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Green Water Evidence: Integrating forensic botany and aquatic ecology for pollution source tracking, illegal disposal investigations, and environmental crime attribution

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Abstract
Aquatic environments serve as critical repositories for anthropogenic pollutants and sites of illicit activities, yet traditional chemical and physical forensic methods often struggle with source attribution, temporal resolution, and linkage of evidence to specific actors or events. This comprehensive review examines the emerging integration of forensic botany with aquatic ecology—collectively framed as "green water evidence"—to address these challenges in environmental crime investigation. Drawing on diatom assemblages, phytoplankton communities, macrophytes, bryophytes in wetland margins, environmental DNA (eDNA), stable isotope signatures, and community ecology principles, the review synthesizes how botanical signatures function as persistent, habitat-specific tracers. Key applications include pollution source tracking through indicator species and bioaccumulation patterns that distinguish industrial, agricultural, sewage, and pharmaceutical inputs; reconstruction of illegal disposal events via transferred plant material and sedimentassociated biota on objects, clothing, or vehicles; and broader attribution of environmental crimes such as unauthorized dumping, habitat degradation, and contamination events. Methodological advances—from classical microscopy and acid digestion protocols to DNA metabarcoding, AI-assisted identification, remote sensing of vegetation change, and GIS mapping of ecological fingerprints—are critically evaluated. Case studies illustrate successful linkages of suspects or materials to specific water bodies and the use of plant-based evidence in supporting civil and criminal environmental enforcement. Challenges remain, including spatial-temporal variability of communities, taxonomic expertise gaps, standardization of protocols, and courtroom admissibility. The review concludes that systematic incorporation of aquatic botanical evidence, supported by interdisciplinary collaboration and reference databases, substantially strengthens environmental forensics. Future priorities include expanded eDNA reference libraries, quantitative transfer and persistence studies, integration with chemical forensics, and training for investigators. By treating aquatic plants and algae as silent, ubiquitous witnesses, this integrative approach offers a powerful, underutilized toolkit for detecting, attributing, and deterring environmental crimes that threaten aquatic ecosystems and public health.

Keywords: Forensic botany, aquatic ecology, environmental forensics, diatoms, phytoplankton, macrophytes, pollution source tracking, illegal disposal, environmental crime, bioindicators, environmental DNA (eDNA), metabarcoding, stable isotopes, trace evidence, forensic limnology, water quality indicators, habitat fingerprinting, bioaccumulation, green water evidence

Introduction

Environmental crimes involving aquatic systems represent one of the most persistent and under-prosecuted categories of offense worldwide. Illegal discharges of industrial effluents, agricultural runoff containing excess nutrients and pesticides, untreated sewage, pharmaceutical residues, microplastics, and deliberate dumping of solid or hazardous waste into rivers, lakes, wetlands, and coastal waters generate cumulative ecological damage that is often diffuse, delayed, and difficult to attribute to specific actors. Traditional investigative approaches rely primarily on chemical analysis of water and sediment, hydrological modeling of contaminant plumes, documentary records of permits and discharges, remote sensing of surface anomalies, and occasional witness or whistleblower testimony. While these tools are indispensable, they frequently encounter fundamental limitations: rapid dilution and transformation of chemical markers, overlapping signatures from multiple legitimate and illegitimate sources, intermittent or pulsed releases that evade routine monitoring, and the absence of direct physical linkage between a suspect vehicle, container, footwear, or tool and a particular water body.Forensic botany has long demonstrated its value in conventional criminal investigations by exploiting the ubiquity, diversity, and transferability of plant material. Pollen grains, seeds, leaf fragments, wood particles, and bryophytes have linked suspects to crime scenes, established postmortem intervals through growth patterns, and reconstructed movements across landscapes. The extension of these principles into aquatic environments creates a specialized interdisciplinary domain that merges forensic botany with limnology and aquatic ecology.

Organisms inhabiting or closely associated with water—diatoms, other phytoplankton, filamentous algae, submerged and floating macrophytes, emergent wetland plants, and margin-dwelling bryophytes—possess a suite of properties that make them exceptionally powerful forensic tracers. Many are microscopic and therefore readily transferred without the awareness of the perpetrator; their cell walls or tissues are often highly resistant to degradation; their species assemblages respond sensitively to finescale differences in water chemistry, substrate, flow regime, light availability, and pollution load; and they leave distinctive

ecological fingerprints that can be matched between evidence items and reference sites. The framing of this body of evidence as

“green water evidence” emphasizes both its botanical character and its aquatic context. Unlike purely chemical markers that may dissipate within hours or days, botanical particles and community signatures can persist on clothing, footwear, vehicle undercarriages, discarded containers, or submerged objects for weeks or longer. Unlike macroscopic debris that may be deliberately cleaned, microscopic diatoms and algal cells often remain after ordinary washing. Unlike generic soil particles, aquatic assemblages frequently exhibit high habitat specificity, allowing investigators to distinguish not only between different water bodies but sometimes between microhabitats within a single lake or river reach. The historical trajectory of this field begins with the classic diatom test for drowning, first systematically explored in the late nineteenth and early twentieth centuries and refined throughout the subsequent decades.

Early forensic limnology casework in the 1990s demonstrated that algal communities recovered from the sneakers of suspects could be statistically matched to pond sediments at an assault scene, providing critical linkage evidence. Subsequent experimental studies quantified transfer and persistence under controlled conditions. Parallel developments in environmental monitoring established diatoms and macrophytes as reliable bioindicators of water quality and pollution gradients. More recently, the advent of environmental DNA metabarcoding, high-throughput sequencing, stable-isotope analysis of plant tissues, automated imaging, and machine-learning classification has dramatically increased taxonomic resolution and analytical throughput. Remote sensing of macrophyte beds and GIS-based mapping of ecological change further expand the spatial scale at which botanical evidence can operate.

This review synthesizes the current state of knowledge and practice. It begins by establishing the biological and ecological foundations that give aquatic botanical evidence its discriminatory power. It then surveys the analytical toolkit, ranging from classical microscopy to cutting-edge molecular and computational methods. Subsequent sections examine concrete applications in three interlocking domains: pollution source tracking, illegal disposal investigations, and the broader attribution of environmental crimes. Challenges of variability, standardization, expertise, and courtroom communication are critically assessed. Finally, the review outlines research and operational priorities necessary to move green water evidence from an occasional specialized resource to a routine component of environmental crime investigation. Throughout, emphasis is placed on the necessity of interdisciplinary collaboration among botanists, limnologists, forensic scientists, environmental chemists, and legal practitioners, and on the development of robust, regionally stratified reference databases that capture both spatial and seasonal dimensions of aquatic plant communities.

Biological and Ecological Foundations of Aquatic Botanical Evidence

The forensic utility of aquatic botanical material rests on a set of interrelated biological and ecological properties that collectively produce transferable, persistent, and informative signatures. Understanding these foundations is essential for designing sampling strategies, interpreting comparative analyses, and assessing the strength of any given match or exclusion. Diatoms (Bacillariophyta) occupy a central position in this toolkit. These unicellular eukaryotic algae construct intricate silica frustules whose morphology is species-specific and highly diagnostic under light or electron microscopy. The siliceous wall confers extraordinary resistance to chemical digestion, enzymatic attack, and physical abrasion, allowing frustules to survive prolonged submersion, drying, and even many cleaning processes.

Diatoms inhabit virtually every aquatic and semi-aquatic environment, from oligotrophic mountain lakes to eutrophic ponds, flowing rivers, estuaries, wet soils, and damp terrestrial surfaces. Their species richness is enormous—tens of thousands of taxa have been described—and many exhibit narrow ecological tolerances with respect to pH, conductivity, nutrient concentrations, organic pollution, salinity, and substrate type.

Consequently, the assemblage of diatom species presents at a given site constitutes a high-resolution ecological fingerprint. Eurytopic taxa that tolerate a wide range of conditions contribute quantitative abundance information, while stenotopic taxa serve as precise indicators of particular habitats or pollution states. Classic studies have shown that the relative proportions of certain genera (for example, Eunotia, Nitzschia, Navicula, Cymbella, and Fragilaria) can discriminate among water bodies only a few hundred meters apart or among different microhabitats within a single pond.

The stakes are high. Aquatic ecosystems provide essential services—drinking water, fisheries, flood regulation, biodiversity support, and recreational value—yet continue to suffer from under-enforcement of environmental regulations. By treating the plants and algae that inhabit these systems as silent, persistent witnesses, investigators gain a powerful additional means of detection, linkage, and accountability. The integration of forensic botany with aquatic ecology does not replace chemical or documentary evidence; rather, it supplies an independent, ecologically grounded line of inquiry that can corroborate, refine, or, in some cases, independently establish attribution where conventional methods fall short.

Ecological theory supplies the interpretive framework. Community composition is the product of regional species pools filtered by local abiotic and biotic conditions. Anthropogenic disturbance—nutrient enrichment, organic loading, metal contamination, altered hydrology, or physical habitat alteration—shifts these filters and produces detectable changes in assemblage structure. Indicator-species analysis, multivariate ordination, and similarity indices allow quantitative comparison between evidencederived assemblages and reference samples. Spatial heterogeneity within and among water bodies requires that reference sampling capture representative microhabitats and, ideally, seasonal variation. Temporal dynamics, including seasonal succession and inter-annual climate effects, introduce additional complexity that must be acknowledged in interpretation.

Transfer and persistence are governed by particle size, surface properties of the substrate (textile weave, leather, rubber, metal), contact duration and pressure, subsequent environmental exposure, and cleaning attempts. Experimental work has demonstrated that diatoms and plant fragments can be deposited after only brief contact with

sediment or vegetation and that a detectable fraction often survives ordinary laundering or weathering. Secondary transfer remains a concern and must be evaluated case by case. Persistence in sediments and on submerged objects can extend for months or years, providing longer-term records of contamination or disposal events.

Collectively, these biological and ecological attributes enable aquatic botanical evidence to function simultaneously as a locator (linking an item or person to a specific water body or microhabitat), a pollution recorder (indicating the type and intensity of contamination), and, in some circumstances, a rough chronometer (through successional stage or growth patterns). The discriminatory power is maximized when multiple lines of botanical evidence—diatom assemblages, macrophyte fragments, isotope signatures, and eDNA profiles—are combined and interpreted within a rigorous ecological and statistical framework.

Analytical Techniques and Methodological Advances

The analytical pathway for aquatic botanical evidence begins with careful scene and evidence sampling and proceeds through preparation, identification, quantification, and comparative statistical analysis. Classical and molecular methods currently coexist and are increasingly integrated.

Reference sampling of suspected water bodies must be systematic. Multiple stations are selected to represent the range of microhabitats (open water, littoral zone, inflow, outflow, different substrates). Both water-column phytoplankton and benthic or epiphytic communities are collected, often with replication. Sediment samples capture longerterm assemblages. Physicochemical parameters (temperature, pH, conductivity, dissolved oxygen, nutrient concentrations) are recorded concurrently to aid interpretation. Seasonal sampling is ideal but frequently constrained by investigative timelines; existing monitoring data or herbarium records can partially compensate.

Evidence items—clothing, footwear, vehicle parts, tools, containers, or recovered waste—are examined under controlled laboratory conditions to avoid contamination. Surface swabbing, scraping, or rinsing recovers adherent particles. For diatom analysis, organic material is removed by acid digestion (commonly nitric acid or a combination of acids with hydrogen peroxide), followed by centrifugation and mounting of the siliceous residue on microscope slides. Light microscopy at high magnification allows morphological identification of frustules; scanning electron microscopy provides higher resolution for critical taxa. Quantitative counts and relative abundances are recorded. Macrophyte fragments are identified morphologically or by DNA barcoding of standard chloroplast markers.

Molecular methods have expanded both sensitivity and taxonomic coverage.

Environmental DNA extracted from water, sediment, or surface swabs can be amplified with primers targeting diatoms, broader phytoplankton, or vascular plants and sequenced on high-throughput platforms. Metabarcoding generates community profiles that include taxa difficult to identify morphologically or present in low abundance. Quantitative PCR can target specific indicator species. Bioinformatics pipelines cluster sequences into operational taxonomic units or amplicon sequence variants and assign taxonomy against reference databases.

Parallel analysis of morphological and molecular data strengthens confidence when the two approaches converge. Stable-isotope ratio mass spectrometry of macrophyte tissues provides independent source information. Elevated $\delta^{15}N$ values often indicate sewage or manure nitrogen, while lower values may reflect synthetic fertilizers. Spatial mapping of isotopic signatures along a watercourse can delineate plume extent and dilution.

Computational advances include automated image-analysis systems and machinelearning classifiers trained on large libraries of diatom and algal images. These tools reduce the time required for routine identification and improve consistency across analysts. Multivariate statistical methods—non-metric multidimensional scaling, principal component analysis, analysis of similarity (ANOSIM), and indicator-species analysis— quantify the degree of assemblage match or mismatch. Similarity thresholds and confidence intervals must be established empirically for the habitats under investigation.

Remote sensing and GIS add landscape context. Multispectral or hyperspectral imagery can detect changes in macrophyte cover or spectral signatures associated with pollution stress. Integration of botanical sample locations with hydrological models and land-use layers supports source attribution at catchment scale.

Quality assurance is paramount. Strict contamination controls, use of blanks, chain-ofcustody documentation, and inter-laboratory validation exercises are required. Reporting must clearly state methods, taxonomic resolution, statistical results, and sources of uncertainty. The combination of classical morphological expertise with molecular and computational tools currently offers the highest overall reliability.

Applications to Pollution Source Tracking

Pollution source tracking constitutes one of the most promising applications of green water evidence. Aquatic plant and algal communities respond to nutrient enrichment, organic loading, metal contamination, and other stressors with shifts in species composition, abundance, and morphological condition that can be diagnostic of particular source types. In eutrophication scenarios, elevated phosphorus and nitrogen favor assemblages dominated by tolerant diatoms such as certain Nitzschia, Navicula, and Cyclotella species, often accompanied by cyanobacterial blooms. Oligotrophic indicators decline. The spatial pattern of these shifts, when mapped along a river or around a lake, can point toward discrete discharge points. Stable-isotope analysis of macrophyte tissues further discriminates sewage/manure nitrogen (higher $\delta^{15}N$) from agricultural fertilizer (typically lower $\delta^{15}N$). Studies using duckweed and other floating plants have successfully mapped nitrogen plumes and identified leaking sewage infrastructure that conventional chemical sampling had missed because of intermittent flow. Industrial effluents introduce metals, acids, or specific organic compounds that select for tolerant taxa or induce frustule deformities in diatoms. Assemblages downstream of known industrial outfalls often differ markedly from upstream reference sites. Macrophytes accumulating elevated metal concentrations in their tissues provide additional confirmation and can serve as time-integrated samplers when water-column concentrations fluctuate. Pharmaceutical and personal-care product residues present a more recent challenge. Certain

macrophytes, notably water hyacinth, have demonstrated the capacity to accumulate these compounds in root and leaf tissues, creating a biological archive that persists after the compounds themselves have been diluted or degraded in the water column. Targeted analysis of plant tissues can therefore reveal intermittent or historical contamination events.

Sediment cores extend the temporal window. Diatom assemblages preserved in sequential layers record the history of water-quality change, allowing investigators to establish when a particular pollution regime began or intensified—information valuable in both regulatory enforcement and civil litigation.

When botanical evidence is recovered from a suspect vehicle, container, or footwear and matches the pollution-indicator assemblage of a specific contaminated site, the linkage supports attribution. Conversely, mismatch can exclude candidate sources. The combination of community data, isotope signatures, and chemical residue analysis produces multi-proxy source attribution of greater robustness than any single method alone.

Applications to Illegal Disposal Investigations

Illegal disposal of waste, hazardous materials, vehicles, or human remains into aquatic environments frequently results in the transfer of botanical particles from the disposal site to the objects or persons involved. The reverse transfer—from disposed material onto the surrounding vegetation or sediment—can also occur but is less commonly exploited. Footwear and clothing are particularly effective collectors.

Experimental studies have shown that walking through littoral sediments or vegetation deposits diatom-rich material that reflects the local assemblage. Even after subsequent walking on terrestrial surfaces or ordinary laundering, a detectable fraction of the original assemblage often remains. Casework has repeatedly demonstrated statistical matches between diatoms recovered from suspects' shoes and reference samples from disposal or assault sites adjacent to water.

Macrophyte fragments and bryophytes provide coarser but still useful geographic indicators and can narrow search areas when the combination of species is rare. Vehicles used to transport waste may retain mud and plant material on undercarriages, wheel wells, or cargo areas. Containers or drums recovered from water bodies can carry epiphytic or sediment-associated biota that link them to specific stretches of river or particular wetlands. Tools used to dig or cut vegetation may transfer characteristic fragments. In cases of body disposal, botanical evidence on the remains or associated clothing can indicate the primary deposition site even if the body has been moved.

Successional colonization of submerged remains by algae and invertebrates has been explored for postmortem submersion interval estimation, although this application remains less standardized than terrestrial entomology. The strength of botanical linkage evidence is greatest when multiple independent markers (diatom assemblage, macrophyte species, bryophytes, isotope ratios) converge on the same location and when reference sampling has adequately captured local heterogeneity. Exclusion of alternative sites further strengthens the inference.

Environmental Crime Attribution and Broader

Enforcement UsesBeyond discrete incidents of dumping or single-point discharges, aquatic botanical evidence contributes to the attribution of cumulative or diffuse environmental crimes. Pattern analysis of community degradation across a catchment can identify the relative contributions of multiple sources and support allocation of responsibility among regulated entities. In civil litigation seeking damages for ecological injury, historical sediment records and contemporary bioindicator data provide objective measures of harm and its onset.

Regulatory agencies can incorporate routine botanical monitoring into compliance programs. Sudden shifts in indicator assemblages may trigger targeted investigations that chemical sampling alone would not prioritize. International or transboundary cases benefit from the fact that many diatoms and macrophyte taxa have well-documented distributional ranges, allowing ecological evidence to complement chemical fingerprinting of contaminants. Remote sensing of macrophyte cover and condition, combined with ground-truth botanical sampling, enables detection of illegal land-use changes, unauthorized dredging, or vegetation clearance that affect aquatic habitats. Integration of these data streams into GIS platforms supports both real-time enforcement and long-term trend analysis.

Challenges, Limitations, and Evidentiary Considerations

Despite its promise, green water evidence faces several practical and scientific challenges. Spatial heterogeneity within water bodies means that inadequate reference sampling can produce false exclusions or weak matches. Seasonal and inter-annual variability requires either multi-season baselines or careful acknowledgment of uncertainty when only single-time-point references are available.

Taxonomic expertise remains limited; accurate morphological identification of diatoms demands specialized training, and reference libraries for molecular approaches are still incomplete for many regions. Transfer and persistence studies, while growing, are not yet comprehensive across the full range of substrates, climates, and post-transfer conditions. Secondary transfer and contamination during collection or laboratory processing must be rigorously controlled and documented. Statistical methods for declaring a "match" require further empirical validation and transparent communication of error rates. In the courtroom, botanical evidence is typically presented as circumstantial.

Clear explanation of ecological principles, methods, and limitations is essential for judges and juries. Standardization of protocols across laboratories and jurisdictions will enhance consistency and admissibility. Resource constraints—both in terms of trained personnel and analytical cost—currently limit routine use.

Future Directions and Recommendations

Realization of the full potential of green water evidence requires coordinated advances on several fronts. Construction of regionally stratified morphological and eDNA reference databases, ideally linked to environmental metadata, is a high priority. Quantitative experimental programs examining transfer efficiencies, persistence under realistic conditions, and secondary transfer rates will strengthen interpretive frameworks. Continued development and validation of AI-assisted identification tools can alleviate the taxonomic bottleneck.

Integration pipelines that combine botanical, chemical, hydrological, and geospatial data will produce more robust

multi-proxy attributions. Training programs for crime-scene investigators, environmental enforcement officers, and forensic scientists should include recognition and proper collection of aquatic botanical evidence. Laboratory capacity building and proficiency testing schemes will support quality assurance. Policy and regulatory frameworks that explicitly recognize the evidentiary value of ecological indicators can encourage their incorporation into standard investigative and compliance protocols.

Research should also explore under-utilized groups (certain filamentous algae, aquatic fungi, and microbial consortia) and novel markers such as plant-associated volatile organic compounds or metabolomic profiles. Collaboration between academic ecologists, forensic practitioners, and enforcement agencies will accelerate translation of scientific advances into operational capability.

Conclusion

The integration of forensic botany with aquatic ecology yields a powerful and still underexploited resource for environmental crime investigation. Aquatic plants and algae generate persistent, transferable, and ecologically informative signatures that complement and often extend the reach of conventional chemical and documentary evidence. Applications to pollution source tracking, illegal disposal investigations, and broader attribution of environmental harm have already demonstrated practical value in casework and experimental studies.

Methodological advances in microscopy, molecular biology, isotope analysis, remote sensing, and computation continue to increase resolution and throughput. Significant challenges of variability, expertise, standardization, and resources remain. Addressing these challenges through database development, experimental research, training, and interdisciplinary collaboration will enable green water evidence to move from specialized application to routine component of the environmental forensic toolkit.

In an era of continuing pressure on aquatic ecosystems, the silent testimony of the plants and algae that inhabit them offers an essential additional means of detection, linkage, and accountability.

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