International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Analysis of Bryophytes as Trace Evidence Using X-Ray Fluorescence

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Abstract
Bryophytes are considered as the trace evidence. Forensic botany comes into play when plants have a link to criminal activity. The application of plant sciences to legal issues is known as forensic botany. This involves using plants or plant-derived products as evidence to help solve crimes like murder, kidnapping, etc., as well as to help determine the cause of death of the victim. Plants are resistant, easily transferable, and easily accessible. When botanical specimens are deteriorated and lacking in physical features, many plant materials cannot be distinguished and identified to the species level by conventional morphological characteristics. Examining bryophytes can help with associative reasoning, investigation, and providing details for crime scene reconstruction. Since they are more abundant, they are easily transferable and can be an important piece of evidence linking a suspect, victim, or crime scene. The percentage of an element inside a plant differs depending on the natural components of each plant, just like the medullary index of a hair is similar and unique. We use this uniqueness to calculate the surface and transit route, estimate the value, and calculate the percentage of the element that is present.
Using bryophyte as trace evidence, the location of a criminal can be determined by analyzing macro elements through XRF. By collecting samples from various regions of the country, we can show the study's consistency. To determine the transit path of the criminal or victim, 25 bryophyte samples were collected and examined using X-Ray Fluorescence. This study focused on identifying the surfaces on which the bryophytes grew based on their nutrient values. It was found that the nutrient values could effectively differentiate between different groups. The final interpretation of the bryophyte surfaces was achieved by utilizing the tabulation developed by Dennis and Jeffrey.

Keywords: trace evidence, forensic botany, X-Ray Fluorescence, uniqueness, Dennis and Jeffrey

1. Introduction

Evidence can be defined as something legally deposited in a court of law as a means of conclusive factual determination. Trace evidence are any artifacts or substances that can serve as a crucial piece of information gathered from the scene of an incident and a correlation between the victim and the criminal. These evidences include not only fingerprints and footprints but also hairs, fibers, blood, and accelerants of arson or anything else that can be deposited as a means of identification. Evidence that exhibits the specific characteristics can be used to identify the offender if thorough study and analysis are conducted. The use of plant evidence in legal proceedings is known as forensic botany. While plant fragments are frequently gathered as trace evidence, they are infrequently identified using microscopy

and are much less frequently evaluated for individualization and source of a sample utilizing molecular biology techniques.

Though flora serve as the foundation of the earth's energy structure, The most varied class of plants outside of angiosperms, bryophytes (mosses, liverworts, and hornworts) are found on every continent in the world. Increasing evidence suggests that bryophytes are the earliest extant lineage of terrestrial organisms and were the first green plants to diversify on land. The term "bryophyte" refers to any nonvascular, seedless plant, including mosses (division Bryophyta), hornworts (division Anthocerotophyta), and liverworts (division Marchantiophyta). Although most bryophytes don't have extensive tissue organization, they exhibit a lot of diversity in form and ecology. They have a wide geographic distribution and are smaller than the majority of plants that produce seeds. From polar and alpine regions to the tropics, bryophytes are found all over the planet. Water must eventually exist in the habitat for the sperm to be able to swim to the egg (see below, Natural history). Although some can be found in perpetually damp conditions in desert regions and a few can be found on seashores above the intertidal zone, bryophytes do not live in severely dry places or in seawater. There are a few aquatic bryophytes. Climates that are consistently humid and equatorial have the highest concentration of bryophytes. At tropical and subtropical latitudes, diversity is at its highest. In many of the cooler regions of the Northern Hemisphere, bryophytes—particularly the moss Sphagnum—dominate the vegetation of peatlands. However, despite their relatively constrained range, certain bryophytes exhibit the same apparent dispersibility and ecological flexibility as more common bryophytes. Others display large interrupted patterns like those found in vascular plants.

Bryophytes are useful for forensic investigations because many of them are clonal and widespread, their pieces may easily attach to things like shoes and clothing, and their DNA can be analyzed for quite some time after the plant has been broken up. Criminal investigations can benefit from using botanical evidence. We lack information, however, regarding the degree to which certain plant species and groupings adhere to articles of clothing or footwear. In addition, it is unclear how long DNA remains intact in plant material that has been separated from its original source. In this study, we tested two aspects of using bryophyte material as a forensic tool: (1) the likelihood of finding plant material, specifically bryophytes, in a person's shoes after they have been walking through a forest; and (2) the degree to which bryophyte material can keep its DNA intact enough to allow DNA profiling when exposed to unfavorable conditions.

To further an investigation, bryophytes can be used as trace evidence to identify the location or transit path of a criminal. Uses for bryophytes also helpful in criminal investigations for a variety of reasons beyond from DNA analysis

2. Objectives:

- To determine the location of the criminal using bryophyte as trace evidence.
- To understand the mechanism of XRF
- To examine the macro elements of Bryophyte
- To describe the collection of samples from various regions of the country in order to show that the study is the same across.

3. Mathodology:

Primary data: It is primary data

Secondary data: The literature review was compiled from a variety of articles and websites.

A. COLLECTION OF SAMPLES:

In India, there are more than 25000 bryophytes. Various common plants have been collected and analyzed in this project.

SPECIES	SAMPLE NO	CLASSIFICATION	REGIONS
Marchantia	1	Liverworts	Tamil Nadu
Riccia	2	Liverworts	Gujarat
Marchantiopsida	3	Liverworts	Bihar
Porella	4	Liverworts	Punjab
Conocephalum conicum	5	Liverworts	Karnataka
Riccia fluitans	6	Liverworts	Delhi
Jungermanniales	7	Liverworts	Uttar Pradesh
Jungermanniopsida	8	Liverworts	Kerala
Hepaticopsida	9	Liverworts	Manipur
Nardia	10	Liverworts	Orissa
Climacium americanum	11	Moss	Andhra Pradesh
Polytrichum commune	12	Moss	Tamil Nadu
Bryoandersonia	13	Moss	Kerala
Rhytidiadelphus squar-rosus	14	Moss	Chhattisgarh
Plagiomnium cupsida-tum	15	Moss	Gujarat
Leucobryum glaucum	16	Moss	Tamil nadu
Dicranum scoparium	17	Moss	Odisha
Rhytidiadelphus triquetrus	18	Moss	Kerala

SPECIES	SAMPLE NO	CLASSIFICATION	REGIONS
Pogonatum aloides	19	Moss	Tamil nadu
Atrichum undulatum	20	Moss	Kerala
Anthoceros	21	Hornworts	Orissa
Dendroceros	22	Hornworts	Telangana
Megaceros	23	Hornworts	Tamil nadu
Nothoceros	24	Hornworts	Tamil nadu
Folioceros	25	Hornworts	Madhya Pradesh

Table 1., Collection of Samples

The preliminary examination was done to determine the chemical makeup of the plants so that the experiment could begin without disruptions.

B. CHEMICALS USED:

1. Ethyl alcohol (C2H5OH): Ethanol can be utilized to create a solution that tends to manage bacteria and microorganisms because of its antibacterial and antimicrobial qualities. In microbiology, ethanol has a variety of applications. It is utilized for biomolecules purification and precipitation, specimen staining and preservation, tissue dehydration prior to embedding, and disinfection. The ethanol which are frequently used in laboratories is Ethanol 95.5% (95.6%)
2. Glycerin (C3H8O3): The physical attributes of glycerin make it the preferable component. Slide mounting involves the application of glycerol to remove bubbles. Glycerin keeps the sample from drying out and aids in obtaining a clean image through the microscope. Additionally, glycerin preserves the specimen's cellular properties and the stain's quality while shielding it from dust and other harm.

C. SAMPLE PREPARATION:

- The entire sample is twice washed with ethanol, which is known as the double washing process. To clean up the sample of any unwanted dirt or particles.
- The entire sample is soaked in glycerin to firm it and to ensure effective results during XRF processing.
- After soaking for 24 hours, the samples are removed and categorized for uncomplicated examination.

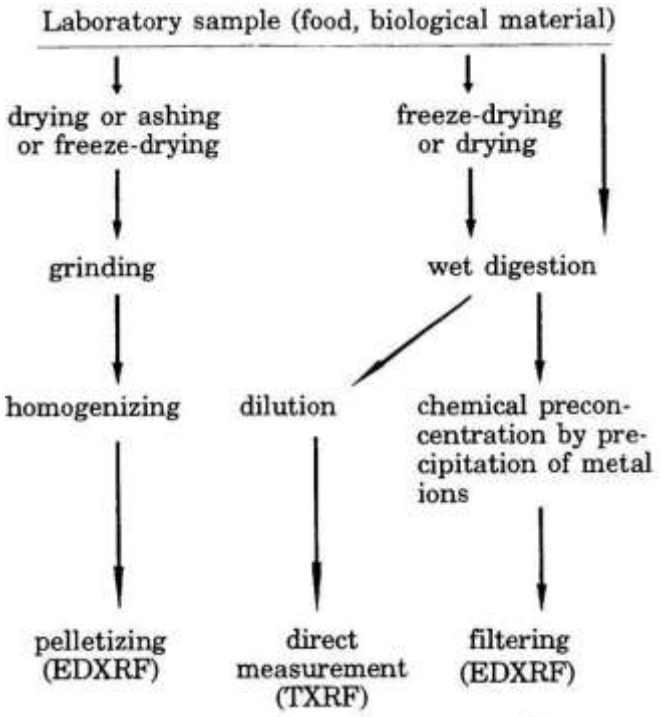

Fig 1., Sample Preparation

- The sample is frequently preconcentrated before measurement by freeze-drying or oven drying.
- The temperature must be kept under control while drying in the oven.
- Over 85 °C is the decomposition temperature for several plant materials.
- Drying biological materials at temperatures more than 100°C is not advised.
- The material is then crushed and homogenized after drying or freeze-drying before being pelletized or taken in aliquots for wet digestion.

D. X-RAY FLUORESCENCE

- An X-ray fluorescence (XRF) spectrometer is a device that uses x-rays to conduct routine chemical analysis of rocks, minerals, sediments, and fluids in a generally non-destructive manner. It operates according to spectroscopic wavelength-dispersive principles, much like an electron microprobe (EPMA). An XRF is primarily utilized for bulk examinations of bigger fractions of geological materials because it is generally unable to do analyses at the small spot sizes typical of EPMA work (2–5 microns). One of the most popular ways for analyzing major and trace elements in rocks, minerals, and sediment is the use of x-ray spectrometers due to their stability and convenience of use, relative ease of sample preparation, and low cost.
- **Principle:** Excitation of atoms in the sample.

Atoms within the sample are excited to produce the X-ray fluorescence effect. A primary X-ray, which is commonly produced in an X-ray tube, strikes an atom's inner shell electron and causes it to be ejected from the atom. An electron from a second outer shell fills the open location, which causes the emission of fluorescence light.

- **Procedure:**
- Fill the sample holders with samples. Put the samples first in alphabetical order. Give one of the Excel spreadsheets a lab number. For important elements, use MAJx00. Sample name, project person's name, LOI, sample weight, and file number are among the entered pieces of information. Enter the

sample name, the project person's name, and the file name when using TRACx00 for trace elements. You must enter the load number for each sample after the sample has run.

- Put a positioning ring of the right size in the sample container for fused discs. Insert the fused disc with the analytical side facing down into the positioning ring. (This is the side that is the opposite of the side that is labelled; the label should be facing up.)
- For pressed powder samples, put an Ultralene sheet on top of a cup of liquid. Utilize a holding ring for liquid cups to hold the film in place. A positioning ring should be placed over the liquid cup. Place the sample in the sample cup with the analytical surface facing down after flipping the cup over. The entire assembly should be put into a sample holder.
- In the appropriate order, insert the sample holders into the sample trays. When looking at each sample tray from the front (the side with the tray number card facing you), position #1 is the position that is furthest to the left.
- Place the sample trays in the sample changer so the tray number cards face the spectrometer.
- Run the XRF and note the results. The results appear to be in graph or sometimes directly in percentage depending on the software.
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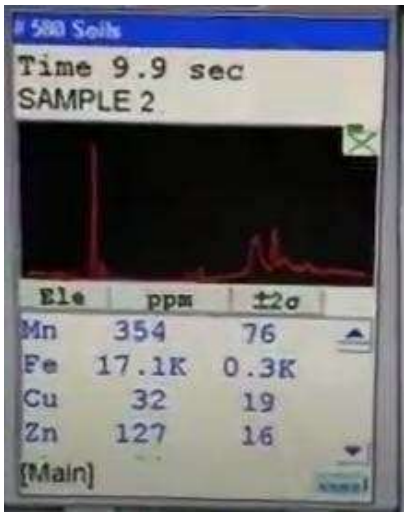


Fig 2., Results obtained on XRF

Below are some pictures of the sample species that were gathered for this project

Fig 3., Atrichum undulatum

Fig 4., Jungermanniales

Fig 5., Dendroceros

Fig 6., Rhytidiadelphus triquetrus

Fig 7., Riccia fluitans

Fig 8., Polytrichum commune

4. Results and Discussion:

The list of outcomes from XRF is shown below:

S. No	SPECIES	GROUP	NUTRIENT	PERCENTAGE (%)
1	Marchantia	Liverworts	Nitrogen	30
2	Riccia	Liverworts	Nitrogen	20
3	Marchantiopsida	Liverworts	Nitrogen	05
4	Porella	Liverworts	Nitrogen	02
5	Conocephalum conicum	Liverworts	Nitrogen	50
6	Riccia fluitans	Liverworts	Nitrogen	87
7	Jungermanniales	Liverworts	Nitrogen	33
8	Jungermanniopsida	Liverworts	Nitrogen	62
9	Hepaticopsida	Liverworts	Nitrogen	93
10	Nardia	Liverworts	Nitrogen	11
11	Climacium americanum	Moss	Nitrogen	20
12	Polytrichum commune	Moss	Nitrogen	34
13	Bryoandersonia	Moss	Nitrogen	67
14	Rhytidiadelphus squarrosus	Moss	Nitrogen	89
15	Plagiomnium cupsidatum	Moss	Nitrogen	40
16	Leucobryum glaucum	Moss	Nitrogen	20

17	Dicranum scoparium	Moss	Nitrogen	03
18	Rhytidiadelphus triquetrus	Moss	Nitrogen	45
19	Pogonatum aloides	Moss	Nitrogen	67
20	Atrichum undulatum	Moss	Nitrogen	40
21	Anthoceros	Hornworts	Nitrogen	10
22	Dendroceros	Hornworts	Nitrogen	25
23	Megaceros	Hornworts	Nitrogen	74
24	Nothoceros	Hornworts	Nitrogen	46
25	Folioceros	Hornworts	Nitrogen	38

Table 2., Results obtained from XRF

Below is a list of the average percentage for each group shown in XRF:

S.NO	GROUPS	AVERAGE PERCENTAGE
1	Liverworts	39.30
2	Moss	42.5
3	Hornworts	38.6

Table 3., Average percentage of each group

According to, **Dennis H. Brown and Jeffrey W. Bates: Bryophytes and nutrient cycling (1991)**

S. No	GROUP	PERCENTAGE (%)	SURFACE
1	Liverworts	01-40	Moist soil
2	Liverworts	40-100	Damp rocks
3	Mosses	01-60	Beneath trees
4	Mosses	60-100	Damp rocks
5	Hornworts	01-35	Tree Trunks
6	Hornworts	35-100	Tree Bark

Table 4., Table of Dennis and Jeffrey Surface identification using Nitrogen Percentage

The information obtained through the above-mentioned table was calculated, the surface was interpreted, and results were obtained.

S. No	SPECIES	GROUP	PERCENTAGE (%)	SURFACE
1	Marchantia	Liverworts	30	Moist soil
2	Riccia	Liverworts	20	Moist soil
3	Marchantiopsida	Liverworts	05	Moist soil
4	Porella	Liverworts	02	Moist soil
5	Conocephalum conicum	Liverworts	50	Damp rocks
6	Riccia fluitans	Liverworts	87	Damp rocks
7	Jungermanniales	Liverworts	33	Moist soil
8	Jungermanniopsida	Liverworts	62	Damp rocks
9	Hepaticopsida	Liverworts	93	Damp rocks
10	Nardia	Liverworts	11	Moist soil

11	Climacium americanum	Moss	20	Beneath trees
12	Polytrichum commune	Moss	34	Beneath trees
13	Bryoandersonia	Moss	67	Damp rocks
14	Rhytidiadelphus squarrosus	Moss	89	Damp rocks
15	Plagiomnium cupsidatum	Moss	40	Beneath trees
16	Leucobryum glaucum	Moss	20	Beneath trees
17	Dicranum scoparium	Moss	03	Beneath trees
18	Rhytidiadelphus triquetrus	Moss	45	Beneath trees
19	Pogonatum aloides	Moss	67	Damp rocks
20	Atrichum undulatum	Moss	40	Beneath trees
21	Anthoceros	Hornworts	10	Tree Trunks
22	Dendroceros	Hornworts	25	Tree Trunks
23	Megaceros	Hornworts	74	Tree Bark
24	Nothoceros	Hornworts	46	Tree Bark
25	Folioceros	Hornworts	38	Tree Bark

Table 5., Interpretation of the surface of bryophytes

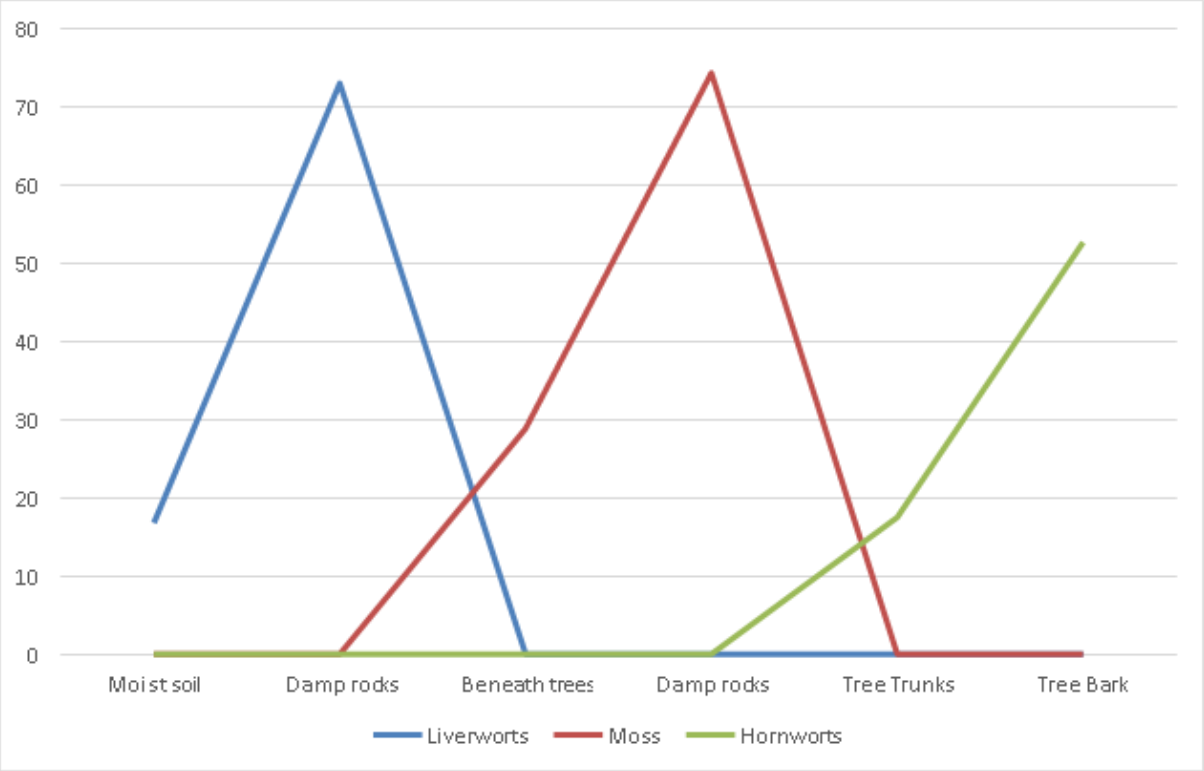
To demonstrate that the results obtained are comparable across the country, several samples of bryophytes from various states in India were collected. The study revealed the macronutrient of each group. The following species were employed as research samples: Marchantia, Riccia, Marchantiopsida, Porella, Conocephalum conicum, Riccia fluitans, Jungermanniales, Jungermanniopsida, Hepaticopsida, Nardia, Climacium americanum, Polytrichum commune, Bryoandersonia, Rhytidiadelphus squarrosus,, Plagiomnium cupsidatum, Leucobryum glaucum, Dicranum scoparium, Rhytidiadelphus triquetrus, Pogonatum aloides, Atrichum undulatum, Anthoceros, Dendroceros, Megaceros, Nothoceros, Folioceros.

All these samples were examined, and using X-Ray Fluorescence and the macronutrient percentage in each sample was determined**.** Only nitrogen was considered among the other nutrients because it is essential for growth and serves as a soil nutrient. Nitrogen is a major component of proteins, vitamins, hormones, and other compounds.

The moss grows usually beneath trees, the hornworts mostly on tree trunks or bark, and the liverworts grow on moist soil or damp rocks. Different amounts of nitrogen are present on each surface, which is where living things grow. It is easily recognizable as a certain plant species grow on a certain surface according to the nutrient.

Based on the nutrient value of bryophytes, the surface on which they grow were identified in this study. This study also demonstrated that the value of the nutrients can be used to distinguish between groups. The tabulation developed by Dennis and Jeffrey is utilized to arrive at the final interpretation of the bryophyte surface.

The graphic below illustrates the difference between the nutrient value of each group i.e., Liverworts, hornworts, and moss.



Graph 1., Graphical representation of the nutrient value of each group

5. Conclusion:

The outcomes of this study indicate that in crucial circumstances, the relationship between soil and plants can be an important component of evidence in a criminal investigation. Plant macro remains (seeds, fruits, flowers, leaves, and other vegetative parts and/or their fragments) can provide detailed information about the ecological and geographic location where the body has been exposed, making them an effective tool for connecting a body or other evidence back to a primary crime scene. Their primary importance has been outlined in forensic-like investigations carried out in the archaeological context.

Plant samples from same place of the same species and those from related species closely overlap. In consideration of this, it has become essential to identify the traits that should be both general and homogenous.

According to the findings of this study, Identifying the nutritional value to determine the plant's location is critical in criminal investigations. Any bryophyte detected on clothing, footwear, or any other object can be utilized to determine the nutrient value.

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