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Environmental Screening of Dewatered Fish-Processing Wastewater Sludge for Land Application: Species-Specific Phytotoxicity and Exposure Limitations

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Abstract

Land application of industrial wastewater sludge may transfer nutrients, salts, metals, and other constituents to soil, creating a complex exposure scenario in which favorable plant responses alone cannot establish environmental safety. We reanalyzed historical seed-bioassay and greenhouse data to evaluate dewatered fish-processing wastewater sludge as an initial environmental screening before land application. A 1:10 sludge-to-water extract was tested with lettuce (Lactuca sativa), cucumber (Cucumis sativus), and arugula (Eruca sativa) across four independent sampling blocks. Lettuce and arugula were also grown in soil under four treatments: lime-amended control (T1), lime plus sludge (T2), lime plus mineral NPK (T3), and sludge without lime (T4). Block-normalized germination indices were 344.7 ± 267.7% for lettuce, 184.3 ± 16.2% for cucumber, and 164.1 ± 13.4% for arugula. Exact inference was inconclusive at four blocks (minimum attainable two-sided $p = 0.125$), and the lettuce interval crossed both the 80% screening threshold and 100%. Thus, cucumber and arugula showed no consistent inhibition at the tested extract strength, whereas lettuce responses were heterogeneous. In the greenhouse, lettuce shoot traits did not differ among treatments, while arugula showed its strongest response under lime plus sludge. Field-equivalent doses, block-matched extract conductivity, pathogens, contaminant bioavailability, leaching, and edible-tissue accumulation were unavailable or not assessed. The results support a tiered pollution-assessment approach in which plant bioassays are used as screening evidence, but dose-defined and exposure-matched tests are required before environmental safety or unrestricted land application can be inferred.

1. Introduction

Fish and seafood processing generates heterogeneous residual streams, including wastewater solids and biological sludge. When such residues are considered for land application, terrestrial organisms may be exposed to complex mixtures whose composition depends on the species processed, wastewater-treatment train, dewatering, and subsequent handling. These matrices may contain recoverable nitrogen, phosphorus, organic carbon, and minerals, but also salts, trace elements, and other constituents of environmental concern. Accordingly, nutrient-recovery potential and environmental safety are separate questions: land-application decisions require biological compatibility, exposure characterization, and contaminant-risk assessment, not nutrient content alone (Ahuja et al., 2020; Madende and Hayes, 2020; Zhang et al., 2023).

Dewatered industrial sludge from fish-processing wastewater treatment is especially understudied compared with fish hydrolysates, composts, or aquaculture effluents. The term industrial sludge is used because regulatory biosolid status has not been established for this material. Unlike more processed fish-derived products, filter-pressed biological sludge is a heterogeneous matrix whose land application may alter soil pH, ionic strength, nutrient availability, and contaminant loading in dose-dependent ways. Sludge-amended soils may simultaneously gain fertility and accumulate potentially toxic elements, particularly as application rates or repeated use increase (Achkir et al., 2023; Seleiman et al., 2020).

Treatment history therefore determines not only potential agronomic value but also the exposure profile encountered by terrestrial organisms (Mponda and Kim, 2023; Pei et al., 2020).

Plant growth alone does not establish environmental safety. Sludge may carry pathogens, trace elements, persistent organic contaminants, microplastics, and antimicrobial-resistance determinants whose mobility and biological effects are not captured by bulk nutrient or metal measurements. Treatment history influences sludge hygiene, repeated applications may enrich resistance determinants, and plant responses depend on species tolerance and contaminant bioavailability (Carraturo et al., 2022; Mokni-Tlili et al., 2024; Wu et al., 2022). Multi-species testing has shown that sewage-sludge amendments can increase plant growth while producing adverse reproductive effects in earthworms at higher doses, illustrating why favorable plant responses alone cannot establish ecosystem safety (Roques et al., 2023). Metals can also remain biologically relevant after thermal or biological processing, so the absence of acute germination inhibition does not imply negligible long-term ecological or dietary risk (Bona et al., 2023). Agronomic performance, environmental authorization, and product eligibility must therefore be considered separately.

Seed germination and radicle-elongation bioassays can serve as a rapid first screening of soluble constituents before soil application. The germination index (GI) combines relative germination success with relative root elongation and can detect responses that germination counts alone miss (Tiquia et al., 1996; Zucconi et al., 1981). GI nevertheless depends on extraction conditions, seed species, salinity, ammonium, and other matrix-specific factors (Hechmi et al., 2020; Wang et al., 2022), and it cannot substitute for dose-defined soil, field, or food-chain assessments. In sewage-sludge soil systems, increasing application ratios can raise water-soluble salt concentrations and metal activity, and salinity may become a dominant driver of early seedling inhibition under some conditions (Chen et al., 2025). Reliable screening therefore requires both correct identification of the independent experimental unit and exposure measurements paired to that unit. Replication must be based on independent sludge collections or sampling blocks rather than multiple plates exposed within the same block; otherwise, precision is inflated by pseudoreplication.

Few studies have examined filter-pressed sludge from fish-processing wastewater treatment by combining short-term seed bioassays with whole-plant responses in soil. Here, the historical dataset is treated as a descriptive environmental screening rather than a confirmatory test of safety or agronomic efficacy. We evaluated (i) the short-term effects of a 1:10 aqueous sludge extract on germination and radicle elongation of lettuce (Lactuca sativa), cucumber (Cucumis sativus), and arugula (Eruca sativa); (ii) lettuce and arugula whole-plant responses in soil receiving sludge with or without lime, relative to lime-amended and mineral-fertilized comparators; and (iii) the exposure and evidence gaps that prevent environmental-risk characterization and routine land-application recommendations. We expected species-specific responses and an influence of liming on the recorded greenhouse endpoints. Raw data were reanalyzed with explicit definition of the experimental unit, block-matched controls for seed assays, and sensitivity analyses for missing or biologically implausible measurements. Beyond this specific residue, the study addresses a broader pollution-assessment problem relevant to heterogeneous

industrial sludges: apparent biological stimulation can coexist with unresolved exposure uncertainty, and pooling non-independent bioassay replicates can conceal heterogeneity that materially changes environmental interpretation.

2. Materials and methods

2.1. Sludge source and physicochemical characterization

Dewatered biological sludge was collected after filter-press treatment at the wastewater treatment plant of a fish-processing facility in Rio Grande, Rio Grande do Sul, Brazil. The experiment and historical records originated from a master's dissertation (Caldasso, 2018); the retained raw and corrected files are available in a data repository, and are publicly available in Mendeley Data (Rosa-Silva & Arsand, 2026). The dissertation describes four 1-kg subsamples obtained after homogenizing 20 field collections over four months, whereas the retained chemistry workbook is structured as two sample groups, each with three analytical replicates calculated from two determinations. Because independence among four subsamples cannot be reconstructed from the retained files, the present reanalysis conservatively treats the two workbook groups as independent sludge samples and does not treat analytical replicates as environmental replicates. Parameters included pH, electrical conductivity, organic matter, total Kjeldahl nitrogen, total phosphorus, potassium, Al, Cd, Pb, Cu, Cr, Zn, Fe, Mn, and Ni. Means, standard deviations, and analytical coefficients of variation were calculated separately for each workbook group.

Results were interpreted with reference to CONAMA Resolutions 498/2020 and 420/2009 and MAPA Normative Instruction 61/2020. Because pathogen data, process documentation, evidence of environmental authorization, and product-registration records were unavailable, no microbiological classification, regulatory compliance, or commercial eligibility was inferred from the dataset.

2.2. Seed bioassays

The raw workbook contained four sampling blocks per species. Within each block and species, three Petri dishes received distilled water (control) and three received an aqueous sludge extract. The extract was prepared at a 1:10 sludge-to-distilled-water ratio, homogenized for 1 h, and filtered; only the undiluted filtrate obtained at this extraction ratio was tested. Ten commercial seeds were placed on qualitative filter paper in each dish, and 5 mL of control water or extract was added. Dishes were incubated in darkness at 25°C for 48 h. Seeds with radicles at least 1 mm long were considered germinated, and radicle length was measured with a digital caliper. Electrical conductivity was not measured in the block-specific extracts. The experiment comprised 96 dishes and 960 seed positions across four species. Watercress (Lepidium sativum) was excluded from the primary analysis because the workbook indicated a one-week incubation rather than the prespecified 48 h. The absence of a dilution series and block-matched conductivity precluded concentration–response modeling, EC50 estimation, and direct salinity–response analysis.

Within each species and sampling block, relative root elongation (RE), germination success (GS), and the germination index (GI) were calculated as follows:

RE(%) = 100 x mean radicle length extract / mean radicle length control (1)

GS(%) = 100 x mean germinated seed extract / mean germinated seed control (2)

GI(%) = RE x GS / 100 (3)

The sampling block, not the individual dish, was the primary unit (n = 4 blocks per species). Stored spreadsheet formulas that referenced a control mean from another block or were blank were replaced by block-specific formulas.

2.3. Greenhouse experiment

The greenhouse experiment was conducted from June to September 2017. Soil classified as a Planossolo Háplico eutrófico arênico was collected from the 0–20 cm layer in Capão do Leão, Rio Grande do Sul, Brazil. The initial soil characterization is reported in Online Resource 1, Table S1 as recorded in the source files. Plants were grown in 5-L pots arranged in triple rows, with 0.30 m between pots, and irrigated every two days. Mean greenhouse temperature and relative humidity were 23.9°C and 46.2%, respectively. Four treatments were reconstructed from the protocol and raw worksheets: T1, soil with dolomitic lime (lime-amended control); T2, lime plus sludge; T3, lime plus urea, triple superphosphate, and KCl (mineral-NPK comparator); and T4, sludge without lime. Lime was applied to raise soil pH to 6.0 according to the SMP index, and nutrient additions followed crop and soil recommendations.

The T2 sludge addition was reportedly calculated from sludge nitrogen and crop and soil requirements, but the final sludge amount and dry-solids dose were not recoverable. No numerical dose was available for T4. T2 and T4 could not be expressed as kg ha^{-1} or t dry solids ha^{-1}. Reconstruction would require the sludge amount per pot, solids concentration, soil mass and bulk density, incorporation depth, dry-mass nitrogen concentration, and the mineralization fraction used in the calculation. Ammonium, nitrate, and the organic-N mineralization fraction were also unavailable, preventing confirmation that the sludge treatments were nitrogen-equivalent to T3. For this reason, the greenhouse trial can describe treatment-associated biological responses under the recorded pot conditions, but it cannot define a field application rate or support a dose-equivalent comparison with mineral fertilization. The recoverable lime and mineral-fertilizer quantities recorded in the source protocol are provided in Online Resource 1, Table S5.

Six pots per treatment and species were planned. Two lettuce plants and one arugula plant assigned to T3 died shortly after transplanting, leaving 22 evaluable lettuce plants (T3, n = 4; all other treatments, n = 6) and 23 evaluable arugula plants (T3, n = 5; all other treatments, n = 6).

Lettuce was harvested 67 d after transplanting and arugula after 54 d. Recorded endpoints were leaf number; maximum leaf length, measured from the plant collar to the tip of the longest leaf; root length; shoot and root fresh mass; and shoot and root dry mass. Shoots and roots were dried at 70°C to constant mass. Because the source files did not retain identifiers linking pots to the original greenhouse blocks, treatment was analyzed as a single fixed factor within each species; no randomized-block or factorial analysis was inferred.

2.4. Data quality and statistical analysis

Analyses were performed in Python 3.12 using SciPy 1.17, pandas 2.2, and NumPy 2.3. Seed-bioassay endpoints were summarized across four sampling blocks as mean ± SD with 95% t confidence intervals. Exploratory one-sample t tests compared normalized endpoints with 100%. An exact two-sided sign-flip test was also reported because the sample size was small. With n = 4, its minimum attainable two-sided p value was 0.125; interpretation emphasized effect magnitude, consistency, and interval width.

Greenhouse endpoints were analyzed separately by species. Residual normality was evaluated with the Shapiro–Wilk test and homogeneity of variance with the Brown–Forsythe version of Levene's test. Homoscedastic normal endpoints were analyzed by one-way ANOVA with Tukey HSD, normal heteroscedastic endpoints by Welch ANOVA with Games–Howell tests, and non-normal endpoints by Kruskal–Wallis tests followed by Dunn pairwise comparisons with Holm adjustment. Effect sizes were η^2 for ANOVA-type summaries and ε^2 for Kruskal–Wallis tests. To account for multiple endpoints, p values were also adjusted by the Benjamini–Hochberg procedure within each species.

One arugula T3 root-fresh-mass value (82.19 g) was retained in the primary analysis but flagged as biologically implausible relative to neighboring observations and its corresponding dry mass; the endpoint was reanalyzed without this value. One negative arugula root-dry-mass value was treated as missing because the recorded gross mass was below the tare. Missing plants were not imputed. Chemistry–plant correlations and principal-component analysis were not performed because only two independent chemistry samples were available and neither was matched to individual pots or bioassay blocks.

Figure 1 Evidence-to-decision framework for the ecotoxicological screening of dewatered fish-processing wastewater sludge. Available evidence comprised physicochemical characterization of two retained sludge sample groups, a 1:10 seed bioassay, and a greenhouse experiment. The observed species-specific responses, together with the absence of field-equivalent sludge doses, pathogen status, nitrogen-mineralization data, contaminant-bioavailability and leaching measurements, and edible-tissue analyses, prevent conclusions regarding environmental safety, agronomic equivalence, or unrestricted land application. Dose-defined experiments with matched sludge, soil, microbiological, and plant-tissue measurements and complementary ecotoxicological tests are required to define an environmentally acceptable application range

3. Results

3.1. Physicochemical characterization and analytical precision

Physicochemical values for the two retained sludge sample groups are reported in Table 1. Most within-sample analytical CVs were below 16%, with the main exception of total phosphorus in Sample 2 (CV 31.46%), reflecting one lower analytical replicate than the other two. Pb was recorded as zero in every replicate, but no reporting limit was available, and the relative magnitudes among trace elements could not be independently verified (Table 1, Notes). With only two conservatively defined sample groups, these data describe the retained material but not batch-to-batch variability or regulatory compliance.

Table 1

Physicochemical characteristics of two retained sample groups of dewatered fish-processing wastewater sludge. Values are mean ± SD of three analytical replicates, each calculated from two determinations

Parameter	Unit	Sample 1	Sample 2	Maximum within-sample CV (%)
pH	-	7.15 ± 0.00	7.12 ± 0.03	0.36
Total phosphorus	mg kg⁻¹ P	2.04 ± 0.03	1.72 ± 0.54	31.46
Total Kjeldahl nitrogen	mg kg⁻¹ N	51.73 ± 0.42	52.02 ± 1.35	2.60
Electrical conductivity	µS cm⁻¹	1222.91 ± 57.17	1243.31 ± 139.43	11.21
Organic matter	mg kg⁻¹ KMnO	540.67 ± 1.53	617.00 ± 84.50	13.70
Al	mg kg⁻¹	1.72 ± 0.13	1.80 ± 0.17	9.40
Cd	mg kg⁻¹	0.0075 ± 0.0009	0.0072 ± 0.0007	11.55
Pb	mg kg⁻¹	0.0000 ± 0.0000	0.0000 ± 0.0000	n/d
Cu	mg kg⁻¹	0.0830 ± 0.0121	0.0797 ± 0.0090	14.61
Cr	mg kg⁻¹	41.05 ± 0.21	45.64 ± 5.07	11.10
Zn	mg kg⁻¹	0.8533 ± 0.0635	0.7567 ± 0.1155	15.26
Fe	mg kg⁻¹	0.4053 ± 0.0439	0.4527 ± 0.0629	13.90
Mn	mg kg⁻¹	0.0184 ± 0.0007	0.0188 ± 0.0007	3.77
Ni	mg kg⁻¹	0.0580 ± 0.0035	0.0567 ± 0.0067	11.75
K	mg kg⁻¹	21.53 ± 1.04	22.04 ± 2.00	9.08

Note. Values are presented in the units and mass basis recorded in the original laboratory files. Method-specific detection and quantification limits were unavailable; zero Pb readings should not be interpreted as confirmed absence. The relative magnitudes among trace elements, including Cr values exceeding Fe, Zn, and Cu, could not be independently verified and may reflect unresolved unit, mass-basis, or reporting inconsistencies in the historical dataset. The higher CV for total phosphorus in Sample 2 reflects one lower analytical replicate

3.2. Block-corrected seed bioassays

Cucumber and arugula showed consistent extract-associated increases in root elongation across the four blocks. Mean RE was 202.6 ± 26.8% for cucumber and 162.6 ± 8.6% for arugula; their mean GI values were 184.3 ± 16.2% and 164.1 ± 13.4%, respectively (Fig. 2). Cucumber germination success was modestly lower than its paired control (91.3 ± 4.2%), whereas arugula germination success was similar to control (101.1 ± 7.8%). Exploratory one-sample t tests for cucumber and arugula RE and GI were below 0.005, but exact sign-flip p values were 0.125 and were inconclusive at four blocks.

Lettuce responses varied widely among blocks. Mean RE was 281.1 ± 114.8%, germination success was 111.7 ± 49.0%, and GI was 344.7 ± 267.7%. The 95% CI for GI crossed both the 80% screening threshold and 100%, indicating that the high mean did not represent a consistent stimulatory response. Although no primary species had a mean GI below 80%, this single-strength assay with four blocks cannot establish the absence of phytotoxicity outside the tested conditions. Because electrical conductivity was not measured in the block-specific 1:10 extracts, the pronounced lettuce variability could not be evaluated in relation to salinity.

3.3. Lettuce whole-plant greenhouse response

Lettuce shoot traits did not differ among treatments: leaf number ($p = 0.0930$), maximum leaf length ($p = 0.7594$), shoot fresh mass ($p = 0.8809$), and shoot dry mass ($p = 0.2965$). Root fresh and dry masses were also non-significant ($p = 0.2908$ and $p = 0.1689$). Root length differed at the unadjusted endpoint level (ANOVA $F = 4.56$, $p = 0.0151$, $\eta^2 = 0.432$); T1 had longer roots than T4 (25.83 ± 6.05 versus 11.00 ± 2.90 cm; Tukey $p = 0.0089$), while T2 and T3 were intermediate. However, the root-length endpoint did not remain significant after Benjamini–Hochberg adjustment across the seven lettuce endpoints (adjusted $p = 0.1059$; Fig. 3).

3.4. Arugula whole-plant greenhouse response

Arugula showed clear treatment-related differences. Leaf number differed by ANOVA ($F = 7.00$, $p = 0.0023$, $\eta^2 = 0.525$): leaf number was lowest under T1 (5.33 ± 1.75) relative to T2 (28.83 ± 9.50), T3 (24.80 ± 12.93), and T4 (21.50 ± 10.71). Root length also differed ($F = 21.08$, $p < 0.0001$, $\eta^2 = 0.769$). Root length was greatest under T2 (40.17 ± 12.51 cm), followed by T4 (26.50 ± 8.02 cm); T1 (7.90 ± 1.60 cm) and T3 (10.82 ± 3.89 cm) formed the lowest group.

Shoot fresh mass differed by Kruskal-Wallis test ($H = 17.64$, $p = 0.0005$, $\varepsilon^2 = 0.770$), with means of 3.49 ± 1.50 g in T1, 61.67 ± 8.58 g in T2, 13.94 ± 6.59 g in T3, and 40.57 ± 27.17 g in T4 (Fig. 4). Shoot dry mass showed the same treatment ordering ($H = 16.71$, $p = 0.0008$, $\varepsilon^2 = 0.722$). Maximum leaf length, root fresh mass and root dry mass were also treatment-associated ($p < 0.0001$, $p = 0.0277$ and Welch $p = 0.0018$, respectively), and all seven endpoint-level tests remained below 0.05 after false-discovery-rate adjustment. Excluding the flagged 82.19-g T3 root-fresh-mass value strengthened the root-fresh-mass result ($H = 13.27$, $p = 0.0041$, $\varepsilon^2 = 0.571$), showing that the overall treatment signal was not created by

that observation. Complete greenhouse endpoint summaries for lettuce and arugula are provided in Online Resource 1, Table S4.

4. Discussion

4.1. Interpretation of the seed bioassays

Correcting each extract value against the control from the same sampling block materially changed several spreadsheet-derived estimates. The original dissertation interpreted the sludge as non-phytotoxic and suitable for agricultural use in the evaluated species (Caldasso, 2018). The present reanalysis leads to a more cautious interpretation because it preserves block-level independence and adjusts for multiple endpoints. The contrast is most visible for lettuce: the original pooled analysis reported GI = 302.34 ± 69.28% as mean ± standard error, whereas the block-normalized reanalysis yielded 344.7 ± 267.7% as mean ± standard deviation, exposing substantial between-block heterogeneity. After correction, cucumber and arugula showed consistent descriptive increases in radicle elongation and GI, whereas lettuce was highly variable. This divergence is biologically plausible because species differ in seed reserves, ion sensitivity, and early-root responses; sludge phytotoxicity also changes with soil or extract properties and the test species used (Hechmi et al., 2020; Wu et al., 2022). The cucumber result further illustrates why germination and elongation should be retained as separate components: germination success was modestly below its control while radicle elongation approximately doubled.

GI values above 100% may reflect stimulation by soluble nutrients or other growth-promoting constituents, but they do not demonstrate a specific biostimulant mechanism or absence of hazard. Salinity deserves particular attention in fish-derived residues. High salinity in thermally dried salmon-farming sludge inhibited germination and constrained soil or substrate application (Pérez-San Martín et al., 2024), and increasing sewage-sludge application ratios have been shown to increase water-soluble salts and metal activity, with salt stress emerging as the principal driver of early seedling inhibition under those tested conditions (Chen et al., 2025). Lettuce germination has likewise declined with increasing electrical conductivity in sludge-amended soils (Hechmi et al., 2020). Other plausible influences include ammonium, pH, nutrient speciation, and contaminant bioavailability. Table 1 reports electrical conductivities of 1222.91 ± 57.17 and 1243.31 ± 139.43 μS cm^{-1} for the two sludge samples. However, these measurements were not confirmed to originate from the same 1:10 extracts used in the bioassay, and the chemical samples were not paired with the four biological blocks. They therefore cannot identify salinity, metals, or any other constituent as the driver of the block-to-block variation in lettuce.

Only the undiluted filtrate produced at the 1:10 extraction ratio was tested. Without a dilution series, EC50 or other ECx values cannot be estimated, and possible transitions between stimulation and inhibition remain unresolved. The assay should therefore be interpreted as a single-strength screening test (OECD, 2006), not a concentration-response assessment. Bio-based fertilizers and waste-derived soil amendments are more robustly evaluated by combining standardized bioassays with exposure measurements and complementary biological models (Albert and Bloem, 2023; Roques et al., 2023).

Fermented or microbially converted fish-waste fertilizers can support plant growth (Mponda and Kim, 2023), but they are not directly equivalent to filter-pressed biological sludge.

The exact four-block analysis preserves the independent sampling unit. Counting the three plates within each block as independent would be pseudoreplication and would produce unjustifiably narrow uncertainty. With four blocks, the smallest attainable two-sided exact sign-flip p value was 0.125, so the large cucumber and arugula effects are best treated as consistent descriptive responses rather than definitive inferential evidence. The 1:10 assay serves only as an initial screen. It did not show consistent mean inhibition in cucumber or arugula under the specified 48-h conditions, though only at the single tested extract strength (see Section 4.4).

4.2. Whole-organism greenhouse responses and exposure uncertainty

Greenhouse responses differed markedly between species, reinforcing that the sludge-associated response was context dependent rather than a general indicator of agronomic performance or environmental safety. Lettuce shoot biomass and leaf production were unchanged, and the nominal T1–T4 root-length difference did not remain significant after multiplicity adjustment. Arugula showed strong treatment-associated differences, with the largest root and shoot responses under lime plus sludge (T2) and generally intermediate responses under sludge without lime (T4). However, the design was not a complete factorial experiment, the original block identifiers were not recoverable, and the applied sludge doses could not be reconstructed. The observed differences therefore cannot be attributed causally to sludge, liming, nutrient supply, salinity, or any single exposure driver.

The retained post-harvest substrate data provide treatment-level context but cannot test mechanism because pot-level measurements, variances, and analytical links to individual plants were not retained. For arugula, T2 combined the highest reported conductivity (445.50 μS cm^{-1}) with the strongest growth, showing that conductivity alone does not explain the treatment pattern; nutrient availability, ionic composition, liming, and species tolerance remain plausible and potentially interacting explanations. Likewise, poorer arugula performance and early mortality in T3 cannot establish that sludge outperformed mineral fertilization because the treatments were not demonstrably N-equivalent and survivorship bias cannot be excluded. Confirmatory work should retain randomization and block identities, express amendments on dry-solids and field-equivalent bases, and pair individual plants with soil and tissue chemistry. In this screening dataset, the greenhouse component is therefore most informative as evidence of species-specific whole-organism response and unresolved exposure attribution. The post-harvest substrate chemistry reported for lettuce and arugula is provided in Online Resource 1, Tables S2 and S3, respectively.

4.3. Environmental implications for agricultural reuse

The present results are most useful as an initial screening of a residue intended for soil application rather than as evidence of agronomic suitability. Cucumber and arugula were not consistently inhibited

at the tested extract strength, lettuce showed substantial between-block heterogeneity, and arugula showed strong greenhouse treatment-associated responses. These biological patterns indicate that the sludge can alter plant performance, but they do not resolve whether the dominant drivers were nutrients, salts, pH-related changes, metals, or other constituents. From a pollution perspective, this distinction is central: a residue may produce favorable short-term plant responses while still introducing constituents whose mobility, persistence, or accumulation could become relevant under repeated application.

Plant-only responses also leave important hazard pathways unresolved. Sludge hygiene varies with processing and target organism, and repeated land application may enrich antibiotic- and metal-resistance determinants in soil (Carraturo et al., 2022; Mokni-Tlili et al., 2024). In a multi-species chronic study, sewage sludge increased plant growth while high-dose exposure inhibited earthworm reproduction, demonstrating that favorable plant responses and ecotoxicological safety are not equivalent endpoints (Roques et al., 2023). In the present study, no data were available for pathogen indicators, antimicrobial-resistance markers, persistent organic contaminants, microplastics, plant-tissue accumulation, or leaching. These gaps are especially relevant for leafy vegetables because the harvested tissue is consumed. Favorable biomass or germination responses therefore cannot be taken as evidence of food-chain or environmental safety. “Industrial sludge” is used here instead of “biosolid” because regulatory status was not established for this material.

Regulatory interpretation was kept secondary to the ecotoxicological evidence. In Brazil, CONAMA Resolution 498/2020 and MAPA Normative Instruction 61/2020 regulate different aspects of land application and product eligibility, while CONAMA Resolution 420/2009 provides soil-quality guidance values. Because pathogen results, process documentation, verified analytical units and limits, field-equivalent dose information, and evidence of authorization or registration were unavailable, the sludge cannot be assigned a microbiological class, declared compliant, or recommended for routine land application. These regulatory gaps reinforce, rather than replace, the exposure limitations identified experimentally.

4.4. Exposure uncertainty and implications for ecotoxicological interpretation

The principal strength of this reanalysis is that it separates observed biological responses from unsupported exposure or safety claims. Correcting the experimental unit, matching seed responses to block-specific controls, and testing the influence of an implausible root-mass value reduced the risk of overstating precision. For pollution studies involving heterogeneous wastes, this distinction is methodologically important because analytical replicates, repeated plates, and independent residue samples represent different levels of inference. The dataset therefore illustrates how quality control in experimental design and reanalysis can change the environmental interpretation of an apparently favorable bioassay.

Online Resource 1, Tables S1–S3 retain the historical soil and substrate values, but treatment means and analytical replicates were not used as independent observations. Confirmatory work should include

independent sludge batches, an extract dilution series with matched conductivity, ammonium, pH, and ion measurements, and randomized blocked greenhouse or field exposure designs using dry-solids-based doses. Pot-matched soil and tissue chemistry, mineral-N release, plant-N uptake, pathogen testing, leaching, relevant emerging contaminants, and complementary terrestrial bioassays are needed to distinguish transient plant responses from an environmentally acceptable land-application range. Taken together, these constraints mean the present dataset cannot establish the absence of phytotoxicity beyond the tested conditions, identify a causal exposure driver, or support unrestricted land application; instead, it defines the specific evidence gaps that the next phase of ecotoxicological testing must close.

5. Conclusions

At the tested extract strength, cucumber and arugula showed no consistent descriptive inhibition, whereas lettuce responses varied among the four sampling blocks. Exact inference was inconclusive, so these findings do not demonstrate absence of phytotoxicity. In the greenhouse, arugula showed strong treatment-associated responses while lettuce was comparatively unresponsive. Taken together, the results show that plant responses to fish-processing wastewater sludge are species- and exposure-dependent and that apparent stimulation cannot be equated with environmental safety. Because field-equivalent dose, block-specific salinity, pathogen status, contaminant bioavailability, leaching, and edible-tissue accumulation were not resolved, unrestricted land application cannot be supported from this dataset. For heterogeneous industrial sludges, seed and greenhouse assays are best positioned as an initial tier of pollution assessment that should be followed by dose-defined, chemically matched, and multi-compartment testing.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

Not applicable. The study involved plant bioassays and did not involve human participants or animals.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The raw workbooks, corrected analysis workbook, statistical outputs, and analysis code supporting this study are publicly available in Mendeley Data: Rosa-Silva and Arsand (2026), https://doi.org/10.17632/j2hf2xxmmn.1.

Author contributions

Daniel Ricardo Arsand: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. Fabiane Araújo Caldasso: Writing – original draft, Investigation, Formal analysis, Conceptualization, Methodology, Data curation. Érico Kunde Correa: Writing – original draft, Methodology, Investigation, Data curation. Michel David Gerber: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. Helen Tais da Rosa-Silva: Writing – review and editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Use of generative AI and AI-assisted technologies

OpenAI ChatGPT and Codex were used to assist with language editing, code-supported reanalysis of author-supplied data, and document preparation. The authors reviewed and edited all outputs, verified the analyses against the raw files, and take full responsibility for the content and conclusions. No generative AI image tool was used to create or modify the figures.

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Figures

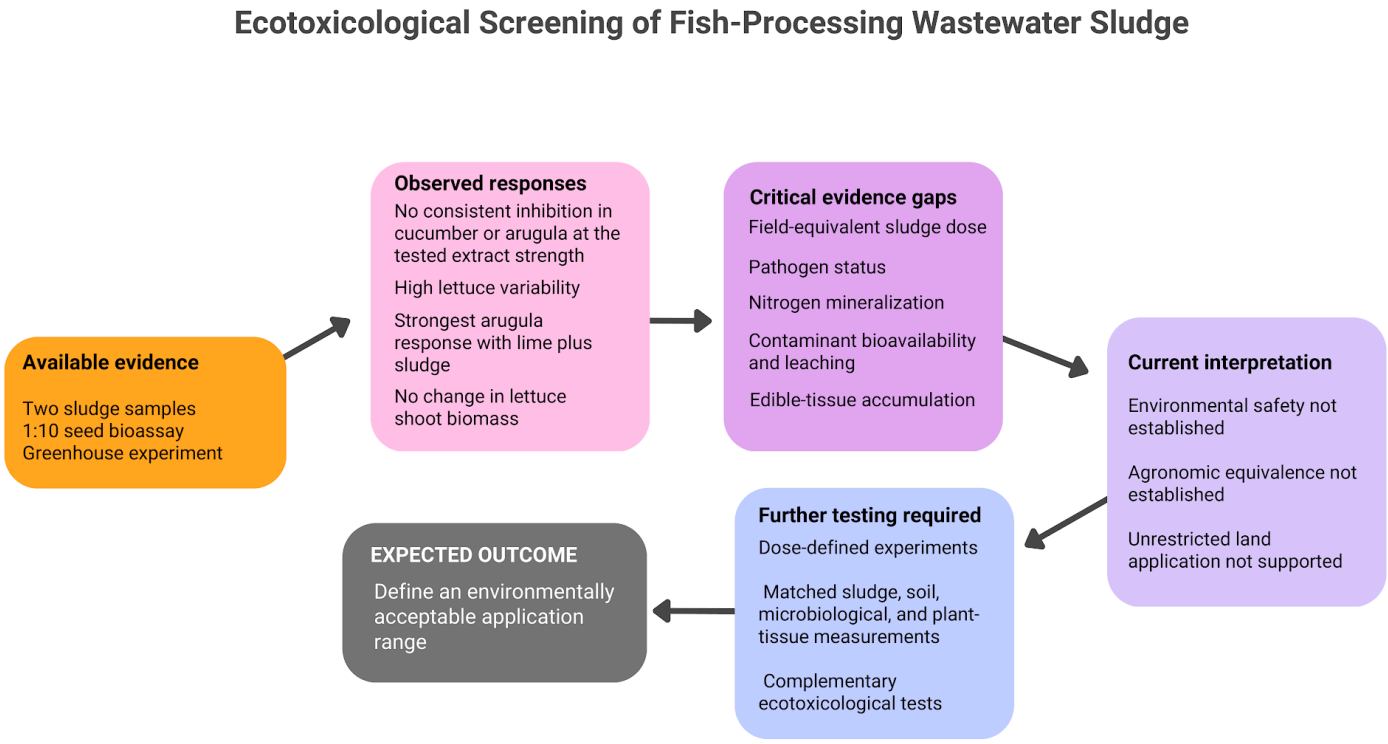


Figure 1

Evidence-to-decision framework for the ecotoxicological screening of dewatered fish-processing wastewater sludge. Available evidence comprised physicochemical characterization of two retained sludge sample groups, a 1:10 seed bioassay, and a greenhouse experiment. The observed species-specific responses, together with the absence of field-equivalent sludge doses, pathogen status, nitrogen-mineralization data, contaminant-bioavailability and leaching measurements, and edible-tissue analyses, prevent conclusions regarding environmental safety, agronomic equivalence, or unrestricted land application. Dose-defined experiments with matched sludge, soil, microbiological, and plant-tissue measurements and complementary ecotoxicological tests are required to define an environmentally acceptable application range

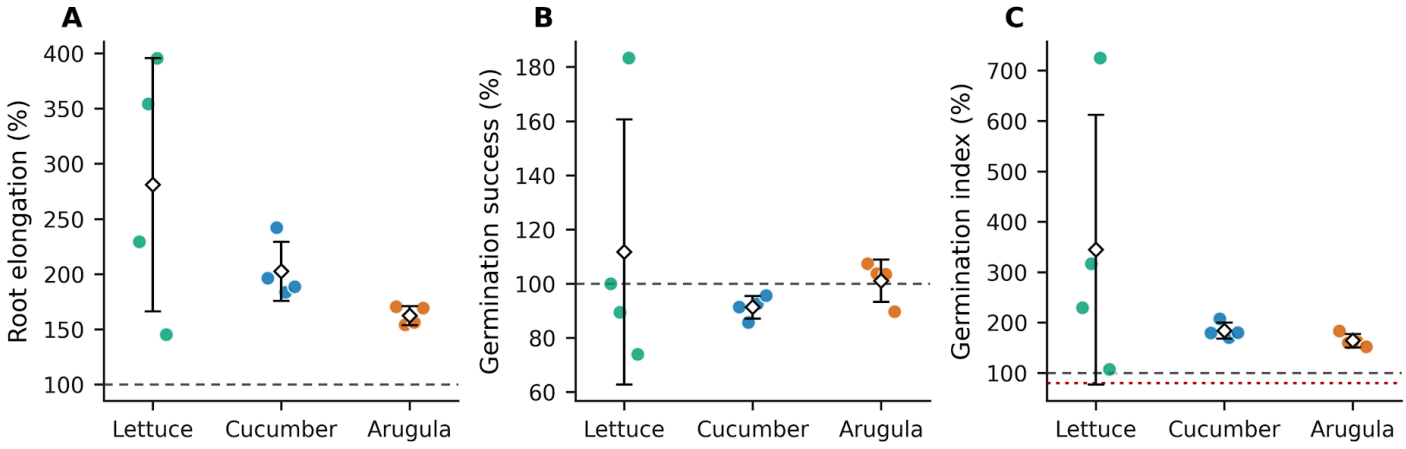


Figure 2

Block-normalized seed-bioassay responses of lettuce, cucumber, and arugula exposed to a 1:10 aqueous extract of dewatered fish-processing wastewater sludge. Panels show (a) relative root elongation, (b) germination success, and (c) germination index. Each circle represents one independent sampling block (n = 4); diamonds and error bars indicate mean ± SD. Values were normalized to the block-matched water control. The gray dashed line denotes the control reference (100%), and the red dotted line in panel C denotes the 80% germination-index screening threshold (Tiquia et al., 1996; Zucconi et al., 1981). Watercress was excluded because its recorded incubation period differed from the prespecified 48-h protocol

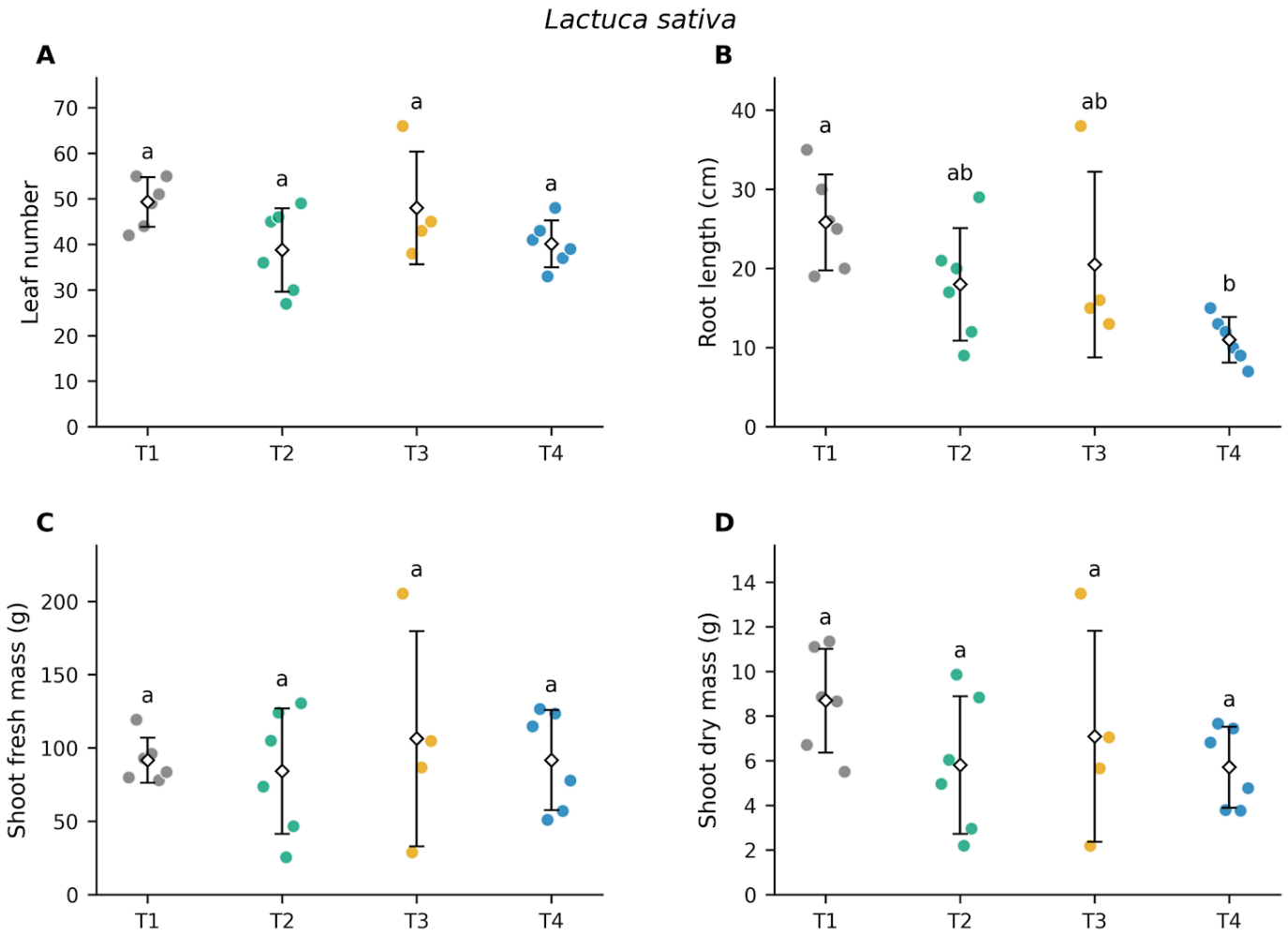


Figure 3

Lettuce responses to soil amendments in the greenhouse experiment. Panels show leaf number, root length, shoot fresh mass, and shoot dry mass. Circles represent individual plants; diamonds and error bars indicate mean ± SD. Treatments were T1, lime-amended soil control; T2, lime plus sludge; T3, lime plus mineral NPK; and T4, sludge without lime. Within each endpoint, treatments sharing a letter did not differ in the applicable unadjusted post-hoc comparison ($p \geq 0.05$). Sample sizes were n = 6 per treatment, except T3 (n = 4). The nominal treatment effect for root length did not remain significant after Benjamini–Hochberg adjustment across the seven lettuce endpoints

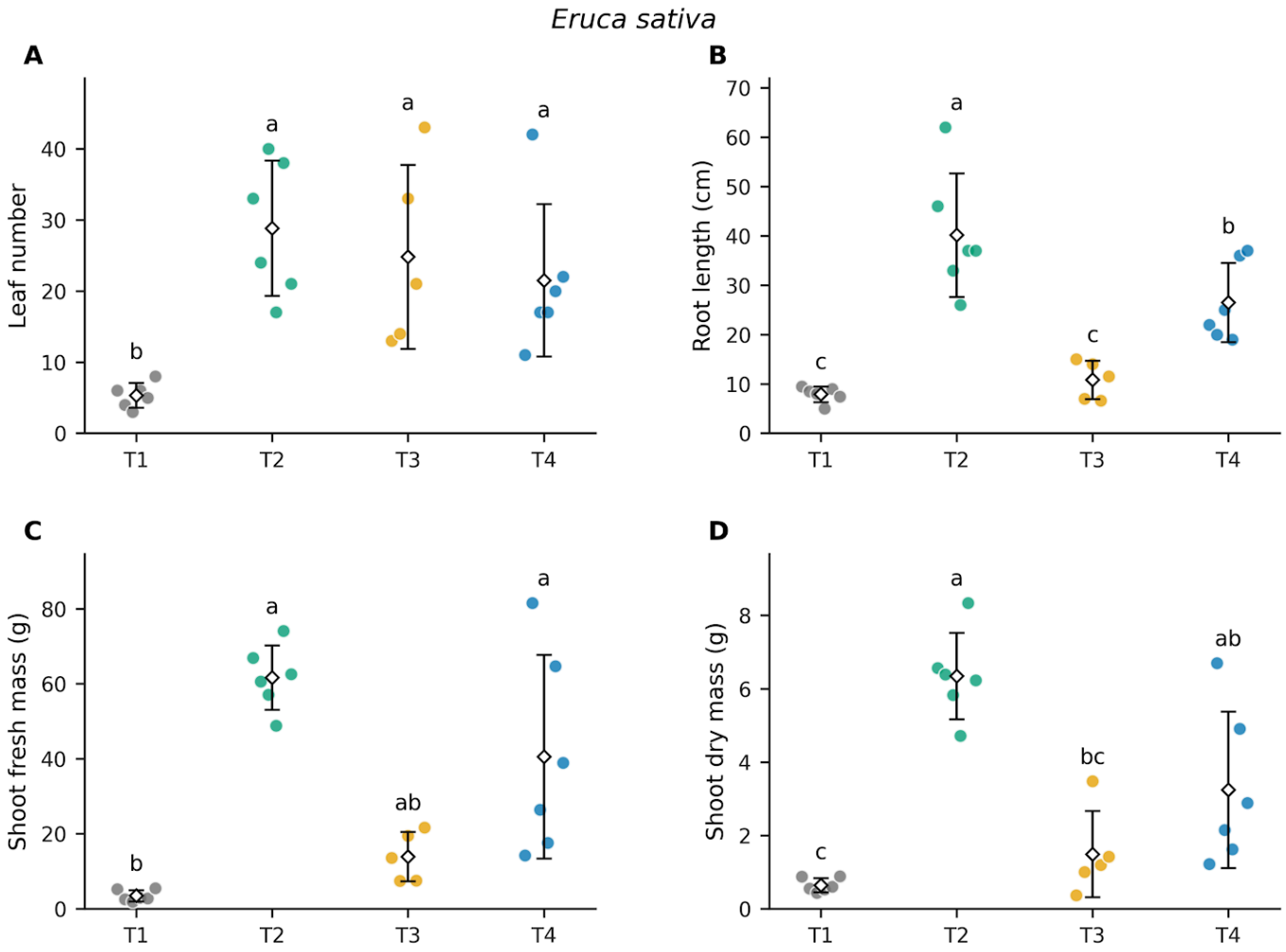


Figure 4

Arugula responses to soil amendments in the greenhouse experiment. Panels show leaf number, root length, shoot fresh mass, and shoot dry mass. Circles represent individual plants; diamonds and error bars indicate mean ± SD. Treatments were T1, lime-amended soil control; T2, lime plus sludge; T3, lime plus mineral NPK; and T4, sludge without lime. Within each endpoint, treatments sharing a letter did not differ in the applicable unadjusted post-hoc comparison ($p \geq 0.05$). Sample sizes were n = 6 per treatment, except T3 (n = 5). All seven endpoint-level treatment tests remained significant after Benjamini–Hochberg adjustment

Supplementary Files

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