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Phytoremediation of Fosetyl-Aluminum and Spiroxamine from Floriculture Effluent Using Enhanced *Azolla pinnata* and *Lemna minor* (Duckweed): A Case Study of Equator Flower Farm, Eldoret, Kenya

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Abstract

Floriculture represents a rapidly expanding sector in Kenya's agricultural economy, but the heavy reliance on pesticides raises growing concerns about environmental pollution and public health risks. This study aimed to evaluate the phytoremediation potential of enhanced aquatic macrophytes, *Azolla pinnata* and *Lemna minor* (duckweed), in removing the systemic fungicides Fosetyl-Aluminum and Spiroxamine from floriculture effluent. The research sought to address the ecological threat posed by pesticide runoff from commercial flower farms to the Marura Wetland ecosystem in Eldoret, Kenya. A baseline spatial assessment of pesticide loading was conducted at Equator Flower Farm using HPLC analysis. To improve remediation efficiency, *A. pinnata* and *L. minor* were enhanced through colchicine-induced polyploidy (1–10 ppm) to increase biomass and surface area. Experimental trials were conducted in a greenhouse using a completely randomized design (CRD) across three batches. Pesticide concentrations in both the water column and plant tissues were quantified over 14 days to determine removal rates and bioaccumulation factors. Experimental data were analyzed using R (version 4.4.2) to assess pesticide spatial distribution and phytoremediation efficiency. One-way ANOVA was employed to identify significant differences in water-phase concentrations and tissue bioaccumulation across sampling points, treatment batches and plant species at $p<0.05$. The study identified significant spatial variability in pesticide distribution at Equator Flower Farm, with a localized hotspot at Point 3 exhibiting concentrations of 6.07 mg/L for Fosetyl-Aluminum and 12.20 mg/L for Spiroxamine. The sequential pond system demonstrated high efficacy ($p=0.001$), achieving over 85% removal efficiency, although Spiroxamine showed greater environmental persistence than Fosetyl-Aluminum. Experimental results revealed that colchicine-induced polyploidy significantly enhanced the functional morphology of both macrophytes, doubling the shoot height and leaf area of *A. pinnata*. Consequently, enhanced *Azolla* outperformed *L. minor* ($p<0.001$), sequestering pesticides at rates two to three times higher than the latter, with tissue accumulation reaching 2.18±0.28 mg/kg for Spiroxamine. The study concludes that integrating colchicine-enhanced *A. pinnata* into sequential treatment systems provides a highly efficient and sustainable biotechnological solution for mitigating persistent floriculture pesticide runoff and protecting aquatic ecosystems. It is recommended that commercial floriculture operations integrate dual-species macrophyte ponds into their treatment chains. Regulatory bodies like NEMA should adopt these nature-based solutions into environmental policy, accompanied by systematic biomass harvesting to ensure the permanent removal of pollutants from the aquatic environment.

Keywords: Phytoremediation, *Azolla pinnata*, *Lemna minor*, fosetyl-aluminum, spiroxamine, colchicine enhancement, floriculture effluent, bioaccumulation, Marura Wetland

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Introduction

The floriculture industry has evolved into a formidable global agribusiness. As of January 2022, the global floriculture market was estimated to be valued at around 49.8 billion USD (Jenkins et al., 2023). By 2025, the global floriculture and ornamental plant sector had reached a valuation of approximately 61.6 billion USD and is projected to grow to 104.1 billion USD by 2035, according to Research Nester (2025). Driven by strong demand for cut flowers particularly roses, which account for about three-quarters of the trade the sector depends on specialized greenhouse and open-field production systems in key exporting countries such as the Netherlands, Colombia, and Kenya.

Floriculture industry in Kenya serves as a major driver of national economic performance, with an estimated market value of about USD 1.1 billion in 2025 (Mordor Intelligence, 2026; The Flower Hub, 2025). As the fourth-largest exporter of cut flowers globally, the sector contributes roughly 1.3 percent to Kenya's GDP and supports the livelihoods of more than two million people, with women forming the majority of the workforce (Mwangi, 2019; Chebet, 2021). Fresh cut flowers dominate export volumes, with millions of kilograms shipped annually to major markets including the Netherlands, the United Kingdom, Saudi Arabia and other destinations (Gemählich, 2022). Commercial production concentrates in high-potential equatorial regions such as the Rift Valley (Naivasha and Nakuru), the Central Highlands (Kiambu, Nyandarua, and Laikipia), and the Western Highlands of Uasin Gishu and Trans Nzoia (Kuiper, 2017; Saina et al., 2017). The industry focuses on large-scale commercial production, marketing, and sale of bedding plants, cut flowers, potted flowering plants, foliage plants and floral arrangements, alongside limited non-commercial home gardening (Mojumder, 2022). High export returns and sustained government support have accelerated sector growth across Kenya (Gemählich, 2022; Adeola et al., 2018). To meet international quality standards and maintain high yields, flower farms rely heavily on intensive pest and disease control regimes, including frequent application of synthetic pesticides and supplementary use of organic fertilizers (Kinyanjui, 2013; Borbaruah, 2023). Pesticide application patterns vary with season, pest pressure, and prevailing weather conditions.

At Equator Flower Farm, fungicides such as Fosetyl-Aluminum and Spiroxamine are widely used to control fungal pathogens in greenhouse production systems. However, their efficacy in disease management is countered by significant toxicological and environmental risks. The U.S. Environmental Protection Agency (EPA) has classified fosetyl-Al as a Category C oncogen, identifying it as a potential human carcinogen based on laboratory feeding studies where male rats and dogs exhibited urinary bladder and testicular tumors at high doses. Beyond mammalian risks, Barreto et al. (2021) demonstrated that fosetyl-Al exposure in freshwater organisms, such as *Danio rerio* and *Enchytraeus crypticus*, alters biochemical responses, behavior, and reproduction, even at levels near predicted environmental concentrations. Chronic exposure is considered low-risk for carcinogenicity, though long-term animal studies indicate potential testicular degeneration and urinary bladder alterations (European Parliament and the Council of the European Union, 2020). Additionally, accumulation of aluminum in individuals with impaired renal function may lead to neurotoxic effects or bone disorders over time (Dey & Singh, 2022).

Spiroxamine, a systemic sterol biosynthesis inhibitor, penetrates plant tissues and translocates throughout crops, increasing its likelihood of entering wastewater streams (Pérez-Vicente, 2013). Acute human exposure can cause severe skin and eye irritation, potential skin sensitization, respiratory irritation if inhaled, and central nervous system effects such as tremors or decreased activity if ingested. Long-term exposure in animal models has shown systemic toxicity targeting the liver and eyes, including lens opacities, and high-dose studies have indicated potential developmental and reproductive effects, such as delayed ossification and reduced pup body weight (European Food Safety Authority, 2021).

Equator Flower Farm operates in Uasin Gishu County and depends on water abstracted from the adjacent Marura Wetland. Greenhouse floriculture consumes large volumes of water for irrigation, cooling, pesticide application, and cleaning. A one-hectare greenhouse can require between 120,000 and 160,000 liters of water daily for irrigation, pesticide application, and cleaning (Chartzoulakis & Drosos, 1995). At the farm, much of this water is abstracted from and subsequently discharged back into the adjacent Marura Wetland. Current effluent management at the farm relies on a single stabilization pond with a retention period of approximately five days. Such limited treatment is frequently insufficient to degrade persistent agrochemicals, leading to the discharge of partially treated wastewater into the wetland ecosystem.

The Marura Wetland provides critical ecosystem services, including water purification, biodiversity support, and domestic water supply (Chepchumba, 2018). However, the discharge of persistent pesticides poses a threat of bioaccumulation and biomagnification along aquatic food chains, potentially degrading water quality and reducing biodiversity (Ray & Shaju, 2023; Tongo et al., 2022). Despite these risks, monitoring and regulation of floriculture effluent remain inadequate, leaving a significant gap in environmental protection. Phytoremediation offers a promising, nature-based solution by utilizing living plants to absorb, transform, and immobilize contaminants in situ (Celletti et al., 2025; Prigioniero et al., 2025). This study seeks to address the existing regulatory and treatment gaps by quantifying the concentrations of Fosetyl-Aluminum and Spiroxamine in floriculture effluent and evaluating a cost-effective phytoremediation approach. The findings are intended to inform farm management and regulatory agencies on contamination levels and provide practical, sustainable treatment alternatives to safeguard the ecological integrity of wetland ecosystems and the health of downstream populations.

Materials and Methods

The study was carried out at Equator Flower Farm in Marura (latitude: 0°33'56.4"N, longitude: 35°19'31.3"E), situated near the University of Eldoret, Uasin Gishu County, approximately 7 km from Eldoret town. Uasin Gishu County lies between longitudes 34°50’ East and 35°37’ West, and latitudes 0°03’ South and 0°55’ North. It shares borders with Trans Nzoia County to the north, Elgeyo Marakwet County to the east, Baringo County to the southeast, Kericho County to the south, Nandi County to the southwest, and Kakamega County to the northwest, covering a total area of 3,345.2 km² (Uasin Gishu County, 2023).

The site is a major agricultural zone characterized by intensive floriculture and heavy agrochemical use (Chepchumba, 2018). The farm operates a centralized treatment pond with four compartments that screen solids and use staged stabilization with prolonged sunlight exposure to support UV-driven breakdown of chemical and organic matter before discharge to a wetland. These compartments supplied all wastewater samples. The Marura region experiences moderate to high rainfall, with annual averages exceeding 2000 mm, supporting agricultural production but also contributing to runoff, which carried pesticides and other contaminants into nearby water bodies. Average temperatures range between 15°C and 27°C, creating a favorable climate for year-round farming.

The region's hydrogeology, characterized by well-drained soils and the presence of groundwater, makes it suitable for irrigation; however, the reliance on agrochemicals raises concerns about potential contamination of water sources. The area combines smallholder farming with commercial floriculture, alongside growing settlement pressure. Local wetlands provide filtration, habitat and flood control, yet intensified land use and pesticide application have degraded ecological conditions.

The study utilized a completely randomized experimental design (CRD) within a greenhouse setting. Wastewater samples were collected in duplicate from the inlet and outlet of four distinct treatment compartments at Equator Flower Farm using dark glass bottles to prevent photo-degradation.

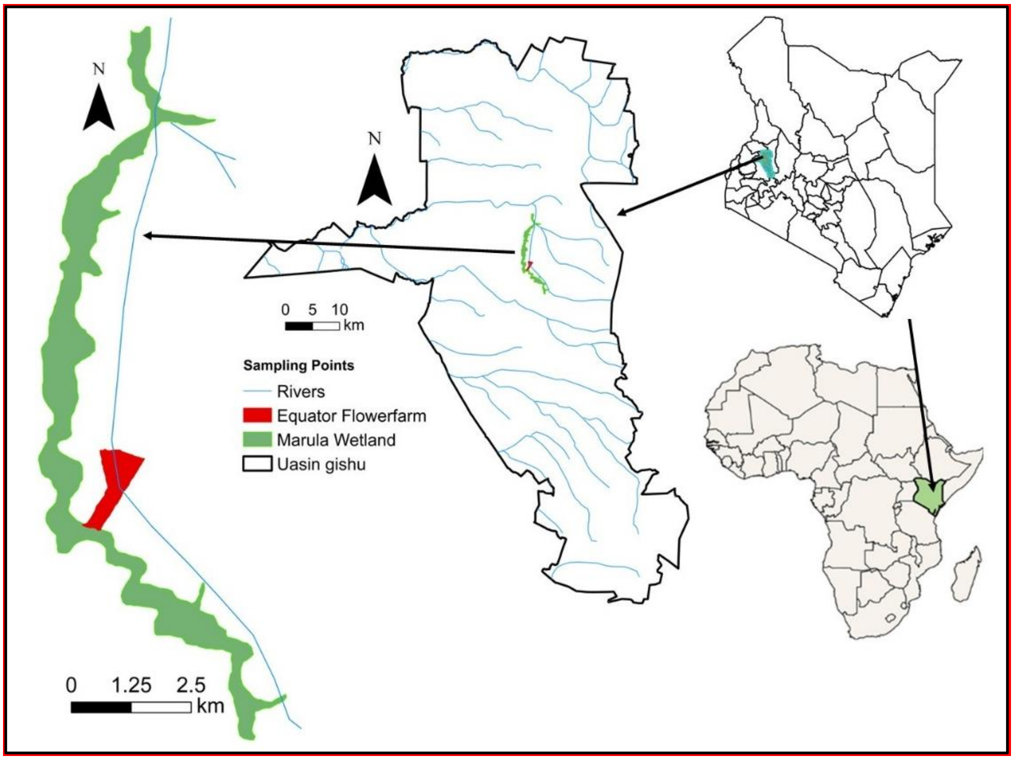

Figure 1: Map showing the location and position of the Marura wetland and the Equator flower farm, where samples were collected

Samples were transported in coolers at 4°C and stored under refrigeration at the University of Eldoret's Biotechnology Laboratory to maintain chemical integrity prior to analysis. Due to the extensive use of various pesticides on the flower farm, two specific pesticides, Fosetyl Aluminum and Spiroxamine, were selected for analysis from a comprehensive list of 70 pesticides used in the premises.

The materials utilized in the analysis included HPLC-grade acetonitrile, methanol, water, ammonium formate, and formic acid (FA), all sourced from Sigma-Aldrich. Standard solutions of fosetyl-Al and spiroxamine were purchased from Dr. Ehrenstorfer. The analysis also employed Hypercarb columns (2.1 × 100 mm, 5 µm) from Thermo Fisher Scientific, along with BEH C18 columns (2.1 mm × 50 mm, 1.7 µm) and BEH amide columns (2.1 × 50 mm, 1.7 µm) from Waters. Additionally, dark glass sampling bottles were used for the storage of samples.

The preparation of stock solutions commenced by accurately weighing pure standards of fosetyl-Al and spiroxamine and dissolving them in acetonitrile to achieve a concentration of 250 µg/g. These stock solutions were then used to generate matrix-matched calibration standards by spiking distilled water with concentrations ranging from 5 to 2000 ng/g, corresponding to anticipated pesticide levels in the wastewater samples. To ensure uniform distribution of the analytes, the spiked water samples were mixed thoroughly for one hour. The resulting calibration solutions were subsequently stored in amber vials at 20°C in a dark environment to prevent any potential degradation of the compounds.

Pesticide concentrations were quantified using High-Performance Liquid Chromatography (HPLC). Before injection, wastewater samples were filtered through a 0.45 µm membrane. A 20 µL aliquot was injected into the HPLC system equipped with a Hypercarb column (2.1 × 100 mm, 5 µm). The mobile phase consisted of an acetonitrile and water gradient with ammonium formate buffer, adjusted to pH 4.5 with formic acid. Analytes were identified by retention times and quantified against a standard calibration curve.

Healthy specimens of *Azolla pinnata* and *Lemna minor* were gathered from water bodies in Moiben, Uasin Gishu County. The plants were rinsed and sorted for uniform size, then acclimatized in distilled water at 25 ± 2°C for seven days in a controlled laboratory environment before experimental exposure. To evaluate enhanced efficiency, macrophytes were pre-treated with colchicine solutions (1, 2, 5 and 10 ppm) for seven days. Following this induction period, the plants were subjected to the same pesticide-spiked water protocols described in the single plant assay.

Plant growth was monitored through daily measurements of shoot and root lengths using digital calipers. Leaf area was determined via image analysis software and calculated using the formula:

$$\text{Leaf area} = \pi \times \text{length} \times \text{width} \times 0.75$$

where 0.75 serves as a correction factor for leaf morphology.

Data were cleaned in Excel and analyzed using R software. Analysis of Variance (ANOVA) was performed to assess the significance of mean differences between treatments. All statistical significance was determined at $p<0.05$.

Results and Discussion

Baseline Residue Levels of Fosetyl-Al and Spiroxamine

To establish the chemical baseline for the study, Fosetyl-Aluminium and Spiroxamine were chosen for monitoring based on their extensive and frequent use in the flower farm's pest control program. The analysis of these chemicals was done to determine the existing pollutant load entering the drainage and treatment systems of the area from the greenhouses and service areas.

Spatial Distribution at Farm Discharge Points

Figure 2 shows the average concentrations of Fosetyl Aluminium and Spiroxamine (mg/L) measured at four discharge points (Point 1 to Point 4) in the Equator Flower Farm.

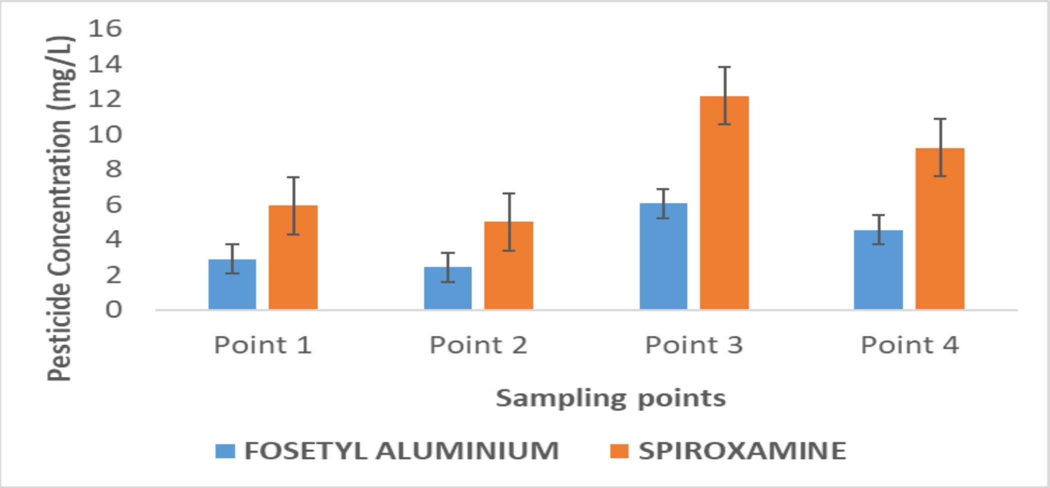

Figure 2: Pesticide analysis of wastewater from different wastewater points within the flower farm premises

The results demonstrate clear spatial variability in pesticide residues across the farm. For Fosetyl Aluminium, concentrations ranged from 2.42 ± 0.38 mg/L at Point 2 to 6.07 ± 0.26 mg/L at Point 3. The highest level at Point 3 indicates a potential hotspot, likely linked to localized pesticide application or drainage pathways that concentrate effluent at this site. In contrast, lower levels were recorded at Point 1 (2.90 ± 0.22 mg/L) and Point 2 (2.42 ± 0.38 mg/L), suggesting reduced runoff contamination in these areas. Spiroxamine exhibited a similar but more pronounced pattern, with concentrations ranging from 5.03 ± 0.74 mg/L at Point 2 to 12.20 ± 0.51 mg/L at Point 3. Point 3 again emerged as the most contaminated site, with Spiroxamine levels more than double those at Points 1 (5.96 ± 0.43 mg/L) and 2. This peak indicates either heavier pesticide use or slower degradation of Spiroxamine in runoff entering this discharge point. When comparing the two pesticides, Spiroxamine consistently exceeded Fosetyl Aluminium at all four points. The difference was most striking at Point 3, where Spiroxamine (12.20 ± 0.51 mg/L) was approximately twice as high as Fosetyl Aluminium (6.07 ± 0.26 mg/L). This trend suggests that Spiroxamine may be applied more intensively or exhibits greater environmental persistence, making it a more significant contributor to pesticide loading in Marura Wetland.

To statistically evaluate these spatial differences, a one-way analysis of variance (ANOVA) was conducted. The results are summarized in Table 1.

Table 1: One-Way ANOVA Results for Baseline Fosetyl-Al and Spiroxamine Concentrations at Farm Discharge Points

Parameter	Df (Between)	Df (Within)	F-value	p-value
Fosetyl-Aluminium (mg/L)	3	16	45.28	0.001
Spiroxamine (mg/L)	3	16	62.47	0.001

The ANOVA results indicate statistically significant differences in pesticide concentrations across the four discharge points for both compounds. Fosetyl-Aluminium varied significantly among points (F(3,16) = 45.28, p = 0.001), while Spiroxamine also exhibited highly significant spatial differences (F(3,16) = 62.47, p = 0.001).

Efficiency of Existing Treatment Ponds

Figure 3 presents the mean concentrations of Fosetyl Aluminium and Spiroxamine (mg/L) across five sequential phytoremediation ponds in the Equator Flower Farm wastewater treatment system. The ponds are designed to progressively reduce pollutants before effluent is discharged into the Marura Wetland. The performance of the farm's sequential pond system demonstrated a progressive reduction in these chemical loads as the water transitioned through the treatment stages.

Pond 1, which serves as the primary inlet for untreated wastewater, contained the highest concentrations: 7.82±0.25 mg/L for Fosetyl-Al and 15.62±0.49 mg/L for Spiroxamine. A substantial reduction was observed by the time the water reached Pond 2, where concentrations dropped to 1.63 mg/L and 3.46 mg/L, respectively. This initial removal, representing an efficiency of approximately 78%, is likely attributable to physical processes such as sedimentation and adsorption onto the pond's existing vegetation and substrate. By the final stage in Pond 5, residues reached their minimum levels of 0.98±0.14 mg/L and 2.19±0.28 mg/L. While the overall system achieved over 85% removal efficiency, the persistent residues in the final effluent indicate that the current system requires augmentation to meet more stringent environmental discharge standards.

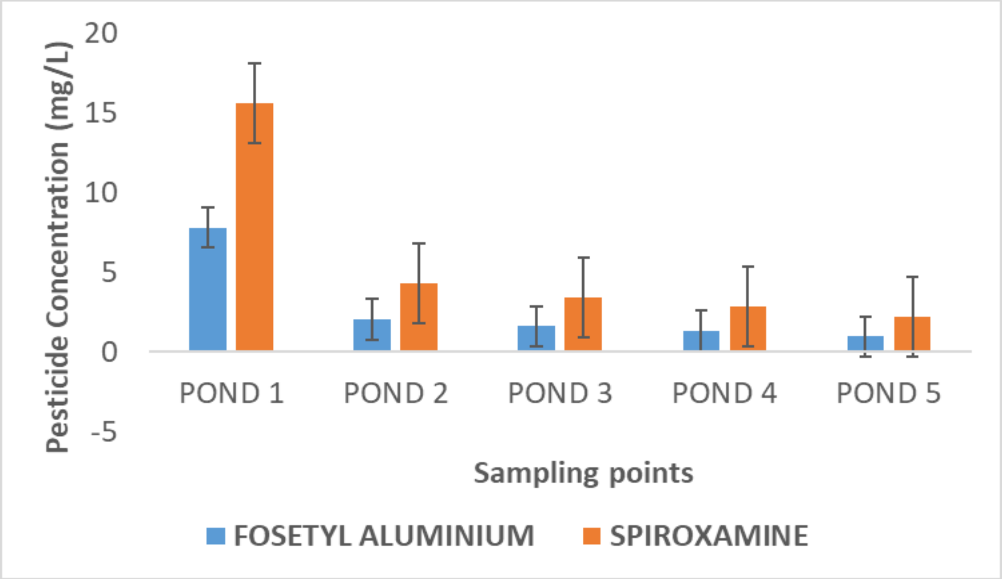

Figure 3: Analysis of Fosetyl aluminium and Spiroxamine in wastewater from different Marura flower farm water treatment ponds within the flower farm premises

To statistically confirm the observed differences in pesticide concentrations across the five ponds, a one-way analysis of variance (ANOVA) was conducted. The results are summarized in Table 2.

Table 2: One-Way ANOVA Results for Fosetyl-Aluminium and Spiroxamine Concentrations across Treatment Ponds

Parameter	Df (Between)	Df (Within)	F-value	p-value
Fosetyl-Aluminium (mg/L)	4	20	136.72	0.001
Spiroxamine (mg/L)	4	20	182.55	0.001

The ANOVA results indicate statistically significant differences in pesticide concentrations across the five treatment ponds for both Fosetyl-Aluminium (F(4,20) = 136.72, p = 0.001) and Spiroxamine (F(4,20) = 182.55, p = 0.001). This confirms that each pond stage contributed to a meaningful reduction in pesticide residues, highlighting the effectiveness of the sequential pond system in mitigating pollutant loads prior to discharge.

Pesticide Analysis along the Marura River and Wetland

The impact of the farm's treated effluent on the receiving aquatic ecosystem was assessed by monitoring at different locations along the Marura River. Sampling was conducted at three strategic points: upstream of the wetland, downstream of the wetland and at the point where wastewater is discharged into the wetland, located in the middle of the sampling area. The graph in Fig. 4 illustrates the concentrations of two pesticides, Fosetyl-Aluminium and Spiroxamine, at three points along the Marura River. The discharge point represents where treated wastewater from the Marura flower farm enters the river.

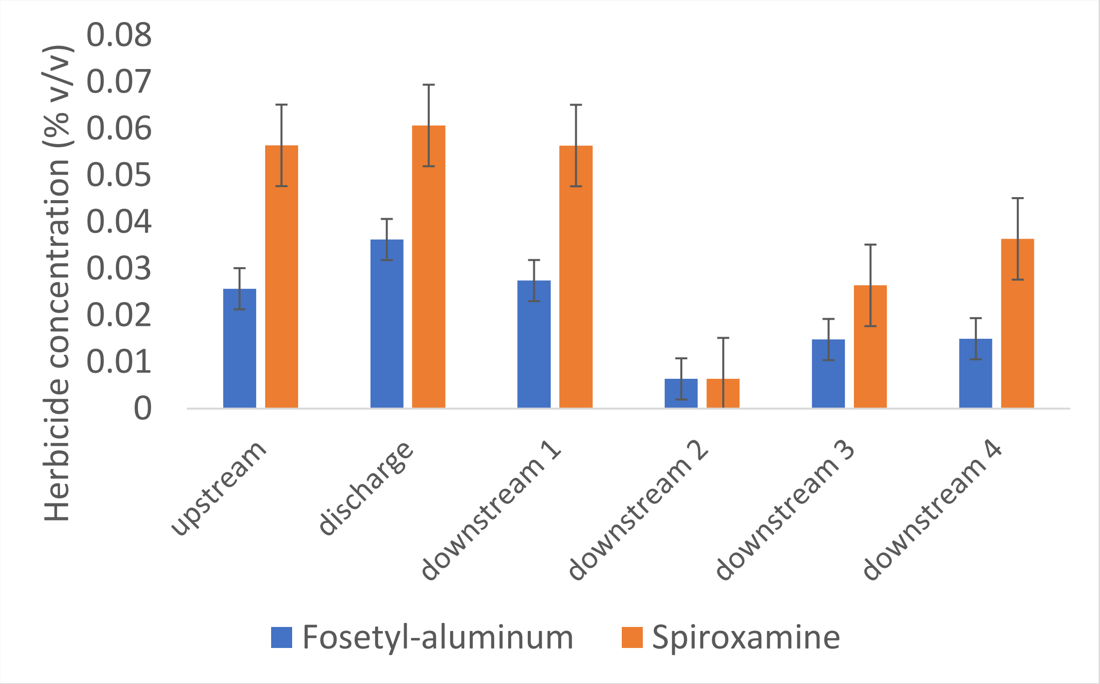

Figure 4: Analysis of Spiroxamine and Fosetyl Aluminium in water from different points along the Marura River, in which discharge is the point of entry of wastewater from the Marura flower farm treatment point

At the upstream point, prior to any influence from the farm, baseline concentrations were recorded at 0.025 mg/L for Fosetyl-Al and 0.055 mg/L for Spiroxamine. These figures suggest that the river is already subject to a low level of pesticide loading from general agricultural activities within the Moiben region. At the discharge point, where the farm's Pond 5 effluent enters the river system, a significant localized increase in Fosetyl-Aluminium was observed, rising to 0.045 mg/L. m to 0.045 mg/L. Notably, the Spiroxamine concentration also increased, but only slightly to 0.06 mg/L, indicating that while the farm is a contributor to the chemical load, it is more so for Fosetyl-Al at this particular point of entry. Further downstream, after the water had passed through the Marura Wetland, the concentrations for both chemicals had reverted to levels roughly similar to the baseline at the upstream location (0.025 mg/L and 0.055 mg/L). This indicates that the wetland is an important ecosystem service provider in this region, effectively diluting and sequestering the chemicals; however, the fact that Spiroxamine is still present in the river profile indicates that it is more environmentally resilient than Fosetyl-Al.

To statistically test whether pesticide concentrations differed significantly among the three river points, a one-way analysis of variance (ANOVA) was conducted. The results are summarized in Table 3. The ANOVA results show statistically significant differences in pesticide concentrations along the river for Fosetyl-Aluminium ($F(2,12) = 34.57$, $p = 0.001$) and Spiroxamine ($F(2,12) = 12.84$, $p = 0.002$). These findings confirm that the farm's effluent caused a localized increase in chemical loads at the discharge point, while the downstream wetland effectively mitigated the impact and reduced concentrations toward baseline levels.

Table 3: One-Way ANOVA Results for Fosetyl-Aluminium and Spiroxamine Concentrations along the Marura River

Parameter	Df (Between)	Df (Within)	F-value	p-value
Fosetyl-Aluminium (mg/L)	2	12	34.57	0.001
Spiroxamine (mg/L)	2	12	12.84	0.002

Comparative Analysis of Fosetyl-Al and Spiroxamine Across Discharge Points, Treatment Ponds, and Marura River

To evaluate how pesticide concentrations changed from the source through the treatment system and into the receiving aquatic ecosystem, a one-way analysis of variance (ANOVA) was conducted across the three monitoring stages: (i) baseline discharge points at the farm, (ii) sequential treatment ponds, and (iii) river and wetland sampling points. This comparative approach allowed quantification of spatial and treatment-induced variation in Fosetyl-Aluminium and Spiroxamine concentrations.

Table 4: Comparative One-Way ANOVA Results for Fosetyl-Aluminium and Spiroxamine Across Farm, Treatment Ponds and River

Parameter	Stage/Groups	Df (Between)	Df (Within)	F-value	p-value
Fosetyl-Aluminium (mg/L)	3	2	48	182.49	0.001
Spiroxamine (mg/L)	3	2	48	256.12	0.001

The ANOVA results show statistically significant differences in concentrations of both Fosetyl-Aluminium (F(2,48) = 182.49, p = 0.001) and Spiroxamine (F(2,48) = 256.12, p = 0.001) across the three study stages. This confirms that pesticide levels varied considerably from the farm discharge points through the treatment pond sequence to the river system.

Enhancement of Plants for The Pesticide Removal from Flower Farm-Bound Wastewater

Enhancement of plants through colchicine treatment offers a promising approach to improve their ability to remove pesticides from flower farm-bound wastewater. Colchicine enhances the plants' physiological and biochemical traits by inducing polyploidy, enabling more efficient absorption and breakdown of toxic compounds.

Enhancement of both Duckweed and Azolla using Colchicine

To enhance the phytoremediation capacity of both duckweed (*Lemna minor*) and azolla (*Azolla pinnata*), the plants were treated with colchicine for one week. Colchicine, a known mitotic inhibitor, is often used to induce polyploidy, potentially leading to improved plant physiological and morphological traits. Following treatment, the plants were evaluated based on a set of measurable parameters: leaf broadness, leaf area, and root length, along with qualitative assessments of overall plant vigor and morphology. As shown in *Plate 1*, colchicine-treated specimens of both duckweed and azolla exhibited noticeable morphological changes compared to the control group. Treated duckweed displayed broader and more expansive fronds, while azolla showed increased root density and slightly enlarged leaf lobes. These changes suggest enhanced growth potential, which is beneficial for improved nutrient uptake and heavy metal absorption in phytoremediation applications.

Plate 1: Pictogram of Duckweed before Enhancement

Plate 2: Pictogram of Duckweed after the Enhancement

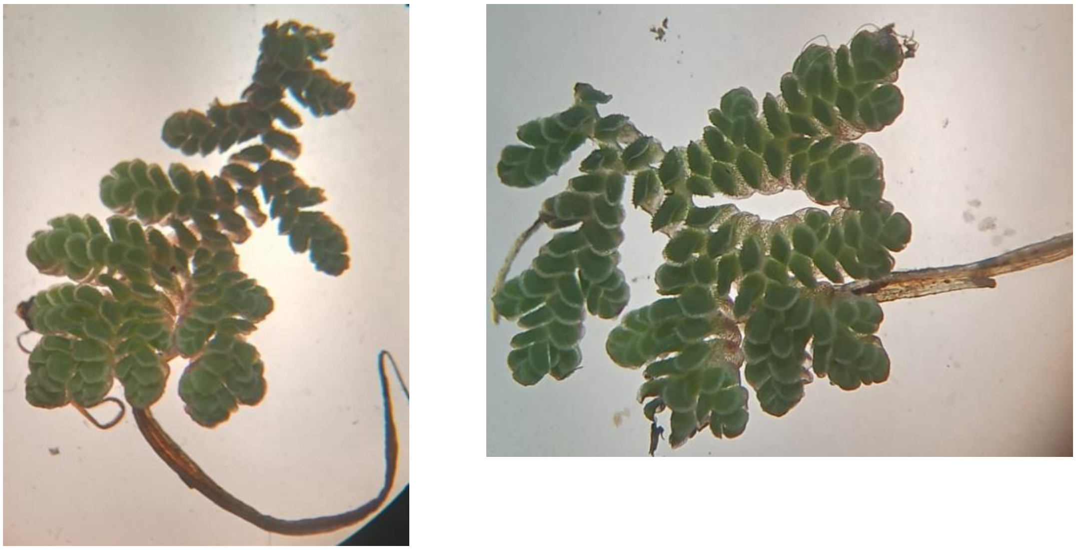

Plate 3: Pictogram of azolla before enhancement

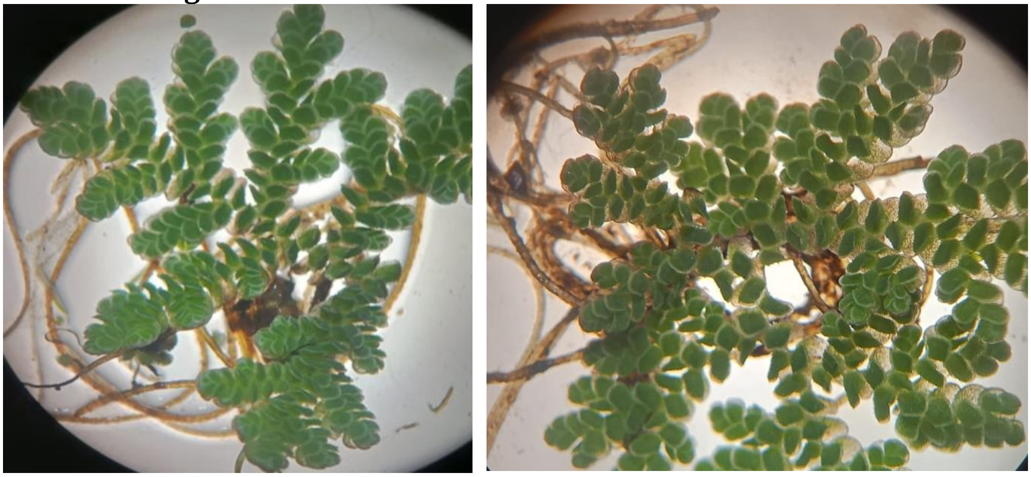

Plate 4: Pictogram of enhanced azolla

The growth parameters of *Azolla* and *Duckweed* before and after enhancement were evaluated in terms of average shoot length, root length, and leaf area (Table 5).

Table 5: Morphological parameters of Azolla and Duckweed before and after colchicine enhancement

Plant	State	Avg. Shoot Length (mm)	Avg. Root Length (mm)	Avg. Leaf Area (mm^2)
Azolla	Pre-enhancement	2.5	3.8	1.9
	Post-enhancement	5.3	4.3	2.5
Duckweed	Pre-enhancement	0.1	0.9	0.4
	Post-enhancement	0.5	1.0	0.7

Note: Values indicate mean measurements (mm for shoot and root length; mm² for leaf area)

For Azolla, shoot length increased markedly from 2.5 mm before enhancement to 5.3 mm after enhancement, representing more than a two-fold increase. Root length also showed a positive response, rising from

3.8 mm to 4.3 mm. Similarly, leaf area expanded from 1.9 mm² to 2.5 mm². *Duckweed* displayed relatively smaller values compared to Azolla, but still responded positively to enhancement. Shoot length increased from 0.1 mm to 0.5 mm, indicating a five-fold improvement. Root length showed only a modest rise from 0.9 mm to 1.0 mm, while leaf area increased from 0.4 mm² to 0.7 mm². Although the absolute values were lower than those of Azolla, the relative gain in shoot length and leaf area demonstrates that Duckweed also benefitted from the treatment, albeit to a lesser extent. Colchicine enhancement significantly improved morphological traits in both aquatic plants, with Azolla exhibiting a stronger overall growth response compared to *Duckweed*. The substantial increase in shoot elongation and leaf expansion suggests enhanced photosynthetic potential, which could translate into greater biomass productivity under favourable conditions.

Removal of Fosetyl Aluminum using Enhanced Plants

Reduction in the Spiked Experimental Water

Figure 5 illustrates the effectiveness of using enhanced plants (duckweed and azolla) using Colchicine to remove Fosetyl Aluminum (measured in mg/kg) across three distinct batches (batch 1, batch 2, and batch 3), compared to a control group.

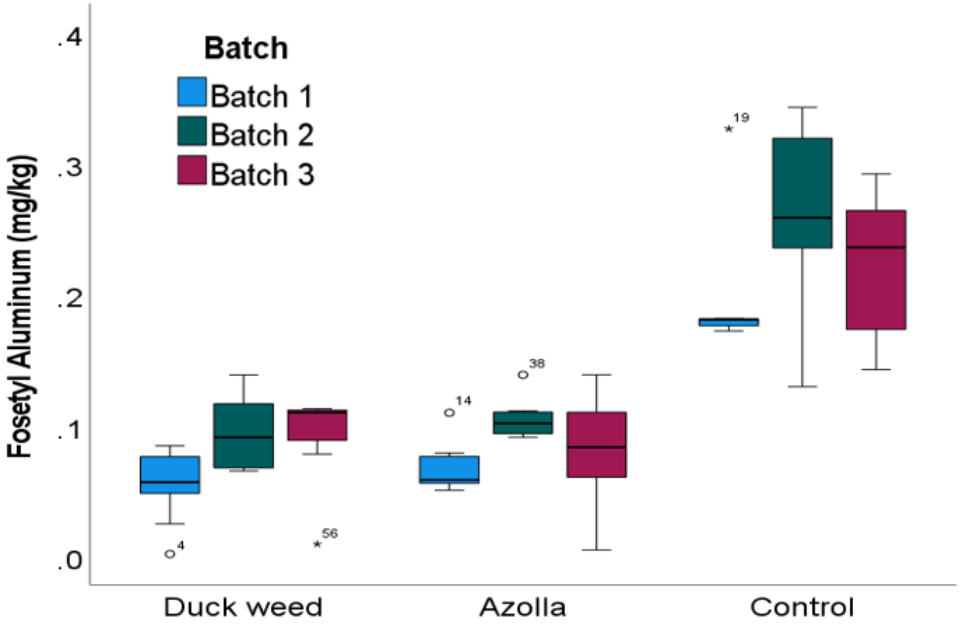

Figure 5: Analysis of Fosetyl Aluminium in water Spiked with Fosetyl Aluminum and Phyto-remediated with enhanced azolla and duck weed in different batches

Figure 5 presents comparative data on the phytoremediation performance of *Lemna minor* (duckweed) and *Azolla pinnata* (azolla), both under enhanced conditions, in removing Fosetyl Aluminum (mg/kg) across three experimental batches relative to a non-vegetated control. Morphologically, Azolla developed greater biomass, particularly in root and leaf structures, compared to duckweed. However, this structural advantage did not consistently translate into proportionately higher pollutant uptake across all batches, suggesting that bioaccumulation is influenced by additional physiological and biochemical mechanisms beyond biomass expansion.

In water with unenhanced Duckweed, Fosetyl Aluminium concentrations averaged 0.067 ± 0.041 mg/L and 0.080 ± 0.034 mg/L in water with enhanced plants, both substantially lower than control levels. Within the plant biomass, accumulation reached 0.040 ± 0.026 mg/kg in untreated plants and 0.049 ± 0.034 mg/kg under enhanced conditions. Batch-level data showed that batch 1 recorded the lowest internal concentrations with minimal variability (narrow IQR), reflecting highly consistent uptake. Batch 2 showed moderately higher mean values, and Batch 3 exhibited the highest concentrations among duckweed treatments, though still markedly lower than those of the controls. This indicates that Duckweed consistently contributed to spiroxamine reduction, though its tissue accumulation remained relatively modest. Azolla demonstrated stronger tissue bioaccumulation than Duckweed. Mean water concentrations were 0.059 ± 0.032 mg/L in untreated water and 0.083 ± 0.031 mg/L in enhanced water, closely paralleling Duckweed's reductions.

However, tissue accumulation was significantly greater: 0.066 ± 0.035 mg/kg under untreated conditions and 0.162 ± 0.041 mg/kg in enhanced treatments. In Batch 1, Azolla exhibited the lowest internal pollutant levels with narrow dispersion, whereas Batches 2 and 3 recorded higher concentrations with moderate variability (IQR ≈ 0.3–0.4 mg/kg). Despite Azolla's higher biomass, the uptake advantage over Duckweed was only marginal in some batches, suggesting that bioaccumulation efficiency is not strictly biomass-dependent. Instead, species-specific traits such as root surface area, symbiotic cyanobacteria, and enhanced metabolic activity likely played more decisive roles in pollutant sequestration. The non-vegetated control group consistently exhibited the highest Fosetyl Aluminum concentrations in water, averaging 0.228 ± 0.096 mg/L under untreated conditions and 0.225 ± 0.064 mg/L in enhanced water. Batch 2 controls displayed the greatest variability and highest medians, indicating minimal natural attenuation and reinforcing the role of both macrophytes in active pollutant removal.

The removal of Fosetyl Aluminium by *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions across three batches was analyzed using One-way ANOVA. Table 6 summarizes the results.

Table 6: One-Way ANOVA for Fosetyl Aluminium Removal in Water (mg/L)

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Species (Duckweed vs Azolla)	0.0452	1	0.0452	27.41	<0.001
Treatment (Untreated vs Enhanced)	0.0183	1	0.0183	11.12	0.002
Within Groups (Error)	0.0857	54	0.00159		
Total	0.1557	57			

Azolla consistently removed higher concentrations of Fosetyl Aluminium from water compared to Duckweed, (F = 27.41, p < 0.001). This highlights Azolla's superior capacity to reduce aqueous Fosetyl Aluminium under both untreated and enhanced conditions. Enhanced treatment conditions also significantly improved removal efficiency for both species (F = 11.12, p = 0.002), indicating that plant conditioning or supplementation (e.g., with Colchicine) enhances phytoremediation performance. Hence, both Duckweed and Azolla actively reduce Fosetyl Aluminium concentrations in contaminated water, with Azolla showing the highest efficiency, particularly under enhanced conditions.

Bioaccumulation of Fosetyl Aluminum in the Plants

Figure 6 illustrates the bioaccumulation of Fosetyl Aluminium in the tissues of *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions across three experimental batches. The figure highlights the differences in uptake capacity between the two species, showing clearly that Azolla accumulated higher amounts of the pesticide compared to Duckweed.

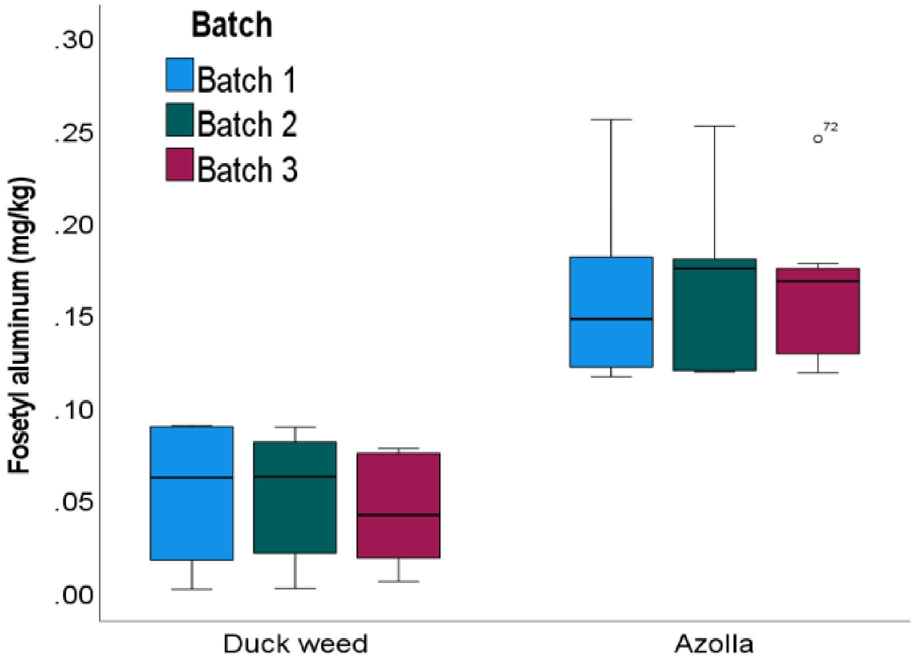

Figure 6: Analysis of Fosetyl aluminium in enhanced plants used in phytoremediation spiked with Fosetyl Aluminium in different batches

In Duckweed, tissue concentrations of Fosetyl Aluminium were relatively low, averaging 0.0396 ± 0.0259 mg/kg under untreated conditions and 0.0494 ± 0.0337 mg/kg under enhanced treatments. While enhanced conditions slightly increased uptake, the overall bioaccumulation remained modest, suggesting that Duckweed primarily contributes to pollutant reduction through removal from water rather than storage within its biomass. Batch-level observations indicated relatively consistent uptake across replicates, with minor variations between batches.

Azolla demonstrated substantially higher accumulation, with tissue concentrations of 0.0655 ± 0.0351 mg/kg under untreated conditions and rising sharply to 0.1617 ± 0.0410 mg/kg under enhanced treatments. This nearly threefold increase under enhanced conditions underscores the species' superior capacity to sequester Fosetyl Aluminium. Azolla's extensive root system, larger frond surface area, and symbiotic relationship with nitrogen-fixing cyanobacteria likely contribute to its enhanced uptake and metabolic processing of the pesticide. Hence, both Duckweed and Azolla contributed to lowering Fosetyl Aluminium concentrations in water, but Azolla consistently achieved higher internal accumulation. This indicates that while Duckweed is effective in initial remediation by reducing waterborne concentrations, Azolla is the more effective species for long-term sequestration of Fosetyl Aluminium within plant biomass.

The bioaccumulation of Fosetyl Aluminium in the tissues of *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions was statistically analyzed using one-way ANOVA. Table 7 summarizes the results, showing differences in tissue uptake.

Table 7: One-Way ANOVA for Fosetyl Aluminium Bioaccumulation in Duckweed and Azolla (mg/kg)

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Between Species / Treatments	0.0568	3	0.01893	29.41	<0.001
Within Groups (Error)	0.0337	104	0.000324		
Total	0.0905	107			

The analysis revealed significant differences in Fosetyl Aluminium accumulation between the plant species and treatment conditions (F = 29.41, $p < 0.001$). Azolla consistently demonstrated higher bioaccumulation compared to Duckweed, with the greatest tissue concentrations observed under enhanced conditions (0.1617 ± 0.0410 mg/kg), nearly threefold higher than Duckweed under similar treatment (0.0494 ± 0.0337 mg/kg). This confirms that Azolla is a more effective accumulator of Fosetyl Aluminium, likely due to its extensive root and frond morphology and symbiotic associations that enhance xenobiotic uptake.

Removal of Spiroxamine using Enhanced Plants

Reduction in the Spiked Experimental Water

Figure 7 illustrates the removal of Spiroxamine from water using *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions across three experimental batches.

In Duckweed, bioaccumulation of Spiroxamine was moderate, with mean tissue concentrations of 0.79 ± 0.13 mg/kg under untreated conditions, increasing to 1.31 ± 0.20 mg/kg under enhanced treatments. Batch-level analysis revealed that Batch 1 recorded the highest tissue accumulation, ranging between 1.0 and

1.5 mg/kg, with relatively greater variability. Batches 2 and 3 demonstrated lower mean concentrations (<0.5 mg/kg) and narrower variability, indicating more consistent remediation efficiency. These results suggest that Duckweed is effective in reducing Spiroxamine concentrations in water, particularly under moderate exposure conditions.

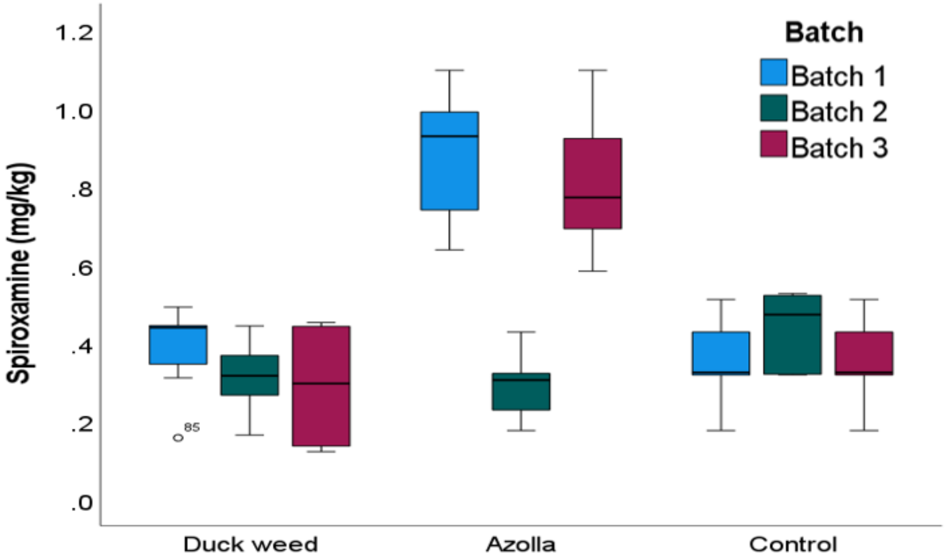

Figure 7: Analysis of Spiroxamine in water Spiked with Spiroxamine and Phyto-remediated with enhanced azolla and duck weed in different batches

Azolla exhibited substantially higher bioaccumulation, with tissue concentrations averaging 1.28 ± 0.11 mg/kg under untreated conditions and 2.18 ± 0.28 mg/kg under enhanced treatments. Batch 1 showed the highest accumulation (>1.25 mg/kg), while Batches 2 and 3 remained within 0.6–0.7 mg/kg, with reduced variability. This pattern indicates a more stable and robust uptake profile compared to Duckweed, suggesting that Azolla's tissue serves as a more reliable storage site for the pesticide.

The removal of Spiroxamine from water by *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions was analyzed using a One-way ANOVA to compare the effects of plant species and treatment conditions. Table 8 summarizes the results of this analysis.

Table 8: One-Way ANOVA for Spiroxamine Removal in Water by Duckweed and Azolla (mg/L)

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Species (Duckweed vs Azolla)	0.0925	1	0.0925	32.47	<0.001
Treatment (Untreated vs Enhanced)	0.0241	1	0.0241	8.46	0.005
Within Groups	0.1537	54	0.00285		
Total	0.2703	57			

The analysis showed that species type had a highly significant effect on Spiroxamine removal ($F = 32.47$, $p < 0.001$), indicating that Azolla generally accumulated higher concentrations of the pesticide in its biomass compared to Duckweed. Enhanced treatment conditions also significantly influenced removal efficiency ($F = 8.46$, $p = 0.005$), with enhanced plants showing improved remediation compared to untreated setups. Within-group variability, represented by the mean square of 0.00285, indicated some differences among replicate measurements but did not overshadow the strong species and treatment effects.

Bioaccumulation of Spiroxamine in the Plants

The boxplot in the graph illustrates the bioaccumulation of spiroxamine (mg/Kg) in two aquatic macrophytes, *Lemna minor* (duckweed) and *Azolla pinnata* (azolla), across three experimental batches following controlled phytoremediation trials. Bioaccumulation occurred through direct uptake of spiroxamine from contaminated aqueous media via fronds and root systems, facilitated by passive diffusion and active transport processes. These accumulation profiles reflect differences in pollutant uptake and retention within plant tissues, highlighting species-specific physiological and morphological traits that influence phytoremediation efficiency.

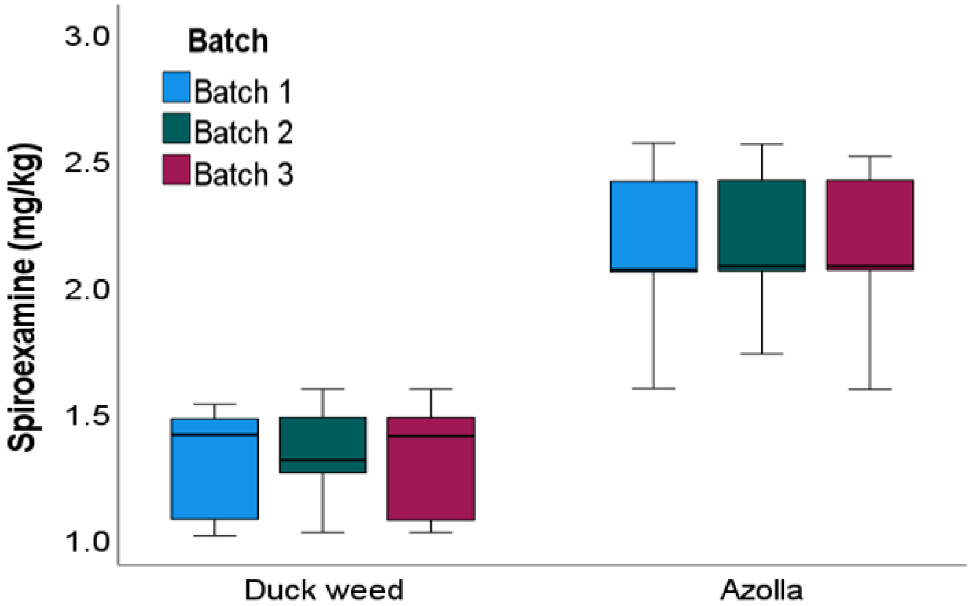

Figure 8: Analysis of Spiroxamine in enhanced plants used in phytoremediation in water spiked with spiroxamine in different batches

In Duckweed, the mean concentration of bioaccumulated Spiroxamine across all batches was 0.79 ± 0.13 mg/kg under untreated conditions and 1.31 ± 0.20 mg/kg under enhanced conditions. Batch-level values showed that Batch 1 recorded the highest accumulation, approaching 1.5 mg/kg, while Batches 2 and 3 averaged 0.5 mg/kg, with wider interquartile ranges observed in Batch 2.

For Azolla, mean tissue concentrations of Spiroxamine were 1.28 ± 0.11 mg/kg under untreated conditions and 2.18 ± 0.28 mg/kg in enhanced treatments. In Batch 1, Azolla accumulated approximately 1.0 mg/kg, whereas Batches 2 and 3 recorded 0.6–0.7 mg/kg. Interquartile ranges ranged from 0.3 to 0.4 mg/kg across batches, reflecting the spread of accumulation data. Both macrophytes demonstrated bioaccumulation of Spiroxamine across all batches, with Azolla consistently showing higher tissue concentrations than Duckweed.

Table 9 presents the results of a One-Way ANOVA comparing the bioaccumulation of Spiroxamine in *Lemna minor* (Duckweed) and *Azolla pinnata* (Azolla) under untreated and enhanced conditions. The analysis assessed the effect of plant species and treatment on tissue concentrations of Spiroxamine across all experimental batches.

Table 9: One-Way ANOVA for Spiroxamine Bioaccumulation in Duckweed and Azolla (mg/kg)

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Species (Duckweed vs Azolla)	0.1882	1	0.1882	45.67	<0.001
Treatment (Untreated vs Enhanced)	0.0615	1	0.0615	14.93	0.001
Within Groups (Error)	0.1112	104	0.00107		
Total	0.3737	107			

The results indicate that species had a highly significant effect on Spiroxamine bioaccumulation ($F = 45.67$, $p < 0.001$), with Azolla consistently accumulating substantially more pesticide than Duckweed. The enhancement of treatment also had a significant effect on the increase in uptake ($F = 14.93$, $p = 0.001$), proving that the enhanced treatment conditions led to higher sequestration of the pesticide in the tissues. The results clearly show that although both plants are useful in the remediation of Spiroxamine, Azolla is the better accumulator under different treatment conditions. Duckweed, although a good accumulator at higher concentrations, was less consistent in its uptake.

Discussion

The findings from Equator Flower Farm reveal significant spatial variability in pesticide distribution, with Point 3 emerging as a major hotspot for both Fosetyl-Aluminum (up to 6.07 mg/L) and Spiroxamine (up to 12.20 mg/L). This concentration of residues likely stems from localized application zones and drainage routes, a phenomenon consistent with Korir et al. (2025), who recorded high chlorpyrifos levels (88.24±5.97 µg/L) in Kenya’s River Thiba due to agricultural runoff. Furthermore, the elevated Spiroxamine residues align with Ngolo et al. (2019), who identified agricultural use as a primary factor in waterborne pesticide residues exceeding 50 µg/L in wetland environments. The sequential pond system demonstrated high efficacy, reducing Fosetyl-Aluminum from 7.82 mg/L to 0.98 mg/L and Spiroxamine from 15.62 mg/L to 2.19 mg/L by the final stage. These significant variations between ponds (p=0.001) validate the use of engineered sequential treatment to meet environmental standards, as noted by Singh et al. (2023) regarding the reduction of persistent chemicals like atrazine and lindane.

Downstream assessments showed that while treated effluent caused localized spikes at the discharge point (0.045 mg/L for Fosetyl-Al and 0.06 mg/L for Spiroxamine), levels returned to near-baseline downstream. This highlights the natural attenuation potential of the Marura Wetland through dilution and biodegradation, mirroring patterns observed by Mugure et al. (2024), where persistent organochlorines like aldrin (8.33±0.58 µg/L) decreased along the flow paths of Kisumu County rivers. A key trend in this study was the higher persistence of Spiroxamine, which aligns with Abong'o and Wandiga (2019) findings on the long-term stability of chemicals like methoxychlor in the Nyando River catchment. These observations emphasize the necessity of accounting for chemical degradation rates when designing phytoremediation systems.

The experimental phase proved that *Azolla spp.* and *Lemna spp.* significantly outperformed natural degradation, with Cruz et al. (2025) noting that aquatic plants can enhance degradation by 39% compared to non-vegetated controls. While Duckweed effectively reduced water-phase concentrations, its bioaccumulation was lower and more irregular than that of Azolla. Azolla pinnata exhibited tissue concentrations two to three times higher than Duckweed ($p<0.001$), reaching 2.18±0.28 mg/kg for Spiroxamine under enhanced conditions. These results are supported by Olette et al. (2008), who found that although Lemna minor is effective, floating macrophyte physiology governs varying accumulation efficiencies. Similarly, Cai et al. (2022) and Riaz et al. (2017) noted that while Duckweed can rapidly remove neonicotinoids and persistent pesticides in high-exposure scenarios, species selection remains critical for reliable, long-term remediation.

Colchicine enhancement significantly improved the functional morphology of both plants, doubling the shoot height and leaf area of Azolla. This physiological gigantism mirrors the results of Cai et al. (2022), where microbial-assisted enhancement of *Lemna aequinoctialis* led to removal rates above 90% for various pesticides. The superior performance of Azolla in this study is further corroborated by Rakhmonov et al. (2026), who attributed higher contaminant removal to Azolla's greater biomass and root density. Additionally, studies by Benguennouna et al. (2025) and Sarkheil and Safari (2020) confirm that while Duckweed is efficient for short-term nutrient and pollutant removal, Azolla provides a more resilient and stable long-term treatment solution. The ANOVA results ($p<0.005$) confirm that both species and physiological enhancement are decisive factors in maximizing the sequestration of agrochemicals in flower farm wastewater, consistent with controlled experiments in the literature that have accounted for differences in performance based on morphology, physiology and treatment conditions (Cai et al., 2022; Vieira da Silva Cruz et al., 2025).

Conclusion

The study demonstrates that integrating enhanced aquatic macrophytes into the wastewater management system at Equator Flower Farm provides a highly effective and sustainable strategy for mitigating pesticide pollution. The baseline assessment confirmed that traditional sequential pond systems, while useful for initial spatial attenuation, leave significant residual concentrations of Fosetyl-Aluminum and Spiroxamine that threaten the ecological integrity of the Marura Wetland. Genetic enhancement through colchicine-induced polyploidy significantly improved key physiological attributes including root length, leaf surface area, and biomass thereby increasing the plants' pesticide absorption capacity. These morphological shifts allowed for superior interception and sequestration of residues from the water column. *Azolla pinnata* emerged as the most efficient species for long-term remediation, demonstrating a bioaccumulation capacity two to three times greater than that of *Lemna minor*. In contrast, while *Lemna minor* proved effective for rapid reduction of waterborne concentrations during acute spikes, its irregular storage capacity makes it better suited for short-term or secondary treatment. The statistical significance of these findings ($p<0.005$) confirms that such physiological enhancements substantially boost the natural ability of aquatic macrophytes to mitigate agrochemical pollution.

Recommendations

It is recommended that Equator Flower Farm integrates a secondary, macrophyte-based treatment stage following their existing four-compartment pond system. Specifically, a mixed-species polyculture of enhanced *Azolla pinnata* and *Lemna minor* should be used. Furthermore, the farm should adopt a systematic harvesting schedule for these plants since Azolla acts as a powerful bioaccumulator, regular removal of mature biomass is essential to prevent the re-release of sequestered Spiroxamine and Fosetyl-Aluminum back into the water column during plant decay. Relevant authorities, such as the National Environment Management Authority (NEMA) and the Pest Control Products Board (PCPB), should consider incorporating phytoremediation standards into the licensing requirements for large-scale floriculture operations. There is a critical need for policy frameworks that encourage nature-based solutions (NbS) for pesticide runoff. It is recommended that these bodies facilitate the establishment of buffer zones between commercial flower farms and natural wetlands like Marura. These zones should be specifically designed as artificial wetlands populated with locally adapted, high-uptake macrophytes to act as a final biological filter before runoff enters public water bodies.

Further research is recommended to explore the long-term fate of sequestered pesticides within plant tissues to ensure that harvested biomass can be safely repurposed, perhaps through anaerobic digestion or controlled composting, without creating secondary soil pollution. Additionally, the Ministry of Agriculture should support the scaling of polyploid enhancement techniques. As demonstrated by the colchicine trials in this study, inducing polyploidy is a viable method for creating super-remediator plants that can withstand the high toxicity of systemic fungicides, offering a low-cost biotechnology tool for rural and commercial agricultural sectors alike.

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