correlation		Wilcoxon test			Z	Sig.
	Spearman's test	Sig.	Car. T. < Cyc.	Car. T. > Cyc.	Car. T. = Cyc.		
Total	0.778**	0.000	29	38	3	− 1.050	0.294
Cupressus	0.826**	0.000	32	30	8	− 0.445	0.656
Urtica	0.765**	0.000	35	24	11	− 0.844	0.399
Mercurialis	0.593**	0.000	18	22	30	− 1.252	0.211
Poaceae	0.492**	0.000	25	29	16	− 0.703	0.482
Olea	0.723**	0.000	24	22	24	− 0.341	0.733
Rumex	0.541**	0.000	21	27	22	− 1.341	0.180
Chenopo.	0.805**	0.000	22	22	26	− 0.035	0.972
Quercus	0.727**	0.000	26	26	18	− 0.073	0.942
Plantago	0.767**	0.000	21	24	25	− 0.074	0.941
Platanus	0.801**	0.000	17	23	30	− 0.499	0.618
Pinus	0.750**	0.000	19	24	27	− 1.667	0.095

Car. T = Carbon tetrachloride, Cyc = cyclohexane

**Correlation is significant at the 0.01 level (2-tailed)

differences are not significant either in comparison with cyclohexane/carbon tetrachloride. The correlation was significant at 0.01.

Similarly, the results of the applied Wilcoxon test for comparing nonparametric paired data have shown that no significant differences ($p > 0.05$) were detected between the count of selected pollen types a using the silicone adhesive containing either CCl₄ or cyclohexane solvent (Table 1).

4 Conclusion

Cyclohexane and carbon tetrachloride used as a solvent for non-volatile silicone fluid did not show statistically significant differences when used as a coating medium for Melinex tape in Hirst-type pollen traps. Because of the toxicity of the CCl₄, and the importance of having a similar efficient solvent, even if it is not the standard solvent used in the aerobiological sampling recommended by the Spanish Aerobiology Network, cyclohexane could be a good alternative used in aerobiological monitoring. Moreover, we have shown that the efficiency of capture was similar using both solvents. Therefore, a change of solvent, at least for the ones tested, did not bring a significant change in pollen count, defined as one which would bring a different interpretation of the results.

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