BIOTEST-BASED TOXICITY EVALUATION OF BIODEGRADABLE FOOD WASTE: CONCLUSIONS BASED ON AN ANALYSIS OF SPENT COFFEE GROUNDS (SCG)

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Abstract

The increasing generation of biodegradable residues from the food service sector highlights the need for ecotoxicological assessment to support circular waste management. This study evaluated the toxicity of spent coffee grounds (SCG) using chemical characterisation and bioassays with plants (*Sinapis alba*, *Lepidium sativum*, *Lactuca sativa*), aquatic invertebrates (*Daphnia magna*) and soil invertebrates (*Eisenia fetida*). The absorbance analysis of SCG aqueous extracts showed increasing DOC with concentration, reaching 869 mg C kg^{-1} at 15% dilution, and indicated the predominance of fulvic compounds. Phytotoxkit assays revealed species-specific responses: *S. alba* was found to be the most sensitive, demonstrating potent inhibition at higher SCG levels, whereas *L. sativum* and *L. sativa* exhibited stimulation at moderate concentrations (maximum GI ≈ 210% at 5% SCG for *L. sativum*). *D. magna* displayed acute sensitivity (EC_{50} ≈ 4.1%), whereas *E. fetida* results were inconsistent due to high control mortality. Overall, the results demonstrate that SCG can act both as a stimulant and an inhibitor depending on concentration and organism, emphasising the importance of multi-species bioassays for risk assessment and potential valorisation of SCG in environmental applications.

Keywords: ecotoxicological assay, environmental application, phytotoxicity, waste management

1. Introduction

The management and valorisation of post-consumer organic residues generated in the food service sector remain a significant challenge and constitute an area of intensive scientific and technological development aimed at mitigating waste-related pollution. As coffee consumption increases globally, especially in cafes and restaurants, waste from all stages in the life cycle of coffee beans has increased significantly (Hoseini et al., 2021). According to the Coffee Market Report from July 2025, published by the International Coffee Organisation, coffee consumption

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estimates reached 177.0 million 60-kg bags in 2023/24, representing a 2.2% increase compared to 2022/23 (ICO, 2025). The main end waste from brewing a cup of coffee is spent coffee grounds (SCG), which represent biodegradable organic waste that still includes valuable compounds that can be useful in lots of areas (Stylianou et al., 2018), but released into the environment could cause toxicity (McNutt & He, 2019). Those compounds comprise mainly alkaloids (e.g., caffeine), aliphatic acids, amino acids, lignin, lipids, melanoidins, minerals, oligo- and polysaccharides, phenolics, and proteins (Campos-Vega et al., 2015), as well as other allelopathic molecules.

To mitigate landfilling and environmental contamination, while simultaneously promoting a circular economy, it is crucial to manage SCG waste properly (Cho et al., 2025) through adequate pre-treatment or valorisation prior to its use as a resource. Recently, many assessment and review studies have focused on the potential applications of SCG waste given its properties and value for a wide range of sectors, including the food industry (Ahanchi et al., 2024; Koay et al., 2023; Masino et al., 2022) and it produces a significant quantity of waste. Spent coffee grounds (SCGs, fuel and energy (Vardon et al., 2013)), cosmetics (Sousa et al., 2018; Marto et al., 2016), or agriculture and fertilisation (Cervera-Mata et al., 2022; Stylianou et al., 2018) several studies have shown that soil application of untreated SCG has detrimental agronomic and environmental effects due to their high degradability and content of noxious compounds (phenols, caffeine, and tannins. At present, research on the use of SCG as a high-value substrate is predominantly experimental; however, several concepts have already been implemented in practice – for example, biodiesel produced from SCG-derived oil is used to power buses in London(Bioenergy International, 2017). When it comes to usage in agriculture there are contradictory results on SCG effects on plants and soil, with some suggesting phytotoxicity and inhibition of growth (Hardgrove and Livesley, 2016) either through direct soil application or after composting with other urban organic wastes. This study investigates the scientific basis for direct application of spent coffee grounds (SCG) while others point to an increase in vital elements like N and K, and organic matter in a soil that contribute to better nutrient profile in plants (Cervera-Mata et al., 2018; Cruz et al., 2014).

Given the wide variety of coffee bean types available on the market, originating from a number of countries and the multitude of coffee processing methods, the characteristics of SCG can vary. As a result, SCG waste often exhibits inconsistent parameters, and each batch should be examined prior to its intended application. Apart from chemical and physical parameters, bioassays can constitute immensely useful tools for assessing properties and toxicity of biodegradable organic residues while low capital and time requirement needs (Sobik-Szotysek and Wystalska, 2020).

This study aims to determine the environmental toxicity of SCG, an example of food biodegradable waste, using standardised biotests to assess its potential risks and benefits in waste management and environmental applications. The environmental effects of SCG depend strongly on the applied concentration, the sensitivity of the exposed species, and the physical form in which the material is introduced (e.g., solid residue or aqueous extract). In this study, we hypothesised that the environmental toxicity of spent coffee grounds (SCG) depends on their concentration and on the sensitivity of the exposed test organisms.

2. Materials and Methods

2.1. Research material

The research material for the present study, and namely spent coffee grounds (SCG), was obtained from a small-scale café on a single working day. The sample consisted of various types of coffee beans, mainly Arabica from the Brazilian Cerrado Mineiro region, which was brewed as espresso (steam extraction under pressure) and also other types brewed by hot water extraction (V60 filter coffee), such as coffee beans grown in Guatemala or Ethiopia. The collected samples have been thoroughly homogenised and dried at 105°C to ensure complete water evaporation. They were keptin the dryer until no appreciable mass change was observed (ISO 11465, 1993), indicating the absence of residual moisture that could otherwise promote mold growth or the proliferation of other microorganisms (Gabrio and Fischer, 2024). The sample was stored at ambient temperature and used in experiments within a period of 5 weeks.

2.2. Experimental analysis

Samples of SCG have been analysed for characteristics such as pH, electrical conductivity (EC), total moisture content, and total organic matter (OM), all according to *International Organization for Standardisation* guidelines. pH (ISO 10523, 2008) and EC (ISO 7888, 1985) were determined using pH meter (Omega, PHH444 portable pH/mV/temperature-meter) at ambient temperature 23.4°C (electrical conductivity was adjusted to the reference temperature 25°C using correction factor as specified in ISO 7888) from SCG aqueous extract (a.e.) which was made by mixing dried SCG and distilled water at a ratio of 1:10 (w/v) in laboratory sample mixer for 30 min and then filtered (Chimanbhai Saypariya et al., 2024; Sudharsan Varma and Kalamdhad, 2015). The obtained aqueous extract was then used as base test solution (100%) for four different dilutions (2.5%, 5%, 10% and 15%, v/v), which were examined upon absorbance of different wavelengths [nm] (254, 355, 465, and 665) using a spectrophotometer (EasyPlus UV/VIS, Mettler Toledo). Furthermore, the associated parameters, such as DOC, SUVA254 and the E4/E6 ratio, were calculated based on the given results using Equations 1-3 (Zhang et al., 2022).

$$DOC = 58.065 + 184.355 \times A_{355} \quad \text{Eq. (1)}$$

where: DOC – dissolved organic carbon, A_{355} – absorbance at 355 nm.

$$SUVA_{254} = \frac{A_{254}}{DOC \times d} \quad \text{Eq. (2)}$$

where: $SUVA_{254}$ – specific ultraviolet absorbance at 254 nm, A_{254} – absorbance at 254 nm, d – optical path length of the cuvette (0.01 m for 1 cm).

$$\frac{E_4}{E_6} = \frac{A_{465}}{A_{665}} \quad \text{Eq. (3)}$$

where: A_{465} – absorbance at 465 nm, A_{665} – absorbance at 665 nm.

The determination of the total moisture content [%] involved the calculation of mass loss of SCG samples dried at 105°C (until achieving of a consistent weight). Total OM [%] was assessed as the mass loss of SCG samples burnt at 400°C for 2 hours compared to those dried at 105°C (ISO 11465, 1993).

2.3. Ecotoxicological assessment

Phytotoxkit F™ (MicroBioTests Inc., Belgium) tests were conducted according to the bench protocol, which strictly adheres to the ISO standard (ISO 18763, 2016), using a standardised laboratory kit provided by the manufacturer. Three different concentrations (5%, 10%, and 15% w/w, dry weight basis) of dried SCG were mixed into test soils and compared to a control soil (0% SCG) (Cruz et al., 2014). Lepidium sativum and Sinapis alba were used in the Phytotoxkit assay; however, the standardised species Sorghum saccharatum was replaced with Lactuca sativa due to the complete absence of germination in the control soil. The substitution was justified by the biological characteristics of L. sativa and its long-standing use in phytotoxicity testing (Diogo et al., 2025). A paper filter was placed on the soil, and once it was completely wet, 10 seeds were placed in a row, equally spaced and approximately 1 cm from the intermediate ridge that separates the two compartments of the test plate. Plates were incubated vertically in the dark at 24.5 ± 1°C for 5 days. All assays were performed in triplicate. The results were expressed as the percentage germination index (GI) and growth inhibition of the young roots (IRG), following the ISO standard (ISO 11269-1, 2012), and were compared to germination and root elongation in thecontrol soil. GI was calculated using Equation 5, and IRG using Equation 4. The results were classified as follows: germination index GI > 110% and negative values of inhibition index IRG < 0%

indicate growth stimulation, GI < 90% and IRG > 0% indicate growth inhibition, and 90% ≤ GI ≤ 110% and IRG equal to 0% indicates no effect or anon-toxic response relative to the control soil (Szara et al., 2020).

$$IRG = \frac{A - B}{A} \times 100\% \qquad \text{Eq. (4)}$$

where: IRG – inhibition of root growth, A – mean root length (mm) in control soil, B – mean root length (mm) in test soil.

$$GI = \frac{G_s \times L_s}{G_c \times L_c} \times 100\% \qquad \text{Eq. (5)}$$

where: GI – germination index, G_c – seed germination in a control soil, L_c – root elongation (mm) in a control soil, G_s – seed germination in a test soil, L_s – root elongation (mm) in test soil.

Daphtoxkit F™ magna (acute immobilisation test using *Daphnia magna*) was performed through a 24-hour exposure under dark conditions at a constant temperature of 20°C ± 1 to the potentially toxic substance, in this case using 10 mL of each of previously prepared SCG a.e. dilutions (2.2.) and comparing it to a distilled water as specified in standard procedures (ISO 6341, 2012; OECD 202, 2004). At the start of the test, all organisms were less than 24 hours old, maintained under culture conditions of 20 ± 1°C, and fed two hours before, but not during, the exposure. After placing five specimens of *D. magna* into each plastic test vessel filled with examined dilutions, they were loosely covered to avoid contamination and water evaporation. After exposure, the immobilisation of organisms was assessed. Immobilisation was determined by whether they remained motionless for 15 seconds after shaking the test vessel, excluding movement of the appendages.

The Standard Earthworm Acute Toxicity Test with *Eisenia fetida* was carried out in analogy to standard procedures (ISO 11268-2, 2023; OECD 207, 1984) by batch test method (PN-EN 12457-2, 2006). The *E. fetida* earthworm species were maintained under culture conditions of 20 ± 1°C, 12/12 h light/dark light regime, and fed with cow dung. To establish the mortality of chosen earthworm species, 10 mL of each SCG a.e. dilution (2.2) was used to hydrate control soils (30 g each), obtaining four different test mixtures and one control soil (30 g) hydrated with the same volume of distilled water. Each soil sample was supplemented with a food source for the organisms, i.e. rolled oats (50 mg), administered only at the start of the test. Next, five specimens of *E. fetida* were placed in each plastic test

vessel with prepared soils, covered with access to air, and left in the dark for 7 days at a constant temperature of 20°C ± 1. After exposure, the mortality of organisms was assessed.

2.4. Statistical analyses

For absorbance, linear regressions were fitted for each wavelength to test the relationship between dilution and absorbance. Coefficients of determination (R^2) and *p*-values for regression slopes were recorded.

For Phytotokxit, two-way analyses of variance (ANOVA) were used to test the effects of species (*S. alba*, *L. sativa*, *L. sativum*) and SCG concentration (0%, 5%, 10%, 15%) on root length, germination index (GI), and inhibition index (IRG). Normality and homogeneity of variance were verified prior to analyses. Significant effects were followed by Tukey's HSD post-hoc tests. The significance level was set at $\alpha = 0.05$.

Immobilisation [%] of *D. magna* and mortality [%] of *E. fetida* were calculated as the proportion of introduced individuals that did not survive after exposure to SCG a.e. dilutions (0–15%). A logistic regression was used to estimate the EC_{50} for *D. magna* (Eq. 6 and 7) and LC50 for *E. fetida* (Eq. 6 and 7) with 95% confidence intervals. For *E. fetida* (7 d), responses across concentrations were irregular, and mortality in the control group exceeded the guidelines (OECD 207, 1984) validity criterion of ≤10%; thus, LC_{50} estimation was prevented, and results were presented descriptively.

$$logit(p) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 C \qquad \text{Eq. (6)}$$

where: p – probability of effect (immobilisation or mortality), C – concentration of SCG a.e. [%], β_0 – intercept estimate from the fitted logistic regression, β_1 – slope estimate from the fitted logistic regression

$$EC_{50}(or\ LC_{50}) = -\frac{\beta_0}{\beta_1} \qquad \text{Eq. (7)}$$

where: β_0 – intercept estimate from the fitted logistic regression, β_1 – slope estimate from the fitted logistic regression

3. Results and Discussion

3.1. SCG characteristics

The measured pH of the SCG aqueous extract was found to be 5.65±0.05, which is consistent with most research (Cervera-Mata et al., 2022, 2020, 2018; Comino et al., 2020). The lower pH level (<6) and certain compounds could impose limitations on the usage of SCG, especially when applied as fertilisers or animal feed; thus, pre-treatment with additives may be necessary (Gil-Gómez et al., 2024; Hardgrove and Livesley, 2016; Yamane et al., 2014). The EC25 value of SCG aqueous extract was found to be 1.75 dS m-1, which is a mere value compared to well-conducting graphite, for which EC25 is above 1.36 S m-1 (Duan et al., 2024). The EC25 value of SCG in this study was also found to be lower than in other research (Cervera-Mata et al., 2022, 2020, 2018; Comino et al., 2020). Since the moisture content of raw SCG can vary depending on brewing methods, coffee bean type, and the time elapsed between brewing and analysis, the results can vary significantly. In this study, the moisture content was established to be 61.5%. SCG mainly consists of organic matter (Zhao et al., 2024; Cervera-Mata et al., 2022, 2018; Comino et al., 2020; Ciesielczuk et al., 2018); this has been confirmed in this study by calculating mass loss of samples, which resulted in a total OM of 98.2 ± 0.1%.

The absorbance at different wavelengths of analysed SCG aqueous extract dilutions and connected parameters are presented in Table 1. Absorbance at a wavelength of 355 nm (determined as CDOM – coloured dissolved organic matter) is highly correlated with DOC and its high values indicate substantial amounts of aromatic compounds, exceeding the availability of nutrients in a soil, and promote microbial activity and buffer abilities (Zhang et al., 2022; Liu et al., 2021; Fasching et al., 2020) which is generally expressed in terms of dissolved organic carbon (DOC). In the present study, values of DOC increase with greater concentration of SCG a.e., which suggests that SCG can elevate OM in soil (Comino et al., 2020). The $SUVA_{254}$ coefficient is helpful in estimating the aromaticity of organic compounds. In this study, for all the analysed SCG a.e. dilutions, the SUVA254 was low (<2 L mg^{-1} m^{-1}) or extremely low (<1 L mg^{-1} m^{-1}), which implies the predominance of humic and hydrophilic low molecular weight compounds (Hua et al., 2020). The E_4/E_6 ratio is used to describe the degree of humification; if it is greater than 3.5, that indicates that fulvic acid predominates (Zhang et al., 2022), which was the case in this study.

As Figure 1 shows, absorbance generally increased with SCG a.e. concentration across all wavelengths. At 465 nm, a significant linear increase was observed ($p = 0.011$, $R^2 = 0.98$). Positive trends were also observed at 254, 335, and 665 nm were also positive ($R^2 = 0.87–0.90$), however, the slopes were only marginally

significant (p = 0.05–0.07), most likely reflecting the limited number of concentrations tested.

Table 1. Absorbance [a.u.] of SCG a.e. dilutions (2.5%, 5%, 10%, and 15%) at selected wavelengths (254, 335, 465, and 665 nm) and parameters (DOC, $SUVA_{254,}$ and E_4/E_6) calculated based on absorbance results

		SCG a.e. dilution [%]			
		2.5	**5**	**10**	**15**
Wavelength	254 nm	2.834	2.882	2.961	3.275
	355 nm	1.164	2.599	4.156	4.399
	465 nm	0.056	0.119	0.176	0.297
	665 nm	0.014	0.028	0.033	0.070
Parameters	DOC [mg C kg^{-1}]	272.7	537.2	824.2	869.0
	$SUVA_{254}$ [L mg^{-1} m^{-1}]	1.039	0.536	0.397	0.341
	E_4/E_6	4.000	4.250	5.333	4.243

Note: DOC - dissolved organic carbon, $SUVA_{254}$ – specific ultraviolet absorbance at 254 nm, E_4/E_6 – ratio of absorbance at 465 nm to absorbance at 665 nm, a.u. – absorbance units, a.e. – aqueous extract

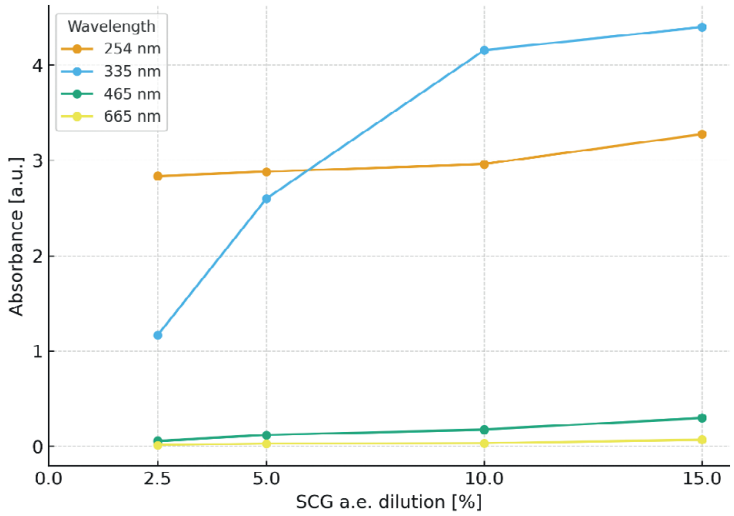


Figure 1. Absorbance [a.u.] of SCG a.e. at selected wavelengths (254, 335, 465, and 665 nm) across dilutions of 2.5%, 5%, 10%, and 15%

3.2. Toxicity biotests

Phytotoxkit results for selected plant species are shown in Figure 2. Analyses have been based on root length, considering that exposure occurred through the plant root system. As shown by Figure 2, the mean length of roots in all scenarios has been found to be the greatest in case of the addition of 5% SCG in a test soil. The lowest analysed contribution of SCG resulted in the greatest plant growth, probably owing to the plant's deprivation of some elements in higher percentages, which corresponds to the literature (Cruz et al., 2014). The least root growth for *S. alba* was measured with the addition of 15% SCG, but for L. sativum and L. sativa, it was the case in the control soil (0% SCG). The analysis of variance has revealed significant effects of species, SCG concentration, and their interaction on root growth (all $p < 0.001$; Figure 2, Table 2). As shown by Table 2, *S. alba* was found to be the most sensitive species, with mean root length decreasing from 33.87 mm at 0% SCG to 13.23 mm at 15% SCG (−60.9%). *L. sativum* exhibited moderate inhibition, with root length declining from 24.67 mm at 0% SCG to 32.57 mm at 15% SCG (−32.0%). In contrast, *L. sativa* has demonstrated relative tolerance, maintaining root growth across concentrations and even a slight increase at 15% SCG (53.32 mm → 57.23 mm; +7.4%). Tukey HSD post-hoc tests have confirmed significant differences among species at all SCG levels and between concentrations within species ($p < 0.05$).

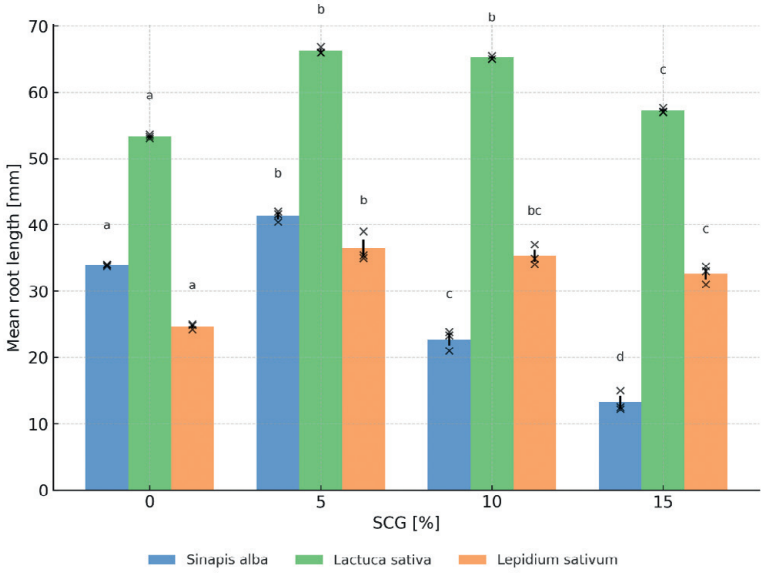


Figure 2. Mean root length [mm] of *S. alba* (blue), *L. sativa* (green), and *L. sativum* (orange) at different SCG dilutions (0–15%). Bars represent means ± SEM. "x" markers indicate individual replicate values. Different letters above bars denote significant differences between treatments according to Tukey HSD post-hoc tests ($p < 0.05$)

Table 2. Mean root length [mm] of *S. alba*, *L. sativa*, and *L. sativum* at 0–15% SCG. Values are means ± SEM (n = 3). Percent change values are relative to the 0% control

Species	Mean 0% (mm)	Mean 5% (mm)	Mean 10% (mm)	Mean 15% (mm)	% change at 5%	% change at 10%	% change at 15%
S. alba	33.87 ± 0.09	41.33 ± 0.48	22.70 ± 0.86	13.23 ± 0.89	22.05%	-32.97%	-60.93%
L. sativum	24.67 ± 0.24	36.47 ± 1.27	35.30 ± 0.89	32.57 ± 0.81	47.84%	43.11%	32.03%
L. sativa	53.32 ± 0.19	66.27 ± 0.27	65.17 ± 0.17	57.23 ± 0.19	24.29%	22.23%	7.35%

Figures 3–5 provide a summary of the results of the impact of SCG on the germination and growth of the analysed plant species. In all cases, the highest germination capacity and root growth stimulation (green zone in the background of Figure 3) are ensured by the addition of 5% SCG, with *L. sativum* exhibiting the best values (-47.8% and 211.2% for IRG and GI, respectively). For the germination index (GI), both species and SCG concentration had significant effects, with a strong interaction effect (all $p < 0.001$; Figure 4). *S. alba* was found to have the strongest inhibitory response, with IRG increasing sharply above 10% SCG (up to 60.93 ± 2.55%) and GI declining below 90% at higher concentrations. *L. sativum* showed growth stimulation at moderate SCG levels, reflected in negative IRG values and GI exceeding 200% at 5–10%. In contrast, *L. sativa* displayed only moderate responses, with GI peaking at 5% SCG (177.56 ± 0.33%) and IRG remaining relatively unaffected at higher doses, which implies limited sensitivity. Tukey HSD tests indicated significant differences among species at each SCG level and within species across concentrations ($p < 0.05$).

The obtained results show that the SCG application significantly influenced germination dynamics and root growth in the tested species. At low concentrations (5%), GI values were elevated in *L. sativum* and *L. sativa*, consistent with a stimulatory effect. However, at 10–15% SCG, strong inhibitory effects on GI and IRG were observed in *S. alba*, suggesting that phytotoxicity is most likely driven by phenolic compounds and caffeine. Research also indicates that the toxic effect of SCG not only depends on dosage but also on the plant species that was exposed (Ciesielczuk et al., 2018; Stylianou et al., 2018; Cruz et al., 2014).

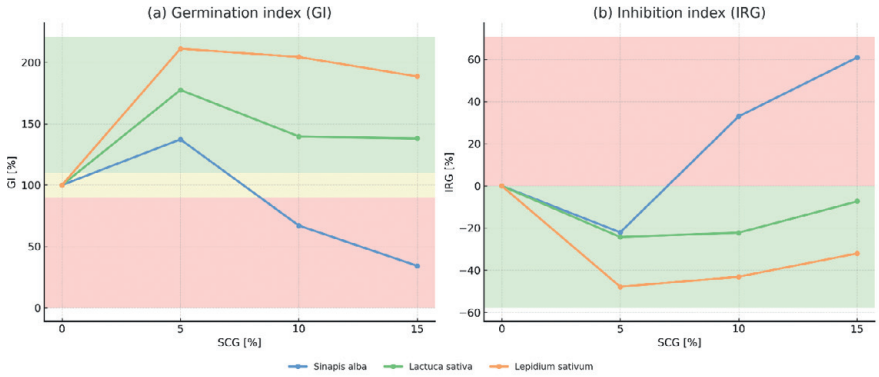


Figure 3. Mean values of the germination index (GI, a) and inhibition index (IRG, b) of *S. alba* (blue), *L. sativa* (green), and *L. sativum* (orange) at different SCG dilutions (0–15%). Background colours indicate growth stimulation (green: GI > 110% and IRG < 0%), growth inhibition (red: GI < 90% and IRG > 0%), and no impact (yellow: 90–110% GI and IRG = 0%)

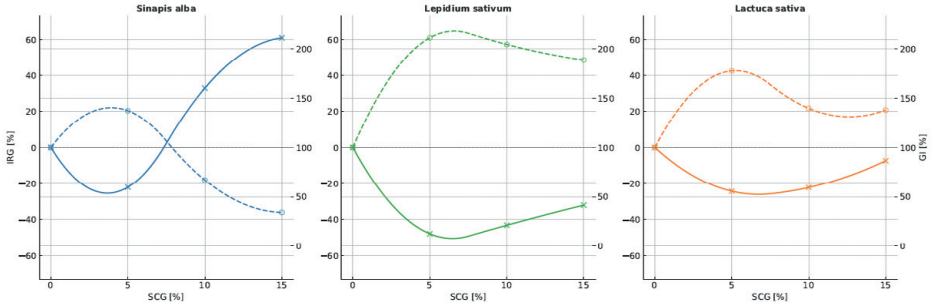


Figure 4. Mean values of the germination index (GI, dashed lines) and inhibition index (IRG, solid lines) in each species *S. alba* (blue), *L. sativum* (green), and *L. sativa* (orange) exposed to an increasing concentration of SCG

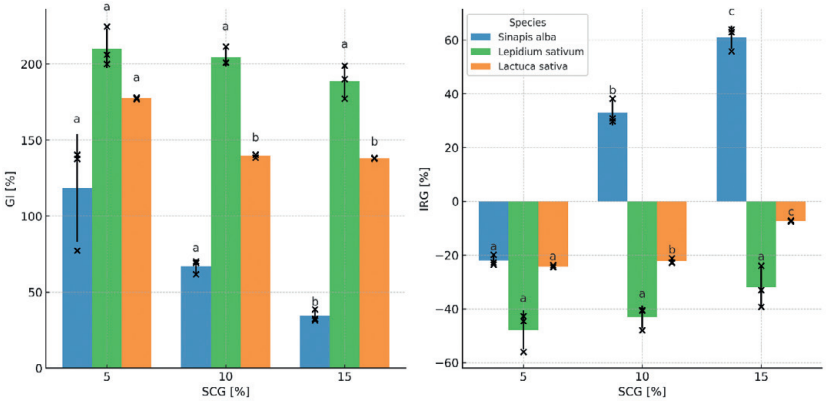


Figure 5. Mean germination index (GI, %) and inhibition of root growth (IRG, %) of *S. alba*, *L. sativa*, and *L. sativum* exposed to SCG (0–15%). Bars show means ± SD, and "x" markers indicate individual replicates. Letters above bars (a, b, c) indicate significant differences at $p < 0.05$ according to Tukey's HSD test

Table 3. Mean germination index (GI, %) and inhibition index (IRG, %) of *S. alba*, *L. sativa*, and *L. sativum* at 0–15% SCG. Values are means ± SEM (n = 3). Percent change values are relative to the 0% control

	Species	Mean 5% SCG	Mean 10% SCG	Mean 15% SCG
GI	*S. alba*	118.39 ± 20.55	67.04 ± 2.65	34.18 ± 2.23
	L. sativum	210.23 ± 7.42	204.39 ± 3.52	188.72 ± 6.33
	L. sativa	177.56 ± 0.33	139.69 ± 0.62	138.02 ± 0.16
IRG	*S. alba*	-22.04 ± 1.11	32.96 ± 2.65	60.93 ± 2.55
	L. sativum	-47.79 ± 4.14	-43.07 ± 2.46	-32.11 ± 4.43
	L. sativa	-24.29 ± 0.23	-22.23 ± 0.54	-7.35 ± 0.12

D. magna immobilisation test results are shown in Table 4. Table 5 presents the results of a toxicity test conducted on the *E. fetida* species. Exposure to SCG a.e. produced distinct responses in *D. magna* and *E. fetida* (Figure 6). After 24 h, *D. magna* has shown 20% immobilisation at 2.5% and again at 10–15% SCG a.e., while no effect was observed at 5%. A logistic model estimated an EC_{50} of 4.10% SCG a.e., but the wide 95% confidence interval (−2.15% to 10.35%) reflects the limited sample size and non-monotonic response pattern. In contrast, *E. fetida* displayed variable responses after 7-day exposure, ranging from 0% at 2.5–5% SCG a.e. to 40% mortality in the control and 20% at 10–15% SCG a.e. This irregular pattern and high control mortality made it impossible to reliably estimate the LC_{50}.

The immobilisation assays have revealed species-specific differences in sensitivity to SCG a.e., with *D. magna* appearing to be more responsive, showing immobilisation at low concentrations, consistent with its well-known sensitivity to toxic substances carried by water. However, the non-monotonic response and wide EC_{50} confidence interval highlight the limitations of small sample sizes in acute toxicity testing. In contrast, *E. fetida* was found to exhibit irregular responses and unexpectedly high control mortality. Together, these findings suggest that while D. magna can serve as an early indicator of aqueous SCG toxicity, additional replicates and refined test conditions are necessary for a robust risk assessment.

Table 4. Results of Daphtoxkit F™ magna, immobilisation test with *Daphnia magna*

SCG a.e. dilution [%]	Number of introduced specimens	Behaviour of introduced individuals	Mobile individuals after exposure	Behaviour after exposure	EC_{50} [mg L^{-1}]
0	5	All very active and mobile, swimming normally	5	All very active and mobile, swimming normally	4,10
2.5	5	All very active and mobile, swimming normally	4	1 immobile, the rest are active, swimming normally	
5	5	All very active and mobile, swimming normally	5	One with limited mobility, the rest are very active and mobile	
10	5	All very active and mobile, swimming normally	4	1 immobile, the rest active and mobile	
15	5	All very active and mobile, swimming normally	4	1 immobile, four mobile but less active than in a control group	

Table 5. Results of Earthworm Acute Toxicity Test with E*isenia fetida*; no LC_{50} value due to irregular pattern and high control mortality

SCG a.e. dilution [%]	Number of introduced specimens	Behaviour of introduced individuals	Alive individuals after exposure	Behaviour after exposure	LC_{50} [mg L^{-1}]
0	5	All active and mobile individuals	3	2 immobile, three less active than before the exposure	NA
2.5	5	All active and mobile individuals	5	A bit less active	
5	5	All active and mobile individuals	5	All active and mobile individuals	
10	5	All active and mobile individuals	4	1 immobile, four active and mobile individuals	
15	5	All active and mobile individuals	4	1 immobile, four active and mobile individuals	

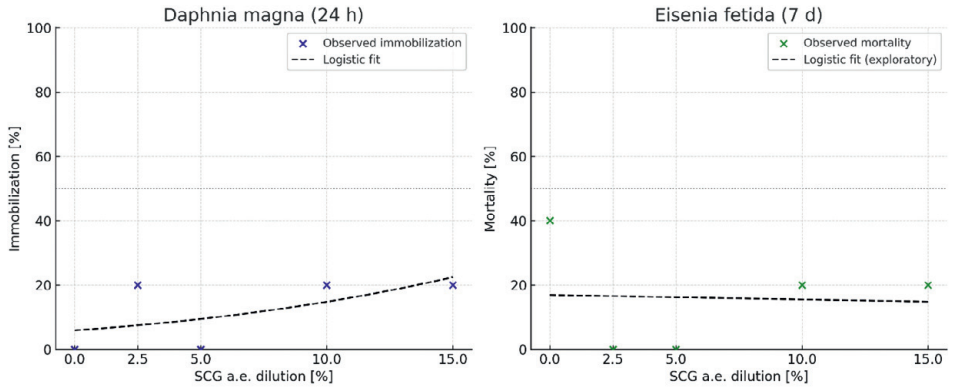


Figure 6. Immobilisation [%] of *D. magna* and mortality [%] of *E. fetida* (not valid) exposed to SCG aqueous extract (a.e.) dilutions (0–15%)

4. Conclusions

The toxicity tests executed on spent coffee grounds, which constitute biodegradable food waste, provided valuable information on the properties and effects they can have when released into the environment. Chemical and physical analyses cannot always accurately reflect the toxic impact of contaminants on living organisms; therefore, supplementation with bioassays can be crucial. Phytotoxkit results in this study suggested that SCG can provoke various responses in plants, depending on the species and the dosage of added SCG. While for *L. sativum* and *L. sativa*, even 15% SCG in the soil still sustained a stimulatory effect on germination and root elongation, for *S. alba,* it led to growth inhibition. The greatest stimulation of germination and growth in all the analysed species was observed in the presence of 5% SCG. While plant-based assays revealed clear phytotoxicity, especially in *S. alba*, aquatic invertebrates provided early warning of aqueous toxicity. The variability observed in earthworm assays highlights methodological constraints that must be addressed before firm conclusions can be drawn. Overall, the combined evidence suggests that SCG contain bioactive compounds capable of inhibiting growth and survival, supporting the need for further research on their safe use and disposal. Taking everything into consideration, this topic requires further investigation to develop strategies that minimise the environmental impacts of SCG while supporting their safe valorisation as a resource. Future research should particularly focus on chronic exposure assessments, multi-generation bioassays, and the evaluation of SCG pre-treatment methods such as composting or co-processing, which may help reduce phenolic toxicity and stabilise bioactive compounds. Such studies are essential to establish evidence-based guidelines for the environmentally responsible use of SCG in circular waste management.

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