MODULE 6.1

Analysis of soils, sediments and biological specimens

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Sampling, Sample Preparation, Extraction Of The Analyte And Determination

In the previous sections, we considered the analysis of analytes that were largely dissolved in water, which required minimum treatment for subsequent stages of chemical analysis. In this section the analysis of solids is considered which includes an addition step involving the extraction of the species of interest. The solids considered for analysis are a) Soils, sediments and sewage sludge b) plant materials c) biological tissues and fluids and d) atmospheric particulates.

a)Soil, Sediments And Sewage Sludge

Eventhough the trace element concentration in soils and sediments are normally much higher than those for water samples, many precautionary steps taken relating to sample container preparations and sampling of waters are equally applicable to soil and sediments. Soil composition may vary greatly over a small area. Samples have to be taken from a number of locations to obtain a suitable average composition studies, the source of contamination and its mobility within the soil should be taken into account. Often pollutants deposited from the atmosphere are immobile and will remain within the surface layer. If the soil is disturbed, sample should be taken from the whole of the disturbed area. If the investigation is concerned with possible uptake by plants or crops, then sampling should be over whole depth that the root system penetrates. For landfill sites, samples should be taken over the complete depth of the land fill. The

samples collected should be representative of the sampled land. In many cases it is important to take sub samples within a defined area in order to provide a representative composite sample. The total amount of mineral or organic soils collected should provide at lest 500g of sieved air dry soil.

Sediment sampling is normally carried out using a tube corer or a grab sampler. Core samplers are used for shallow areas. The tube is immersed with valve system open. The valve is then closed to permit the sample to be withdrawn. Just before breaking the surface of the water, the tube is sealed to preserve the sediment structure so that sections corresponding to different depths in the sediments can be analysed. Grab samples are used where the sediment is loose so that there is no vertical structure.

Sample preparation

Soil and sediment samples should be stored in sealed polythene containers at 40^{o}C until arrival at the laboratory. The samples are seperated into particle sizes by wet or dry sieving using 63µm or 20µm nylon sieves. Moist sediments are often used for metal specification analysis. Such samples should be sealed under the nitrogen in polyethylene containers and frozen. Before chemical analysis, the samples should be homogenised and dried (30^{o}C - 60^{o}C) to a constant weight. Homogenisation is achieved by a grinding mill or agate pestle and mortar. The powdered sample is poured into a cone shaped heap, divided into four equal parts. Two opposite quarters are combined and reconed. The process is repeated until the amount of the sample is reduced to that required for analysis.

Extraction of the analyte and its determination:

Determination of pH of the soil:

The soil pH is frequently monitored since the transport of the material in the soil is influenced by the acidity or alkalinity of water in the soil structures. Although soil contains water as an essential constituent, it is of course predominantly a solid. In the pH determination of soil, the added water should be such that there is minimum disturbance to the solution equilibria. A salt solution, usually potassium or calcium chloride is often used to form a paste, and this is left for one hour for the equilibria to be re-established.

Extraction of the available ions in the soil:

The concentrations of available trace metals and nutrients in the soil would be expected to be in the mg kg^{-1} level. These ions many only be released into solution from the soil, if the total charge remains constant. This release will be dependent upon the soil type and the chemical composition of the extraction water. Analytical procedures attempt to reproduce the environmental conditions by suitable choice of extracting solution.

The procedure is simply to shake the soil with the extracting solution for a fixed period typically 1h. A range of extracts have been used, including ammonium acetate, dilute acetic acid, dilute hydrochloric acid and EDTA solution, to mimic local conditions. Once in solution most ions can be analysed by the methods such as AAS, ICP-AES or spectrophotometry.

Analysis of nitrogen content in the soil:

The nitrogen content in the soil can be classified into organic nitrogen, nitrate nitrogen, nitrite nitrogen and ammoniacal nitrogen. Only the last three constitute the readily available nitrogen which is also called the ionic form of nitrogen.

The ionic form of nitrogen is extracted with potassium chloride solution which on reduction with titanium(III) sulphate gives ammonia. The released ammonia can then be determined by standard methods. The standard method of ammonia analysis involves increasing the pH of the solution with sodium hydroxide, distilling the ammonia into a known excess of acid and titrating the excess acid with standard alkali.

Organic nitogen is measured after a preliminary conversion into ammonia. This is achieved by boiling with concentrated sulphuric acid for several hours in a kjeldahl's flask. Potassium sulphate is added to raise the boiling point of the sulphuric acid, along with a catalyst(selenium or mercury is often used).

Determination of total metal concentrations in soil:

For the determinations of total metal concentration, the soil samples are either dissolved by treatment with hydrogen fluoride / perchloric acid mixture or fused with sodium carbonate followed by dissolution in dilute acid. Once in solution the metal concentrations can be determined by standard techniques such as AAS, ICPAES or spectrophotometry.

Determination of organic contaminants in soil:

The organic contamination is typically in the $mgkg^{-1}$concentration range. The simplest method for the extraction of organics is to shake a sub-sample with an extracting solvent(e.g hexane or light petroleum for neutral organics) and to leave the two phases in contact for several hours. Alternatively soxhlet apparatus can be used in which fresh solvent is continously refluxed through a finely divided sample contained in a process thimble and a siphon system removes the extract into the refluxing solvent. A extraction time is 12 hours. However this technique is only applicable to analytes which can withstand the reflux temperature of the solvent. Appropriate solvent should be chosen for the extraction of the analyte.

After the extraction of the contaminants in organic solvent, the analysis of most of the organic materials can be done by gas chromatography as described previously.

Extraction and determination of trace metals from sediments:

Since the ions which are readily available to living species are only the loosly adsorbed metal ions, care has to be taken to dissolve only adsorbed metal ions and not to dissolve the bulk sediment itself. A suitable acid mixture therefore would be concentrated nitric acid / hydrogen peroxide. Concentrations in the $mgkg^{-1}$ range could be expected in the sediment. However for the analysis of less soluble portion of the sediment, extreme solubilisation techniques have to be used such as treating with hydrogen fluoride under pressure in a Teflon-lined Parr bomb. Once in solution the metal ions can be analysed by the methods such as AAS, ICP-AES or spectrophotometry.

Extraction and determination of organic contaminants in sediments:

The organic contaminant in the wet homogenised sediments is extracted with polar solvents like acetone or acetonitrile inorder to overcome the problem of water-solvent emulsions. The extraction is performed in a soxhlet apparatus.

The organic materials after extraction into the organic solvents are then analysed either by gas chromatography or HPLC.

Analysis of sewage sludge:

This requires similar treatment for trace level organics to those discussed above for sediments samples. However, the material contains a high organic content and digestion is necessary before any metal analysis. This involves heating with concentrated nitric acid in kjeldahl apparatus and extraction of the metal ions after dilution with water. The metal ions in solution can quite often be estimated by techniques such as AAS, ICP-AES or spectrophotometry.

B) Plant Materials:

sampling: The collection of plant materials may relate both leaves and / or root system. The aerial parts are obtained by cutting with precleaned secateurs at least 3cm above the soil level. Aerial parts must be stored separately from root samples for transport to the laboratory. Care must be taken to ensure that the plant is representative of the area being studied. Plant contamination from spraying, industrial or transportational particulate fall out or the application of fertiliser requires a carefully planned sampling programme. Composite sampling should provide 0.5 kg of fresh weight material. Wet plant material should be transferred to the laboratory immediately after field collection.

Washing is desirable for terrestrial plant material, in order to remove surface contamination, and in some studies to provide and evaluation of surface particulate contamination. Some pollutants may have been deposited from the atmosphere on to the leaf surface. If the uptake of the pollutant by plant,is to be studied,this would have to be removed by washing. If however the transfer of the pollutant along the food chain is to be studied, then this should be included or determined separately. Dioxins, for instance, are not taken up by plants, but can enter the food chain by deposition on leaves, which are then eaten by herbivores. All root material should be washed with double distilled deionised water to remove any trace element contribution from soil particles.

Sample preparation:

The sample may be freeze-dried to remove moisture which involves deep freezing the sample, reducing the pressure and removing water by sublimation. Drying the samples lessens the possibility of change due to biological activity. A second advantage is that homogenisation of the bulk sample becomes easier if the sample is dry. Freeze dried plant is often powdered using an agate mortar and pestle, grinder or blender. Care should be taken to avoid any sources of metal contamination. All material should be homogenised and dried to a constant weight before acid digestion or solvent extraction is undertaken.

Extraction and determination of organic contaminants in plant samples:

The organic contaminants in the plant material if present are likely to be in the µg kg^{-1} concentration range or below. The simplest method for the extraction

of organics is to shake a sub-sample with an extracting solvent (e.g. Hexane or light petroleum for neutral organics) and to leave the two phases in contact for several hours.

Alternatively soxhlet extraction apparatus can be used in which fresh solvent is continuously refluxed through a finely divided sample contained in a process thimble and a siphon system removes the extract back into the refluxing solvent. A typical extraction time is 12 hours. However this technique is only applicable to analytes which can withstand the reflux temperature of the solvent. Appropriate solvent should be chosen for the extraction of the analyte. The organic materials after extraction into organic solvents can then be analysed by GC or HPLC.

Extraction and determination of trace metals in plant samples:

Trace metals in the plant samples are likely to be in the mg kg^{-1} concentration range. For the extraction of metals the organic matter is decomposed by dry or wet ashing. Dry ashing consists of heating the sample in a muffle furnace, typically at 400 - 600 ^{o}C for 12 - 15h. The resulting ash is then dissolved in dilute acid to give a solution of the metal ions. The volatilisation of metals such as Hg, Cd, Cu, As and Ag can result in inaccuracies.

Wet ashing involves heating the sample with oxidising agents to break down the organic matter. A typical procedure would be heating with concentrated nitric acid followed by perchloric acid. Alternative combinations include sulphuric acid/ hydrogen peroxide and nitric /sulphuric acids. Only ultra pure acids should be used for digestion inorder to reduce the reagent blank. Greater care has to be taken with methods using perchloricacids . The problem of volatility of metals is overcome by using pressurised decomposition

(0.1-0.2g or 0.5-1.0 ml) with nitric acid in Teflon digestion bombs. In addition there is increased digestion efficiency through smaller acid volumes and pressure digestion. However such digestion systems require strict attention to general safety rules inorder to prevent explosive type reactions. The metal ions once in solution can be analysed by AAS, ICP-AES or spectrophotometry.

(C)Biological Tissues And Fluids:

Sampling:

Biological samples are more liable to decomposition than plant samples and should be preserved by freezing below 0^{o}C. The major concern in the collection of biological samples is contamination. Blood samples should be taken using Teflon catheters. Urine collection requires mid-stream samples to be delivered into a covered acid-washed polythene container in order to prevent dust contamination. Animal or human organ samples should be sampled using a tantalum blade to prevent contamination.

Sample preparation:

Sample prepartion methods for biological tissues and fluids can be major sources of trace element contamination. In particular, sample homogenising is normally undertaken with a laboratory homogeniser or blender. Hair samples require a washing procedure to remove exogenous elements. They are then dried under an infrared lamp with the material enclosed in a quartz container to prevent dust contamination.

Freeze drying is also used for blood, urine, milk and tissue samples, which are then stored. Most biological material is stored frozen, although whole blood

samples can hemolyse. On thawing, the sample should be thoroughly mixed before an adequate sample is taken for acid digestion or solvent extraction. The biological samples are routinely digested by dry ashing in a muffle furnace at 500-550^{o}C or by wet digestion on a hot plate or by decomposing under pressure with mineral acids in Teflon bombs, or by low temperature plasma ashing or by microwave digestion.

Extraction of organic and inorganic analytes in biological samples:

Organic compounds are extracted without drying the sample. The bulk sample is often homogenised in a blending mill with water and sub-samples are taken from slurry for extraction. An alkaline digestion stage is also often included before organic extraction to break down any fatty tissue. Metals are once again extracted after wet or dry ashing which is similar to that explained for plant samples.

General analytical determination of biological samples:

The analysis of most organic materials in the organic extract can be done by gas-liquid chromatography or HPLC after sample work up. Atomic absorption spectroscopy is the most common simple technique for the determination of most of the metal ion concentrations.