



High, Buffered pH as the Primary Driver of Ecotoxicity in Construction and Demolition Waste (CDW) Leachates

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Abstract The ecotoxicological evaluation of construction and demolition waste (CDW) is essential for its sustainable reuse, but assessment is challenged by the characteristically high pH of its leachates. This study performed a comparative evaluation of three plant-based bioassays (*Lemna minor*, *Desmodemus subspicatus*, *Sinapis alba*) to identify the most robust method for assessing the phytotoxicity of seven distinct CDW leachates under environmentally relevant, unadjusted pH conditions. Furthermore, a validation experiment with model compounds (CaCl_2 , $\text{Ca}(\text{OH})_2$, NaOH) was conducted to unequivocally isolate the primary drivers of the observed growth inhibition.

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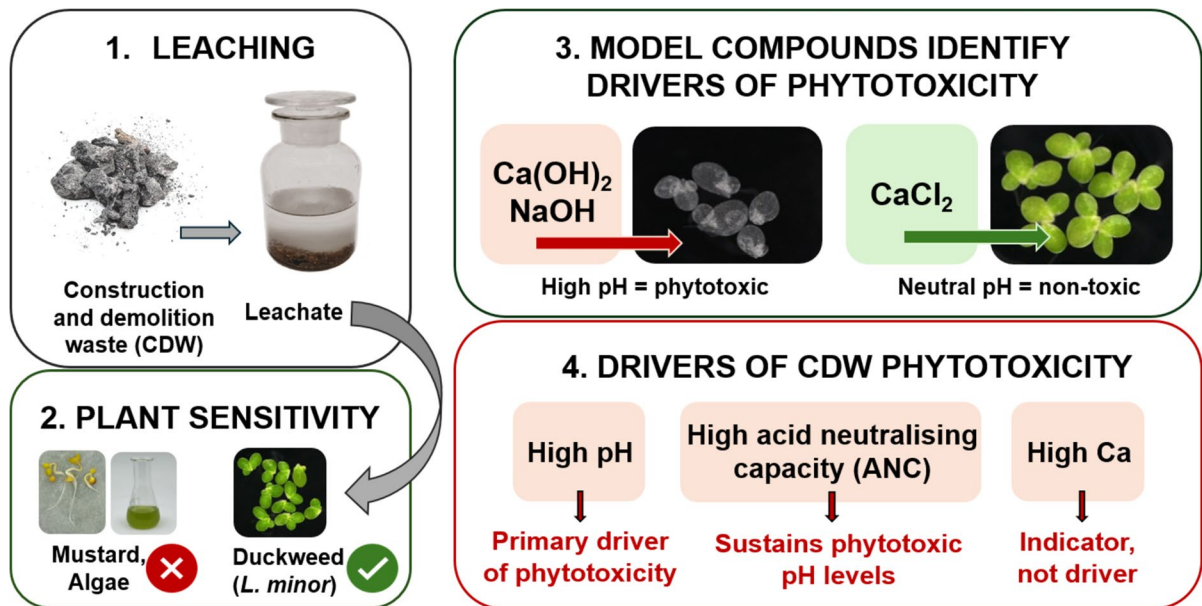
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The *L. minor* bioassay demonstrated superior performance, showing clear, dose-dependent inhibition where the algal and mustard seed assays showed limited applicability due to pH tolerance or methodological constraints. Chemical analysis and validation experiments confirmed that the severe phytotoxicity of fresh CDW leachates is driven primarily by high alkalinity ($\text{pH} > 11$) and the associated buffering capacity, rather than by leached trace elements or specific ion phytotoxicity. High calcium concentrations at neutral pH induced only negligible, sublethal stress. We conclude that the *L. minor* bioassay is a sensitive and discriminating tool for this challenging matrix. Our results demonstrate that high, stable pH is the primary factor of phytotoxicity in CDW leachates, and that calcium concentration serves as a reliable indicator for this hazard. Testing unadjusted leachates provides an environmentally relevant approach for guiding the safe and sustainable management of CDW.

Graphical Abstract



Keywords Duckweed · Alkalinity · Acid neutralising capacity · Concrete · Calcium

Abbreviations

ANC	Acid Neutralising Capacity
CDW	Construction and Demolition Waste
Chls	Total Chlorophyll Content
d0	Day 0 (initial measurement)
d7	Day 7 (final measurement)
GR	Growth Rate
MC	Model Compounds
+P	Assays with plants
-P	Assays without plants (abiotic control)
TOC	Total Organic Carbon

1 Introduction

The construction sector is a major consumer of natural resources and a significant producer of waste. The recycling of construction and demolition waste (CDW), particularly concrete, is therefore a cornerstone of the circular economy, offering a path to conserve primary materials and reduce landfilling (Bampanis & Vasilatos, 2023; Menegaki & Damigos, 2018). However, for recycled materials to be used

sustainably and safely, their potential environmental impact must be thoroughly assessed. While many studies focus on the mechanical properties of recycled aggregates (Junior et al., 2025; Xie et al., 2018), the ecotoxicological risks associated with their leachates are often overlooked.

The primary environmental concern with concrete-based CDW is the leaching of contaminants into soil and groundwater. These leachates are characteristically highly alkaline ($\text{pH} > 11$), a property primarily attributed to the dissolution of portlandite (Ca(OH)_2) from the cement matrix (Bonen, 1994; Engelsens et al., 2010). While numerous studies have characterized the chemical composition of CDW leachates (e.g., Andrade et al., 2025; Butera et al., 2014; Molla et al., 2025), a comprehensive ecotoxicological assessment is often necessary to understand the true environmental risk. Integrated assessment approaches, combining chemical analysis with a battery of bioassays, have been successfully applied to other complex wastes, such as incineration bottom ash (Bandarra et al., 2023), providing a holistic evaluation of their potential hazards.

When ecotoxicological testing of such alkaline materials is performed, a key methodological debate arises concerning the adjustment of the leachate's

natural pH. A common approach involves neutralizing the pH prior to testing to isolate the toxicity of other leached constituents (e.g., Legan et al., 2025; Tsiridis et al., 2012). Adjusting the leachate pH from alkaline to neutral can alter the speciation of both heavy metals and nutrients, which in turn may modify their bioavailability to the test organisms (Janssen & Heijerick, 2003). Moreover, this practice can be misleading for an environmentally relevant risk assessment, as it masks the primary toxicity factor – the high, buffered pH itself (Er & Kayış, 2022; Lapa et al., 2007). However, studies specifically linking the calcium-driven alkaline reserve of CDW to ecotoxicological outcomes remain limited.

Therefore, this study aimed to address this knowledge gap through a dual approach. The first objective was to perform a comparative evaluation of three plant-based bioassays (*Lemna minor*, *Desmodemus subspicatus*, and *Sinapis alba*) to identify the most sensitive and robust method for assessing the phytotoxicity of unadjusted CDW leachates. The second, and principal, objective was to unequivocally identify the primary drivers of this phytotoxicity by employing a novel validation experiment with model compounds (CaCl_2 , $\text{Ca}(\text{OH})_2$, and NaOH) that systematically isolate the effects of calcium concentration from the effects of extreme alkalinity.

2 Materials and Methods

2.1 Preparation of Test Solutions

2.1.1 CDW Leachates

Seven samples of concrete construction and demolition waste (CDW) were used in this study. Five samples of pre-consumer concrete waste (C1–C5), originating from hardened production residues not incorporated into construction structures, were obtained from a construction company in the Czech Republic. Two samples of demolition concrete waste (D1–D2) were collected from a building waste landfill (DTA Group, Prague, Czech Republic). Sample D1 was a mixture of crushed concrete, bricks, and tiles, while D2 consisted solely of concrete waste. All solid materials were air-dried, crushed, homogenised, and sieved to determine their particle size distribution (1, 2, 5, and 10 mm sieves). Leachates were prepared

from the 0–10 mm fraction according to the European standard EN 12457–4 (CEN, 2002), using a solid-to-liquid ratio of 1:10 (100 g of material to 1 L of deionized water). The mixture was agitated on an overhead shaker (7 rpm, 24 h), after which the leachate was centrifuged ($2360\times g$, 10 min, 25 °C) and filtered through a 5 µm paper filter.

2.1.2 Model Compound Solutions

To investigate the mechanisms of ecotoxicity observed in the CDW leachates, solutions of three model compounds were prepared: calcium chloride (CaCl_2), calcium hydroxide ($\text{Ca}(\text{OH})_2$), and sodium hydroxide (NaOH). Stock solutions of CaCl_2 and $\text{Ca}(\text{OH})_2$ were prepared to a nominal Ca concentration of 400 $\text{mg}\cdot\text{L}^{-1}$. This target concentration was selected to approximate the highest concentration measured in the most phytotoxic CDW leachate sample (C5, which contained 384.4 $\text{mg}\cdot\text{L}^{-1}$ of Ca). To maximize the solubility of $\text{Ca}(\text{OH})_2$ and minimize carbonate precipitation, all solutions and media were prepared using deionized water pre-chilled to 4 °C.

The 100% stock solutions were used to create a dilution series (50%, 25%, 12.5%, and 6.25%) with chilled Steinberg's medium. The NaOH series served as a pH-matched control to isolate the effect of high pH. It was prepared by first creating mixtures of chilled water and Steinberg's medium in the same volumetric ratios as the calcium series, and then titrating NaOH solutions into each mixture until its pH precisely matched that of the corresponding $\text{Ca}(\text{OH})_2$ dilution. To ensure the added volume was negligible (<1%), different concentrations of the NaOH titrant were used.

2.2 Ecotoxicity Bioassays

All bioassays were performed using the 100% test solutions and their dilutions (50%, 25%, 12.5%, and 6.25%) with the appropriate nutrient medium, which also served as the control.

2.2.1 Duckweed (*Lemna minor*) Growth Inhibition Bioassay

The test was conducted with a sterile Steinberg strain of *Lemna minor* (Federal Environmental Agency, Germany) following the standard ISO 20079

(ISO, 2005) Steinberg medium ($\text{pH } 5.5 \pm 0.2$) was used as the control and dilution medium ISO 20079 (ISO, 2005). The growth rate (GR), based on frond area, and the total chlorophyll content (Chls) per unit area were determined as described previously (Mariaková et al., 2021). To assess changes in solution chemistry during the 7-day exposure of the model compounds, a parallel setup was employed. For each test concentration, identical vessels containing the test solution but no plants were incubated under the same conditions, serving as an abiotic control.

At the end of the 7-day exposure period (Day 7), final pH (pH_{d7}) was measured in all vessels. Additionally, for the model compound experiment, dissolved calcium concentrations (Ca_{d7}) were determined in both the standard test vessels (with plants, hereafter +P) and the abiotic control vessels (without plants, hereafter -P). To analyze the dissolved fraction, samples were carefully pipetted from the supernatant to avoid disturbing any sediment before acidification, as described in Section 2.3.1.

2.2.2 Algal (*Desmodesmus subspicatus*) Growth Inhibition Bioassay

The algal growth inhibition test was performed with *D. subspicatus* (strain SAG 86.81, CICALA, Czech Republic) according to the standard ISO 8692 (ISO, 2012) with modifications. Bold Basal Medium ($\text{pH } 6.6 \pm 0.2$) served as the control and dilution medium (Rathnayake et al., 2021). The algal growth rate was determined from changes in optical density at 750 nm (UV/Vis spectrophotometer, Shimadzu UV 1900, Japan).

2.2.3 Mustard (*Sinapis alba*) Seed Germination Bioassay

The germination and root elongation test was performed with *S. alba* seeds (variety Merkantil, Forestina, Czech Republic). The test conditions and methodology are described in previous studies (Mariaková et al., 2021; Soudek et al., 2010).

2.3 Physico-Chemical Characterization

Initial pH (pH_{d0}) and electrical conductivity were measured in all freshly prepared test solutions at room temperature. Acid neutralising capacity (ANC) was

determined according to ISO 9963–1 (ISO, 1994). Both phenolphthalein alkalinity ($\text{ANC}_{8.3}$) and total alkalinity ($\text{ANC}_{4.5}$) were measured by titration with 0.1 M HCl. Total organic carbon (TOC) was determined in the CDW leachates using a Vario TOC select analyser (Elementar, UK) following the standard EN 1484 (CEN, 1997).

2.3.1 Elemental Analysis (ICP-OES)

Elemental concentrations were quantified using an ICP-OES spectrometer (iCAP 7000 Duo, Thermo Scientific, UK). For the CDW leachates, a broad range of elements were screened. Elements that were consistently below the detection limit (Cd, Co, Cu, Mn, Mo, and Sb) are not presented in the results. However, the complete elemental dataset and specific LOD values are available in the associated data repository.

In contrast, for the model compounds, the analysis focused solely on Ca. All samples were prepared for analysis by acidification and filtration (0.45 μm PTFE filter). However, two different acidification protocols were used: CDW leachates were acidified with 65% HNO_3 to a pH of 2.0 ± 0.1 , whereas model compound solutions were prepared by adding 1 mL of 65% HNO_3 to a 10 mL sample aliquot. Samples of the model compounds for total elemental analysis (Day 0) were homogenized before acidification to include any precipitates. Calibration was performed using a multielement certified stock solution (100 $\text{mg}\cdot\text{L}^{-1}$, CPA chem, Bulgaria; Analytika, Czech Republic) diluted daily in 2% v/v HNO_3 .

2.4 Data Evaluation

Ecotoxicity endpoints were expressed as percentage inhibition relative to the control. EC50 values were calculated using a four-parameter nonlinear regression model. NOEC and LOEC values were determined based on a one-way ANOVA followed by Dunnett's post-hoc test ($\alpha=0.05$) to compare treatments with the control. Differences in physico-chemical data among the samples were assessed using a one-way ANOVA followed by Tukey's HSD test.

Two separate Principal Component Analyses (PCA) were performed: one for the CDW dataset and one for the model compound dataset. For the CDW analysis, the input variables were EC50-GR,

EC50-Chls, pH_{d0} , pH_{d7} , conductivity, $\text{ANC}_{4.5}$, Ca, K, Na, Al, Sr, Ba, S, and P. The first two principal components (PC1 and PC2) explained 86.02% of the total variance. For the model compound analysis, the input variables were EC50-GR, EC50-Chls, pH_{d0} , $\text{pH}_{\text{d7+P}}$, $\text{pH}_{\text{d7-P}}$, conductivity, $\text{ANC}_{4.5}$, $\text{ANC}_{8.3}$, Ca_{d0} , $\text{Ca}_{\text{d7+P}}$, and $\text{Ca}_{\text{d7-P}}$. In this case, PC1 and PC2 explained 100% of the total variance. All statistical analyses were performed using GraphPad Prism (Version 10.2, San Diego, CA, USA).

3 Results

3.1 Ecotoxicological Response and Species Sensitivity

The ecotoxicological effects of the seven CDW leachates varied significantly depending on the test organism (Fig. 1; Fig. S1, Table 1). The algal (*Desmodesmus subspicatus*) and mustard (*Sinapis alba*) bioassays showed limited sensitivity and did not reveal clear dose–response relationships. Algal growth was generally unaffected or even stimulated at lower concentrations (Fig. S1a), while the mustard root elongation test yielded ambiguous results (Fig. S1b), preventing the calculation of reliable EC50 values (Table 1).

In stark contrast, the duckweed (*L. minor*) bioassay proved to be highly sensitive and effective in differentiating the phytotoxicity of the leachates. A clear dose–response trend was observed for both endpoints measured: frond area growth rate and total chlorophyll content (Fig. 1a, b). All undiluted C-series leachates (C1–C5) were acutely lethal, causing complete growth inhibition and total leaf necrosis. The D-series leachates were also highly phytotoxic: D2 induced near-total necrosis (> 90% chlorosis), while D1 was sublethal, resulting in severe chlorosis but allowing for minimal survival (see Fig. S2 for representative photographs of these effects).

Upon dilution, distinct differences in phytotoxicity among the leachates became apparent. The inhibitory effect was generally more pronounced for the growth rate endpoint than for chlorophyll content, as reflected by the lower EC50 values for growth rate (ranging from 7.8 to 53.8%) compared to chlorophyll (ranging from 12.9 to > 100%) (Table 1). Based on the EC50 values for the growth rate of *L. minor*, the leachates

could be ranked in increasing order of ecotoxicity: $\text{D1} < \text{C1} < \text{D2} < \text{C2} \approx \text{C3} < \text{C4} < \text{C5}$. This wide spectrum of ecotoxicological responses provided an ideal basis for investigating the underlying physico-chemical drivers.

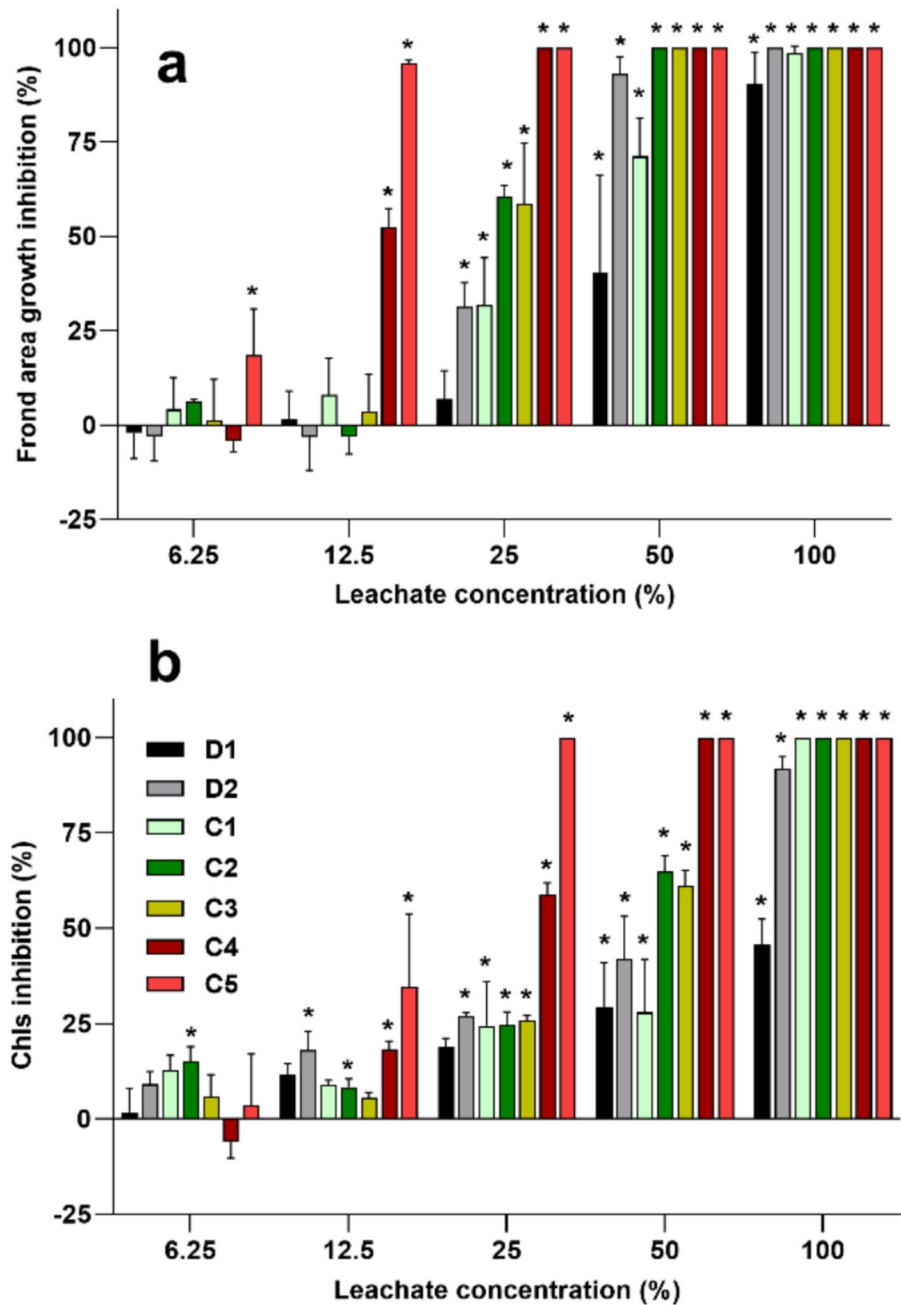
3.2 Ecotoxicity Linked to High pH, ANC, and Key Elemental Content

The physico-chemical properties of the seven CDW leachates showed substantial differences, which correlated strongly with the observed ecotoxicity patterns (Fig. 2). While some parameters, such as the particle size distribution of the solid waste (Fig. S3b) and total organic carbon (TOC) in the leachates (Fig. S3a), did not show a clear relationship with phytotoxicity, the key drivers were identified among the inorganic chemical parameters.

The most phytotoxic leachates (C4, C5) were characterized by the highest values of electrical conductivity and, crucially, the highest acid neutralising capacity (Fig. 2a, b). This high buffering capacity was directly linked to their ability to maintain a highly alkaline pH. Although a decrease in pH was observed over the 7-day period in the diluted samples, the final pH values for the most phytotoxic leachates remained significantly higher than those of the other leachates, demonstrating their exceptionally high buffering capacity (Fig. 2c, d).

The elemental analysis revealed that the concentrations of several major and minor elements also correlated with the observed ecotoxicity patterns. As shown in Fig. 2e, calcium was the most abundant cation, and its concentration largely followed the order of phytotoxicity, where the least phytotoxic leachate (D1) had the lowest Ca concentration and the most phytotoxic (C5) had the highest (384.4 mg.L^{-1}). Similar increasing trends were generally observed for K, Na, Ba, and Sr also reaching its peak in the most phytotoxic leachate (Fig. 2f, g, i, j). Conversely, the concentration of Al and Si showed a notable inverse trend, with the lowest values found in the most phytotoxic leachates (Fig. 2h, Fig. S4b). A detailed overview of all other measured elements is provided in the Supplementary Information (Fig. S4). These results strongly suggest that the concentration of major cations, particularly Ca, serves as a robust proxy for the overall leaching potential and associated ecotoxicological properties of the CDW.

Fig. 1 Ecotoxicity of CDW leachates on *Lemna minor*. Inhibition of **a** frond area growth rate and **b** total chlorophyll content (Chls) in duckweed exposed to CDW leachates. Negative values indicate growth stimulation. Asterisks (*) denote a significant difference from the control (Dunnett's test, $p < 0.05$)



3.3 Uncoupling the Effects of pH and Calcium using Model Compounds

The validation experiments with model compounds robustly confirmed the hypothesis that high, stable pH is the primary driver of phytotoxicity, rather than the Ca concentration itself. The ecotoxicological responses of *L. minor* to the model solutions

mirrored the patterns observed with the CDW leachates (Fig. 3a, b).

Both hydroxide solutions ($\text{Ca}(\text{OH})_2$ and NaOH) exhibited 100% growth inhibition at the highest tested concentrations ($\text{pH} \approx 12.6$). The 100% and 50% solutions were effectively lethal, causing complete growth inhibition and near-total to total necrosis of the fronds (Fig. 3a, b; Fig. S2d, f). High phytotoxicity persisted

Table 1 Ecotoxicity indices for three plant-based bioassays exposed to CDW leachates. Values shown are the 50% effective concentration (EC50), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC), all expressed in % (v/v) of leachate. GR – growth rate; Chls – chlorophyll content; n.c. – not calculable due to lack of a clear dose–response

Sample	D1	D2	C1	C2	C3	C4	C5
Algae (<i>D. subspicatus</i>) GR							
NOEC	50	50	50	50	50	50	50
LOEC	100	100	100	100	100	100	100
EC50	> 100	> 100	> 100	> 100	> 100	> 100	90.8
Mustard (<i>S. alba</i>)							
NOEC	500	50	25	25	< 6.25	< 6.25	6.25
LOEC	100	100	50	50	6.25	6.25	12.5
EC50	> 100	> 100	> 100	> 100	n.c	> 100	n.c
Duckweed (<i>L. minor</i>) GR							
NOEC	25	12.5	12.5	12.5	12.5	6.25	< 6.25
LOEC	50	25	25	25	25	12.5	6.25
EC50	53.8	29.2	33.9	24.5	23.4	12.5	7.8
Duckweed (<i>L. minor</i>) Chls							
NOEC	25	6.25	12.5	< 6.25	12.5	6.25	6.25
LOEC	50	12.5	25	6.25	25	12.5	12.5
EC50	> 100	44.7	59.1	38	39.5	21.6	12.9

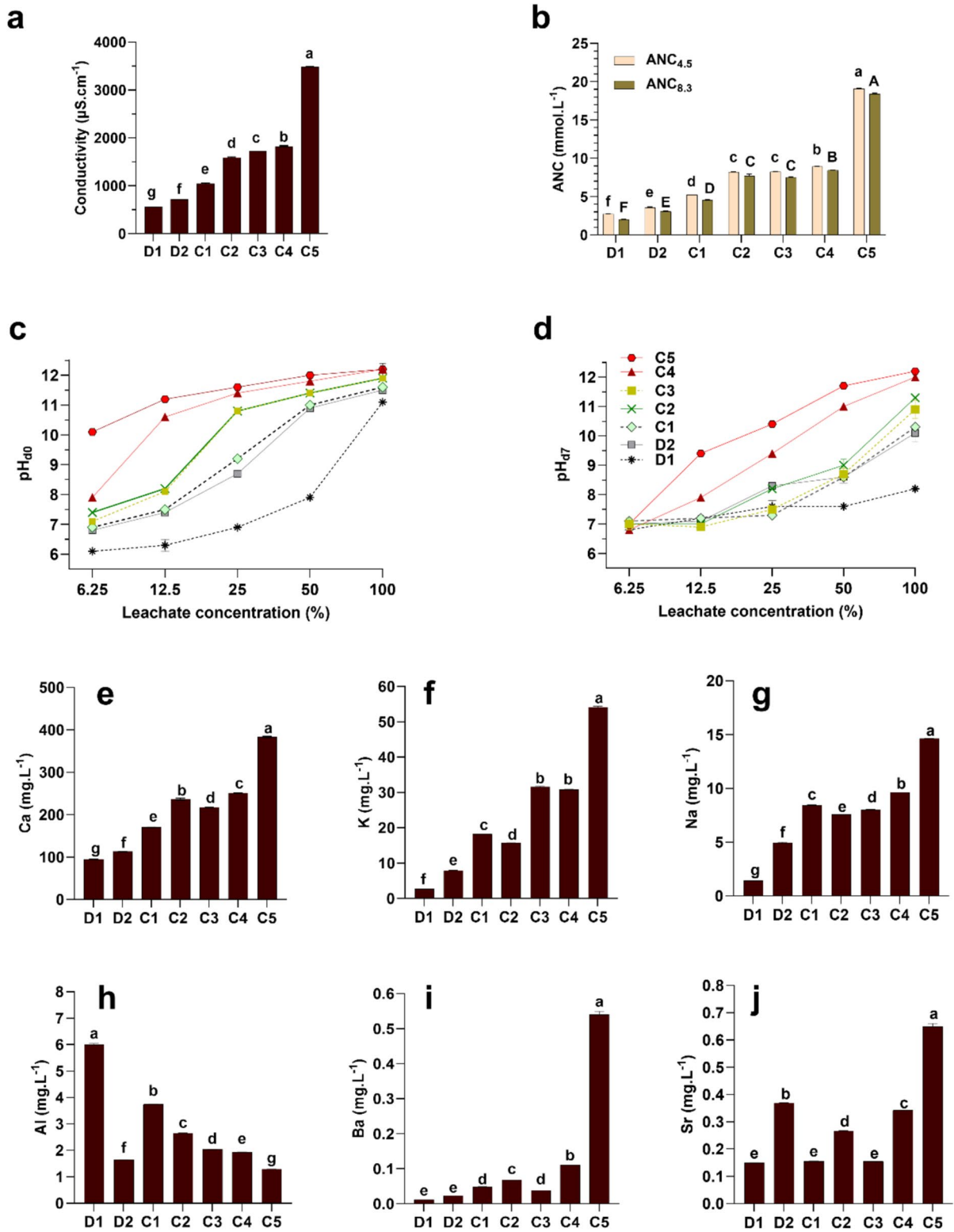
even at 25% dilution, with growth rate inhibitions reaching 94% for $\text{Ca}(\text{OH})_2$ and 90% for NaOH. This response directly corresponded to the extreme alkalinity of these solutions. The initial pH of the 100% and 50% solutions for both hydroxides was above 11 (Fig. 3c). Crucially, while the pH of the 50% solutions decreased over the 7-day period, it remained above the critical tolerance threshold of pH 11, similar to the highly stable pH of the 100% solutions (Fig. 3d). A significant mitigation of adverse effects for both hydroxide solutions was only observed at the 6.25% concentration, which was the first dilution level where the final pH dropped to near-neutral values (Fig. 3d). The presence of plants generally had a minor effect on the final pH, causing a slightly greater decrease (up to 0.5 units) in some dilutions compared to the abiotic controls.

Conversely, the CaCl_2 solution, which had a comparable Ca concentration to the $\text{Ca}(\text{OH})_2$ solution but a low buffering capacity, exhibited negligible phytotoxicity across the entire concentration range (Fig. 3a). The only notable effect was a significant chlorosis observed in the 100% solution, likely attributable to a combination of nutrient limitation and mild osmotic stress from elevated chlorides (Fig. 3b, Fig. S2e). Remarkably, despite this visible stress, the fronds were still able to grow; the growth rate inhibition was minimal (8.2%) and not statistically significant. The pH of the diluted CaCl_2 solutions remained

stable in the near-neutral range (pH 5.6–6.1) throughout the experiment. The undiluted 100% stock solution was a notable exception; it exhibited an initially elevated pH of 8.5, which then neutralized to a final value of 6.6 by Day 7 (Fig. 3d). For the CaCl_2 series, the presence of plants led to a slightly higher final pH in the diluted solutions compared to the abiotic controls, though the values remained within the neutral range.

3.4 Calcium as a Proxy for Acid Neutralising Capacity

The analysis of the model compound solutions revealed that while Ca concentration in CDW leachates correlated with phytotoxicity, its behaviour in the model systems points to an indirect mechanistic role (Fig. 4). The initial (Day 0) conditions clearly differentiated the three types of solutions. While the total Ca concentrations in the $\text{Ca}(\text{OH})_2$ and CaCl_2 series were consistent with the expected values from the dilution scheme (Fig. 4a), their underlying chemical properties varied significantly. The key difference lay in their buffering capacity and conductivity (Fig. 4c, d). Both hydroxide solutions ($\text{Ca}(\text{OH})_2$ and NaOH) were characterized by an extremely high total alkalinity ($\text{ANC}_{4.5}$ ranging from 14.9 to 18.4 mmol.L^{-1}) and high initial conductivity, which both decreased sharply upon dilution. On the contrary, the



◀**Fig. 2** Physico-chemical properties driving the observed phytotoxicity of CDW leachates. Panels show: **a** conductivity and **b** acid neutralising capacity (ANC) of the undiluted leachates; **c** initial pH (Day 0) and **d** final pH (Day 7) of the leachates after dilution for the *Lemna* test; and concentrations of key major cations **e** calcium, **f** potassium, **g** sodium, **h** aluminium, **i** barium, and **j** strontium in the undiluted leachates. For ANC, ANC_{4.5} represents total alkalinity (to pH 4.5), and ANC_{8.3} represents phenolphthalein alkalinity (to pH 8.3). Within each panel, samples labelled with different letters are significantly different (Tukey's HSD, $p < 0.05$)

CaCl₂ solution exhibited negligible ANC_{4.5} (0.255 mmol.L⁻¹) and a moderate, more stable conductivity across the dilution series.

This fundamental difference in buffering capacity directly explains the divergent behaviour of dissolved Ca observed after the 7-day exposure period (Fig. 4b). In the negligibly phytotoxic, unbuffered CaCl₂ solutions, the concentration of dissolved Ca remained high and stable. Conversely, in the severely phytotoxic and strongly buffered Ca(OH)₂ solutions, a substantial decrease in dissolved Ca occurred, particularly at the higher concentrations, consistent with the visual observation of precipitate formation (Fig. S2d). In the NaOH solutions, the presence of plants led to a noticeable uptake or adsorption of the available Ca from the medium, a phenomenon not observed in the abiotic controls.

3.5 Multivariate Analysis of Leachate Properties and Ecotoxicity

To synthesize the complex relationships within the CDW dataset, a Principal Component Analysis (PCA) was performed (Fig. 5). The first two principal components (PC1 and PC2) explained 86.02% of the total variance. The resulting biplot clearly illustrates the main drivers of phytotoxicity and separates the leachate samples based on their properties.

A strong negative correlation was observed between the ecotoxicity indices (EC50-GR and EC50-Chls) and the pH values, confirming that higher phytotoxicity is directly associated with alkalinity. Furthermore, the vectors for pH, acid neutralising capacity (ANC_{4.5}), conductivity, and major cations (Ca, K, Na, Sr, Ba) were all positively correlated, forming a distinct cluster associated with the highly phytotoxic samples. While the presence of these cations reflects the high mineral load of the leachates, the

high alkaline reserve (ANC_{4.5}) is the critical factor; it buffers the system and sustains extreme pH levels, preventing rapid neutralisation.

This is further supported by the grouping of samples: the most phytotoxic leachate, C5, was isolated in the quadrant driven by highest alkaline reserve (ANC), and cation concentrations. Conversely, the least phytotoxic leachate, D1, occupied a distinct space on the opposite side of the biplot, strongly associated with higher EC50 values and, notably, a higher concentration of dissolved Al. Other elements, such as S, showed little to no correlation with the primary drivers of inhibition.

Principal component analysis (PCA) of the model compounds confirmed that adverse biological effects clustered exclusively with high pH and hydroxide treatments, while neutral Ca solutions showed distinct characteristics (Fig. S5). This analysis supported the key findings: a strong negative correlation was observed between the ecotoxicity indices (EC50 values) and the pH and ANC variables. Conversely, and in stark contrast to the CDW data, no correlation was found between phytotoxicity and the initial total Ca concentration (Ca_{d0}). This powerfully illustrates the role of Ca as an indirect proxy in complex leachates versus its substantially lower phytotoxic potential as a neutral salt. Furthermore, the analysis highlighted a strong negative correlation between the final dissolved Ca (Ca_{d7}) and conductivity, visually confirming the precipitation process in the highly conductive hydroxide solutions.

4 Discussion

4.1 *Lemna minor* as a Sensitive and Discriminating Bioindicator for CDW Leachate Phytotoxicity

The selection of an appropriate bioassay is critical for the environmental risk assessment of complex matrices like CDW leachates. In this study, the *Lemna minor* bioassay proved to be a significantly more sensitive and discriminating tool than the algal or mustard seed assays. This was most evident in the tests with the undiluted (100%) leachates: while nearly all were acutely lethal to *L. minor*, the same solutions only caused partial growth inhibition (max. 62.9%) in the alga *Desmodesmus subspicatus* (Fig. 1, Fig. S1). This direct comparison, performed without

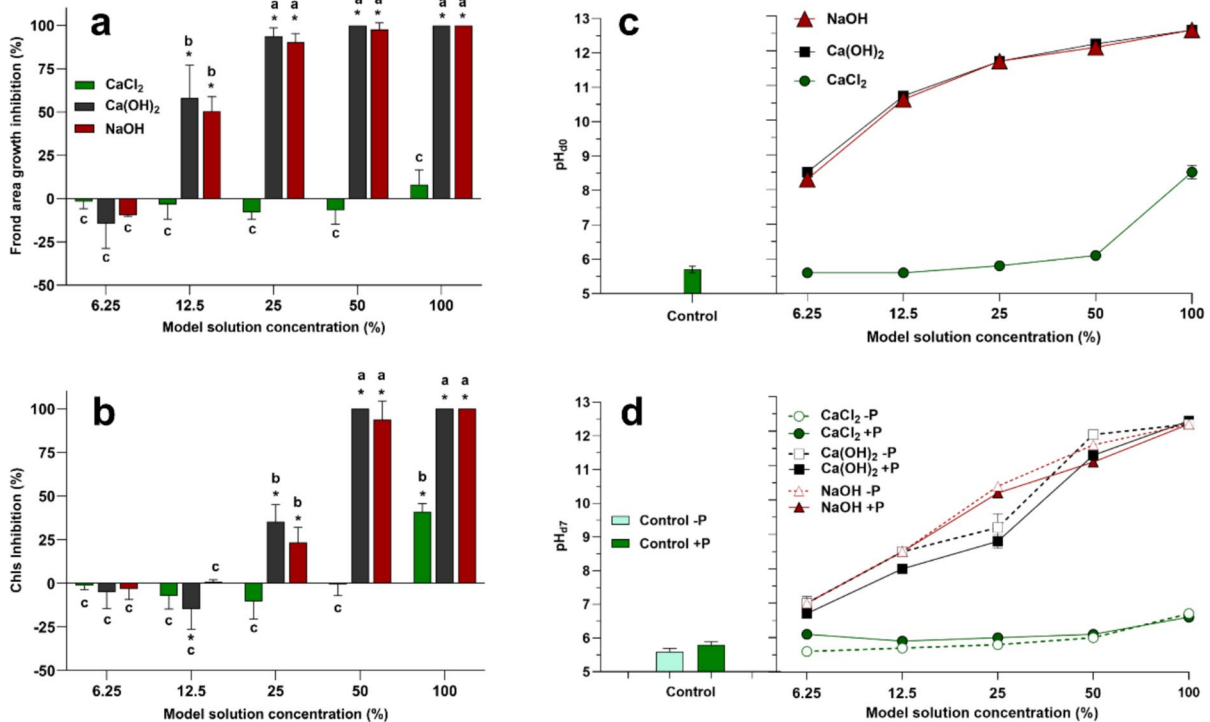


Fig. 3 High pH as the primary driver of phytotoxicity in model compound solutions. Ecotoxicological response of *Lemna minor* to the model compound solutions, showing **a** inhibition of frond area growth rate and **b** inhibition of total chlorophyll content (Chls). Panels **c** and **d** show the initial (Day 0) and final (Day 7) pH of the dilution series, respec-

tively. The final pH was measured in parallel assays with (+P) and without (-P) the presence of *Lemna minor*. Asterisks (*) denote a significant difference from the control (Dunnett's test, $p < 0.05$). Different letters indicate significant differences between the model compounds at a given concentration (Tukey's HSD, $p < 0.05$)

the addition of any external growth medium, strongly suggests an inherently higher tolerance of the alga to this specific type of inorganic, high-pH stress.

The lower sensitivity of the algal assay in the diluted samples was likely amplified by the composition of the Bold's Basal Medium (BBM). While BBM is a standard cultivation medium, its high concentration of complexing agents (phosphates and EDTA) is known to reduce the bioavailability of metal ions (Rathnayake et al., 2021), potentially mitigating the observed phytotoxicity. This highlights a critical methodological consideration: for testing highly inorganic and alkaline wastes, standard nutrient-rich algal media may lead to an underestimation of the actual environmental hazard. This does not imply that microalgae are universally unsuitable for assessing waste materials, but rather underscores the importance of matching the test system (organism and medium) to the specific chemical characteristics

of the matrix. While algae are effective in remediating nutrient-rich, near-neutral effluents (Hu et al., 2021; Sisman-Aydin, 2022), our results suggest that for the specific stress profile of concrete leachate — extreme alkalinity and high inorganic ion content — macrophytes like *L. minor* provide a more sensitive assessment.

Consequently, the *L. minor* bioassay proved to be a robust tool, providing clear dose-response curves that allowed for a distinct ranking of leachate phytotoxicity, which was not possible with the other assays (Fig. 1). This finding is consistent with previous work where duckweed was also a more sensitive indicator for recycled aggregates than algae or mustard (Mariaková et al., 2021). Its utility for cementitious materials has been noted by others as well, though often in studies where the leachate pH was neutralized prior to testing, for example, in the study by Legan et al. (2025). As our study demonstrates, leaving the pH

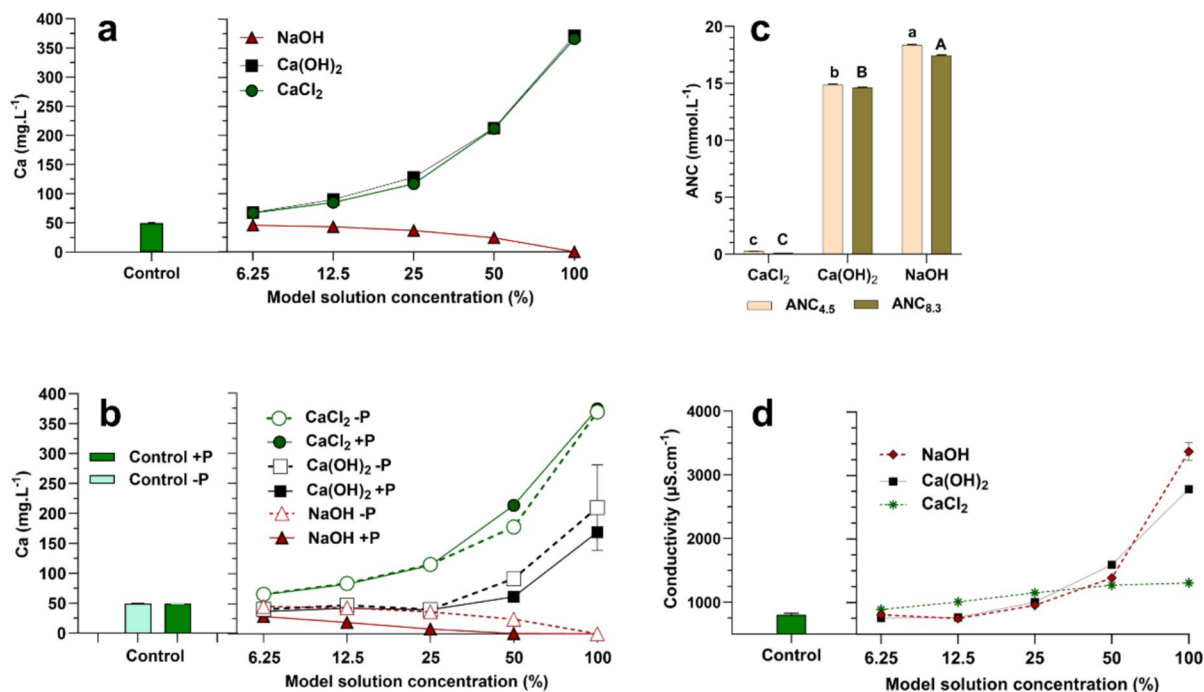


Fig. 4 Calcium as a proxy for acid neutralising capacity in model solutions. Evolution of key physico-chemical properties in the model compound solutions. Panels show: **a** initial total calcium at Day 0; **b** final dissolved calcium at Day 7 in assays with (+P) and without (-P) *Lemna minor*; **c** acid neutralising

capacity (ANC) of the 100% stock solutions; and **d** conductivity of the dilution series. For ANC, ANC_{4.5} and ANC_{8.3} are defined as in Fig. 2. Different letters indicate significant differences between the stock solutions (Tukey's HSD, $p < 0.05$)

unadjusted is critical for a realistic risk assessment. The key advantage of the *Lemna* assay for this matrix lies not only in its sensitivity but also in its methodological suitability. The 7-day static exposure allows for the observation of chemical changes, such as precipitate formation, while the multi-faceted endpoints (frond area, pigments) provide a richer, more ecologically relevant dataset than the single endpoints of the other assays.

4.2 The Role of pH and Buffering Capacity in Observed Ecotoxicity

A critical point of discussion in the ecotoxicology of alkaline materials is the role of pH. Some standard protocols suggest pH adjustment prior to testing (e.g., ISO, 2005), a common practice in studies aiming to isolate specific adverse effects of individual leached constituents like heavy metals (e.g., Legan et al., 2025; Tsiroidis et al., 2012). However, we argue that for an environmentally relevant risk assessment of

materials like CDW, this step is misleading (Van Der Sloot, 2002), as it can mask the full adverse potential of the material under realistic leaching conditions by altering the chemical speciation and bioavailability of various elements. Indeed, pH is widely recognized as the single most important environmental variable controlling the solubility, speciation, and thus the bioavailability of metals in aquatic systems (De Paiva Magalhães et al., 2015).

Our results with the CDW leachates clearly demonstrate this: the lower phytotoxicity of D1 was not a result of a less alkaline initial pH (which was also > 11), but a direct consequence of its low buffering capacity, which allowed for a significant pH drop over the 7-day exposure (Fig. 2c, d). In contrast, the severe phytotoxicity of the C5 leachate was directly linked to its exceptional ANC (Fig. 2b), which maintained a lethal pH throughout the test period. This finding is consistent with the general principle observed for other alkaline waste materials, such as wood ash, where adverse biological effects were also

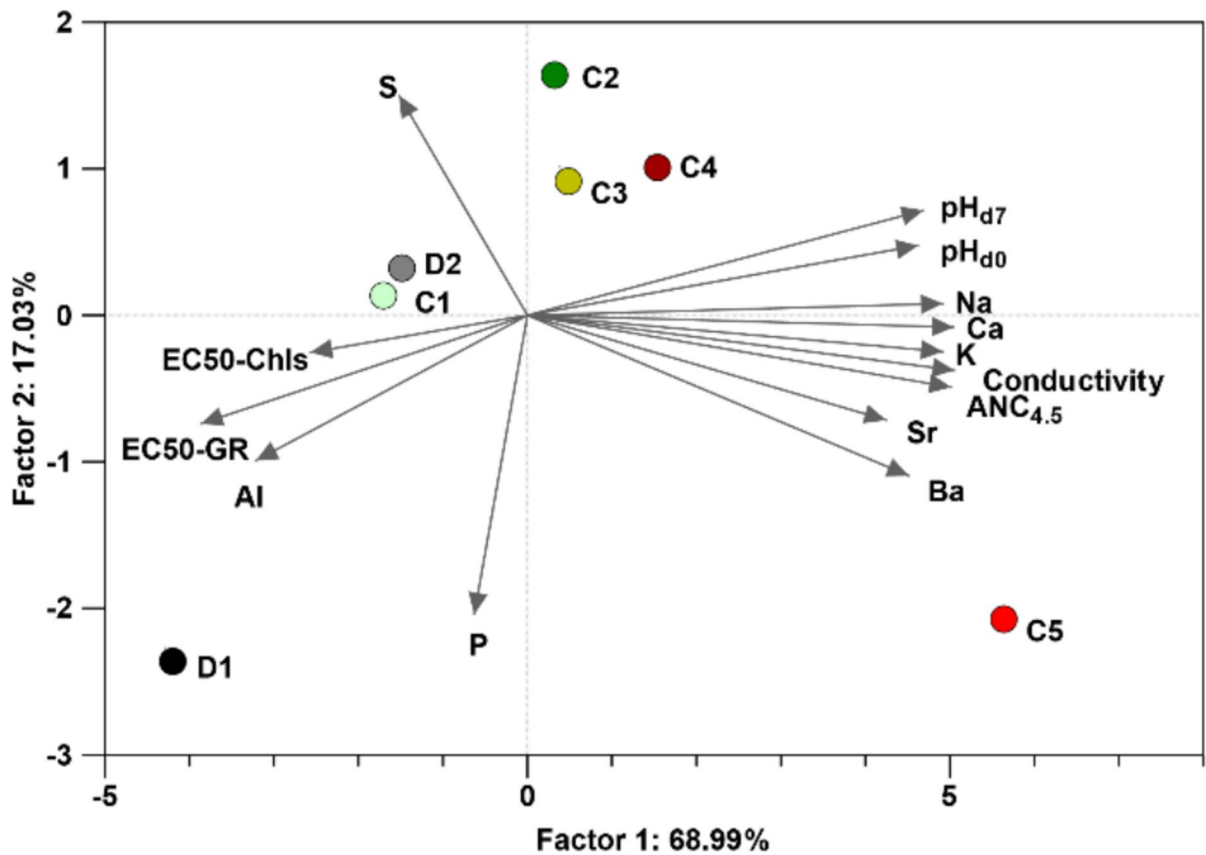


Fig. 5 Principal Component Analysis (PCA) biplot of CDW leachate properties and ecotoxicity indices. The plot shows loadings of variables (arrows) and scores of individual leachate samples (points). The analysis included physico-chemical parameters (Conductivity, $ANC_{4.5}$ – total alkalinity; pH_{d0} and

pH_{d7} – initial and final pH), ecotoxicity indices (EC50-GR and EC50-Chls – EC50 values for duckweed growth rate and chlorophyll content, respectively), and concentrations of key elements (Ca, K, Na, Al, Sr, Ba, S, and P)

primarily driven by high, unadjusted pH (Arronson & Ekelund, 2005). For concrete specifically, it is well-established that the material's high initial alkalinity naturally decreases over time as it weathers and undergoes carbonation (Engelsen et al., 2010; e.g., Mariaková et al., 2022), which further underscores that the fresh, unweathered state represents the worst-case scenario for pH-driven phytotoxicity. Indeed, the observed pH decrease in diluted samples over the 7-day period is primarily driven by atmospheric carbonation and the depletion of buffering capacity. While the undiluted leachate maintains a high ANC that resists pH changes, dilution reduces this reserve alkalinity, making the solution more susceptible to

pH reduction caused by CO_2 absorption and precipitation reactions.

The dominant role of high, buffered pH was unequivocally confirmed by our validation experiments with model compounds. The $Ca(OH)_2$ and NaOH solutions, defined by their high pH and ANC, induced severe phytotoxicity, mirroring the effect of the C5 leachate. Conversely, the $CaCl_2$ solution, with a comparable Ca concentration but negligible ANC, showed negligible adverse effects. This definitively demonstrates that the high, stable pH, maintained by a high buffering capacity, is the primary driver of the observed phytotoxicity in fresh concrete leachates.

4.3 Calcium as a Proxy and the Complex Behaviour of Co-Leached Elements

Although Ca^{2+} itself is not the direct cause of the observed inhibition, its concentration in CDW leachates serves as a robust proxy for the presence of leachable portlandite $\text{Ca}(\text{OH})_2$ and, consequently, for the material's potential to generate ecotoxic, high-pH conditions (Bonen, 1994). While alkali metals (K and Na) also showed strong correlations with phytotoxicity due to their high solubility, Ca is uniquely tied to the solid-phase buffering reserve, making it the most reliable indicator of the material's capacity to maintain lethal pH levels. This Ca^{2+} release thus creates a specific alkaline aquatic environment that profoundly influences the leaching and bioavailability of other ions.

Chemical analysis confirmed that the elemental composition played a secondary role in the observed phytotoxicity. Several hazardous trace elements, such as Cu and Cd, were consistently below detection limits (e.g., $\text{LOD} < 0.005 \text{ mg.L}^{-1}$ for Cu). Other potentially toxic metals, including Pb ($0.052\text{--}0.063 \text{ mg.L}^{-1}$; Fig. S4h) and Zn ($0.032\text{--}0.041 \text{ mg.L}^{-1}$; Fig. S4i), were detected only at trace levels. Their low concentrations are attributed to the highly alkaline conditions, which promote the precipitation of metal cations as hydroxides or carbonates (De Paiva Magalhães et al., 2015). Other minor components and nutrients (e.g., Cr, Fe, B, Mg, and P) also remained at very low concentrations (see Fig. S4 and the associated data repository), further confirming that the leachate's toxicity is primarily driven by pH rather than chemical contamination.

Beyond the primary drivers of phytotoxicity, the leaching of other elements revealed more complex patterns. For instance, the concentrations of Ba (up to 0.54 mg.L^{-1}) and Sr (up to 0.65 mg.L^{-1}) showed a positive correlation with the phytotoxicity gradient and clustered with pH and ANC in the PCA (Section 3.5, Fig. 5). However, their concentrations remained well below reported phytotoxic thresholds for aquatic macrophytes. This confirms that their presence is a marker of cement matrix dissolution (Vollpracht & Brameshuber, 2016) rather than the cause of the observed growth inhibition.

This complexity is further underscored by the behaviour of Ba and S. Sulfur concentrations, likely in the form of soluble sulfates (De Paiva

Magalhães et al., 2015), ranged from 6.5 to 47.5 mg.L^{-1} (Fig. S4a). Notably, the lowest S concentration (6.5 mg.L^{-1}) was found in the C5 leachate, coinciding with the highest Ba concentration. This inverse relationship strongly suggests the precipitation of highly insoluble barium sulfate (BaSO_4), as Ba^{2+} is a known exception to the general solubility of metal sulfates (De Paiva Magalhães et al., 2015). The presence of Ba itself is likely linked to the cement content, a characteristic shared with other trace elements historically associated with cement, such as Cr (Vollpracht & Brameshuber, 2016). While older cements were known to leach significant amounts of hazardous Cr(VI), the low Cr concentrations observed in modern samples are consistent with European regulations requiring the addition of reducing agents to immobilize chromium in a less soluble form (EC, 2003).

Further complex trends were observed for aluminium and silicon (Fig. 2h, Fig. S4b). The highest concentration of Al was found in the least phytotoxic D1 leachate (6.0 mg.L^{-1} Al with 2.4 mg.L^{-1} Si), a mixture containing crushed bricks and tiles rich in aluminosilicates (Dill, 2016). Conversely, sample D2 exhibited the highest Si levels (6.0 mg.L^{-1} with 1.6 mg.L^{-1} Al). Despite these differing elemental profiles, both samples showed the lowest overall phytotoxicity. This lack of toxicity, even in the presence of elevated Al in D1, can be explained by the protective role of Si, which is known to alleviate metal stress by forming non-toxic hydroxyaluminosilicate complexes and enhancing plant antioxidant defences (Hodson & Evans, 2020; Vaculík et al., 2020).

Finally, a particularly intriguing finding concerns the potentially protective role of Ca in mitigating other environmental stressors. This was suggested by results from the 100% CaCl_2 solution, where *L. minor* demonstrated growth despite severe nutrient limitation that caused significant chlorosis. As calcium acts as a crucial secondary messenger in plant cellular signaling, particularly in response to abiotic stress (White & Broadley, 2003). We hypothesize that high Ca^{2+} availability may have partially compensated for the lack of other nutrients. This hypothesis is further supported by the observed greater uptake of Ca by the plants in hydroxide solutions compared to abiotic controls. However, a definitive confirmation of this protective role would require further targeted experiments.

4.4 Methodological Implications and Environmental Outlook

The results of this study have important implications for the environmental risk assessment of cement-based materials. While standard protocols often mandate pH neutralisation to isolate the effects of specific chemical constituents, our data demonstrate that for fresh CDW, the extreme pH and its associated buffering capacity represent the dominant environmental stressor. Testing unadjusted leachates therefore provides a more realistic representation of the immediate 'chemical shock' that aquatic ecosystems might experience. However, it is important to note that these results reflect a 'worst-case scenario' for fresh, unbuffered material. In real-world applications, factors such as carbonation, dilution in receiving waters, and soil interaction will gradually moderate this hazard. Our findings confirm that detailed elemental analysis, while important for chemical characterization, plays a subordinate role to pH and buffering capacity in predicting immediate environmental hazard.

Finally, this study identifies several avenues for future research. While pH is the primary factor in fresh leachates, its role in controlling the speciation and bioavailability of elements in aged or carbonated materials deserves further investigation. Future work should focus on simulating long-term environmental behaviour, such as the interaction of alkaline materials with the natural buffering capacity of soils in road sub-bases. Additionally, investigating how the gradual neutralisation of these materials—whether through natural weathering or during leaching—affects the overall ecotoxicological risk would be a crucial step towards a comprehensive understanding of CDW sustainability.

Conclusions

1. The *Lemna minor* bioassay was identified as the most robust and sensitive tool for the ecotoxicological assessment of unadjusted CDW leachates, providing reliable dose–response data where algal and mustard seed assays showed limited applicability.
2. The severe phytotoxicity of fresh CDW leachates is driven primarily by extreme alkalinity (pH > 11) and the associated buffering capacity

of the cement matrix, rather than by leached heavy metals or specific ion toxicity.

3. Chemical analysis and validation experiments confirmed that hazardous trace elements remained below detection limits or toxic thresholds, and that equivalent calcium concentrations at neutral pH (CaCl₂) caused only negligible, sublethal stress.
4. We conclude that standard protocols requiring pH neutralization are inappropriate for fresh CDW, as they remove the primary environmental stressor. A more holistic risk assessment should consider both the immediate pH-driven hazard and the long-term impact after carbonation.

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Data Availability The datasets generated and/or analysed during the current study are available in the Zenodo repository, <https://doi.org/10.5281/zenodo.14587224>.

Declarations

Competing Interests The authors declare no competing interests.

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