PLANT SCIENCE USING LABORATORY MICROPROBE X-RAY FLUORESCENCE: NEW TECHNIQUES AND CASE REPORTS

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Abstract:

In vivo and microchemical analytical methods play crucial roles in advancing our understanding of plant metabolism and development. These methods allow researchers to study biochemical processes and molecular interactions within living organisms at a cellular or subcellular level, providing valuable insights into plant physiology. Benchtop microprobe X-ray fluorescence spectroscopy (m-XRF) is indeed a promising tool in this regard. m-XRF provides high spatial resolution, allowing researchers to analyze elements within specific regions of plant tissues. This capability is essential for understanding the distribution and accumulation of elements involved in plant metabolism. Unlike some traditional analytical techniques, m-XRF is non-destructive, meaning it can analyze samples without altering their integrity. This feature is particularly valuable when studying living organisms like plants, where preserving sample viability is essential for accurate results.They can detect and quantify a wide range of elements present in plant tissues. This capability is useful for studying nutrient uptake, mineral accumulation, and elemental composition changes during various stages of plant development.By mapping the distribution of multiple elements simultaneously, m-XRF enables researchers to correlate elemental patterns with physiological processes. This holistic approach provides comprehensive insights into the dynamics of plant metabolism and development.Benchtop m-XRF instruments offer relatively fast analysis times compared to some other techniques. This efficiency is advantageous for high-throughput studies or when analyzing multiple samples, allowing researchers to generate data more rapidly.

Introduction

XRF spectroscopy is widely used in various fields such as chemistry, geology, environmental science, archaeology, forensics, and materials science. It offers several advantages, including non-destructive analysis, high sensitivity, and the ability to analyze.manyelements simultaneously. By leveraging the capabilities of m-XRF, researchers can investigate fundamental questions in plant biology, such as nutrient transport mechanisms, metal ion homeostasis, and stress responses. Furthermore, integrating m-XRF with other analytical techniques, such as mass spectrometry and microscopy, can enhance the depth and breadth of plant metabolic studies. Benchtop microprobe X-ray fluorescence spectroscopy holds significant promise for advancing our understanding of plant metabolism and development. Its high spatial resolution, non-destructive nature, elemental analysis capabilities, multielemental imaging, and time efficiency make it a valuable tool for unravelling the complexities of plant biology.

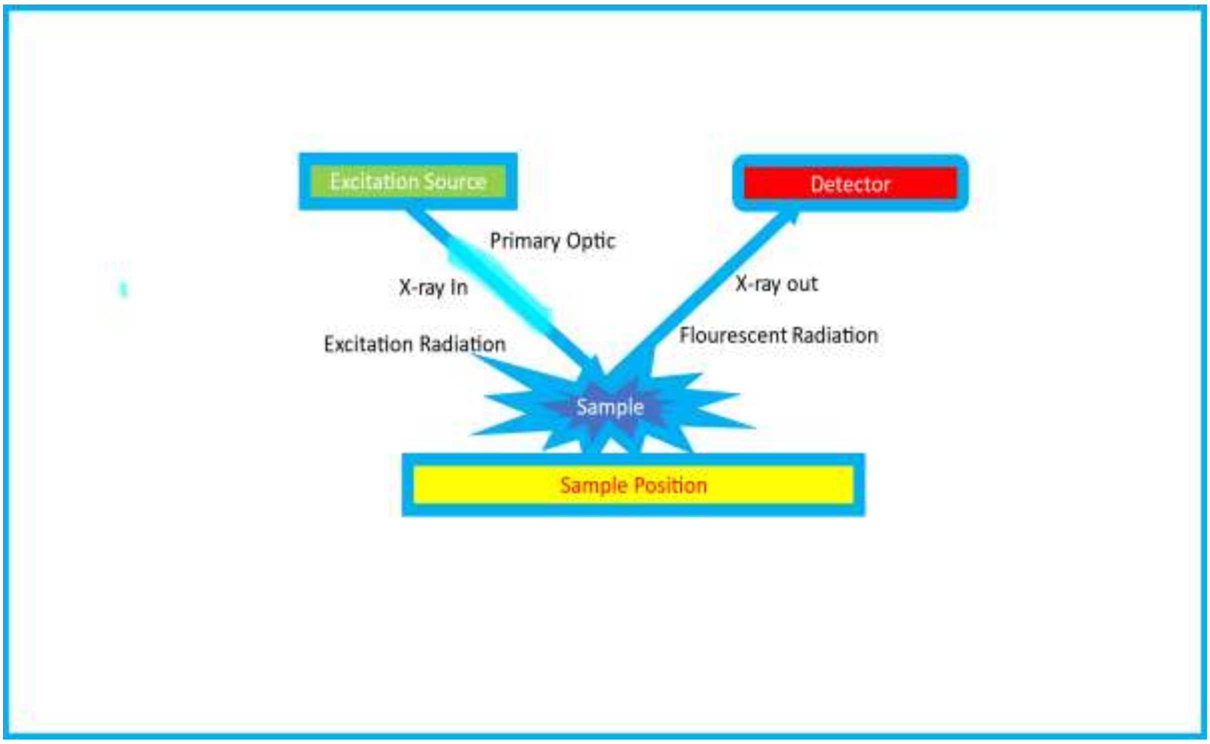
X-ray fluorescence (XRF) spectroscopy is a powerful technique used for qualitative and quantitative analysis of materials. In qualitative analysis, the emitted X-ray spectra are compared to a database of known elemental signatures. Each element produces characteristic X-ray emissions at specific wavelengths, known as characteristic X-ray lines or peaks. By identifying these peaks in the spectrum, the elements present in the sample can be determined. This is because each element has its own characteristic set of X-ray emission lines.

In quantitative analysis, the intensity of the emitted X-rays is indeed proportional to the concentration of the elements in the sample. By calibrating the instrument with known standards of the elements of interest, it's possible to study the concentrations of those elements in the sample being analyzed.

XRF works based on the principle of ionization of inner-shell electrons in the atoms of the material when exposed to high-energy X-rays or gamma rays. When these inner-shell electrons are ejected, outer-shell electrons transition to fill the vacancies, emitting characteristic X-rays in the process. The energy of these emitted X-rays corresponds to specific elements, allowing for identification and quantification. When an inner-shell electron is ejected from its orbit by the incoming high-energy radiation, an electron from a higher energy level may drop down to fill the vacancy, emitting energy in the form of X-rays. By analyzing the energies of the emitted X-rays, scientists can identify the elements present in a sample and quantify their concentrations.

Photon-induced atomic excitation results in relaxation events, such as ejecting an inner shell electron, filling a vacancy, and releasing excess energy as a photon. This unique energy serves as a fingerprint for chemical element identification.Matrix effects pose a significant challenge in m-XRF analysis, which uses X-ray radiation to excite atoms in a sample, detecting and analyzing characteristic X-rays to determine its elemental composition.

Figure 1Microprobe X-ray fluorescence spectrometer scheme.



However, the other elements in the sample matrix can interfere with this process in several ways:

1. **Penetration Depth Variation**: The ability of the excitation X-ray beam to penetrate the sample depends on its energy and the density and composition of the matrix. Different elements have varying absorption coefficients, meaning some elements will absorb X-rays more readily than others. This can result in variations in the depth to which the excitation radiation penetrates the sample.
2. **Escape Probability of X-ray Photons**: Similarly, the probability of an emitted X-ray photon escaping the sample without further interaction is influenced by the matrix composition and density. If the matrix contains elements that absorb X-rays, there is a higher chance that emitted X-ray photons will be absorbed before reaching the detector, leading to reduced signal intensity.
3. **Spectral Overlaps**: Elements present in the sample matrix may produce overlapping X-ray emission spectra with the elements of interest. This can complicate the analysis and interpretation of the emitted X-ray signals, especially in cases where the concentrations of interfering elements are high.

4. **Secondary Excitation and Fluorescence**: Some elements in the matrix may undergo secondary excitation or fluorescence under the primary excitation radiation, leading to additional X-ray emission signals that can interfere with the analysis of the analyte elements.

The Orbis PC EDAX model is a benchtop m-XRF system from the US, featuring a Rhodium anode with a power rating of 50 kV and 1000 mA.The machine offers flexibility in terms of collimation and optics:

- It operates with 1 mm and 2 mm collimators, which are used to control theX-ray beam thickness.
- Additionally, it utilizes a 30 mm polycapillary optic, which is a type of X-ray optic designed to focus X-rays into a small spot.

Several optional primary filters are available to modify the X-ray beam's characteristics:

- 25 mm Al (Aluminum)
- 25 mm Ti (Titanium)
- 25 mm Ni (Nickel)
- 100 mm Rh (Rhodium)
- 127 mm Nb (Niobium)
- 250 mm Al (Aluminum)

These filters are utilized to adjust the energy spectrum of the X-rays emitted by the anode, allowing for specific elemental analysis based on the sample's composition.

The detection system of this setup employs a silicon drift detector. The detector's energy resolution is specified as 140 eV FWHM (Full Width at Half Maximum) at the 5.9 keV Mn-Kα line. This indicates the detector's ability to distinguish between different X-ray energies and thus detect specific elements present in the sample.

Case Reports

Investigation of P, S, K, and Ca distribution in fungi-injured soybean leaves in vivo.

Colletotrichum truncatum spores are obtained, suspended in deionized water on an agar plate, and filtered to remove debris and agar, resulting in a clean suspension.The filtered spore suspension is spread onto the adaxial (upper) surface of soybean leaves using a Drigalski spatula. This ensures that the spores are evenly distributed across the leaf surface. After inoculation, the leaves are moistened by spraying water onto them. This step is crucial for creating a favourable environment for the fungal spores to germinate and infect the plant tissue.The inoculated plants are then placed inside plastic bags to create a controlled environment with constant and adequate humidity. This environment is essential for the growth and proliferation of the fungal pathogen on the plant tissue. Plants are then incubated, assembled in a homemade acrylic sample holder, and their leaves stretched for analysis. Symptoms are monitored daily, and maps recorded.

In vivo Transportation of Feand Mn in Root

Microprobe X-ray fluorescence (μ-XRF) is a non-destructive analytical technique used to map the distribution of elements within a sample. It works by irradiating a sample with X-rays, which causes the elements within the sample to emit characteristic X-ray fluorescence.The context of studying root uptake and transport, researchers typically grow plants hydroponically (in a nutrient solution) or in soil containing specific isotopes of Fe and Mn. These isotopes act as tracers, allowing researchers to track the uptake and movement of these elements within the plant.Plant roots are carefully harvested and prepared for analysis. Depending on the specific research question, researchers may choose to study entire roots or specific regions of interest (such as root tips or lateral roots).

The prepared root samples are then placed under the microprobe XRF instrument. The instrument irradiates the sample with X-rays, causing the Fe and Mn within the roots to emit characteristic fluorescence. By scanning the sample and analyzing the emitted fluorescence at each point, researchers can generate spatial maps showing the distribution and concentration of Fe and Mn within the roots.Once the microprobe XRF scans are complete, researchers analyze the resulting data to extract information about the transportation of Fe and Mn within the roots. This may involve quantifying the concentration of these elements at different locations along the root, as well as studying how their distribution changes over time or in response to various experimental conditions (e.g., nutrient availability, stress). The data obtained from microprobe XRF analysis can provide valuable insights into the mechanisms underlying Fe and Mn uptake and transport in plants. This information is essential for understanding how plants acquire and utilize these micronutrients, as well as for developing strategies to improve nutrient uptake efficiency and crop productivity.

Distribution of Pb in Eucalyptus Hybrid Leaf Cultivated in vitro using m-XRF

m-XRF (Micro X-ray Fluorescence) is a technique used for elemental analysis, capable of providing distribution maps of elements within a sample. In the case of studying thedistribution of lead (Pb) in Eucalyptus hybrid leaf cultivated in vitro, m-XRF would involve the following steps:

The Eucalyptus hybrid leaf samples need to be prepared for analysis. This might involve drying, grinding, and possibly embedding the samples in a suitable matrix for stability during analysis. The m-XRF instrument needs to be set up for the analysis. Calibration standards containing known concentrations of lead and possibly other elements are used to calibrate the instrument's response. This ensures accurate quantification of the elemental concentrations in the sample.

The prepared sample is placed under the X-ray beam of the m-XRF instrument. The X-rays excite the atoms in the sample, causing them to emit characteristic fluorescent X-rays. The emitted X-rays are then detected by an energy-dispersive detector.The m-XRF instrument scans the sample in a grid pattern, collecting X-ray fluorescence spectra at each point. These spectra contain information about the composition of elements of the sample at each point.The collected spectra are processed to extract the intensity of the lead fluorescence signal at each point on the sample. This data is then used to create a spatial distribution map of lead concentration within the Eucalyptus hybrid leaf.The resulting spatial distribution map allows researchers to visualize and interpret the distribution of lead within the leaf sample. This information can be valuable for studying the uptake and transport of lead in plants grown in vitro and understanding the potential impacts of lead contamination on plant health.

Results & Discussion

XRF can detect the presence of various elements within a sample. This can be useful in identifying elements associated with pathogens. XRF microprobes can provide spatial information about the distribution of elements within a sample. This can help in understanding the localization of elements associated with pathogens or changes in elemental composition within infected tissues. XRF can provide qualitative information about the presence or absence of certain elements in a sample. For example, it can detect the presence of metals or other elements that may be associated with bacterial metabolism or virulence factors. With appropriate calibration and standards, XRF can also provide quantitative information about the concentration of elements within a sample. This can be valuable in determining the extent of elemental changes associated with infection.

Proper sample preparation is critical for XRF analysis. Samples need to be properly prepared to ensure representative results and to minimize artifacts that could affect the interpretation of the data. Interpreting XRF data from biological samples, particularly infected tissues, can be challenging due to the complexity of biological matrices and the potential presence of interfering elements. Careful consideration and validation of results are necessary to ensure accurate interpretation. XRF analysis is often used in conjunction with other analytical techniques, such as scanning electron microscopy (SEM) or transmission electron microscopy (TEM), to provide comprehensive information about the composition and structure of infected tissues.

Conclusion

Benchtop m-XRF covers a larger sample area compared to SEM and TEM. It can analyze sample areas ranging from hundreds of square millimeters to square centimeters. This is particularly useful in plant science where the elemental composition of a larger area can provide valuable insights into nutrient distribution, mineral uptake, and plant health. Theycan produce elemental maps and images in two dimensions.. For example, they can see how nutrients are distributed within plant tissues or how pollutants are distributed in soil samples.

. This information is crucial for understanding plant physiology, nutrient transport, and environmental interactions.Benchtop m-XRF typically provides superior detection limits for most elements compared to SEM-EDS and TEM-EDS. This means it can detect trace elements at lower concentrations, providing more sensitive analysis. Sensitivity detection is crucial in plant science for accurate analysis of low element concentrations. It complements other elemental analysis techniques like SEM-EDS and TEM-EDS, providing detailed information on micro- and nano-scale morphology and structure, enabling a more comprehensive understanding.

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