Refining wastewater-based epidemiology for cannabis consumption monitoring: relevance of analysing both the aqueous phase and suspended solids of influent wastewater

Natan Van Wichelen[1], Marina Celia Campos-Mañas[2], Claudia Simarro-Gimeno[2], Adrian Covaci[1], Alexander L.N. van Nuijs[1], Christoph Ort[3], Sara Castiglioni[4], Frederic Béen[5] ,Félix Hernández[2], Lubertus Bijlsma[2]

[1]Toxicological Centre, University of Antwerp, Antwerp, Belgium
[2]Research Institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain
[3]Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland
[4]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Department of Environmental Health Sciences, Milan, Italy
[5]KWR Water Research Institute, Nieuwegein, the Netherlands

Wastewater-based epidemiology & Cannabis use

- **Wastewater-based epidemiology (WBE)** employs the analysis of human metabolic excretion products of xenobiotics in influent wastewater (IWW) with the aim of estimating their community use

- **Cannabis** is worldwide the most commonly consumed illicit drug
  - Δ9-tetrahydrocannabinol (THC) is the major psychoactive ingredient of cannabis, further metabolised (microsomal hydroxylation) to 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) and its main human metabolite 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)
  - Careful monitoring of THC use is necessary to detect changes in consumption patterns and to understand shifts in the drug markets

- **THC-COOH detection** in the liquid phase of IWW in the context of WBE is **complex** although no problems were faced with other illegal substances (e.g. cocaine) and with THC-COOH detection in methanol (interlab study, SCORE, 2018)
  - Relatively high lipophilicity of cannabis biomarkers → Consider partition between liquid phase and suspended solids (SS) in IWW?

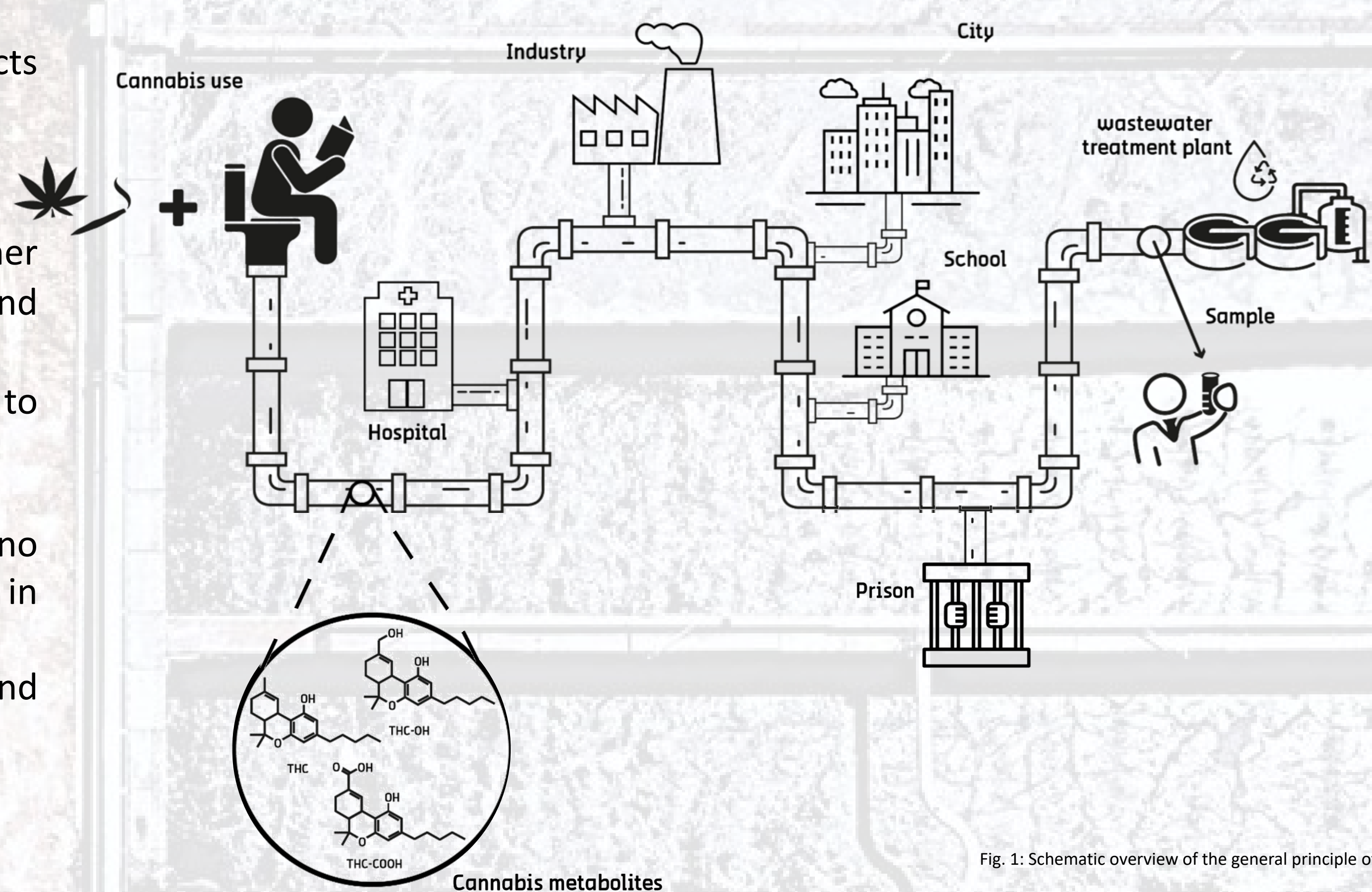→ **Objective: Improve the estimation of cannabis use through WBE**

Fig. 1: Schematic overview of the general principle of WBE

Methods

- Conventional method to measure cannabis biomarkers: **solid-phase extraction (SPE)**
  - Filtration is necessary → suspended solids are not considered
  - By applying **liquid-liquid extraction (LLE),** both liquid and suspended solids are considered
  - By applying **solid-liquid extraction (SLE)**, only the suspended solids are considered

- Seven daily IWW samples (24-h composite, time proportional with a time interval of 10 min) were collected from wastewater treatment plant (WWTP) of Castellon, Spain

- The different validated methods were applied to different fractions of the IWW samples, as illustrated in figure 1 and 2.

- UPLC-MS/MS sample analysis was performed with a Xevo TQS (Waters) for THC, THC-OH and THC-COOH
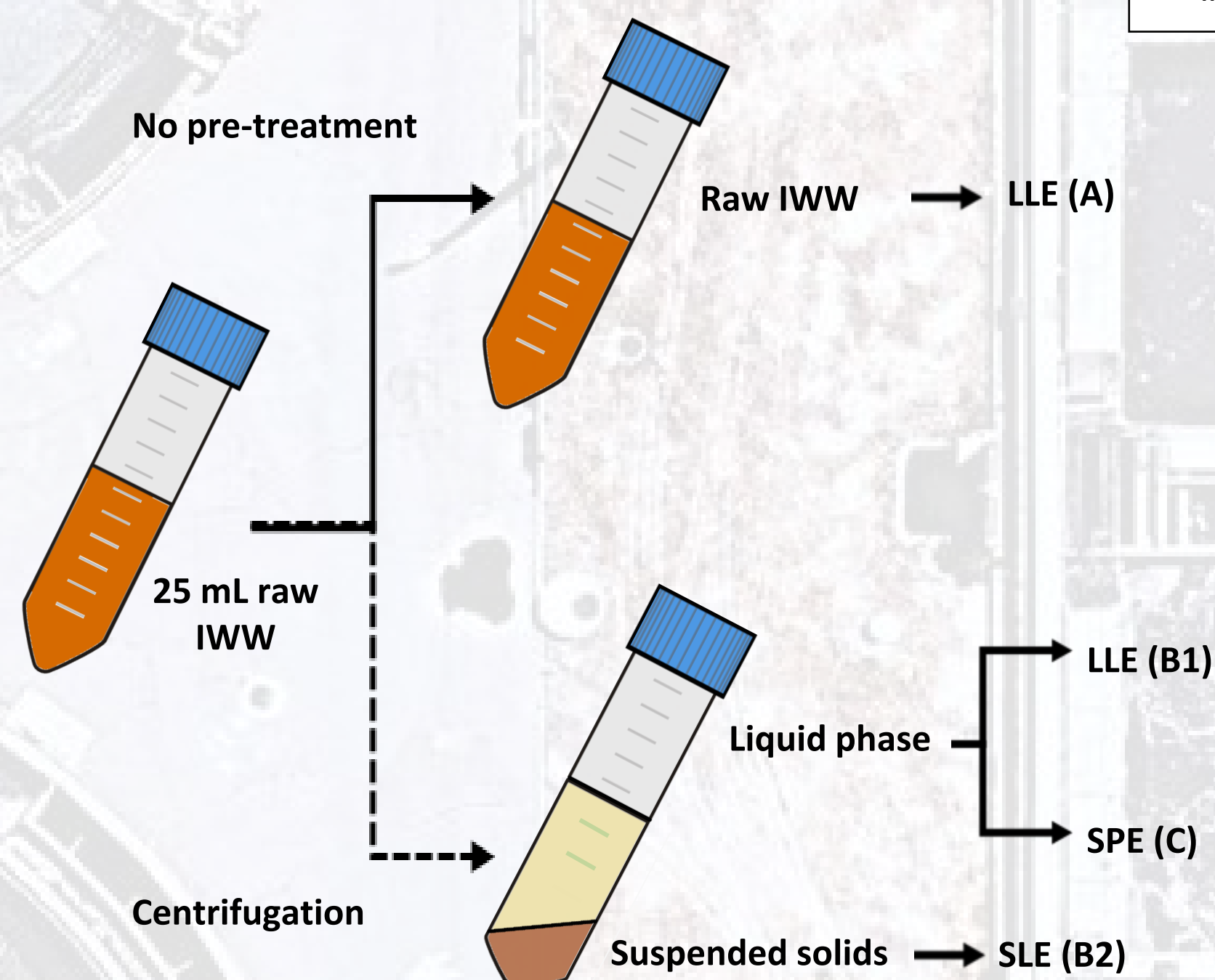

Fig. 2: Sample preparation for the analysis of the liquid and solid phase by different methods: SPE, LLE and SLE.
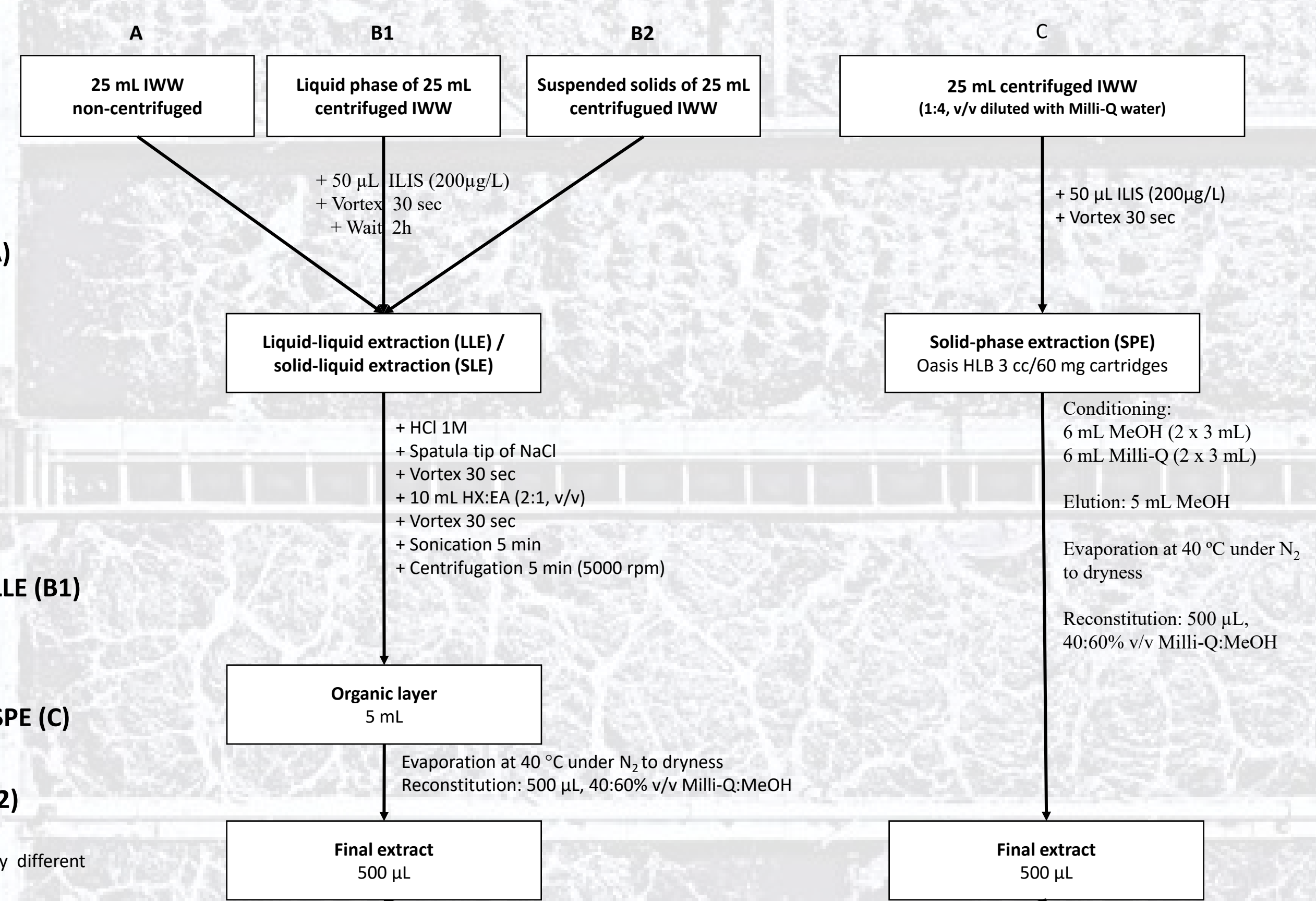

Fig. 3: Graphical workflow of the analytical procedure for SPE, LLE and SLE

Results and Discussion

- **Liquid phase only**: no substantial difference between SPE (Recovery% = 90%) and LLE (Recovery% = 75%) for THC-COOH analysis
  - Deviations <30% in 6 out of 7 samples analysed (Table 1)
  - LLE was considered as a good alternative taking into account the higher cost and time consumption of SPE

- **Suspended solids (SS)**: substantial amounts of cannabis biomarkers detected (Fig. 4)
  - In agreement with the polarity characteristics of the three compounds (higher lipophilicity favours presence in SS)

- **Raw IWW**: Results support the hypothesis that data obtained analysing the raw IWW by LLE without previous removal of suspended solids is similar to the sum of biomarkers in the suspended solids (SLE) and the liquid phase (LLE or SPE) (Fig. 4)

Sample	THC-COOH (ng/L)		Deviation SPE/LLE (%)
	LLE	SPE	
IWW 1	239	336	+41
IWW 2	270	328	+22
IWW 3	262	308	+18
IWW 4	221	208	-6
IWW 5	327	348	+6
IWW 6	281	356	+27
IWW 7	332	308	-7

Table 1: Determination of THC-COOH by LLE and SPE in seven centrifuged IWW samples from a one-week sampling
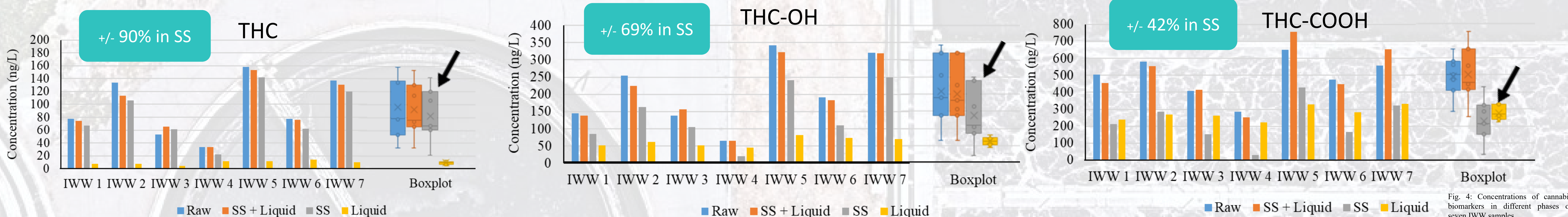

Fig. 4: Concentrations of cannabis biomarkers in different phases of seven IWW samples

Conclusions

- The **suspended solids of IWW** contain a **high percentage (+/- 42% for THC-COOH)** of cannabis biomarkers
- **SPE** without correction factor for the amount of biomarkers in the suspended solids would lead to an **underestimation** since it only considers the liquid phase of IWW
- The analysis of the **IWW without separation of the solid phase** offers **more realistic information** on the biomarker concentration in IWW
- More research is required to calculate consumption estimates

Future research

- Sampling uncertainty related to solids
- Partition of the cannabis biomarkers between liquid and solid phases
- Extrapolating to WBE applications

Natan.vanwichelen@uantwerpen.be