# Preparation of Samples of Plant Material for Chromatographic Analysis

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## Abstract

**The selection, collection, and preliminary treatment of plant material samples are discussed. Stages of sample preparation and extraction techniques presently used are described, and the most recent examples of sample preparation for chromatographic analysis are reviewed. Recent applications of gas chromatography for the assessment of emission of volatile organic compounds by plants are also described.**

## Introduction

Plants are omnipresent in the earth's environment and play an important role in food webs, cycles of numerous chemical elements, and environmental quality. Plants can integrate various mediators of environmental quality into their tissues, and they can accumulate and metabolize hazardous substances emitted into the environment. Aims of analyzing plants are to: (*a*) monitor the content of hazardous substances in the environment and answer the question of mass balance, metabolic turnover, and accumulation of these substances in food webs; (*b*) assess plant pollution; (*c*) evaluate plant capability to remove pollutants from the environment; and (*d*) observe nutrients supply to and distribution within a plant.

Plants can also emit substances into the environment, sometimes in such quantities that the ecological equilibrium is disturbed to a detrimental level; in other words, they can be a source of environmental pollution.

The most common application area of vegetation analysis is environmental monitoring (1–3). In Germany, for example, many samples of plant material have been collected for a long-term monitoring and banking project (4).

The intended information can be drawn from analytical results if an environmental matrix from a naturally heterogeneous real world is properly selected and all steps of the analytical process are well planned and correctly performed. The basic steps of vegetation analysis include the selection of material to be sampled, sampling and preliminary sample handling, sample preparation for the proper analysis, final analysis, and data treatment.

The reliability of data should be tested by the application of appropriate procedures of quality assurance and quality control (5).

Plant materials are very complex matrices, often components of very low concentration are of interest, and increasingly specific speciation is frequently required. In such a situation, it is necessary to use analytical methods that are characterized by high selectivity, sensitivity, and resolution. Very often, this means the application of chromatographic techniques for the final analysis (6,7).

### Sample selection, collection, and preliminary treatment

The selection of species and the number of individuals depend on the intended aim of the study. If a given plant is to be characterized, then the whole plant or its particular parts are sampled. For the characterization of a given area occupied by one or more plant species, individual samples are collected from points representative of the area. Although they can be analyzed separately, most often they are combined to give a composite sample.

Herbaceous plants or young plants are usually collected whole. Alternatively, only a part of a plant is taken for analysis (e.g., leaves, needles, stems, twigs, and branches; fruits; seeds and grain; and bark). Underground plant parts that are typically collected are bulbs, tubers, corms, and roots (commonly divided into taproots and fibrous roots).

Higher plants are typically collected during blooming and lower plants as mosses and lichens in autumn. Leaves are generally collected during growth periods, and needles are collected after a period of growth from the outer part of the middle of the tree head. Generally, older needles are collected to measure long-term effects of pollution. If the study's aim is the assessment of the assimilation of given substances during a growing period, then needles from that period are gathered.

In order to determine the total quantity of components accumulated by cereals and oil plants during a growing period, full-grown individuals should be collected. Seeds and straw are analyzed as separate samples.

For the determination of pollution, needles are taken from the windward side (i.e., from the direction of pollutants influx).

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When insulation is highly differentiated, samples from shaded and nonshaded sides should be blended.

Great care should be taken during sampling to avoid external contamination (e.g., special gloves and stainless steel tools).

Depending on a species, components of interest, and other parameters, samples may be freeze-dried at a sampling site or packed in linen or paper bags and transported to a laboratory for further handling.

For establishing a relationship between polycyclic aromatic hydrocarbons (PAHs) accumulation in plant tissues and its concentration in a surrounding environment, Kipopoulou et al. (8) collected five vegetable species (cabbage, lettuce, carrot, leek, and endive) during two harvest periods (autumn and spring).

Holoubek et al. (9) proved that vegetation can be used to show spatial differences in the atmospheric burden of a range of persistent organic pollutants (e.g., PAHs). They collected mosses (outside the crown projection of a tree) and pine needles. Samples were wrapped in aluminum foil, air-dried at ambient temperature, and stored in paper and sealed polyethylene bags in darkness.

Howsam et al. (10) studied the differences in PAH content between oak, ash, and hazel, which represented 85% of the leaf biomass in the area they studied. The sampling of leaves was conducted in midgrowing season using a stratified random strategy. Oak and ash leaves were collected from the middle of the canopy (~ 15–20 m above the ground) and hazel leaves from the middle of the understory (~ 7–10 m above the ground). Small twigs were cut from three or four branches of each species at each sampling location using a long-handled pruner. The leaves were removed by hand from the twigs, and care was taken to minimize contact with the leaf surface. Leaves were transported to the laboratory in jars at approximately 4ºC and stored at approximately –20ºC until analysis. The same transport and storage conditions were applied to the leaves of great plantain and mixed species of grass collected for the purpose of PAH pollution monitoring (11).

For the monitoring of air pollution with PAHs in the urban area of Naples, leaves of an evergreen Mediterranean oak were analyzed by gas chromatography (GC)–mass spectrometry (MS) (12). Branches were sampled around lower foliage and brought in plastic bags to the laboratory immediately. The representative samples of healthy mature one-year-old leaves (deprived of petioles) were cleaned in order to take away spider webs or aphids and then prepared for analysis.

The extent of polychlorobiphenyl (PCB) pollution resulting from the accidental release of PCBs from the Alberta Special Waste Treatment Center (Swan Hills, Canada) was assessed on the basis of PCB determination in white spruce needles and a lichen of witches' hair (13). Samples were frozen (–50ºC) until extraction. For the pollution with PCB and other semivolatile organic compounds (SOCs), a great variety of species were sampled (11,14,15).

In order to study the aquatic dissipation rate of the acidic herbicide triclopyr, different matrices including plants have been analyzed (11). For this purpose, rectangular test plots divided into quadrants were established. Plant samples were collected from suitable vegetation stands with the use of a rake-type device. Plants were separated into target and nontarget species, rinsed with distilled water, and transported in sample containers to a local laboratory in which they were immediately frozen.

Sea grass samples from shallow waters collected for organochlorine pesticides (OCPs) and herbicides monitoring have also been frozen following collection (17).

Schuhmacher et al. (18) and Domingo et al. (19) used vegetation for monitoring pollution with polychlorinated dibenzo-*p*-dioxines/dibenzofurans (PCDD/F). Herbage samples were obtained by cutting at a height of approximately 4 cm above the soil level and immediately packed in aluminum foil. Subsequently, they were dried at room temperature, kept in double aluminum foil, packed in labeled plastic bags, and stored at room temperature prior to analysis.

In studies of the global distribution of PCDD/Fs, tree bark was used in which the samples were chiseled into a clean glass jars (20). The shipping and storing of sealed samples at ambient temperature and at –20ºC gave analytical results that were not different to a statistically significant degree.

Ok et al. (21,22) used vegetation and pine needles as indicators of local pollution with PCDD/Fs. Samples were dried at room temperature and then cut into a size of approximately 1 cm before the proper preparation for chromatographic analysis.

The determination of arsenic compounds offers the possibility of elucidating the cycling and metabolic pathway of arsenic in the environment. Kuehnelt et al. (23) found out that organic arsenic compounds formally attributed only to marine organisms and mushrooms are present also in terrestrial green plants and lichens. The grass and lichen samples collected were rinsed with tap water, frozen at –20ºC, and freeze-dried for 24 h at –10ºC and another 24 h at 10ºC at 0.1 mbar.

Periphyton mat samples were collected by supporting floating material with an open hand, drawing the material from water, and transferring it to a ziplock plastic bag for monitoring copper, lead, mercury, and zinc (24), and for the studies of the distribution of and relation among mercury and methylmercury, organic carbon, carbonate, nitrogen, and phosphorus in periphyton (25).

For studying traffic pollution, the epicuticular wax of spruce needles and spruce shoot aphid were collected from spruce seedlings placed on roadsides (26,27). Roadside Scots pines have also been applied as an indicator of deicing salt use (28).

**Sample preparation for final analysis**

Plant material is a very complex matrix composed of a great variety of organic compounds. Sample preparation, even for chromatographic techniques of high separation power, is a difficult process composed of many operations. All of these operations have been presented in a previous paper (29). The basic operations include (not necessarily all are applied in a given analysis) prewashing, drying plant material or freeze drying, grinding [which is aimed at obtaining a homogenous sample and often improving the kinetics of analyte extraction—the problem is thoroughly discussed by Rossbach (31), dividing a sample in such a way that representative subsamples are obtained, digestion, isolation and preconcentration, decanting and centrifuging, drying the extract, solvent evaporation, and extract cleanup.

A crucial step of sample preparation is selective isolation and enrichment requiring very sophisticated operations when trace components in inherently complex plant matrices are to be determined. Principles and the application of most common extraction

techniques applied in plant material analysis have been discussed in a previous paper (29). The literature search during this study indicated that traditional methods are still widely used. The most recent studies on the chromatographic analysis of plant material have been reviewed and the corresponding extraction procedures described.

**Conventional liquid extraction**

If the analytes of interest are easily extractable, then shaking a pure solvent or solution with a solid plant sample can be used for effective extraction. For the improvement of extraction kinetics, shaking is intensified using mechanical shakers or rotors. The extraction process is sometimes performed at an elevated temperature in order to improve recovery and kinetics.

Marth et al. (31) used poplar trees and pine shoots (among other bioindicators) to monitor local and regional pollution with chlorinated hydrocarbons (CHC). For the isolation of CHC, either a frozen (< –150ºC) single sample or the powdered subsample (material ground and homogenized) were mixed with anhydrous sulfate/sea-sand in order to form a free-flowing product, which was subsequently extracted with *n*-hexane–acetone in an extraction column. Before chromatographic analysis, this raw extract had to be cleaned up. In the case of poplar leaves, a majority of the lipid and biogenic material were removed using size exclusion chromatography (BIO-Beads S-X 8). In order to eliminate more polar impurities, the pesticide fraction was precleaned in a high-performance liquid chromatographic (HPLC) column packed with Hypersil silica gel. Because of the high content of the coextracted impurities from pine needles, the *n*-hexane–acetone extract was purified by chromatography on florisil. Recoveries for the extraction processes were satisfactory, which means that the pesticides looked for were not strongly bonded to the plant matrices.

Chlororganic pesticides from sea grasses have been extracted at ambient temperature using an acetone–*n*-hexane mixture (17). The raw extracts were subjected to liquid–liquid partitioning to remove some impurities, solvent exchange, and additional cleanup and fractionation in order to obtain reliable GC data.

For the determination of $SO_4^{2-}$, $NO_3^-$, and $PO_4^{3-}$ by means of ion chromatography (IC), dried and milled pine needles were extracted with deionized water on a shaker (32). After centrifuging the membrane, filtered supernatant was ready for IC analysis.

For the speciation analysis of organic arsenic compounds in green plants and lichens from an old arsenic smelter site, freeze-dried and pulverized samples were extracted with water or water–methanol (9:1) on a cross-shaped rotor at 25ºC (23). Before ion-exchange chromatographic determination, methanol–water extracts were centrifuged and the supernatant was evaporated to dryness and redissolved in water.

Liang et al. (33) efficiently recovered methylmercury from sea plants using simple solvent extraction, which was confirmed by tests with reference materials. The first step was digestion with KOH–methanol at an elevated temperature (75ºC). An acidified digestive was extracted with dichloromethane, and MeHg was back-extracted to water prior to aqueous-phase ethylation. The authors investigated the effect of different parameters on the recovery in order to optimize the extraction.

In the determination of the herbicide triclopyr and its metabolites in aquatic plants, digestion at an elevated temperature of 130ºC with a sodium hydroxide methanol–water solution was applied in order to release any bound analytes (11). The successive steps included cleanup of the acidified extract using a C18 solid-phase extraction (SPE) column, extraction to 1-chlorobutane, and derivatization. With such sample preparation and final analysis by means of GC–MS, the limits of quantitation for triclopyr in different aquatic plants ranged from 8 to 17 ng/g.

**Soxhlet extraction**

Soxhlet extraction (SE) is a very old extraction technique and still quite widely used in plant analysis, as can be seen even from the cursory search of the most recent literature. Fundamentals of the technique are comprehensively described in review studies on the sample preparation of solid samples for analysis (34–36). The method is generally time-consuming and uses large amounts of solvents, and it is often expensive and environmentally unfriendly. However, it is very effective and often is used as a reference method for newer extraction methods.

SE has been used to isolate PCDD/Fs from tree bark for their GC–MS determination (20). Bark was broken into small pieces (dimensions of a few millimeters) with a precleaned hammer and chisel. The pieces were placed into a glass Soxhlet thimble plugged with glass wool and extracted with a mixture of acetone–hexane (1:1). The extract contained numerous interfering compounds and was subjected to many solvent exchange, cleanup, and fractionation operations using solvent evaporation, liquid–liquid extraction (polar components extracted from the hexane concentrate with water), and silica gel and alumina liquid chromatography. The final concentrate that was reduced to a volume of 20 mL was sufficiently cleaned in order to obtain reliable results by GC–MS analysis. Recoveries of PCDD/Fs for the entire procedure were in the range of 65–110%.

SE has also been used to isolate PCDD/Fs from vegetation that was ground into a fine powder (18). Again, the Soxhlet extract was subjected to multistep treatment before analysis by means of high-resolution (HR) GC–HRMS.

Other more recent applications of SE in the chromatographic analysis of organochlorine compounds in plant material include: (*a*) PCDD/Fs and PCBs in samples of burned and unburned vegetation such as the wood and bark of pines and bushes (37); (*b*) PCBs in a variety of vegetation (e.g., grasses, climbers, trees, moss, and fungus) [dichloromethane was used for extraction and silica gel columns and gel permeation chromatographic (GPC) columns were used for cleanup (14)]; (*c*) SOCs such as PCBs and PAHs in grasses, deciduous tree leaves, pine needles, and lichens [The effects of solvents and other parameters on extraction efficiency have been investigated (15). It was found that the efficiency strongly depends on preliminary sample preparation for extraction, species, solvent, and analyte (the behavior of PCBs and PAHs is very different).]; (*d*) SOCs in the leaves of an evergreen Australian native tree [The Soxhlet extract was split into two parts and treated in different ways. One portion was prepared for PCDD/Fs, PCBs, and hexachlorobenzene analysis and the other for PAHs analysis (15).]; (*e*) interlaboratory tests for the determination of organochlorine compounds (PCBs and OCPs) and petroleum hydrocarbons in a seaweed sample in which the

majority of participants (57%) used SE (38); (*f*) organochlorine pollutants in cordgrass from two different habitats [Lipids were removed from the extracts by GPC and sulfur from a selected fraction by boiling with prewashed copper turnings (39).]; (*g*) persistent organic pollutants (PAHs, PCBs, and OCPs) in mosses and pine needles [partially dried samples were minced and weighed in glass extraction thimbles, extracted with dichloromethane, cleaned up with sulfuric acid, fractionated by florisil column chromatography, and analyzed by HRGC–mass-selective detection (MSD) (9)]; (*h*) PAHs associated with the leaves of some deciduous tree species (oak, hazel, and ash) [for extraction purposes whole leaves and powdered sodium sulfate were placed in a large Soxhlet body and the extract, as usual, subjected to multistep treatment (10)]; and (*i*) PAHs in vegetables grown in an industrial area [a freeze-dried vegetable sample was mixed with anhydrous sodium sulfate, Soxhlet extracted with $CH_2Cl_2$, rotary evaporated to approximately 2 mL, extract cleaned up in a florisil packed cartridge, sorbates eluted with $CH_2Cl_2$, eluate concentrated and then evaporated to dryness under a $N_2$ stream, and analytes redissolved in acetonitrile and analyzed by HPLC (8)].

A higher extraction temperature is achieved when the extracted material is immersed in boiling solvent (this should improve extraction efficiency). In such a case, however, the sample is in contact not with pure solvent (the case of SE) but with an extract that can have a negative effect on extraction. Such an approach, based on extraction with toluene under reflux, was applied by Ok et al. in order to isolate PCDD/Fs from dried vegetation (22) and pine needles (21).

### Sonication

The simpler way of furnishing extra energy to facilitate the transfer of analytes from sample to solvent is to use ultrasound. In such an approach, a vial containing a sample and an appropriate solvent is placed in an ultrasonic bath for a preselected, generally short period of time.

Iridoid glycosides were efficiently extracted from the pulverized dried root of *Scrophularia ningpoensis*, which is cultivated as a medical plant in China, and from prepared pieces for decoction with three portions of methanol if sonicated for 30 min each (40).

In order to isolate PAHs from the leaves of an evergreen Mediterranean oak, the leaves were immediately subjected to ultrasonication extraction with dichloromethane at 25ºC (12). The extracts obtained were concentrated in a vacuum rotary evaporator at 30ºC, filtered for water separation, and further concentrated under a gentle stream of nitrogen. The concentrate was then ready for GC–MS analysis.

For the determination of PCBs in conifer forest vegetation, Froese et al. (13) used ultrasonic extraction with an acetone–hexane mixture. Before GC–electron capture detection (ECD), the extract needed further treatment based on solvent exchange and cleanup by means of SPE (silica gel and florisil).

Powdered Scots pine needles (applied as an indicator of deicing salt use) were treated with distilled water in an ultrasonic bath in order to extract chloride ions (28).

### Accelerated solvent extraction

Accelerated solvent extraction (ASE) is the most recent invention in the area of extraction techniques. It has been internationally applied for approximately five years (41–43). In a wider sense [ASE is a Dionex (Sunnyvale, CA) trade name], it is termed pressurized fluid extraction (PFE) (44). The effectiveness of ASE can be even better than that of SE because it can be conducted at higher or much higher temperatures. In order to keep solvent in a liquid state at increased temperatures, an appropriately high pressure (generally 0.3–20 MPa) is required. Unlike supercritical fluid extraction (SFE), the polarity of the extractant in PFE can be changed over a wide range and the consumption of the extractant is much lower than in SE, which diminishes costs and environmental burden.

The extraction system consists of a solvent reservoir, a pump, an extraction cell placed in an oven with a capability to control temperature, extract collection vials, and a cylinder of nitrogen gas used to force the extract out and wash the solvent through the cell. The successive steps of the extraction process are loading the cell with a sample, filling the cell with a solvent, heating the cell to a preset temperature and maintaining it for a preset time (the cycle can be repeated) (this is the proper extraction), flushing the extract to the collection vial and washing the sample with fresh solvent, and finally purging the cell with nitrogen gas.

Hubert et al. (45) performed extensive studies on the extraction of persistent orgnaic pollutants (POPs) and PAHs from different plant materials including mosses and needles. They carried out optimization experiments with respect to solvent and extraction parameters and compared ASE extraction effectiveness with other known extraction techniques. They found that hexane is the optimum extractant for POPs and PAHs from the plant material they studied. Depending on the material, pollutant, and level of pollution, two optimal temperatures were found to be 40ºC and 120ºC. The extraction efficiencies for PAHs and POPs with the use of ASE were two orders of magnitude higher than with ultrasonic extraction. They concluded that ASE is a very successful technique for the extraction of POPs and PAHs from plant samples. Extraction efficiencies obtained can be much higher than with the use of SE and ultrasonic extraction. However, operating variables should first be optimized with respect to the matrix and analytes of interest.

### SFE

SFE is a relatively new extraction technique whose application steadily increases because of more favorable kinetics, relatively good extraction efficiency mainly for nonpolar and slightly polar analytes, and an environmentally friendly extractant ($CO_2$, possibly with methanol or acetonitrile additives for more polar analytes) that is used. The fundamentals of SFE and instrumentation and its applications have been discussed in many studies (29,33,46,47) and also for plant material (29).

Supercritical carbon dioxide modified with 5% or 7.5% methanol was used to extract methylmercury from periphyton (24,25). Certified samples routinely run showed that the extraction procedure was consistently extracting methylmercury from samples. Simon et al. (48) showed that the efficiency of the extraction of methylmercury from periphyton ranged from 87% to 113%.

Subcritical $CO_2$ extraction has been used to isolate secondary metabolites from the leaves, flowers, stems, and roots of Colombian rue (49). The method applied had low environmental

impact and produced solvent-free extracts in a good yield with no pigments, waxes, resins, or high-molecular-mass compounds that could interfere with the HRGC–MS separation and detection of the alkaloids responsible for the rue's biological activity. These observations demonstrated all the principal advantages of SFE in plant material analysis. The extraction was performed at 40–45°C and 6.3–6.7 MPa. Dichloromethane (2 mL) was used to trap the analytes from $CO_2$.

Also, for the determination of steviol glycosides in the plant *Stevia rebaudiana* by means of capillary electrophoresis, SFE using $CO_2$ modified with methanol proved sufficiently efficient (11).

**Headspace**

Headspace (HS) is a gas extraction technique in which a plant material is placed in a vial sealed with an elastic membrane through which the needle of an injection syringe can be pierced without sealing damage. An analyte partitions between the solid plant sample and gas phase (HS) over the sample. After equilibration is reached, HS samples are analyzed by GC. The analyte concentration in the plant sample is derived from its concentration in the HS. The approach is simple, sample preparation is minimized, and the injected samples have a very simple matrix (only volatile components of a plant sample can be transferred into the HS).

Cahill et al. (50) developed a sensitive and simple analytical procedure for trifluoroacetic acid in plant materials by means of HSGC. The species of plants included an herbaceous annual (monkey flower), an annual grass (beard grass), a deciduous tree (black locust), a coniferous tree (Panderosa pine), and a cereal grain (rice). Preliminary treatment of the plant material depended on the species. Monkey flower, beard grass, and rice samples were frozen in liquid nitrogen and then homogenized into a single powder, and black locust and Panderosa pine samples were powdered and freeze-dried. After this preliminary treatment, plant samples were processed by adding 0.05 g of dry plant and 0.4 g of sodium sulfate to a 22-mL HS vial and then 3 mL of 9M sulfuric acid was added. The acid was part of a derivatization solution and helped cleave amide bonds and free trifluoroacetic acid (TFA). Sodium sulfate was used to help displace TFA anions from the plant material and "salt out" the methyl ester of TFA from the solution. After digestion, samples were frozen at –10°C and methanol was added in order to obtain a derivatization solution of proper composition. HS samples were analyzed by means of a GC using an HS autosampler and an ECD.

An HS technique has also been used to determine volatile chlorinated hydrocarbons (VCHs) and chlorinated acetic acids in conifer needles, in which these pollutants were found to accumulate (52). The needles were transported at a temperature of 0–5°C in aluminum-laminated screw-capped vials filled to the maximum in order to avoid large gas volumes over the needles. In the laboratory, pine needles were immediately cut to shorter pieces and placed in HS vials, which were then sealed with a poly(tetrafluoroethylene) (PTFE)-laminated silicon rubber septum. Trichloroacetic acid (TCA) is not suitable for HSGC analysis because it is thermally decarboxylated to trichloromethane (TCM). In the procedure used by Plimucher and Renner (51), the vials with needles were thermostated at 65°C. The injection of the HS sample after 3 h equilibration gave the original content of TCM in a sample. Because TCA was found to decarboxylate completely to TCM within 72 h, the second injection of the HS was taken after 72 h. Thermostating gave the total amount of TCM originally present in the sample and formed from TCA. The content of TCA can be calculated from the TCM amount difference (first and second injection). SE 54 fused-silica columns were used for separation and ECD used for detection. For the pine needles from Berlin, minimum concentrations of VCH were on the level of 10 ng/kg, and maximum values ranged from 1 to 17 mg/kg. TCA concentrations were in the range of 0.7–175 mg/kg.

HSGC has also been used to determine TCA in the vegetation of polluted and remote areas of both hemispheres (52). All of the sampled needles were two years old. They were taken from the sunny side of the trees. They were packed airtight, transported at 5°C, and stored at –65°C until TCA determination. Again, TCA was converted to TCM in HS vials, and the HS was analyzed by GC as described by Weissflog et al. (53).

In HS techniques, analytes partition between a solid sample and inert gas. A better recovery of analytes from a plant sample can be expected when vacuum is applied. Hiatt (54) applied vacuum distillation followed by cryogenic trapping in order to isolate airborne-originating volatile organic compounds (VOCs) from the leaf samples of grass, mock orange, rosemary, and juniper. The addition of water helped suppress the recovery of alcohols, which otherwise would decrease chromatographic resolution. A vacuum distiller was online coupled with a GC–MS measuring system.

Steam distillation under atmospheric pressure was used to isolate methylmercury from leaf and moss samples, which were homogenized beforehand with the use of a ball mill (material frozen at –30°C was ground) (55).

**Sampling for the chromatographic determination of biogenic emissions of VOCs**

Living vegetation releases a large number of VOCs (56,57). Compound classes that emit in the largest quantities include isoprene, terpenes, alkanes, alcohols, esters, carbonyls, and acids. Biosynthesis, emission inventories, the relationship between emission and plant physiology as well as temperature and radiation, and ecophysiological functions have been comprehensively reviewed by Kesselmeier and Staudt (58). That study presented a large number of data on the emission rates of different VOCs from a wide spectrum of plants. The fluxes were strongly influenced by stress effects such as experimental effects, injuries, parasites, and environmental pollution. In some situations plant emission can significantly alter the chemistry of the atmosphere (59,60), and VOC fluxes are often a subject of studies.

Obtaining meaningful information requires sound and careful planning of a sampling process. Samples are generally collected in glass vessels or bags made of suitable plastic material. The other approach (indispensable when concentrations are rather low) is isolating sampling based on the selective trapping of analytes (without air matrix) on an adsorbent or cryogenically (61,62).

Kauppi et al. (63) studied the production of ethylene by exposing the lichen *Claudina stellaris* to sulfur and a heavy-metal-containing solution under acidic conditions. After exposure, samples were placed in test tubes from which, after

incubation, a 1-mL sample was withdrawn with a gas-tight syringe and injected into a GC. A Porapak Q packed column was used for separation and flame ionization detection (FID) used for detection.

Darmais et al. (61) measured vertical fluxes of monoterpenes emitted by orange trees using two different micrometeorological–chemical approaches: relaxed eddy diffusion (REA) (64,65) and vertical gradient (VG) (66). Terpenes were sampled in stainless steel adsorbent tubes packed with Tenax TA by passing air at 80 mL/min (60 min). Analysis was done by means of thermal desorption–GC–MS with a cryofocusing step for band narrowing.

Baker et al. (67) made flux measurements of 2-methyl-3-butyl-2-ol (MBO), a compound whose concentration over some forests is higher than the concentration of isoprene. They used the REA technique whose theory was explained by Businger and Oncley (68). In this method, two air samples are collected over a statistically meaningful time period (~ 30 min), one consisting of updrafts and the other of downdrafts. The frequency of sample collection for each reservoir is related to the speed at which wind eddies change vertical direction. In that study, the relationship that permitted the calculation of the flux from experimental values of concentrations in the up and down reservoirs was given.

The sampling set proposed by Baker et al. (67) consisted of a computer, sonic anemometer, pump, sample inlet and standard inlet lines, shutoff, vent, segregation valves, and up and down bags. The Tedlar bags of a volume of approximately 10 L (which were covered to protect photochemistry during and after sampling) were used. Analytes from sampling bags were trapped cryogenically in a loop directly coupled with a GC. A BD-WAX coated capillary column was used for separation and FID used for detection. For calibration, a GC linked to an atomic emission detector was used.

VOCs can be emitted as well as taken up by plant foliage. Staudt et al. (69) investigated emissions of formic and acetic acids from young orange trees in an orchard and in laboratory (a potted tree). In order to determine exchange rates, the distal end of a branch was mounted in a dynamic plant enclosure system. Air was passed through chambers consisting of cylindrical Teflon bags sustained by a frame. Volatile acids in air entering and exiting the chambers were trapped cryogenically at –70ºC and analyzed by ion-exchange chromatography with conductivity detection. Exchange rates were calculated from the concentration difference between the air entering and leaving the chamber.

Also, Kesselmeier (70) used enclosure (cuvette) measurements in studies of the exchange rates of formic and acetic acids and their homologous aldehydes between tree species and the atmosphere. Organic acids were trapped cryogenically, and aldehydes were trapped on dinitropheynylhydrazine-coated C18 sorbents in glass columns.

## Conclusion

Although chromatographic techniques are characterized by high separation power, plant material generally needs extensive sample treatment before it can be injected into GC for final analysis. Usually, sample preparation is a multistage process that takes much time and effort. For analytical tasks that can be done with the use of HS sample preparation, it is quite simple and should be preferred.

Conventional extraction methods are still widely used, and the application of new extraction methods is steadily increasing, especially because they often offer better extraction efficiency, lower environmental impact, and sometimes are simpler and shorter. Plant materials are very complex matrices composed of different tissues, and selecting the proper plant part for analysis and ensuring repeatable and efficient extraction requires great care. In order to ensure reliability of the results, rules of good laboratory practice should be followed. In testing method accuracy, it should be noted that it is strongly affected by a sample preparation step of analysis, and quality-control-certified reference materials play an important role. Fortunately, quite a few are available and new ones have been introduced (24,71,72). For precision-testing experiments, in-house or laboratory reference materials can be used (72).

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