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Assessment of the water quality index and pesticide genotoxicity via the Single-Cell Gel Electrophoresis (SCGE) assay utilizing aquatic plants

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Abstract

Pesticides pose significant threats to the integrity and functionality of aquatic ecosystems worldwide, necessitating thorough assessment and mitigation by all stakeholders. Chemical analysis alone cannot predict the synergistic effects of various contaminants found in aquatic ecosystems. This study assesses water quality indicators, including the Water Quality Index (WQI) for physicochemical and biological parameters, alongside the human health risks associated with pesticide exposure. The investigation focuses primarily on pesticide toxicity, persistence, and the impacts on water resources. Gas Chromatography-Mass Spectrometry (GC-MS/MS) is employed to quantify pesticides in aquatic ecosystems. Chemical analyses revealed detectable pesticide concentrations in water supplies, albeit at relatively low levels. Additionally, the single-cell gel electrophoresis (SCGE) assay was utilized to evaluate DNA damage in aquatic plants. Genotoxic effects were assessed using four species as bioindicators: *Ceratophyllum demersum* L., *Eichhornia crassipes* (Mart.) Solms, *Ipomoea aquatica* Forssk., and *Salvinia cucullata* Roxb. ex Bory, to evaluate aquatic ecosystem health. The results demonstrated that DNA fragmentation increased in proportion to pesticide exposure levels, with maximum damage reaching 17.49% and statistically significant differences from control specimens ($p < 0.01$). Complex species-specific responses were revealed during analysis, with trends suggesting potential phytoremediation mechanisms rather than simple bioaccumulation patterns. The SCGE assay proved effective for assessing DNA migration rates under conditions of low-level pesticide contamination, establishing aquatic plants as valuable biomonitoring tools for environmental risk assessment.

Keywords: Aquatic plants, Genotoxicity, Pesticides, Single-cell gel electrophoresis assay, Water Quality Index, Biomonitoring

1. Introduction

The environmental hazards posed by pesticide-induced biochemical contamination of rivers, soil, and sediment are becoming more severe. The widespread use of pesticides, characterized by their long half-lives and resistance, has led to various environmental issues. Agricultural pesticides are effective in controlling crop pests and diseases, but their initial introduction into rivers is detrimental to ecological systems and the environment [1]. If pesticide residues are absorbed by soil and sediment, they can be transported into the river through discharge [2]. The spatial dispersion of pesticides can exacerbate issues by affecting river quality. A greater degree of persistence increases the likelihood of contamination to rivers, soil, sediment, vegetation, animals, and humans [3].

From 1990 to 2022, pesticide usage increased significantly, with a 94% rise per farmland area, 5% per agricultural output value, and 35% per capita. In 2022, 3.70 million tons of active substances were used, doubling since 1990 and marking a 4% increase from 2021. The total pesticide exports reached approximately 6.9 million tons, a slight decrease from 2021, but their value rose by 13% to USD 48.8 billion, with Asia leading in exports [4]. In Thailand, by the end of 2024, of the 40.3 million people employed, around 12.1 million were in the agriculture sector, with the country ranking 18th globally in pesticide usage, despite lower agricultural yields compared to other nations [5, 6]. Pesticide-related health issues are a major concern, with the World Health Organization (WHO) estimating around one million unintentional poisonings and over 11,000 deaths annually [7]. In Thailand, from 2001 to 2020, there were nearly 47,000 reported pesticide poisoning cases, averaging 2,344 annually, with the northern region experiencing the highest rates [8].

Pesticides are a significant environmental pollutant, affecting soils, sediments, and surface waters on a scale of parts per trillion to parts per million, the toxicity and effects of which are influenced by their transport and degradation mechanisms, including volatilization, aerosol dispersion, rinsing, discharge, leaching, and lateral drainage [2, 9]. Sediment temperature, depth, location, and season significantly influence pesticide half-lives. Abiotic processes also promote pesticide breakdown, with certain herbicides exhibiting increased biodegradation following several applications. Soil and sediment pollutants adversely affect aquatic habitats, with leaching determined by pesticide composition, physicochemical properties, irrigation methods, hydrogeological processes, and precipitation patterns [10, 11].

Commercial formulations have unique leakage properties, while sediments and suspended particles affect pesticide dissemination. Pesticides can lead to contamination of the surface and groundwater via diffuse or non-point-source pollution, with concentration levels

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influenced by light, temperature, pH, sulfur dioxide, and suspended particulates. Pesticide transformation products can surpass their parent substances, presenting significant hazards to human and environmental health. Toxicity is influenced by their properties, molecular structure, dosage, and time of exposure. Pesticides, some biocidal, have been linked to chronic health issues like cancer and genetic disorders [12]. However, these concerns are unsubstantiated due to challenges in epidemiological studies. To assess carcinogenicity and mutagenicity, regulatory bodies use chronic animal bioassays, short-term assays, and human biomonitoring. Agencies like the US Environmental Protection Agency, the International Agency for Research on Cancer, and the National Toxicology Program use the weight-of-evidence approach. Short-term assays assess genetic toxicity, while genetic toxicology examines the harmful effects on DNA and other living organisms [13, 14].

Biomarkers have become increasingly important in assessing environmental quality due to their ability to detect pesticide contaminants [15, 16]. Plant bioassays, which use single-cell gel electrophoresis (SCGE) technology, have become essential for ecotoxicology risk assessments. These bioassays measure DNA migration levels from various environmental contaminants, using plant components like leaves, stems, and roots [17]. The foliage and roots absorb naturally occurring toxins from the environment, which can be transported to the foliage and released into the environment in small quantities. The foliage also assimilates hazardous compounds, converting them into less toxic substances [18]. Plant leaves have been used as biomonitors of environmental pollution due to respiration [19, 20]. The SCGE is used to detect DNA fragmentation at the genetic level, evaluating the genetic health of aquatic organisms in both vertebrate and invertebrate groups [21-23]. However, there is a lack of research on in situ bioassays using aquatic plants as models for genotoxicity investigation. Therefore, it is crucial to conduct research on aquatic vegetation to develop a new biomarker for identifying water contamination caused by hazardous substances.

This research endorses and promotes the Sustainable Development Goal SDG 6 (clean water and sanitation) of the United Nations. The current study evaluates the quality of water resources and the possible health risks associated with water contaminants in a few Thai cities in watershed areas impacted by pesticide contamination. The indices for assessing water quality parameters in this study comprise pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total coliform bacteria (TCB), fecal coliform bacteria (FCB), ammonia nitrogen (NH_3-N), and residual pesticides. This study seeks to ascertain the degree to which pollution induces DNA mobility by conducting an inquiry to assess the genotoxicity of pesticides on aquatic flora. The indigenous aquatic plants selected for the genotoxicity investigation in the specified region are *Ceratophyllum demersum* L. (Hornwort), *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth), *Ipomoea aquatica* Forssk. (Swamp Morning Glory), and *Salvinia cucullata* Roxb. ex Bory (Floating Moss), achieved by assessing the extent of DNA damage occurring.

2. Materials and methods

2.1 Sampling locations

Environmental samples were collected from the Wang River in Lampang Province, Thailand, for comparative analysis. The comprehensive Water Quality Index (WQI) was used to monitor the investigated areas, thereby minimizing the confounding effects of water quality variation on the molecular composition of aquatic plants. The study area encompassed three primary locations: Mueang Lampang (W1, W2), Ko Kha (W3, S3, C1, C2, C3, C4), and Sop Prap (W4, S1, S2). Water samples (W) were collected from Mueang Lampang (W1, W2), Ko Kha (W3), and Sop Prap (W4) to delineate the screening target zone as illustrated in Figure 1. The selected study area was characterized by superior surface water quality and representative, diverse aquatic vegetation communities. Aquatic plant samples (C) were gathered exclusively at Ko Kha, with sediment samples (S) obtained from both Ko Kha and Sop Prap locations.

To conduct an ecological risk assessment of pesticide dispersion in surface water, aquatic plant samples were selected as bioindicators. The specimen collection included four indigenous species: *Ceratophyllum demersum* L. (Hornwort: C1), *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth: C2), *Ipomoea aquatica* Forssk. (Swamp Morning Glory: C3), and *Salvinia cucullata* Roxb. ex Bory (Floating Moss: C4). The study's overarching conceptual framework is presented in Figure 2.

All collected samples were immediately wrapped in sterile zip bags and preserved in an icebox during transportation. Subsequently, samples were stored at 4 °C prior to conducting laboratory analysis to maintain sample integrity and prevent the degradation of target compounds.

2.2 Water quality index application

According to the WHO [24], over 115 million people worldwide rely on contaminated surface water sources, posing significant health risks. This issue is particularly prevalent in developing countries like Thailand, where rural populations rely on surface or groundwater for their daily needs [25]. Assessing river water quality is crucial for human health and ecosystem conservation. Numerous methodologies and models have been developed to evaluate the diverse water quality characteristics of rivers affected by pollutants [26]. However, integrating these methods and assessing diverse samples with multiple parameters presents challenges. The WQI was created to streamline this process and is widely recognized as the most precise instrument for assessing water quality, including surface water quality and categorization based on various water applications [27].

The parameter determination principle is based on water quality standards for surface water sources, enabling evaluation of their nature. The WQI can fluctuate rapidly due to water contamination, making it vulnerable or potentially problematic. Various water quality indicators have been developed for various applications, including potable use and agriculture [28, 29]. For this study, the WQI calculation employs the standard methodology of the Pollution Control Department (PCD). The WQI for rivers in Thailand is based on five core parameters: dissolved oxygen (DO), biochemical oxygen demand (BOD), total coliform bacteria (TCB), fecal coliform bacteria (FCB), and ammonia nitrogen (NH_3-N). The WQI score ranges are compared with the standard values established for various water source classifications. The PCD sets the standards for water quality assessment using the following equation [30, 31]:

$$WQI = \frac{DO\ \left(\frac{mg}{L}\right) + BOD\ \left(\frac{mg}{L}\right) + FCB\left(\frac{mpn}{100mL}\right) + TCB\ \left(\frac{mpn}{100mL}\right) + NH_3\text{-}N\ \left(\frac{mg}{L}\right)}{5} - PS \quad (1)$$

The penalty score (PS) adjusts the average of the five parameter scores to align with surface water classification standards: PS = 0 (same level), PS = 10 (differing by one level), PS = 15 (differing by two levels), PS = 20 (differing by three levels). These empirically

derived values are subtracted from the parameter average to calculate the final WQI for each monitoring point.

To interpret the WQI results, water quality status is categorized into five distinct classes: Excellent (91–100), Good (71–90), Fair (61-70), Poor (31–60), and Very Poor (0–30). This classification system enables the standardized assessment and comparison of water quality conditions across different monitoring locations and time periods.

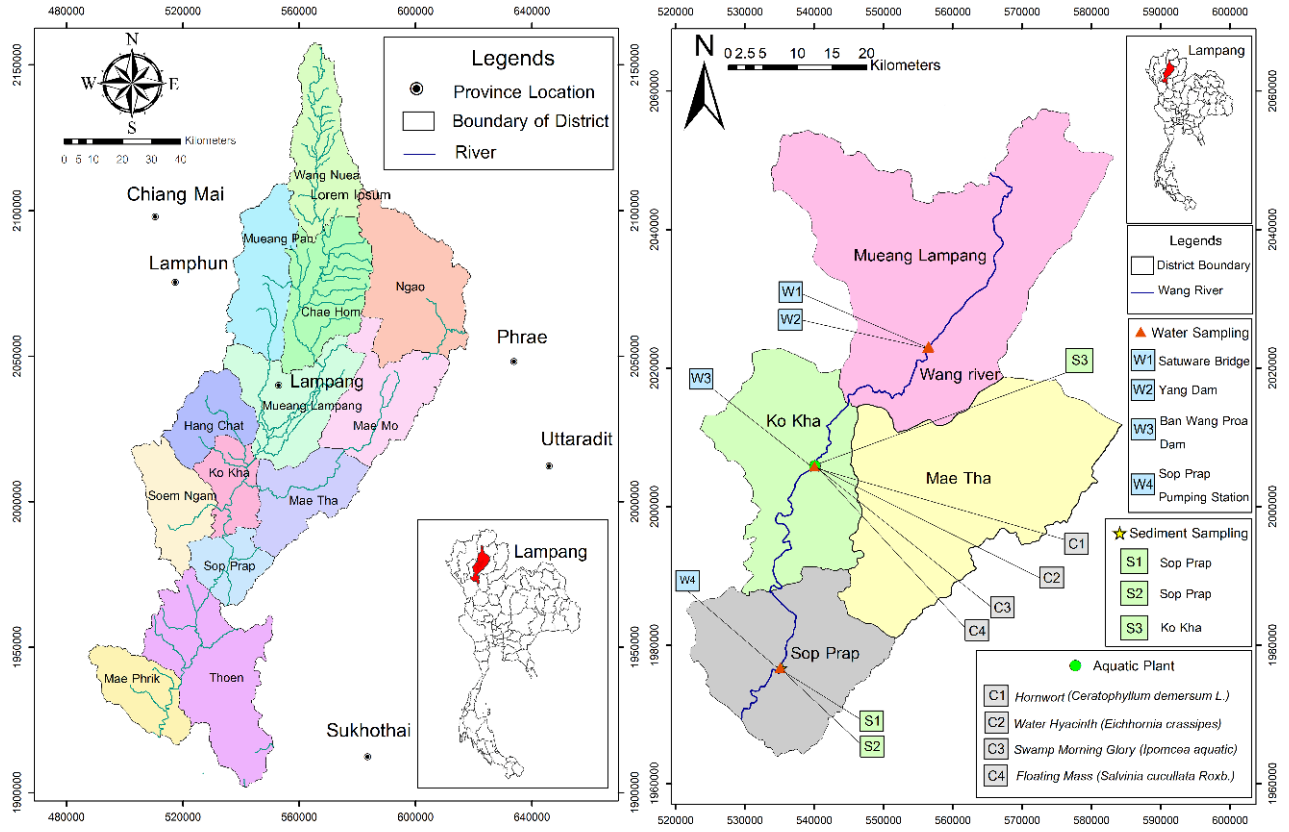

Figure 1 Location of the study area in the Wang River

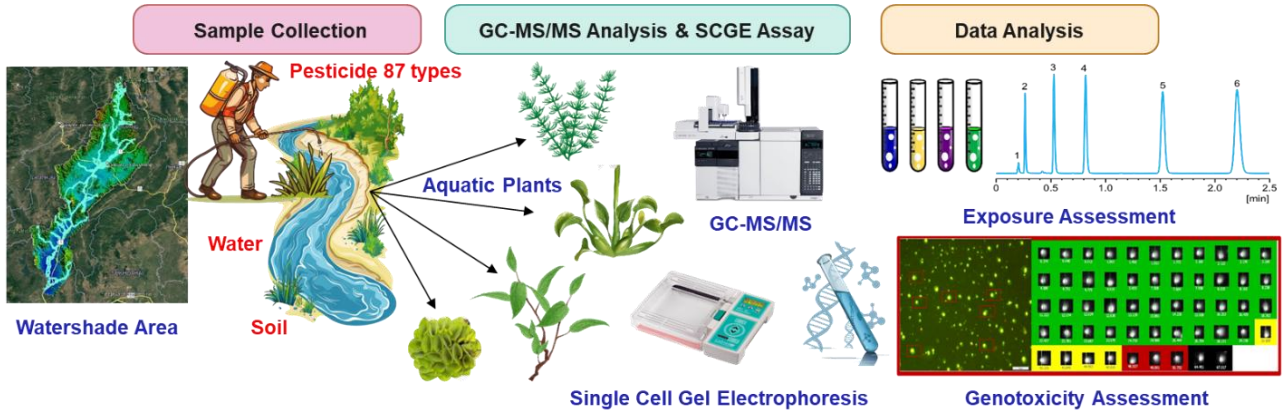

Figure 2 Conceptual framework of this study

2.3 Analysis of pesticides

Plant specimens were prepared using a systematic sectioning technique, with samples cut into small pieces to maximize the surface area for extraction. Subsequently, 5 grams of each sample were transferred into clean Erlenmeyer flasks for further analysis. Five milliliters of high-performance liquid chromatography (HPLC)-grade methanol from Sigma-Aldrich was added to both process control and spike solutions, followed by extraction for 30 minutes [32, 33]. This procedure was designed to effectively leach the pesticide residues present in the plant samples.

Prior to pesticide analysis, all extract samples were filtered through 0.2 µm syringe filters and transferred into clean analytical bottles to remove particulate matter and ensure sample purity. Detection and quantification were performed using a Thermo-Scientific gas chromatography triple quadrupole mass spectrometer (TSQ 8000 Evo Triple Quadrupole GC-MS/MS, Thermo-Scientific, USA) operating in positive ion electron impact (EI) mode under selective reaction monitoring (SRM) conditions. The instrumental parameters were optimized as follows: ion source temperature was maintained at 250 °C, with the transfer line temperature set to 290 °C. Chromatographic separation was achieved using a TR-Pesticide II capillary column (Thermo-Scientific), specifically designed for pesticide analysis, with dimensions of 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness.

A temperature program was employed to optimize the separation of target pesticides in the samples. The program was initiated at 80 °C with a 0.5-minute hold time, followed by a temperature ramp to 200 °C at 15 °C min^{-1}. The temperature was then increased to 280 °C at 5 °C min^{-1} with a two-minute hold period and finally ramped to 300 °C at 5 °C min^{-1}. The injection was performed in splitless mode with a one-minute surge injection. The injection volume was 1 µL, and the injector temperature was maintained at 290 °C. Helium was used as the carrier gas with a constant flow rate of 1.0 mL min^{-1} and an emission current of 50 amperes (A). For GC-MS/MS analysis, argon was employed as the collision gas at a pressure of 1.5 mTorr. The analytical method demonstrated exceptional performance characteristics with calibration curves for 87 pesticides showing high sensitivity (0.1 µg L^{-1}), excellent reproducibility (relative standard deviation <10% at 5 µg L^{-1}), and strong linearity ($R^2 > 0.99$) within the concentration range of 0.1–100 µg L^{-1}.

Comprehensive pesticide screening encompassed four major categories: organophosphate, organochlorine, persistent organic pollutants (POPs), and pyrethroid compounds. A total of 87 different pesticides were analyzed as part of this multi-residue method [34], as detailed in Table 1. This extensive coverage ensures the comprehensive assessment of potential pesticide contamination across multiple chemical classes commonly used in agricultural applications.

Table 1 Categories and types of pesticides

Pesticide Category	Substance Type
Organophosphate	Azinphos ethyl, Azinphos methyl, Bromophos ethyl, Bromophos methyl, Bromopropylate, Carbophenothion, Chlorfenvinphos, Chlorpyrifos, Chlorpyriphos methyl, Coumaphos, Diazinon, Dichlofenthion, Dichlorvos, Dicrotophos, Dimefox, Disulfoton, Ethion, Etrimfos, Fenchlorphos, Fenitrothion, Fonofos, Formothion, Iodofenphos, Malaoxon, Malathion, Methacrifos, Methidathion, Mevinphos, Monocrotophos, Paraoxon ethyl, Parathion ethyl, Parathion methyl, Phosalone, Phosphamidon, Pirimiphos ethyl, Pirimiphos methyl, Profenophos, Propetamphos, Pyrazophos, Sulfotep, Tetrachlorvinphos, Triazophos
Organochlorine	Aldrin, cis-Chlordane, trans-Chlordane, Chlorothalonil, o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDT, Dicofol, o,p'-Dicofol, Dieldrin, alpha-Endosulfan, beta-Endosulfan, Endosulfan sulfate, Endrin, HCB, alpha-HCH, beta-HCH, delta-HCH, Heptachlor, cis-Heptachlor epoxide, Lindane, Oxychlordane
Pyrethroid	Bifenthrin, Cyfluthrin, Lambda-cyhalothrin, Cypermethrin, Deltamethrin, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Fluvalinate, Permethrin, D-trans-phenothrin, Tetramethrin
POPs	Aldrin, cis-Chlordane, o,p'-DDT, p,p'-DDT, Dieldrin, Endrin, Heptachlor, cis-Heptachlor epoxide, Oxychlordane, PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180

2.4 Single-Cell Gel Electrophoresis (SCGE) assay

The SCGE assay for assessing DNA migration ratios was conducted in accordance with the Standard Technique for Examination of Water and Wastewater procedure 8071B [35]. The study involved mixing 50–75 µL of suspended aquatic plant cells with 100 µL of low-melting-point agarose at 35–40 °C. The cell-agarose suspension was immediately pipetted onto frosted slides pre-coated and covered with coverslips. Two replicate slides were prepared for each sample and maintained at 4 °C for five minutes to allow solidification.

Following coverslip removal, the slides were immersed in a cold lysis solution and subjected to alkaline electrophoresis for 20 minutes in a horizontal electrophoresis chamber. To prevent photodegradation-induced DNA damage, electrophoresis was conducted in darkness at 25 V and 300 mA for 20 minutes. Slides were subsequently rinsed three times with neutralizing buffer, fixed for five minutes using cold 100% methanol, and air-dried at ambient temperature.

DNA visualization was achieved by applying 10 µL of Cyber Safe Green fluorescent dye to each slide. Slides were coded for blinded analysis and examined using a fluorescence microscope equipped with appropriate excitation and barrier filters. DNA damage was objectively evaluated using Tritek COMET Score software (version 2.0) for automated image analysis. For each sample, 100 randomly selected cells were analyzed to ensure statistical robustness.

DNA damage was quantified by calculating the DNA migration ratio (tail area divided by total comet area), as this method provides a clear representation of comet morphology and demonstrates linear correlation with the DNA break frequency across damage levels. The percentage of DNA damage was computed using the following equation [18, 19].

$$\% \text{ of DNA damage} = \frac{\sum_{i=1}^{n} (T/(T+H))}{n} \times 100 \quad (2)$$

where T is the tail area of the comet image, H is the head area of the comet image, and n is the number of comet images.

For negative control specimens in the SCGE assay, *Ceratophyllum demersum* L. (Hornwort) plants were cultured in the laboratory under controlled conditions. Control plants were maintained in glass containers of dechlorinated tap water under ambient temperature with 12-hour light/dark cycles. Prior to use as controls, the cultured plants underwent preliminary pesticide screening using the same GC-MS/MS analytical protocol to confirm the absence of detectable pesticide residues. This laboratory cultivation approach ensured that control specimens had no prior exposure to environmental contaminants, providing a clean baseline for comparison with field-collected samples.

2.5 Statistical analysis

Data analysis was conducted using SPSS software (version 18.0 for Windows, license number 5082357; SPSS Inc., Chicago, IL, USA) and Microsoft Excel (version 365; Microsoft Corporation, Redmond, WA, USA). The Shapiro-Wilk test confirmed the non-normal distribution of DNA damage data ($p < 0.05$), necessitating non-parametric statistical methods for all comparisons. The Mann-Whitney U test was used for pairwise comparison between control and experimental groups to evaluate whether two independent

groups differed significantly. The U-statistics, Z-score, and two-tailed p-values were reported. The Kruskal-Wallis test assessed the overall heterogeneity among five groups (one control and four aquatic plant species), reporting the H-statistic, degrees of freedom, and p-value.

3. Results and discussion

3.1 Assessment of wang river water quality

Water quality data on the Wang River, sourced from the Environment and Pollution Control Office 2, encompassed five parameters (DO, BOD, TCB, FCB, and NH3) as presented in Table 2. This secondary data was utilized for the calculation of the WQI [30, 31]. Among the monitoring stations, Sop Prap Pumping Station (W4) demonstrated the highest water quality with its physicochemical parameters classified as Class 2 (good quality). However, bacterial contamination parameters at two stations exceeded the acceptable thresholds. At Satuware Bridge (W1) and Ban Wang Proa Dam (W3), intermediate contamination levels were observed for TCB (9,200.00 ± 9,289.78 and 1,700.00 ± 10,619.72 MPN 100 mL^{-1}, respectively) and FCB (2,200.00 ± 8,609.27 and 390.00 ± 3,733.73 MPN 100 mL^{-1}, respectively), resulting in Class 3 (fair quality) classification, as shown in Table 2. The most concerning water quality conditions were recorded at Yang Dam (W2), where bacterial parameters substantially exceeded optimal standards. Specifically, TCB concentrations reached 54,000.00 ± 41,068.83 MPN 100 mL^{-1} while FCB levels were 17,000.00 ± 26,147.98 MPN 100 mL^{-1}, both well above the recommended limits for safe water use.

The comprehensive WQI assessment conducted in 2024 revealed significant spatial variations in water quality across the Wang River system. The WQI scores ranged from 57 (poor water quality) at both Yang Dam (W2) and Satuware Bridge (W1) to 72 (good water quality) at Sop Prap Pumping Station (W4), with Ban Wang Proa Dam (W3) achieving an intermediate score of 70 (fair water quality). Based on these results, the Ban Wang Proa Dam (W3) was selected as the optimal sampling location for aquatic plant collection, as it provided the best balance between moderate water quality conditions and diverse aquatic vegetation communities.

Agricultural activities along the Wang River corridor, particularly intensive pesticide application, contribute significantly to water quality degradation through surface runoff and subsurface transport of agricultural chemicals. This contamination pathway introduces suspended organic particles containing pesticide residues, creating potential ecological risks throughout the aquatic ecosystem. Such agricultural pollution represents a widespread environmental challenge in developing regions like Thailand, where intensive farming practices often occur near major water bodies.

To address these contamination concerns, aquatic plants were employed in this study as sensitive bioindicators for pesticide bioaccumulation assessment within the aquatic system. The WQI-based site selection methodology ensured that variations in fundamental water quality parameters would not confound the molecular-level responses of target plant species. Consequently, all aquatic plant specimens were collected at the Ban Wang Proa Dam monitoring station (W3): *Ceratophyllum demersum* L. (Hornwort), *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth), *Ipomoea aquatica* Forssk. (Swamp Morning Glory), and *Salvinia cucullata* Roxb. ex Bory (Floating Moss).

Table 2 Water quality parameters of the Wang River 2019–2024

Collection Site	Co-ordinates	Water Quality Parameters (Minimum: Maximum: Mean ± SD)					
		pH	DO ($mg\ L^{-1}$)	BOD ($mg\ L^{-1}$)	TCB (MPN 100 mL^{-1})	FCB (MPN 100 mL^{-1})	NH_3 ($mg\ L^{-1}$)
W1 (WQI:57)	X: 554357 Y: 2023117	6.70 – 8.20 7.60 ± 0.33	4.80 – 11.00 7.20 ± 1.53	0.50 – 5.60 1.20 ± 1.13	790.00 – 160,000.00 9,200.00 ± 9,289.78	110.00 – 35,000.00 2,200.00 ± 8,609.27	0.01 – 0.61 0.17 ± 0.15
W2 (WQI:57)	X: 551749 Y: 2023004	6.50 – 8.30 7.60 ± 0.37	3.70 – 8.60 6.10 ± 1.50	0.80 – 6.10 1.40 ± 1.14	9,200.00 – 160,000.00 54,000.00 ± 41,068.83	230.00 – 92,000.00 17,000.00 ± 26,147.98	0.13 – 0.85 0.41 ± 0.18
W3 (WQI:70)	X: 543773 Y: 2005869	7.10 – 8.40 7.60 ± 0.31	1.80 – 11.00 6.70 ± 2.63	0.70 – 3.70 1.10 ± 0.95	45.00 – 35,000.00 1,700.00 ± 10,619.72	18.00 – 16,000.00 390.00 ± 3,733.73	0.01 – 0.46 0.18 ± 0.13
W4 (WQI:72)	X: 535160 Y: 1976689	6.00 – 9.00 7.80 ± 0.73	6.00 – 11.00 7.80 ± 1.22	0.60 – 4.90 1.00 ± 1.05	18.00 – 22,000.00 1,700.00 ± 5,897.42	18.00 – 5,400.00 330.00 ± 1,615.49	0.01 – 0.39 0.18 ± 0.13

Source: Environment and Pollution Control Office 2 [30]

3.2 Distribution of pesticides in environmental specimens

Bioindicators, comprising aquatic plants, plankton, and bacterial communities, serve as essential tools for assessing environmental health within ecosystem frameworks. Aquatic plants function as particularly sensitive indicators of environmental changes and provide reliable biomarkers for evaluating water quality and detecting aquatic pollution. The effectiveness of these bioindicator species depends on their responsiveness to contaminant exposure [19].

Pesticide distribution assessment in the study region was conducted through the systematic collection of environmental samples, including water and sediment matrices. Indigenous aquatic plant species were selected from Wang River habitats based on their established presence and ecological relevance. The comprehensive screening of 87 pesticides across four major categories, organophosphates, organochlorines, pyrethroids, and POPs, was performed using GC-MS/MS analysis.

Organophosphate pesticides enter aquatic systems through multiple pathways, including improper application practices, agricultural runoff, precipitation-mediated transport, accidental spills, industrial discharges, and irrigation return flows.

These compounds pose significant threats to aquatic ecosystems and environmental integrity. Water sample analysis revealed detectable organophosphate concentrations, specifically azinphos ethyl (29.62 $\mu g\ L^{-1}$) and chlorfenvinphos (7.22 $\mu g\ L^{-1}$) at Yang Dam (W2) (Table 3). Historical monitoring studies have documented the presence of various organophosphates in aquatic environments, including dimethoate, chlorpyrifos, endosulfan, methyl parathion, diazinon, and malathion. These compounds are particularly hazardous to aquatic organisms, causing reduced biodiversity, disrupted photosynthesis, impaired cellular development, compromised respiratory function, and toxicity to specialized aquatic microorganisms [10].

Sediment analysis revealed considerable pesticide contamination across sampling locations. At the Sop Prap location S1, parathion methyl (15.23 μg kg⁻¹) and methidathion (16.70 μg kg⁻¹) were detected. Sample S2 from the same area showed greater contamination diversity, with bromophos methyl (12.99 μg kg⁻¹), azinphos ethyl (16.81 μg kg⁻¹), chlorfenvinphos (14.36 μg kg⁻¹), triazophos (13.70 μg kg⁻¹), and D-trans-phenothrin (19.95 μg kg⁻¹). At the Ban Wang Proa sediment site (S3), coumaphos was detected at 30.99 μg kg⁻¹. The extensive use of organophosphate insecticides for agricultural pest control has historically contributed to enhanced crop productivity and quality improvements. However, the capacity of soils and sediments to effectively sorb organic pollutants creates persistent contamination reservoirs that pose ongoing health risks to both human and animal populations [12, 13].

Organophosphate contamination is particularly detrimental to soil organisms, threatening biodiversity and contributing to soil acidification. Contaminant introduction into soil and sediment environments occurs through direct application, surface water runoff from treated areas, and atmospheric deposition.

Aquatic plant tissue analysis identified three compounds: coumaphos, malathion, and bromophos methyl. The concentrations of these compounds are shown in Table 3. Beyond agricultural pesticide contamination in surface water bodies, non-agricultural pyrethroid compounds were also detected, indicating the influence of diffuse pollution sources. Bioassays utilizing aquatic plants have become integral components of comprehensive water quality assessment protocols.

Pesticide residue analysis revealed differential accumulation patterns among plant species. Residues were detected in three of the four tested species: *Ceratophyllum demersum* L. (Hornwort: C1), *Ipomoea aquatica* Forssk. (Swamp Morning Glory: C3), and *Salvinia cucullata* Roxb. ex Bory (Floating Moss: C4). Notably, no pesticide residues were detected in *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth: C2). Chemical analysis identified three compounds in plant tissues: bromophos methyl, coumaphos, and malathion. A particularly significant finding was the nearly identical coumaphos concentrations detected in *Ceratophyllum demersum* L. (Hornwort: C1) at 30.87 μg kg⁻¹ and the corresponding sediment sample (S3) at 30.99 μg kg⁻¹, suggesting equilibrium partitioning between biotic and abiotic environmental compartments.

To establish the relationships among water quality parameters, pesticide concentrations, and environmental impacts, correlation analysis was performed using data collected across all sampling sites. The WQI values demonstrated an inverse relationship with both pesticide detection frequencies and contamination levels. Sites with lower WQI scores (indicating degraded water quality) consistently showed higher pesticide concentrations and greater environmental stress indicators. Yang Dam (W2), exhibiting the poorest water quality (WQI = 57), demonstrated the highest pesticide concentrations in water samples (total: 36.84 μg L⁻¹). The nearby sediment samples (S1, S2) showed substantial pesticide accumulation ranging from 31.93–77.80 μg kg⁻¹, indicating significant contamination potential. Conversely, Sop Prap Pumping Station (W4), with the best water quality (WQI = 72), showed no detectable pesticide residues in water samples, consistent with its upstream location and limited agricultural influence. Ban Wang Proa Dam (W3), exhibiting intermediate water quality (WQI = 70), displayed moderate pesticide bioaccumulation in plant tissues, reflecting its position in the agricultural corridor. The analysis of spatial distribution patterns showed that sites with lower WQI scores (W1, W2: WQI = 57) were associated with detectable pesticide concentrations, while locations with better water quality (W4: WQI = 72) showed no pesticide detection. However, statistical correlation analysis did not reveal significant relationships due to the limited sample size and heterogeneous contamination patterns.

This research demonstrates the effectiveness of aquatic plants as bioindicators for assessing river water contamination, particularly persistent pesticides with extended biological half-lives. The bioaccumulation capacity of these organisms enables the detection of contamination even in the absence of continuous exposure. However, the absence of pesticide residues in *Ipomoea aquatica* Forssk. (Swamp Morning Glory: C3) tissue and the Ko Kha water sample (W3) may be attributed to dilution effects in the aquatic system at that location. While water and sediment sampling provide an instantaneous assessment of environmental conditions, aquatic plants offer integrated exposure information across their lifespan due to the persistence of accumulated pesticides.

Table 3 Pesticide distribution in the environmental samples from the study area

Environmental Samples	Pesticide Type	Concentration Detected	Organophosphate	Pyrethroid	Total
C1: Hornwort (μg kg^{-1})	Coumaphos Bromophos methyl	30.87 5.48	36.35	ND	36.35
C2: Water Hyacinth (μg kg^{-1})	ND	ND	ND	ND	ND
C3: Swamp Morning Glory (μg kg^{-1})	Malathion	23.05	23.05	ND	23.05
C4: Floating Moss (μg kg^{-1})	Bromophos methyl	5.47	5.47	ND	5.47
W1 Satuware Bridge (μg L^{-1})	ND	ND	ND	ND	ND
W2 Yang Dam (μg L^{-1})	Azinphos ethyl Chlorfenvinphos	29.62 7.22	36.84	ND	36.84
W3 Ban Wang Proa Dam (μg L^{-1})	ND	ND	ND	ND	ND
W4 Sop Prap Pumping Stations (μg L^{-1})	ND	ND	ND	ND	ND
S1 Sop Prap sediment 1 (μg kg^{-1})	Parathion methyl Methidathion	15.23 16.70	31.93	ND	31.93
S2 Sop Prap sediment 2 (μg kg^{-1})	Bromophos methyl Chlorfenvinphos Trizophos D-trans-phenothrin Azinphos ethyl	12.99 14.36 13.70 19.95 16.81	57.85	19.95	77.80
S3 Ban Wang Proa sediment 3	Coumaphos	30.99	30.99	ND	30.99

ND: Not detected

3.3 Aquatic plants genotoxicity

Following pesticide application, residual compounds persist in agricultural environments and subsequently appear in food products and various environmental matrices, particularly aquatic systems. Contaminated surface and groundwater directly affect non-target and beneficial organisms within both aquatic and terrestrial ecosystems. While point-source pollution has declined due to regulatory interventions over recent decades, non-point-source pollution from agricultural and urban runoff remains a major concern for water quality degradation [28, 36]. Pesticides enter aquatic environments at varying concentrations and interact with diverse aquatic components, complicating comprehensive toxicological assessment [11, 37]. Two primary methodologies are employed to evaluate potential threats to aquatic ecosystem health: (a) development of standardized water quality criteria, and (b) predictive risk assessment for aquatic organisms.

The SCGE assay is an effective method for evaluating both single and double-strand DNA breaks, as well as quantifying the damage induced by various genotoxic agents. Cells exhibiting greater DNA damage demonstrate enhanced migration of chromosomal DNA from the nucleus toward the anode, creating a comet-like morphology that directly correlates with the extent of DNA damage (Figure 3). This methodology facilitates the rapid assessment of genotoxicity in aquatic plant populations exposed to environmentally hazardous chemicals.

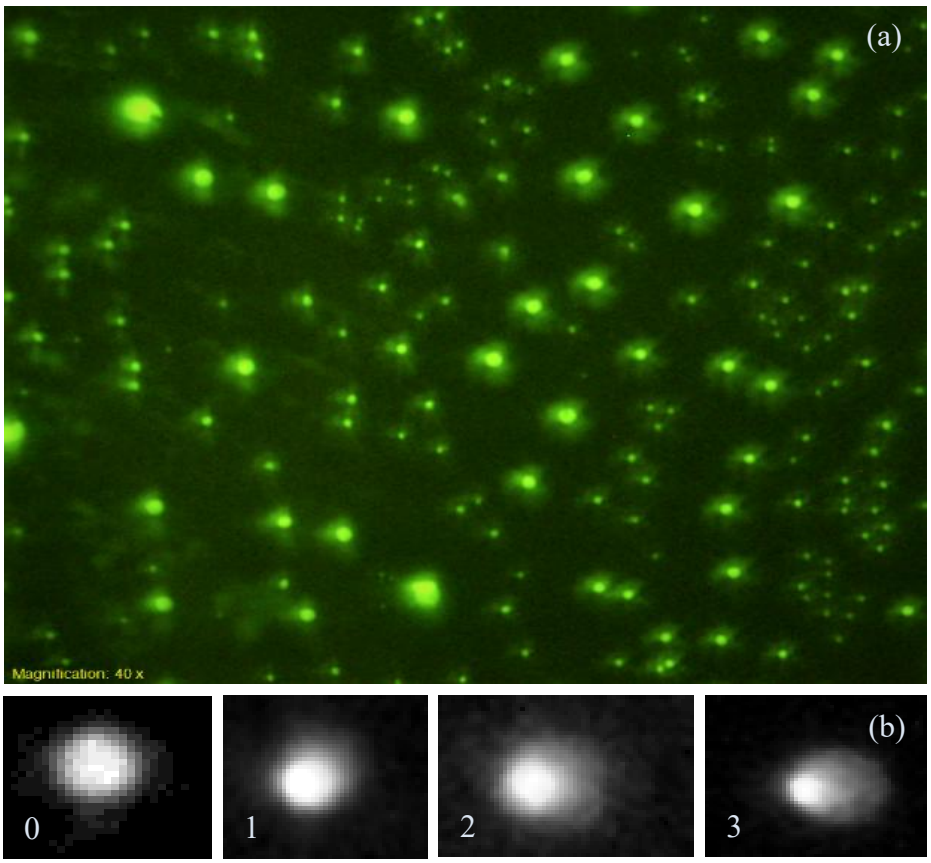

Figure 3 Representative images of cells processed using the single-cell gel electrophoresis protocol. (a) *Ipomoea aquatica* Forssk. (Swamp Morning Glory) cells and (b) Damage categories of aquatic plant cells: 0; nuclei without DNA damage (without tails) and 1, 2, 3; nuclei with ascending DNA damage (40X).

The morphological parameters of DNA damage in aquatic plant species are presented in Table 4. Different aquatic plant species exhibited varying degrees of DNA damage susceptibility when exposed to environmental contamination. Mean DNA tail percentages from 100-cell analysis varied among species: *Eichhornia crassipes* (Mart.) Solms (C2) displayed mean values of 14.33 ± 15.81%, followed by *Salvinia cucullata* Roxb. ex Bory (C4) (10.15 ± 12.66%), *Ceratophyllum demersum* L. (C1) (9.71 ± 19.00%), and *Ipomoea aquatica* Forssk. (C3) (8.83 ± 18.48%). The high standard deviations observed reflect the heterogeneous nature of cellular DNA damage responses under environmental contamination conditions, where individual cells within populations exhibit varying degrees of susceptibility to genotoxic stress.

DNA damage data from SCGE assays typically exhibit non-normal distribution patterns due to the inherent characteristics of cellular responses to genotoxic stress. Most cells demonstrate low levels of DNA damage, while a smaller subset exhibits significantly higher damage levels, resulting in right-skewed distributions. This non-normal distribution was confirmed by the Shapiro-Wilk test ($p < 0.05$), necessitating the use of non-parametric statistical methods. Consequently, both mean values (for comparison with previous literature) and median values (from box plot analysis) are presented in this study to provide comprehensive data interpretation.

Box plot analysis (Figure 4) demonstrated median DNA damage values of 16.58% for *Ceratophyllum demersum* L. (C1), 17.49% for *Eichhornia crassipes* (Mart.) Solms (C2), 16.92% for *Ipomoea aquatica* Forssk. (C3), and 12.91% for *Salvinia cucullata* Roxb. ex Bory (C4), compared to 2.19% for laboratory-cultured control specimens. These median values reflect the central tendency of data distribution without influence from extreme values, while the mean values presented in Table 4 provide the arithmetic averages used for comparison with previous studies. The reference line in Figure 4 represents the overall median threshold of 15.97% across all experimental samples, indicating the baseline for DNA damage assessment under environmental contamination conditions.

Mann-Whitney U tests revealed highly significant differences in DNA damage between laboratory-cultured control specimens (Median = 2.19%, IQR: 0.15–8.17%) and all field-collected plants. *Eichhornia crassipes* (Mart.) Solms (C2) exhibited the highest median DNA damage at 17.49% (IQR: 5.40–27.54%, U = 2088, Z = -7.115, $p < 0.001$), followed by *Ipomoea aquatica* Forssk. (C3): Median = 16.92%, IQR: 1.37–31.60%, U = 3063, Z = -4.733, $p < 0.001$), *Ceratophyllum demersum* L. (C1): Median = 16.58%, IQR: 1.64–24.63%, U = 2976, Z = -4.945, $p < 0.001$), and *Salvinia cucullata* Roxb. ex Bory (C4): Median = 12.91%, IQR: 3.61–17.94%, U = 2752, Z = -5.493, $p < 0.001$). All pairwise comparisons achieved statistical significance at $p < 0.001$. The Kruskal-Wallis test demonstrated significant overall differences among the five groups ($H = 53.36$, $df = 4$, $p < 0.001$), confirming species-specific variations in DNA damage responses.

The observed differences between mean and median DNA damage values reflect the typical characteristics of comet assay data, where cellular populations exhibit heterogeneous responses to environmental stressors. This distribution pattern is consistent with the established principles of genotoxicology, where a subset of cells demonstrates heightened sensitivity to DNA-damaging agents while the majority exhibits more moderate responses. The presentation of both statistical measures ensures comprehensive data interpretation and addresses the inherent variability in cellular genotoxic responses observed in environmental biomonitoring studies. Such heterogeneous cellular responses are well-documented in the literature and reflect the natural biological variation in DNA repair mechanisms, antioxidant capacity, and cellular stress tolerance among individual cells within aquatic plant populations [21-23].

The experimental findings confirmed that even low-level pesticide contamination (0–80 μg kg^{-1}) was sufficient to induce significant DNA damage in all tested aquatic plant species. All Mann-Whitney U comparisons demonstrated highly significant differences from the control (all $p < 0.001$), with DNA fragmentation reaching the maximum level of 17.49%. The Kruskal-Wallis test confirmed significant overall heterogeneity among species ($H = 53.36$, $p < 0.001$), indicating differential susceptibility to genotoxic stress. These results establish the SCGE assay as a sensitive and effective methodology for assessing genotoxicity in contaminated aquatic environments, enabling in situ monitoring and screening capabilities. Aquatic plants demonstrate considerable potential as biomonitoring tools for identifying genetic damage induced by polluted waters, industrial effluents, and agricultural runoff.

Table 4 Morphological parameters of DNA damage in aquatic plant species

Aquatic Plants	Mean ± SD % Head DNA	Mean ± SD %Tail DNA	Mean ± SD Tail Moment	Mean ± SD Tail Olive Moment
C1: Hornwort	90.29 ± 19.00	9.71 ± 19.00	0.22 ± 2.56	1.08 ± 1.91
C2: Water Hyacinth	85.66 ± 15.82	14.33 ± 15.81	0.48 ± 2.33	1.25 ± 1.56
C3: Swamp Morning Glory	91.17 ± 18.48	8.83 ± 18.48	0.61 ± 3.92	1.03 ± 2.36
C4: Floating Moss	89.85 ± 12.66	10.15 ± 12.66	0.97 ± 3.05	1.70 ± 2.05
*Control***	97.81 ± 8.04	2.19 ± 8.04	0.01 ± 1.46	0.29 ± 0.91

Note: Direct morphological measurements from the Tritek COMET Score software analysis

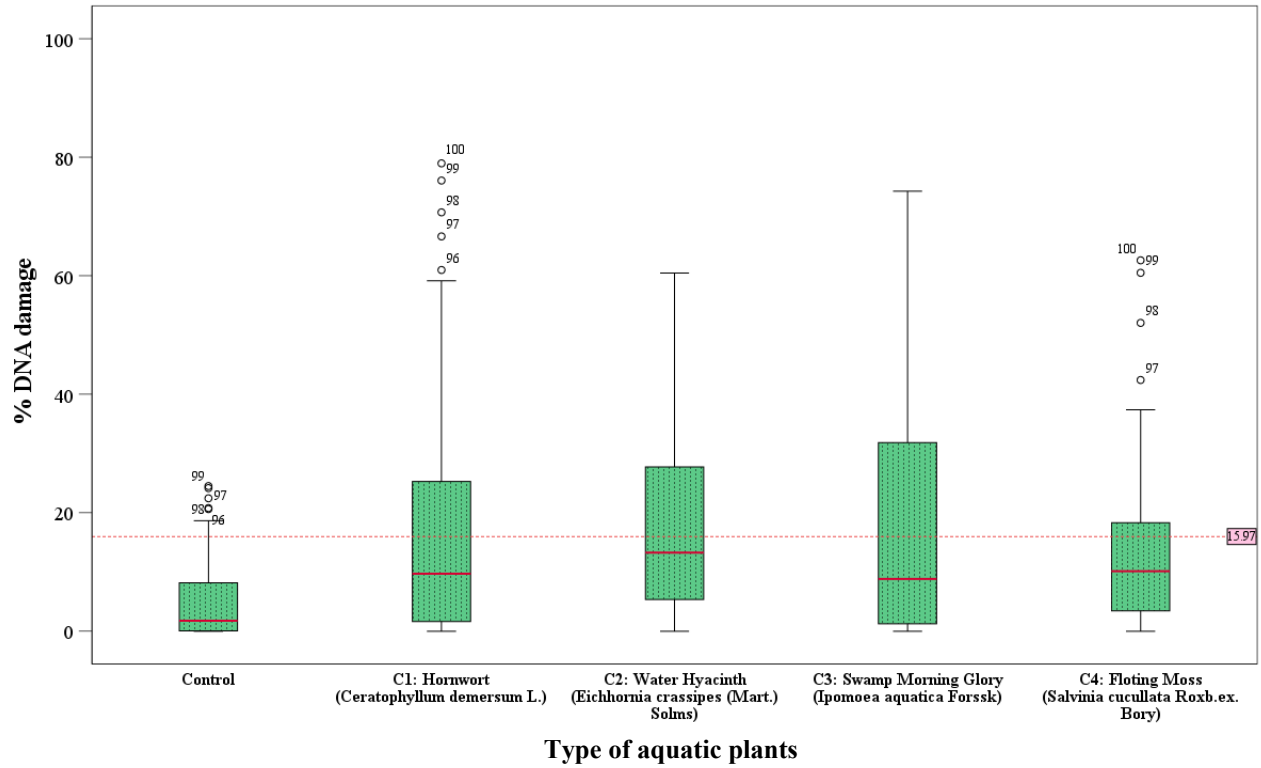

Figure 4 DNA migration of aquatic plants (n = 100 cells/aquatic plant)

3.4 Bioaccumulation potential and food safety implications

The detection of pesticide residues in aquatic plants raises significant concerns regarding bioaccumulation through food webs, particularly for edible species consumed by local communities. Plant crops can uptake environmental pollutants from contaminated water and sediments, subsequently bioaccumulating and biomagnifying these compounds in their tissues, thereby making aquatic vegetables harvested from polluted water bodies a potential health concern for consumers.

Among the investigated species, *Ipomoea aquatica* Forssk. (Swamp Morning Glory) represents a widely consumed vegetable throughout Thailand. The detection of malathion at 23.05 μg kg^{-1} in this edible plant indicates potential dietary exposure pathways for populations consuming vegetables harvested from contaminated aquatic environments. The differential accumulation patterns observed among plant species reflect their distinct physiological characteristics and contaminant uptake mechanisms. *Ceratophyllum demersum* L. (Hornwort) exhibited the highest compound diversity, accumulating both coumaphos (30.87 μg kg^{-1}) and bromophos methyl (5.48 μg kg^{-1}), likely attributable to its completely submerged growth habit that maximizes direct contact with contaminated water. Conversely, *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth) demonstrated no detectable residues despite sharing the same aquatic environment, suggesting species-specific metabolic capabilities for pesticide degradation or active exclusion mechanisms.

The nearly identical coumaphos concentrations in *Ceratophyllum demersum* L. (Hornwort) (30.87 μg kg^{-1}) and corresponding sediment sample S3 (30.99 μg kg^{-1}) suggest equilibrium partitioning between plant tissues and the sediment compartment, indicating prolonged exposure conditions. This equilibrium relationship establishes these plants as potential vectors for pesticide transfer to herbivorous fish and other aquatic consumers within the food web. Agricultural activities along riverine systems contribute to persistent environmental contamination, with pollutants being transported into water bodies through surface runoff and erosion processes [38].

4. Conclusions

Pesticide residues are detectable in food and the environment, especially in aquatic ecosystems. Urban and agricultural runoff exemplify non-point sources of pollution that significantly jeopardize water quality. Two methods for assessing possible dangers to aquatic health are the establishment of standardized water quality criteria and the forecasting of risks to aquatic organisms. The SCGE test is an effective method for assessing the extent of damage inflicted by various genotoxic substances and the occurrence of single and double-strand breaks in DNA. In cells exhibiting elevated DNA damage, the translocation of chromosomal DNA from the nucleus to the anode occurs more swiftly, like a comet. This enables the rapid assessment of genotoxicity in aquatic plants among populations exposed to environmentally detrimental substances. The DNA of the samples analyzed exhibited above 5% damage despite relatively low amounts of total pesticides, with a larger percentage of DNA in the tail compared to uncontaminated settings. The DNA of the examined aquatic plants degrades by less than 15.97% when subjected to low levels of pollution, exhibiting various degrees of DNA damage. The SCGE test serves as an effective method for assessing the genotoxicity of contaminated settings, facilitating in situ screening and monitoring.

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