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Laboratory visual phytotoxicity screening of tropical macrophytes exposed to high-leachate pollution index landfill leachate: Exploratory benchmarks for species selection

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ABSTRACT

Inadequate landfill leachate management threatens aquatic ecosystems in rapidly urbanising tropical regions. This study conducted a preliminary laboratory visual-phytotoxicity screening of four tropical aquatic macrophytes (*Eichhornia crassipes*, *Eleocharis dulcis*, *Salvinia molesta*, and *Hydrilla verticillata*) exposed to high-strength leachate from the Sukawinatan Landfill, Indonesia. Raw leachate had a mean leachate pollution index (LPI) of 44.2. Plants were exposed to 0%, 6.25%, 12.5%, 25%, 50%, and 100% leachate for 14 days, and ordinal visual injury was assessed on Days 7 and 14. The verified dataset comprised 144 observations from 72 experimental units. Repeated ordinal responses were analysed using weakly regularised Bayesian cumulative-link mixed-effects models, with experimental unit included as a random intercept; exact paired sign tests and day-specific Kruskal-Wallis/Dunn analyses were used as sensitivity analyses. Increasing concentration consistently increased the odds of severe visual injury for all species, whereas concentration-by-day interactions remained uncertain. After Holm correction, paired analyses did not establish a general deterioration between Days 7 and 14. *Eleocharis dulcis* showed the widest visual-tolerance range, while *Salvinia molesta* was the most sensitive. Approximate moderate-injury benchmarks were 3.13%, 4.17%, 8.33%, and 33.33% for *S. molesta*, *H. verticillata*, *E. crassipes*, and *E. dulcis*, respectively. These values are exploratory visual-effect benchmarks, not physiological EC50 values or field-operational limits. Raw or weakly diluted high-LPI leachate therefore requires pretreatment or substantial dilution before pilot-scale macrophyte testing.

Keywords: constructed wetland, leachate pollution index, phytotoxicity, species screening, tropical macrophytes, visual injury benchmark.

INTRODUCTION

Landfill leachate is a complex effluent containing dissolved organic matter, ammonium nitrogen, heavy metals, xenobiotics, and microbial pathogens. Inadequate containment can threaten aquatic ecosystems through oxygen depletion, eutrophication, and systemic toxicity, particularly in rapidly urbanizing tropical regions where waste generation often exceeds treatment capacity (Lindamulla et al., 2022; Teng et al., 2021). The Sukawinatan Landfill in Palembang, Indonesia, receives large volumes of municipal waste, and its leachate frequently exceeds regulatory limits for BOD5, COD, TSS, Fe, total nitrogen, and total coliform. The leachate pollution index (LPI) was used to integrate these physicochemical and microbiological indicators into a screening metric. A mean LPI of 44.2 confirmed that the leachate represented a high-strength exposure matrix for controlled phytotoxicity testing (Bisht et al., 2025; Rodriguez-Cardenas et al., 2025; Singh and Singh, 2024).

Previous studies have mainly evaluated individual macrophytes through pollutant-removal performance or tolerance to specific contaminants. Direct comparisons among tropical macrophytes with contrasting growth forms under the same high-strength landfill-leachate matrix remain limited. This study addresses that gap by integrating station-specific LPI characterisation, replicate-level ordinal visual-injury assessment, longitudinal analysis, and species-specific tolerance ranking. The contribution is therefore contextual and screening-oriented rather than the development of a new toxicological endpoint or a validated field-operational threshold.

The objectives were to, (i) characterise the physicochemical profile and LPI of Sukawinatan leachate, (ii) evaluate concentration-dependent visual phytotoxicity in *Eichhornia crassipes*, *Eleocharis dulcis*, *Salvinia molesta*, and *Hydrilla verticillata*, (iii) analyse the repeated ordinal observations and derive exploratory visual-effect benchmarks, and (iv) identify preliminary candidates for subsequent constructed-wetland testing while explicitly limiting laboratory-to-field extrapolation.

MATERIAL AND METHODS

Study site and leachate collection

Leachate samples were collected from the retention pond of Sukawinatan Landfill, Palembang, Indonesia (2°58′S, 104°46′E), an active facility discharging toward the Sedapat River system. To capture spatial variation, depth-integrated grab sampling at 0.5–1.0 m depth was conducted at three stations: L1 (inflow), L2 (central), and L3 (outflow), with five replicates per station (n = 15). Samples were collected in pre-cleaned containers, transported in cooled boxes at approximately 4 °C, and analyzed within 24 h following APHA-based procedures (Rice et al., 2012; USEPA, 2002). The preparation of the composite stock is determined as follows: equal volumes of L1, L2, and L3 are homogenised only after verifying that the sample container is intact, the label is complete, the holding time is not exceeded, and there are no visible foreign materials unrelated to the leachate sampling. The composite is gently mixed before dilution to reduce sedimentation bias (Figure 1).

Physicochemical analysis, LPI calculation, and quality control

Parameters relevant to regulatory compliance and plant stress were analysed, including pH, total suspended solids (TSS), biochemical oxygen demand (BOD5), chemical oxygen demand (COD), iron (Fe), cadmium (Cd), lead (Pb), mercury (Hg), total nitrogen (TN), and total coliform. Analyses followed APHA Standard Methods (Rice et al., 2012), and results were benchmarked against Indonesian Regulation P.59/2016 for landfill-leachate discharge. LPI values were calculated using standard parameter weights and sub-index rating curves (Kumar and Alappat, 2005; Rodriguez-Cardenas et al., 2025; V. P. Singh and Singh, 2024).

Analytical quality control (QA/QC) follows the APHA Standard Methods (Rice et al., 2012). Instruments are calibrated before each analysis batch according to standards, pH meters and

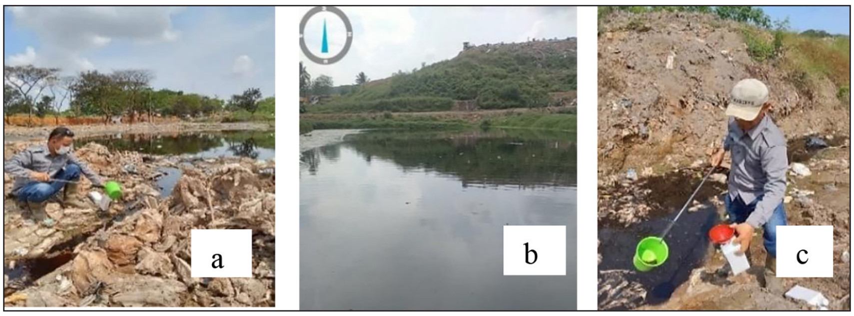

Figure 1. Leachate sampling locations, a. L1 Leachate Station, b. L2 Leachate Station, c. L3 Leachate Station

dissolved oxygen probes are calibrated against zero buffer/oxygen references and saturated air references, and spectrophotometric/titrimetric methods for BOD5, COD, TSS, Fe, Cd, Pb, Hg, and total nitrogen are verified using method blanks, duplicate samples, and calibration standards conducted in certified laboratories (BLKM and Baristand Lab) where leachate sample inspections are carried out as data for LPI calculations.

Dilution media and composite exposure stock

A geometric dilution series of 0%, 6.25%, 12.5%, 25%, 50%, and 100% (v/v) leachate was prepared from the homogenised composite stock using quality-controlled freshwater. The dilution medium was inspected for visible contamination, turbidity, oil films, and suspended debris, and its initial pH, dissolved oxygen, and temperature were recorded before use. The same dilution medium was used across all treatments to minimise variation unrelated to leachate concentration.

Plant materials, size standardisation and acclimation

The selected macrophytes represent various forms of functional growth: *Eichhornia crassipes* and *Salvinia molesta* that float freely, *Hydrilla verticillata* that is submerged, and *Eleocharis dulcis* that is emergent (Agarry et al., 2020; Shrivastava and Srivastava, 2021; Zahari et al., 2023). Healthy specimens were collected from uncontaminated freshwater habitats and acclimated for 7–14 days under controlled conditions. Initial plant material was standardised as 20 g fresh weight for *S. molesta* and *H. verticillata*, one approximately 200-g clump for *E. crassipes*, and one 30-stem clump for *E. dulcis*. Plants showing visible necrosis, severe chlorosis, pest damage, or abnormal morphology were excluded before exposure (Figure 2).

Bioassay design and exposure monitoring

The experiment followed a completely randomised design comprising four species, six leachate concentrations, and three independent experimental units per species–concentration combination, giving 72 experimental units. Each unit was assigned a unique identifier based on species, concentration, and replicate number. Static exposure lasted 14 days without aeration. The same units were assessed non-destructively on Days 7 and 14; therefore, the two observations were treated as repeated measurements rather than independent replicates. Temperature, pH, and dissolved oxygen were monitored on Days 0, 7, and 14, and no pH adjustment was performed.

The measured water pH, dissolved oxygen (DO), and temperature for each leachate concentration treatment on Days 0, 7, and 14 are reported in Table S3 (mean values, n = 3 replicate containers per treatment). During the exposure period, pH values ranged from 7.1 to 8.6, showing a gradual increase at higher leachate concentrations, particularly in the 100% leachate treatment at Days 7 and 14. Dissolved oxygen concentrations ranged from 7.2 to 7.9 mg/L, remaining relatively stable throughout the exposure period across all treatments. Water temperature ranged from 30.55 to 32.00 °C, indicating consistent environmental conditions during the experiment. Overall, the monitored physicochemical parameters showed only minor variations among treatments and exposure periods. Therefore, the observed morphological injury responses of macrophytes were considered primarily associated with differences in leachate concentration rather than substantial changes in water-quality conditions.

Figure 2. Natural habitats of the macrophyte species used in this study: a) *Eleocharis dulcis,* b) *Eichhornia crassipes,* c) *Hydrilla verticillata,* d) *Salvinia molesta*

Exposure was conducted in 5-L containers at a water depth of approximately 50 cm using a static protocol adapted for tropical macrophytes (OECD, 2006, 2014; USEPA, 2002). The six treatments were prepared from the composite stock with the same freshwater diluent (ERA, 1998; USEPA, 2002). Initial biomass and stem number were standardised by growth form as described above. The endpoint was restricted to visual injury; destructive biomass harvesting, physiological measurements, tissue accumulation, and pollutant-removal efficiency were not assessed. Consequently, the derived concentrations are visual-effect benchmarks rather than conventional physiological EC50 values or operational wetland limits.

Visual scoring and endpoint assessment

Plant condition was assessed independently for each experimental unit on Days 7 and 14 using a five-point ordinal visual-injury scale adapted from established protocols (ERA, 1998; Menone et al., 2023): 1 = healthy; 2 = mild stress (slight chlorosis, <10% of visible tissue affected); 3 = moderate stress (chlorosis and partial necrosis, 10–50% affected); 4 = severe stress (extensive necrosis, >50% affected, with apparent growth cessation); and 5 = near-death/dead (≥90% tissue loss or severe collapse). Original replicate-level scores were retained for statistical analysis. Treatment medians and minimum–maximum ranges were calculated only for descriptive presentation.

Statistical analysis

Ordinal visual-injury scores were analysed separately for each species using weakly regularised Bayesian cumulative-link mixed-effects models with a cumulative-logit link. Ordered leachate concentration, observation day, and their interaction were specified as fixed effects, and experimental unit was included as a random intercept to account for paired Day 7 and Day 14 observations. Concentration was coded as an ordered six-level variable corresponding to 0%, 6.25%, 12.5%, 25%, 50%, and 100%. Models were implemented in Python 3.11 using NumPyro 0.21.0 with four chains, 750 warm-up iterations, and 750 retained draws per chain. Results are reported as odds ratios (ORs) with 95% Bayesian credible intervals (CrIs). Weak regularisation was used because extensive ties and upper-category scores produced complete or quasi-complete separation in conventional maximum-likelihood cumulative-link models.

Within-unit changes from Day 7 to Day 14 were additionally evaluated using exact paired sign tests, with Holm adjustment across the four species. Day-specific Kruskal-Wallis tests followed by Dunn-Bonferroni comparisons against the control were conducted as sensitivity analyses. Day 7 and Day 14 observations were analysed separately in these tests and were not pooled as independent replicates. The Kruskal-Wallis eta-squared statistic was reported only as a rank-based descriptive index, particularly because tied scores can generate boundary estimates.

Convergence was evaluated using divergent transitions, R-hat, and effective sample sizes. Across the species-specific models, no divergent transitions occurred; maximum R-hat ranged from 1.0021 to 1.0041, minimum bulk effective sample size ranged from 690 to 1175, and minimum tail effective sample size ranged from 812 to 1109. Approximate moderate-injury benchmarks were obtained by bracketing interpolation of treatment medians at a visual-injury score of 3.0. These interpolated values were used only as descriptive screening estimates and were not interpreted as EC50 values, physiological thresholds, regulatory limits, or field-operational criteria.

Conceptual workflow and numbered equations

The analytical workflow linking leachate characterisation, repeated ordinal observations, longitudinal modelling, sensitivity analyses, and exploratory benchmark interpretation is summarised in Figure 3.

The principal calculations used to connect leachate characterisation with descriptive visual-effect benchmarks are outlined below. Because the endpoint was ordinal, the interpolation equations are screening-oriented and do not represent conventional physiological toxicity models.

$$LPI = \frac{\sum_{i=1}^{m} w_i p_i}{\sum_{i=1}^{m} w_i} \tag{1}$$

where: *LPI* is the leachate pollution index, *wi* is the weight assigned to parameter *i, pi* is the corresponding sub-index score, and *m* is the number of parameters included in the index calculation, following the standardized weighting and rating-curve procedure of Kumar and Alappat (2005).

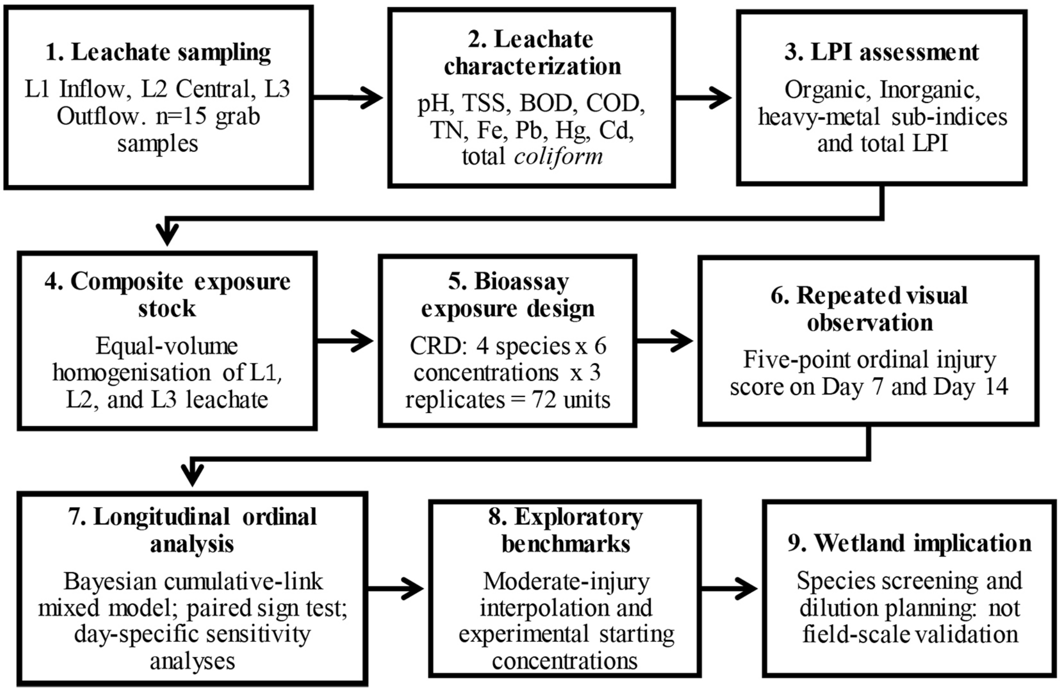

Figure 3. Conceptual workflow linking leachate characterisation, repeated ordinal visual observations, longitudinal analysis, and exploratory benchmark interpretation

$$C_{comp,j} = \frac{(C_{L1,j}+C_{L2,j}+C_{L3,j})}{3} \quad (2)$$

$$L_c(\%) = \left[\frac{V_L}{V_L+V_F}\right] \times 100 \quad (3)$$

where: *Ccomp,j* represents the parameter *j* concentration in the composite stock averaged from the inflow *CL*1,*j*, central *CL*2,*j*, and outflow *CL*3,*j* stations per APHA standards (Rice et al., 2012); while Lc denotes the target bioassay concentration derived from the volumes of composite leachate VL and dilution freshwater VF following standard static dilution-series guidelines (OECD, 2006, 2014; USEPA, 2002).

$$S_{median}(s,c,t) = median[S(s,c,t,1), S(s,c,t,2), S(s,c,t,3)] \quad (4)$$

where: *Smedian*(*s,c,t*) is the median visual injury score for species *s* at concentration *c* and observation time *t*, *S*(*s,c,t,r*) is the ordinal injury score recorded for replicate r, and r = 3 experimental replicates, scored according to the ordinal phytotoxicity rubric adapted from ERA (1998) and Menone et al. (2023).

The median, rather than the arithmetic mean, is used to aggregate replicate scores because the injury scale is ordinal, consistent with the non-parametric analytical framework.

$$H = \frac{12}{N(N+1)} \times \sum_{g=1}^{k} \frac{R_g^2}{n_g} - 3(N+1) \quad (5)$$

where: *H* is the Kruskal–Wallis statistic, *k* is the number of concentration groups, *ng* is the number of observations in group *g*, *Rg* is the sum of ranks for group *g*, and *N* is the total number of observations, calculated following the original rank-based non-parametric test formulation of Kruskal and Wallis (1952).

Tie correction should be applied by the statistical software when tied ordinal scores occur.

$$\eta^2 H = \frac{(H-k+1)}{(n-k)} \quad (6)$$

where: $\eta^2 H$ is the Kruskal–Wallis eta-squared effect size, *H* is the Kruskal–Wallis statistic, *k* is the number of groups, and *n* is the total number of observations, calculated using the non-parametric effect-size formula of Tomczak and Tomczak (2014).

$$C_{MI,s} = C_a + \left[\frac{3-S_a}{S_b-S_a}\right] \times (C_b - C_a) \quad (7)$$

where: $C_{MI,s}$ is the approximate moderate-injury threshold for species s, defined as the interpolated concentration corresponding to a median visual injury score of approximately 3.0; C_a and C_b are the lower and upper adjacent concentrations surrounding the score of 3.0, and S_a and S_b are their corresponding median visual injury scores.

Where the reported median already equals exactly 3.0 at a tested concentration, $C_{MI,s}$ is taken directly as that concentration and no interpolation is required. This simple bracketing linear-interpolation procedure was used only to describe an approximate visual-effect threshold and should not be interpreted as a conventional EC_{50} or physiological inhibition estimate.

$$C_{screen} = SF \times min(C_{LVIC,s}) \quad (8)$$

where: C_{screen} is an exploratory starting concentration obtained by applying a precautionary factor of 0.5 to the lowest concentration producing visible injury.

Equations 1–3 describe leachate characterisation and dilution; Equation 4 defines median ordinal-score aggregation; Equations 5–6 describe the day-specific rank-based sensitivity analyses; and Equations 7–8 provide descriptive visual-effect benchmarks. The longitudinal Bayesian model was fitted directly to the replicate-level ordinal observations. All derived concentrations are preliminary laboratory screening values rather than physiological EC50 values, ecological safety thresholds, pollutant-removal measures, or field-operational limits.

RESULTS AND DISCUSSION

Leachate characteristics

The physicochemical and microbiological characteristics of Sukawinatan leachate are summarized in Table 1. The leachate exhibited high pollution strength, with exceedances for TSS, BOD5, COD, total nitrogen, Fe, and total coliform relative to Indonesian regulatory limits. These results confirm that the leachate is not suitable for direct environmental release or direct macrophyte introduction without pretreatment.

As outlined in Table 1, Sukawinatan leachate exhibited high pollution strength, with multiple parameters significantly violating Indonesian regulatory limits. While its overall physicochemical profile aligns with regional Southeast Asian landfill characteristics, the concurrent violation of organic, particulate, nutrient, and microbial thresholds confirms its absolute unsuitability for direct environmental release, rendering robust containment and integrated pretreatment mandatory prior to biological treatment or final discharge.

Leachate pollution index

The total LPI ranged from 42.2 to 47.4, with a mean of 44.2, far above the commonly used high-contamination threshold of 7.5. The organic sub-index was the main contributor, reflecting high organic and nitrogen loading. This result supports the use of Sukawinatan leachate as a high-strength stress matrix for preliminary tolerance screening.

As presented in Table 2, the total Leachate Pollution Index (LPI) values ranged from 42.2 to 47.4 (mean: 44.2), drastically exceeding the critical contamination threshold of 7.5 (V. P. Singh and Singh, 2024). Spatial variation was minimal, with all stations consistently falling within the severe pollution category. The organic sub-index was the primary driver of this high severity (mean: 70.40), reflecting substantial BOD_5, COD, and total nitrogen loads, followed by the heavy-metal sub-index, which remains ecologically critical due to elevated Fe levels (Essien et al., 2022; Kou et al., 2024). Consistent with recent recommendations for transparent sub-index reporting to improve diagnostic accuracy (Bisht et al., 2025; Chaudhary et al., 2021), this station-specific breakdown confirms that Sukawinatan leachate provides a high-strength organic and nitrogen-driven exposure baseline ideal for macrophyte tolerance screening.

Phytotoxicity bioassay responses

Visual damage increased with the concentration of leachate and varied among species. All controls remained healthy. *Salvinia molesta* reached maximum damage at the lowest concentration tested, while *Eleocharis dulcis* maintained the widest visual tolerance window. The results are explained as a visual response because biomass, pollutant removal, and physiological endpoints were not measured.

As shown in Table 3, responses were strongly concentration-dependent and species-specific. *Salvinia molesta* reached score 5 at 6.25% on both days. *Eichhornia crassipes* retained only a mild response at 6.25% and reached score 5 from 12.5% upward. *Hydrilla verticillata* showed moderate injury at 6.25% on Day 7 and severe injury at the

Table 1. Characteristics of Sukawinatan Landfill leachate compared with those of other landfills in Indonesia and Southeast Asia.

Parameter	Sukawinatan	Other Indonesian landfills[1)]	Southeast Asian landfills[2)]	Standard
pH	8.54	7.6–8.5	7.4–8.46	6–9
Temperature (°C)	28	25.8–29.3	27.5–31	–
TSS (mg/L)	321.5	≥100–>1,000	>2000	100
BOD5 (mg/L)	723.5	423–4,700	84–2.073	150
COD (mg/L)	>1,650	143.5–4,000	590–2,920	300
Total N (mg/L)	98.6	91–4,955	<10 – >1.000	60
Fe (mg/L)	8.95	0.6–1.8	1.447–12.81	5.0
Pb (mg/L)	0.020	0.01–0.1	0.001–0.1	0.1
Hg (mg/L)	<0.0004	<0.05–0.01	0.001–0.02	0.005
Cd (mg/L)	<0.0031	0.01–0.03	0.01–0.05	0.1
Total coliform (MPN/100 mL)	37,396	10^3–10^7	10^3–10^7	3.000

Note: [1)] (Arliyani et al., 2023; Bagastyo and Anggrainy, 2024; Emalya et al., 2020; Isnadina et al., 2019; Putri and Wardiha, 2020), [2)] (Bisht et al., 2025; Hoai et al., 2021; Mohd-Salleh et al., 2020; Xaypanya et al., 2018).

Table 2. Leachate Pollution Index by station and sub-component at Sukawinatan Landfill

Station	LPI organic sub-index	LPI inorganic sub-index	LPI heavy-metal sub-index	Total LPI
L1	75.43	14.81	25.19	47.4
L2	67.31	8.43	27.94	42.2
L3	68.46	11.38	24.97	42.9
Mean	70.40	11.54	26.03	44.2

Note: values represent LPI sub-index scores, not pollutant concentrations. LPI > 7.5 indicates high contamination requiring intensive treatment.

Table 3. Visual injury scores [Median (min–max), n = 3] by species, concentration, and exposure day

Species	Day	0%	6.25%	12.5%	25%	50%	100%
Eichhornia crassipes	D7	1 (1–1)	2 (2–2)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)
Eichhornia crassipes	D14	1 (1–1)	2 (2–2)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)
Eleocharis dulcis	D7	1 (1–1)	1 (1–1)	2 (2–2)	2 (2–2)	3 (2–4)	5 (5–5)
Eleocharis dulcis	D14	1 (1–1)	1 (1–1)	2 (2–2)	2 (2–2)	5 (5–5)	5 (5–5)
Salvinia molesta	D7	1 (1–1)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)
Salvinia molesta	D14	1 (1–1)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)
Hydrilla verticillata	D7	1 (1–1)	3 (2–4)	4 (4–5)	5 (5–5)	5 (5–5)	5 (5–5)
Hydrilla verticillata	D14	1 (1–1)	4 (4–5)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)

Note: scores represent ordinal visual injury: 1 = healthy; 2 = mild stress; 3 = moderate stress; 4 = severe stress; and 5 = near-death/dead. D7 = Day 7; D14 = Day 14. Values are median (minimum–maximum) across three independent experimental units. Verification of the replicate-level SAV dataset confirmed that the three scores were identical within every species × concentration × day cell; therefore, the minimum and maximum equal the median.

same concentration on Day 14, with score 5 from 25% on Day 7 and from 12.5% on Day 14. *Eleocharis dulcis* showed no injury at 6.25%, mild injury at 12.5–25%, and the broadest tolerance range.

Table 4 indicates that *Salvinia molesta* had the lowest approximate moderate-injury benchmark (3.13% on both days). *Hydrilla verticillata* decreased descriptively from 6.25% on Day 7 to 4.17% on Day 14, while *Eichhornia crassipes* remained at 8.33%. *Eleocharis dulcis* had the highest benchmark, decreasing from 50.00% on Day 7 to 33.33% on Day 14. Because the values were interpolated from ordinal medians across broad concentration intervals, they should be read as

Table 4. Median-based approximate moderate-injury threshold derivation for each species and observation day

SpecieS	Day	Bracketing concentrations (Ca–Cb)	Median scores (Sa–Sb)	CMI,s,t (%)	Method
Eichhornia crassipes	D7	6.25–12.5	2–5	8.33	Interpolated
Eichhornia crassipes	D14	6.25–12.5	2–5	8.33	Interpolated
Eleocharis dulcis	D7	Exact match	median = 3 at 50%	50.00	Exact match
Eleocharis dulcis	D14	25–50	2–5	33.33	Interpolated
Salvinia molesta	D7	0–6.25	1–5	3.13	Interpolated
Salvinia molesta	D14	0–6.25	1–5	3.13	Interpolated
Hydrilla verticillata	D7	Exact match	median = 3 at 6.25%	6.25	Exact match
Hydrilla verticillata	D14	0–6.25	1–4	4.17	Interpolated

Note: CMI,s,t denotes the approximate concentration at which the median ordinal visual-injury score reaches 3.0 for species s at observation time t. The value is a descriptive visual-effect benchmark and is not a conventional EC50, physiological inhibition threshold, regulatory limit, or field-operational criterion.

approximate response-transition locations rather than precisely measured toxicological thresholds. The concentration profiles supported the visual-tolerance hierarchy *Eleocharis dulcis* > *Eichhornia crassipes* > *Hydrilla verticillata* > *Salvinia molesta* (Figure 4).

Species-level interpretation

Eleocharis dulcis exhibited the widest visual-tolerance range, showing no injury at 6.25% and only mild injury at 12.5–25%. Its emergent growth form may reduce direct contact between photosynthetic tissues and the leachate matrix, although rhizosphere buffering and tissue-level defence mechanisms were not measured (Khan et al., 2024; Prihatini, 2021; Santosa and Zaman, 2021). *Eichhornia crassipes* showed a narrow low-dose tolerance window and deteriorated abruptly at concentrations of 12.5% or higher. *Hydrilla verticillata* displayed the clearest tendency toward greater injury on Day 14, but this temporal signal did not remain statistically significant after Holm correction and is therefore suggestive rather than confirmatory. *Salvinia molesta* was the most visually sensitive species because the lowest non-zero concentration already produced maximum injury. The resulting hierarchy is restricted to short-term visual injury and must not be extrapolated to physiological tolerance, pollutant-removal capacity, or field-scale wetland performance.

Longitudinal ordinal analysis and sensitivity analyses

The verified dataset comprised 144 ordinal observations from 72 experimental units, each measured on Days 7 and 14, with no missing observations. All three replicate scores were identical within each species × concentration × day cell, and 84 of 144 observations (58.3%) reached the maximum score of 5. This discrete structure generated extensive ties, a pronounced ceiling effect, and complete or quasi-complete separation in conventional cumulative-link models, supporting the use of weak Bayesian regularisation.

The Bayesian cumulative-link mixed-effects models consistently supported a strong concentration effect. For every species, the 95% CrIs for the concentration OR were entirely above 1 on both observation days (Table 5), indicating that successive increases in the ordered leachate series raised the probability of belonging to a more severe visual-injury category. The concentration-by-day interaction remained uncertain for every species because each 95% CrI included 1. *Hydrilla verticillata* showed the clearest model-based temporal signal at the average concentration (Day 14 vs Day 7 OR = 11.47; 95% CrI: 1.86–87.83), but the interval was wide because of limited replication, ties, and ceiling scores. This estimate was therefore interpreted together with the exact paired analysis rather than as stand-alone confirmation of deterioration.

Holm adjustment was applied across the four species. A worsened unit had a higher injury score on Day 14; an improved unit had a lower score.

No experimental unit of *Eichhornia crassipes* or *Salvinia molesta* changed between Days 7 and 14 (Table 6). *Eleocharis dulcis* worsened in 3 of 18 units, all at 50% leachate, but the pattern was not statistically supported (exact $p = 0.2500$; Holm-adjusted $p = 0.7500$). *Hydrilla verticillata* worsened in 6 of 18 units and had an unadjusted exact p-value of

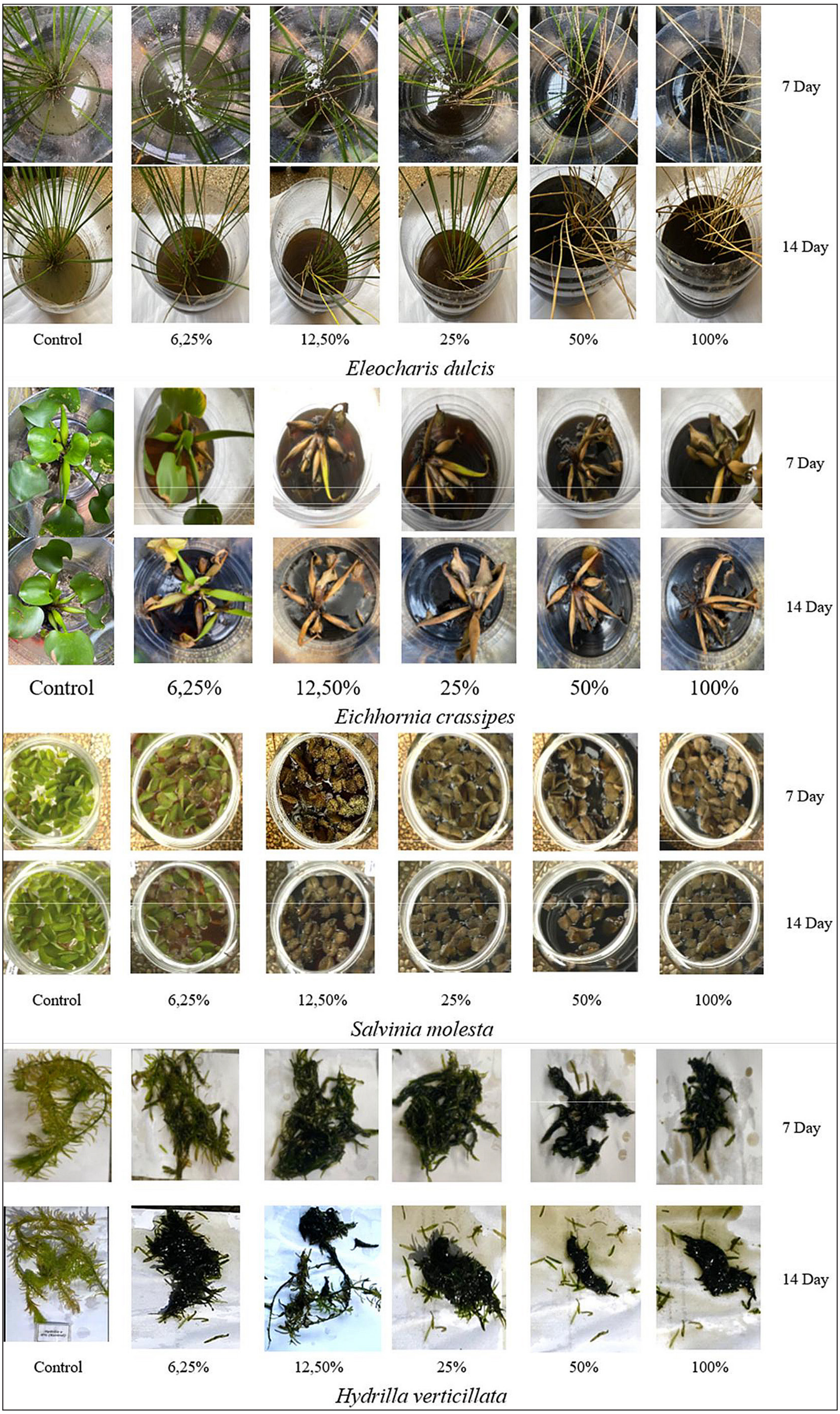

Figure 4. Visual phytotoxicity response of tropical macrophytes exposed to various leachate concentrations

Table 5. Weakly regularised Bayesian cumulative-link mixed-effects model estimates for ordinal visual-injury scores

Species	Concentration OR, Day 7 (95% CrI)	Concentration OR, Day 14 (95% CrI)	Day 14 vs Day 7 at average concentration, OR (95% CrI)	Concentration × day OR (95% CrI)
Eichhornia crassipes	11.53 (4.42–37.02)	22.64 (6.22–114.75)	4.17 (0.78–29.64)	1.97 (0.58–7.34)
Eleocharis dulcis	10.15 (4.22–27.50)	25.35 (8.50–107.92)	0.84 (0.21–3.26)	2.49 (0.84–9.12)
Hydrilla verticillata	10.10 (4.15–32.92)	17.09 (5.21–69.29)	11.47 (1.86–87.83)	1.71 (0.49–5.76)
Salvinia molesta	4.12 (1.58–12.54)	7.01 (2.11–28.76)	4.44 (0.64–34.81)	1.70 (0.48–6.09)

Note: OR > 1 indicates higher odds of belonging to a more severe visual-injury category. The concentration OR represents a one-step increase in the ordered series 0%, 6.25%, 12.5%, 25%, 50%, and 100%. CrI = Bayesian credible interval. An interaction CrI that includes 1 indicates insufficient evidence that the concentration-injury slope changed between Day 7 and Day 14.

Table 6. Paired changes in visual-injury scores between Day 7 and Day 14

Species	Paired units	No change	Worsened	Improved	Exact p-value	Holm-adjusted p-value
Eichhornia crassipes	18	18	0	0	1.0000	1.0000
Eleocharis dulcis	18	15	3	0	0.2500	0.7500
Hydrilla verticillata	18	12	6	0	0.0312	0.1250
Salvinia molesta	18	18	0	0	1.0000	1.0000

Note: Exact paired sign tests were based on the within-unit change from Day 7 to Day 14.

Table 7. Day-specific Kruskal-Wallis and Dunn-Bonferroni sensitivity analyses

Species	Day 7: concentrations different from control	Day 14: concentrations different from control
Eichhornia crassipes	12.5%, 25%, 50%, and 100% (all p_adj = 0.0195)	12.5%, 25%, 50%, and 100% (all p_adj = 0.0195)
Eleocharis dulcis	100% (p_adj = 0.0062)	50% and 100% (both p_adj = 0.0178)
Hydrilla verticillata	25%, 50%, and 100% (all p_adj = 0.0153)	12.5%, 25%, 50%, and 100% (all p_adj = 0.0195)
Salvinia molesta	6.25%, 12.5%, 25%, 50%, and 100% (all p_adj = 0.0070)	6.25%, 12.5%, 25%, 50%, and 100% (all p_adj = 0.0070)

Note: For each species on each observation day, the Kruskal-Wallis result was H = 17.0, df = 5, and p = 0.0045. The corresponding $\eta^2 H$ value was 1.00, but this boundary estimate primarily reflects identical replicate scores within treatment cells and extensive tied ranks; it should not be interpreted as a precise biological magnitude. p_adj = Bonferroni-adjusted p-value.

0.0312; however, the evidence did not remain significant after Holm correction (adjusted p = 0.1250). The paired analysis therefore did not establish a general temporal deterioration between the two observation days (Table 7).

Day-specific analyses corroborated the concentration-response pattern without treating repeated observations as independent. *Salvinia molesta* differed from the control at every non-zero concentration on both days. *Eichhornia crassipes* differed from the control from 12.5% upward. *Hydrilla verticillata* differed from the control from 25% on Day 7 and from 12.5% on Day 14. *Eleocharis dulcis* retained the widest visual-tolerance window, with significant differences confined to 100% on Day 7 and 50–100% on Day 14. These comparisons support the species-sensitivity ranking but remain conditional on the ordinal visual endpoint and three replicates per treatment (Table 8).

Salvinia molesta had the lowest approximate moderate-injury benchmark, followed by *Hydrilla verticillata* and *Eichhornia crassipes*, whereas *Eleocharis dulcis* had the highest benchmark and the widest visual-tolerance range. The 3.125% multi-species value should be used only as an exploratory starting concentration for confirmatory bioassays. Because it was derived from a simple precautionary factor and ordinal visual observations, it does not establish long-term survival, treatment efficiency, or environmental safety.

Table 8. Species-level visual-effect benchmarks and exploratory starting concentrations derived from ordinal visual-injury scores

Species	No-visible-injury boundary	Lowest visible-injury concentration	Conservative species-level approximate moderate-injury threshold, C(MI,s) (%)	Safety-adjusted preliminary screening value (%)	Sensitivity interpretation
Salvinia molesta	<6.25%	6.25%	3.13	3.125	Highest sensitivity
Hydrilla verticillata	<6.25%	6.25%	4.17	3.125	High sensitivity; suggestive temporal worsening
Eichhornia crassipes	<6.25%	6.25%	8.33	3.125	Moderate sensitivity; narrow low-dose tolerance
Eleocharis dulcis	6.25%	12.5%	33.33	6.25	Lowest sensitivity; most tolerant species

Note: the approximate moderate-injury benchmark is the lower Day 7 or Day 14 interpolation for each species. Exploratory starting concentrations were obtained by applying a precautionary factor of 0.5 to the lowest concentration producing visible injury. The multi-species value of 3.125% is an experimental starting concentration, not an ecological safety threshold, regulatory limit, EC50, or constructed-wetland design criterion.

Preliminary implications for constructed wetland species selection

Raw or weakly diluted high-LPI Sukawinatan leachate rapidly produced severe visual injury, supporting the need for pretreatment or substantial dilution before macrophyte introduction. *Eleocharis dulcis* retained the widest visual-tolerance range and is therefore identified as a candidate for further confirmatory testing rather than as a species already demonstrated to be superior for constructed-wetland application. This preliminary ranking is based solely on short-term visual injury; it does not establish which species would perform best in a constructed wetland, since that determination additionally requires physiological stress responses, biomass production, pollutant-removal efficiency, and longer-term field or mesocosm trials that were not assessed here. *Salvinia molesta* and *Hydrilla verticillata* were more sensitive and may have value as early-warning taxa under lower-strength exposure. These interpretations remain conditional on the static laboratory system because constructed-wetland performance also depends on hydraulic retention time, substrate interactions, microbial activity, nutrient availability, seasonal conditions, and pollutant transformation. The repeated-measures analyses did not demonstrate a general deterioration from Day 7 to Day 14 after multiplicity correction. Visual scoring is appropriate for initial screening but cannot substitute for biomass, chlorophyll, root and shoot growth, oxidative-stress biomarkers, tissue accumulation, or pollutant mass-balance measurements.

Study limitations

Several limitations define the evidential scope of this study. First, exposure was restricted to a 14-day static laboratory bioassay. Second, phytotoxicity was assessed using an ordinal visual endpoint without quantitative biomass, physiological, biochemical, tissue-accumulation, or pollutant-removal measurements. No biomass, chlorophyll content, root/shoot length, or other quantitative growth endpoints were recorded in this study as defining limits, and the conclusions below are limited to visual phytotoxicity and do not extend to physiological tolerance, growth performance, or pollutant removal capacity. Third, only three experimental units were available per treatment. Identical within-cell scores and the 58.3% ceiling frequency produced extensive ties and separation, so Bayesian regularisation yielded finite estimates but could not replace the biological information obtainable from greater replication and continuous endpoints. Fourth, the study used leachate from a single landfill and therefore does not establish broadly generalisable concentration limits.

CONCLUSIONS

Sukawinatan Landfill leachate exhibited severe pollution, with a mean LPI of 44.2 and multiple exceedances of Indonesian discharge limits. Longitudinal ordinal analysis supported a strong concentration-dependent increase in visual injury in all four macrophytes, whereas concentration-by-day interactions remained uncertain and exact paired analyses did not establish a general deterioration from Day 7 to Day 14 after Holm correction. The short-term visual-tolerance hierarchy was *Eleocharis dulcis* > *Eichhornia crassipes* > *Hydrilla verticillata* > *Salvinia molesta*. Approximate concentration values derived from ordinal medians are exploratory visual-effect benchmarks for designing confirmatory experiments and are not physiological EC50 values, ecological safety thresholds, or wetland-operational limits. Raw or weakly diluted high-LPI leachate should not be introduced directly into macrophyte-based treatment systems without prior treatment or substantial dilution.

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