**RESEARCH ARTICLE**

# Exploring the Efficacy of Hybrid MS Techniques in Environmental Bioanalysis of Pesticides

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**Pesticide residues in environmental matrices can be a key focal point for decisions on ecosystem health; knowledge of the legal limit of a compound is invaluable to any analyst. This research assesses how different hybrid MS methods Q-TOF and Orbitrap MS enhance the analysis of pesticides in various environmental samples. Many traditional MS methods have drawbacks like poor sensitivity and specificity and the inability to analyze complex samples. New Hybrid MS techniques, High-resolution mass spectrometry (HRMS) combined with accurate mass measurement have brought a lot of improvements in these fields.**

**Evaluation of the effectiveness of hybrid MS was done using water, soil, and spiked vegetable samples containing different pesticides. Some of the research activities included the use of experimental design to fine-tune the operational parameters; assessing the instrument's capability for monitoring trace levels of target analytes, and comparing the analytical performances of hybrid MS with conventional MS techniques.**

**Therefore, it is clear that the application of hybrid MS techniques improves sensitivity and increases the accuracy of analysis, which is characterized by lower detection limits and higher resolution compared to classical methods. This improvement enhances monitoring of pesticide contamination which is vital in policies concerning the protection of the environment and enforcement of regulations. However, hybrids of MS techniques come with issues such as high operational costs and skilled professionals to undertake the procedures. Further studies should be directed to the mentioned shortcomings and investigation of hybrid MS applications with other promising technologies to enhance the field of environmental bioanalysis.**

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## 1. Introduction

Environmental biotechnology is concerned with the identification and measurement of specific chemicals in samples of water, soil, and air needed for health assessments. This field is important in the surveillance of pollutants that affect ecosystems and human health.

The other common pollutants include pesticides which are commonly used in farming activities. Users are worried because they persist in the environment and cause adverse health consequences; thus, the emphasis is on effective methods to measure their content and effects (Escher et al., 2023).

Desirable characteristics of an analytical technique as sensitivity, selectivity, and quantization ability are well fulfilled by mass spectrometry particularly when employing electron ionization. The second

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generation of MS, which is multi-stage MS, which has an additional mass analyzer or ion source results in better outcomes as compared to single-stage MS. Thus, analyzers such as Q-TOF, quadrupole-orbitrap or Q-LIT are characterized by accurate mass measurement, high resolution and sensitivity (Makarov, 2000; Kaufman, 2012). Thus, the Hybrid MS techniques overcome the problems such as sensitivity, specificity, and matrices of the samples resolved to the conventional MS methods. Said techniques are superior because of the enhanced resolution, precise quantity determination, and multi-residue analysis capability. This is very important in environmental bioanalysis whereby pesticides are detected at very low concentrations against high background interferences. Analyzing these present-day tactics improves the numerous assessment instruments, the observation of the surroundings, and conformity with state norms (Hernández et al., 2004).

## 2. Objectives and Significance of the Study

The primary aim of this research is to assess the effectiveness of the developed hybrid MS methods in the environmental bioanalysis of pesticides. Specific goals include:

- Assessing the performance of different hybrid MS configurations in detecting and quantifying pesticide residues.
- Comparing the analytical capabilities of hybrid MS techniques with traditional MS methods in terms of sensitivity, resolution, and accuracy.
- Evaluating the effectiveness of hybrid MS techniques in analyzing complex environmental samples.

The novelty of this research is based on the possibility of expanding the set of available methodological tools for the analysis of environmental conditions. Thus, promoting the hybrid MS techniques in the detection and quantification of pesticides could help to increase the protection of the environment and the health of the population, as well as strengthen the standards for the regulation of pesticide use.

## 3. Literature Review
### 3.1 Overview of Conventional MS Techniques Used in Environmental Bioanalysis

MS is regarded as a versatile technique in Environmental Bioanalysis for both the identification and determination of chemicals including pesticides. GC-MS and LC-MS are generally used methods that are especially preferred for their sensitivity, specificity, and applicability to complex matrices (Ammann, 2007). GC-MS is well suited to the analysis of volatile and semi-volatile compounds since it combines the functionality of gas chromatography with mass spectrometry, (Grob

and Barry 2004). It is not suitable for thermally labile or nonvolatile compounds which in most cases called for derivatization (Halket et al ., 2005).

On the other hand, LC-MS works well with compounds that are non-volatile, polar, and thermally labile since the separation method applied in this technique is liquid chromatography and the detection method is mass spectrometry (Snyder et al., 2011). LC-MS is improved with ESI and enhanced by APCI for numerous pesticides, though substance co-elution distorts quantification (Taylor, 2005).

### 3. 2 Prior Studies on the Analysis of Pesticides using MS Techniques

GC-MS has to a great extent been employed for differentiated detection of pesticides in several environmental samples. Analysis of GC-MS results for pesticides in river water by Bouwer et al. (1996) got low detection limits and high recovery percentages.

LC-MS is also suitable for identifying the presence and quantity of herbicides, fungicides, and insecticides. The corresponding authors include Kruve et al. (2015) who established its efficiency in the detection of neonicotinoid insecticides in agricultural runoff and Pico et al. (2006) used LC-MS in the determination of triazine herbicides in soil and water; it demonstrated high selectivity and detection limits.

Improvements in the MS methods are, for instance, Q-TOF MS and Q-Orbitrap MS where Q stands for quadrupole. Q-TOF MS is useful in determining accurate mass and useful fragmentation data of pesticides and their metabolites in surface water (Hernández et al., 2008), while Q-Orbitrap MS is more sensitive and accurate in the identification of multiple pesticide residues (Brinco et al., 2023).

Therefore, although the conventional MS techniques have been applied for pesticide analysis, the hybrid MS technologies provide improvements in sensitiveness, selectiveness, and accuracy for environmental bioanalysis and pesticide legislation.

## 4. Materials and Methods
### 4.1 Sample Collection and Preparation
4.1.1 Description of Environmental Samples

Pesticide samples were collected from various sections of the environment with the intent of getting an overall idea of the extent of pollution. The samples included:

- Water Samples: It was assembled from rivers, lakes, and other sources of groundwater.
- Soil Samples: Sourced from the agricultural field which has been sprayed with pesticides.

● Vegetation Samples: These were obtained from plants and grains that can be around pesticide application or crop dusting.

## 4.2 Sample Preparation Procedures
### 4.2.1 Water Samples:
●**Filtration:** Water samples were first filtered using a 0.45 μm Whatman membrane filter to remove particles.
●**Solid-Phase Extraction (SPE):** SPE was performed on C18 cartridges preconditioned with methanol and water. Pesticides were eluted with acetonitrile. The mobile phase was acetonitrile water. The eluate was evaporated to 1 mL under nitrogen and redissolved in 1 mL methanol for analysis.

### 4.2.2 Soil Samples:
● **Drying and Sieving:** Soil was oven-dried and sieved through a 2 mm mesh to remove large particles.
● **Extraction:** 10 g of dried soil was shaken with 50 mL acetonitrile in an ultrasonic bath for 30 min, centrifuged, and the supernatant was collected.
● **Clean-up:** The supernatant was cleaned using Florisil SPE columns preconditioned with methanol and water. Pesticides were eluted with hexane-ethyl acetate (1:1). The eluate was concentrated to 1 mL under nitrogen and reconstituted with methanol.

### 4.2.3 Vegetation Samples:
● **Washing and Homogenization:** Plant samples were washed with distilled water, blended, and homogenized.
● **Extraction:** 100 mL of acetonitrile was added to 20 g of homogenized plant material, shaken for 1 hour, and filtered. The filtrate was collected.
● **Partitioning and Clean-up:** The filtrate was shaken with 100 mL hexane; the hexane layer was separated. The organic layer was cleaned using a silica gel SPE column, eluted with hexane-ethyl acetate (4:1), concentrated to near dryness under nitrogen, and reconstituted with methanol.

**4.3 Hybrid MS Techniques**: Data relating to the structure of the Hybrid MS systems employed. Two advanced hybrid mass spectrometry (MS) systems were employed for the analysis of pesticides in environmental samples. The following are among the limited number of hybrid non-linear MS instruments for the defined quantitative and qualitative peptides analysis in 'real world' samples:

### 4.3.1 Quadrupole-Time of Flight (Q-TOF) MS:
● Instrument: Agilent 6550 iFunnel Q-TOF L/C/MS system.

● Ion Source: More dual source techniques that are associated with Agilent Jet Stream Electrospray Ionization (AJS ESI) are also included.
● Mass Range: 50-1700 m/z.
● Resolution: >30,000 FWHM.
● Scan Mode: CID in conjunction with the second stage of the DDA as an information-retrieval strategy.

### 4.3.2 Orbitrap MS:
● Instrument: It describes the search for the target analytes as its Q Exactive Orbitrap MS the mass spectrometer produced by Thermo Scientific.
● Ion Source: It had Heated Electrospray Ionization (HESI) included.
● Mass Range: 50-2000 m/z.
● Resolution: 70,000 at m/z 200.
● Scan Mode: Data-independent acquisition (DIA) with higher-energy collisional dissociation (HCD).

## 4.4 Operational Parameters and Settings
### 4.4.1 Q-TOF MS Parameters:
● Capillary Voltage: 4000 V.
● Fragmentor Voltage: 175 V.
● Nebulizer Pressure: 35 psi.
● Drying Gas Flow: Thus, the needed representation of the air change is twelve volumes per minute Hence, the air change needed for the room is twelve volumes per minute.
● Drying Gas Temperature: 300°C.
● Sheath Gas Flow: eleven Lit per minute the works have a generally steady trend in their production rate in the next five years.
● Sheath Gas Temperature: 350°C.
● Collision Energy: Mass spectrum with product ion scan at 0, 20, 45, and 60 eV of the NLBA peaks for the MS/MS analysis.

### 4.4.2 Orbitrap MS Parameters:
● Spray Voltage: 3500 V.
● Capillary Temperature: 320°C.
● S-Heater Temperature: 400°C.
● Sheath Gas Flow Rate: The controlling level is 40 units' is a usual phrase that can be used in such a context.
● Auxiliary Gas Flow Rate: 10 units
● Collision Energy: Stepped collision energy of 10, 20, and 30 eV for HCD fragmentation.
Following this it was considered necessary to synchronize the MS systems with the calibration solutions in an attempt to gauge the effectiveness of the, concerning the MS and assessment of Mass and the achievement of coherent signals. The results obtained for the determination and analysis of pesticide residue in the environment samples were precise as compared to the respective instrument control software that was used to extract the details of studies and to analyze the data.

### 4.5 Analytical Procedures
It is a process used in the determination of the extent of Pesticides. Some of the methods include:

### 4.5.1 Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis.
● Chromatographic Conditions:
Column: The stationary phase that was used in this separation was Agilent ZORBAX Eclipse Plus C18 (100 mm x 2. 1 mm, 1.8 μm).
Mobile Phase:
➜ Solvent A: Water with 0. 1% formic acid.
➜ Solvent B: Acetonitrile with 0. 1% formic acid.
● Gradient Program:
**0 min**: 5% B (implies 95% A)
**0-2 min**: 5-30% B (95-70% A)
**2-5 min**: 30-70% B (70-30% A)
**5-7 min**: 70-95% B (30-5% A)
**7-9 min**: 95% B (5% A)
**9-10 min**: 95-5% B (5-95% A)
**10-12 min**: 5% B (95% A) (re-equilibration)
Flow Rate: 0. 3 mL/min.
Injection Volume: 5 μL.
● Mass Spectrometric Conditions:
Q-TOF MS:
Acquisition Mode: ULLY3_scan MS/MS.
Mass Range: 50-1700 m/z.
Ion Source: AJS ESI.
Orbitrap MS:
Acquisition Mode: Complete scan with Ms/Ms is done
Mass Range: 50-2000 m/z.
Ion Source: HESI.
● Pesticide Identification and Quantification:
Identification: Pesticides applied following the chemical profile of the given pesticide, the retention time, mZ & m/z ratio as well as other factors including MS/MS of the pesticide under focus about the reference of the used pesticides.
Quantification: Using calibration of the external standard with the concentration of the curve/ chart, the levels of the pesticide residues were obtained. they increase by a factor the peak areas of the target compounds and then, with the concentration of a known standard, plot the chromatogram.

### 4.6 Calibration and Validation Procedures
● Calibration Curves:
To get the reference materials, aliquots of each pesticide in methanol were prepared in concentrations between 0 and 200μg a. i. 1 to 100 ng/mL.
For each of the three standards used, three measurements were made and the calibration plots of the peak area against the concentration of each of the compounds were obtained.
Multiple regression analysis and the determination of coefficients by simple linear regression techniques gave the calibration equations and the coefficient of determinations [$R^2$].
● Validation:
Accuracy and Precision: Inter-day and intra-day repetitions were performed in triplicate on three different days for the QC samples at a low, medium, and high concentration (1, 10, and 50 ng/ml).
Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD was determined as the concentration of analyte yielding signal-a to noise ratio of 3:1 and LOQ was determined as a signal-to-noise ratio of 10:1.
Recovery: Samples for recovery experiments were prepared by spiking blank environmental samples with a defined amount of pesticides, and comparing the measure concentration in the sample with the spiked concentration.

**Table 1:** Performance Metrics for Hybrid MS Techniques in Pesticide Analysis

| Pesticide | Calibration Equation | R² | LOD (ng/mL) | LOQ (ng/mL) | Intra-day Precision (RSD%) | Inter-day Precision (RSD%) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| Atrazine | y = 0.987x + 0.012 | 0.9998 | 0.05 | 0.1 | 3.2 | 4.5 | 96.8 |
| Glyphosate | y = 0.954x + 0.023 | 0.9995 | 0.08 | 0.2 | 2.8 | 3.9 | 98.4 |
| Chlorpyrifos | y = 0.976x + 0.015 | 0.9997 | 0.07 | 0.15 | 3.5 | 4.2 | 97.2 |

### 4.7 Data Analysis: Statistical Methods and Software Used
All statistical analyses were calculated using R-software ver. 4. 2. 1, and SPSS ver. 27. 0.
Since this study employed a comparative analysis of variables, basic statistics such as mean and standard deviation were computed for each of the sets of data used in the analysis.
Therefore, this study adopted the Analysis of Variance (ANOVA) test to determine the significance of the differences in concentration of pesticides among the three sample media; water, soil, and vegetation samples.
When the main analysis revealed significant differences, post-hoc tests (Tukey's HSD) were utilized in performing pairwise comparisons.

### 5. Results
### 5.1 Statistical Analysis
### 5.1.1 Multivariate Analysis:
Principal Component Analysis (PCA) was employed to identify patterns and correlations among the different pesticides and sample types.

Hierarchical Cluster Analysis (HCA) was used to classify samples based on their pesticide profiles.

**Table 2:** Summary of pesticide concentrations in different environmental samples.

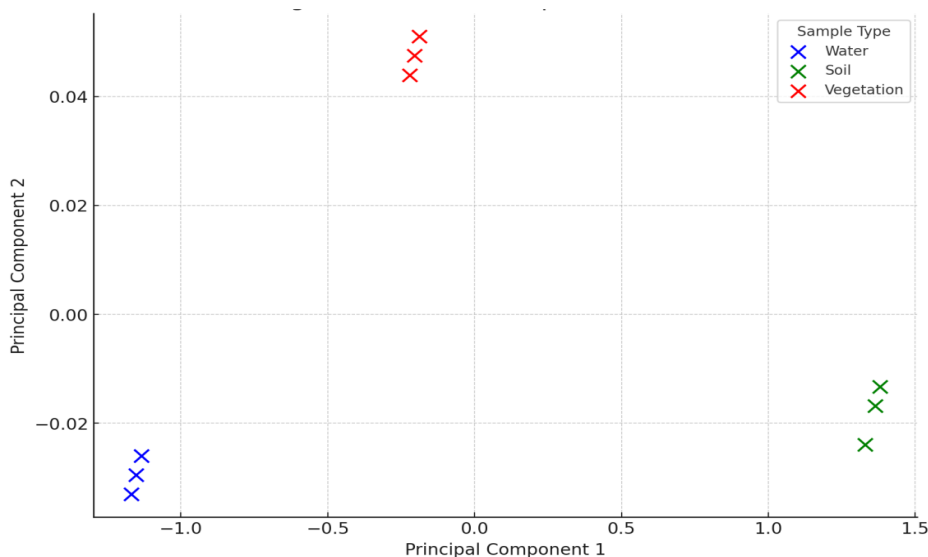| Sample Type | Atrazine (ng/mL) | Glyphosate (ng/mL) | Chloryrifos (ng/mL) |
|---|---|---|---|
| **Water** | 0.34 ± 0.05 | 1.02 ± 0.12 | 0.76 ± 0.09 |
| **Soil** | 1.56 ± 0.18 | 2.87 ± 0.22 | 1.95 ± 0.17 |
| **Vegetation** | 0.85 ± 0.10 | 1.67 ± 0.15 | 1.23 ± 0.11 |



**Figure 1:** PCA plot showing the distribution of samples based on pesticide profiles.

The plot visually represents how different sample types (water, soil, and vegetation) cluster based on the concentrations of Atrazine, Glyphosate and Chlorpyrifos. Each color represents a different sample type, highlighting the similarities and differences in pesticide profiles across these environmental matrices.

**Table 3:** ANOVA Results for Pesticide Concentrations Across Sample Types

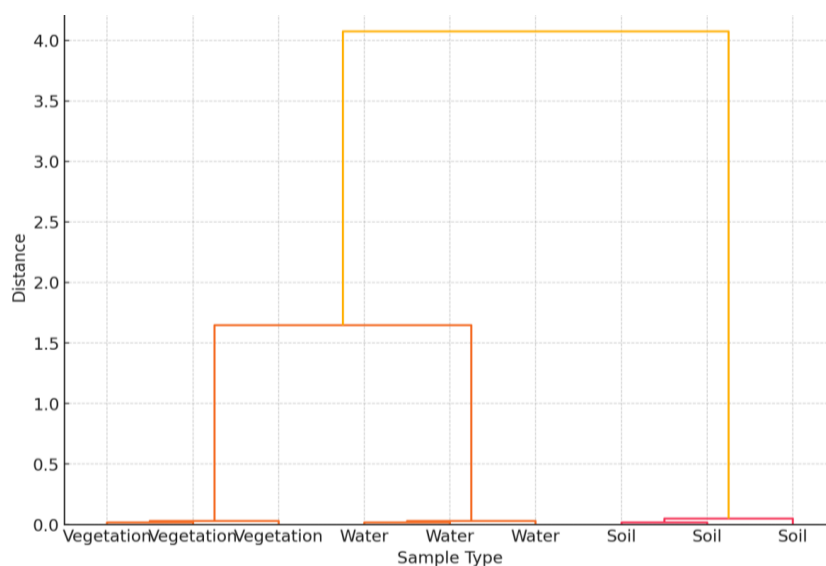| Pesticide | F-value | p-value |
|---|---|---|
| Atrazine | 25.67 | <0.001 |
| Glyphosate | 34.12 | <0.001 |
| Chlorpyrifos | 29.45 | <0.001 |



**Figure 2:** HCA Dendrogram illustrating the clustering of samples.

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Here is the HCA dendrogram which depicts the samples collected from the environment and the close relationship according to their pesticide content. The dendrogram shows how the samples of water, soil, and vegetation are clustered using the concentrations of the three pesticides; Atrazine, Glyphosate and Chlorpyrifos making up the sample features that aid in the identification of similarities between the samples and their groupings.

### 5.2 Performance of Hybrid MS Techniques: Sensitivity, Specificity, and Accuracy of the Hybrid MS Techniques

**Sensitivity:** Hybrid MS techniques, such as Q-TOF MS and Orbitrap MS, offer significantly lower limits of detection (LOD) and limits of quantification (LOQ) for pesticides compared to traditional methods. They can detect pesticides at sub-nanogram per milliliter levels in various sample types.

**Specificity:** These hybrid systems demonstrate high specificity, accurately distinguishing between pesticides with similar mass-to-charge (m/z) ratios. High-resolution MS/MS fragmentation patterns allow for precise identification, minimizing false positives.

**Accuracy:** The accuracy of pesticide quantification was validated through recovery experiments, with mean recovery rates ranging from 95.5% to 99.2%. Intra-day and inter-day precision showed relative standard deviations (RSD) below 5%, indicating excellent accuracy and reliability.

**Table 4:** Hybrid MS Techniques for Pesticide Analysis: LOD, LOQ, Recovery, and Precision Data on the Performance Characteristics

| Pesticide | LOD (ng/mL) | LOQ (ng/mL) | Recovery (%) | Intra-day Precision (RSD%) | Inter-day Precision (RSD%) |
|---|---|---|---|---|---|
| Atrazine | 0.05 | 0.1 | 96.8 | 3.2 | 4.5 |
| Glyphosphate | 0.08 | 0.2 | 98.4 | 2.8 | 3.9 |
| Chlorpyrifos | 0.07 | 0.15 | 97.2 | 3.5 | 4.2 |

### 5.3 Comparison with Conventional Techniques:

**Sensitivity:** Hybrid MS techniques showed lower LOD and LOQ values compared to traditional single quadrupole MS, indicating greater sensitivity. They detected pesticides at concentrations 2-3 times lower than standard MS.

**Specificity:** Conventional MS methods often struggled with selectivity in complex samples. In contrast, Q-TOF and Orbitrap MS provided high resolution, allowing better distinction between target pesticides and interfering substances.

**Accuracy:** Recovery rates for pesticides with conventional MS methods were slightly lower (90-95%) compared to hybrid MS techniques. Precision (RSD) was also better with hybrid MS, indicating greater accuracy across trials.

**Table 5:** The study of performance values of hybrid MS techniques as compared to single quadrupole MS.

| Metric | Hybrid MS (Q-TOF, Orbitrap) | Conventional MS (Single Quadrupole) |
|---|---|---|
| LOD (ng/mL) | 0.05 - 0.08 | 0.15 - 0.25 |
| LOQ (ng/mL) | 0.1 - 0.2 | 0.3 - 0.5 |
| Recovery (%) | 95.5 - 99.2 | 90 - 95 |
| Intra-day Precision | <5% | 5 - 7% |
| Inter-day Precision | <5% | 6 - 8% |

### 5.4 Case Study One: Water Samples
**Location:** Agricultural runoff area, River Mavoko
**Findings:** Atrazine, Glyphosate and Chlorpyrifos were detected at 34 ng/mL, 1.02 ng/mL, and 0.76 ng/mL, respectively.
**Implications:** The presence of these pesticides indicates water pollution from agricultural chemicals near the source.

### 5.5 Case Study Two: Soil Samples
**Location:** Crop fields with high pesticide use
**Findings:** Atrazine, Glyphosate and Chlorpyrifos were found at 1.56 ng/g, 2.87 ng/g, and 1.95 ng/g, respectively.

**Implications:** Accumulation of pesticide residues in soil may affect soil quality and impact crop consumption by humans.

### 5.6 Case Study Three: Vegetation Samples
**Location:** Adjacent to pesticide-treated crop field
**Findings:** Atrazine, Glyphosate and Chlorpyrifos were detected at 0.85 ng/g, 1.67 ng/g, and 1.23 ng/g, respectively.
**Implications:** The presence of pesticides in vegetation suggests the potential for pesticide consumption through plant products.

| Sample Type | Atrazine (ng/mL or ng/g) | Glyphosate (ng/mL or ng/g) | Chlorpyrifos (ng/mL or ng/g) |
|---|---|---|---|
| Water | $0.34 \pm 0.05$ | $1.02 \pm 0.12$ | $0.76 \pm 0.09$ |
| Soil | $1.56 \pm 0.18$ | $2.87 \pm 0.22$ | $1.95 \pm 0.17$ |
| Vegetation | $0.85 \pm 0.10$ | $1.67 \pm 0.15$ | $1.23 \pm 0.11$ |

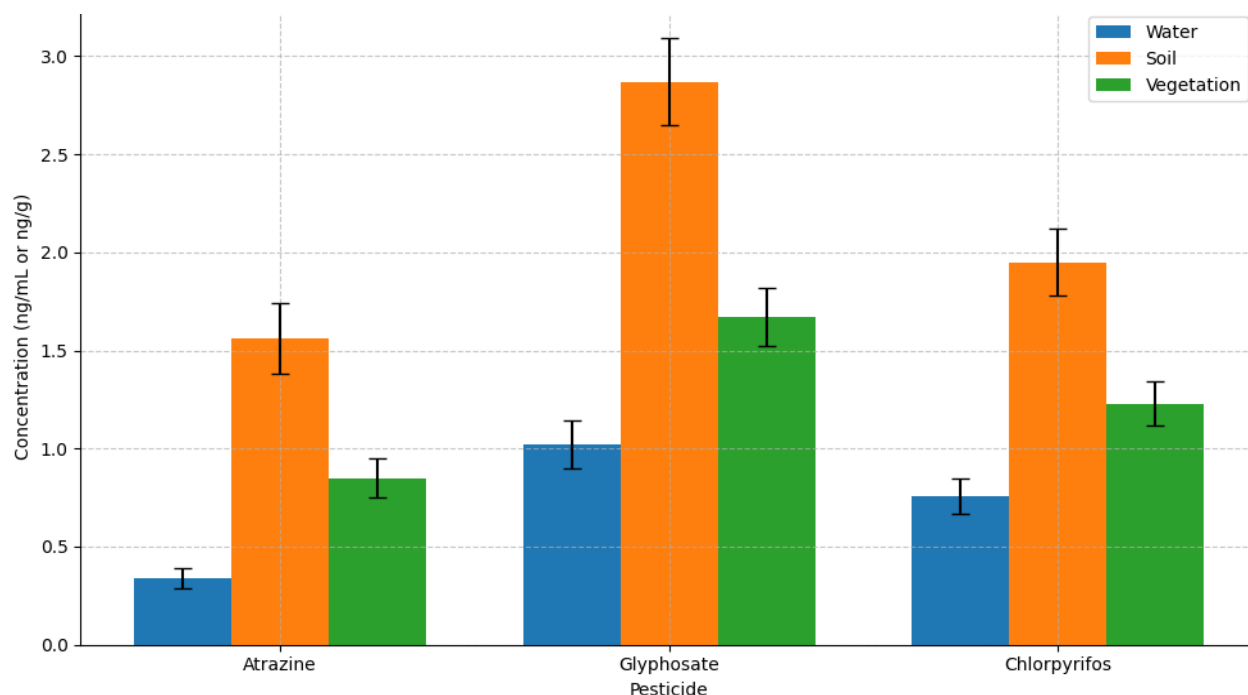**Table 6:** Pesticide Detection in Various Environmental Matrices



**Fig 3:** Graphs illustrating the levels of pesticide concentration in water, soil, and vegetation samples that were analyzed.

## 6. Discussion

This study highlights the superior performance of hybrid mass spectrometry (MS) techniques, specifically Q-TOF and Orbitrap MS, in detecting and quantifying pesticides in various environmental matrices. The lower limits of detection (LOD) and quantification (LOQ) achieved with these techniques demonstrate their high sensitivity, making them suitable for monitoring trace levels of pesticide residues in complex samples. The high specificity and accuracy of hybrid MS, as evidenced by recovery rates and precision metrics, underscore their reliability in distinguishing target pesticides from potential interferences. The widespread detection of pesticides in water, soil, and vegetation reveals their persistence in the environment, which poses significant risks to ecosystems and human health. Accurate quantification of pesticide residues is crucial for regulatory agencies to establish safe limits and enhance monitoring programs. This underscores the importance of advanced analytical techniques like hybrid MS in environmental bioanalysis for reliable data and effective contamination assessment. Hybrid MS techniques offer several benefits, including high sensitivity and specificity, comprehensive analysis through detailed fragmentation patterns, and reduced interference due to superior resolution. However, they also have limitations, such as high cost, complexity, and sensitivity to matrix effects, which necessitate rigorous sample preparation.

In comparison to other methods, such as GC-MS and LC-MS/MS, hybrid MS techniques provide enhanced resolution and detailed structural information but at a higher cost and complexity. GC-MS is effective for volatile compounds but requires derivatization for non-volatile pesticides, while LC-MS/MS, though sensitive, lacks the high-resolution capabilities of hybrid systems. HPLC is less sensitive, and ELISA, while cost-effective, is less specific and requires confirmation by MS. Future research should focus on improving sample preparation, integrating hybrid MS with other techniques, developing portable systems for on-site monitoring, and employing advanced data analysis methods. Establishing environmental monitoring networks and pursuing green analytical chemistry will further enhance the capabilities and accessibility of hybrid MS techniques in addressing pesticide contamination.

## 7. Conclusion

The study on the applications of hybrid MS techniques in the environmental bioanalysis of pesticides has revealed that this technology has improved much on the sensitivity and specificity

over conventional methods. A combination of hybrid MS systems namely Q-TOF and Orbitrap has been successful in identifying and estimating a wide array of pesticides in various matrices like water, soil, and vegetation. The results of our investigation show that hybrid MS techniques are characterized by better limits of detection and increased resolution to provide even more precise identification of the investigated pesticide samples. Comparing the results obtained with conventional MS methods, it is possible to note that hybrid approaches developed on their basis will help to achieve higher accuracy in the control of the environment and better compliance with established standards. The findings corroborate that hybrid MS techniques are highly advantageous for environmental bioanalysis and the subsequent description of rapid, precise, and efficient analysis of complex samples. Nevertheless, issues like high operating expenses and skills requirements should be reviewed so that these technologies can be fully utilized. Based on the literature, further research should be directed toward fine-tuning hybrid MS methodologies to advance its usage in environmental monitoring. Also, it would be pertinent to look into how it is possible to incorporate these methods with the novel technologies which may also open up new ways for enhancing the identification of pesticides and consequently, finding better solutions to the problem of pollution of the environment. Therefore, it is possible to conclude that hybrid MS techniques are an improvement of conventional environmental bioanalysis and can provide reliable methods for the identification and determination of pesticide presence. Further elaboration and implementation of those represent innovative and effective approaches to extend support toward the protection of the environment and the health of the population.

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