

## Characterization of Landfill Leachate and their Toxic Effects on Germination and Seedling Growth of Various Plant Species – A Case Study

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### ABSTRACT

Leachate generated from landfills contains many toxic contaminants, such as dissolved organics, inorganic salts, ammonia, and heavy metals, which impact the surrounding environmental systems. This study characterized the AL-Mufarrihat Sanitary Landfill (MSL) leachate in Al-Medinah Al-Munawwarh (MM) province, Saudi Arabia, by analyzing important physicochemical parameters. Phytotoxicity was assessed using various higher plant bioassays, namely, cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* L.), cabbage (*Brassica oleracea* var. *capitata* L.), and corn (*Zea mays* L.). The effective concentration of seed germination represented by EC<sub>50</sub> was calculated using a USEPA computer program based on Finney's Probit analysis method. Selected phytotoxicity test endpoint parameters, namely relative seed germination (RSG), relative root elongation (RRE), and germination index (GI) were determined. The tested leachate exhibited low concentrations of heavy metals, whereas high levels of chemical oxygen demand (COD) and ammonia nitrogen (NH<sub>3</sub>-N) were recorded. The mean EC<sub>50</sub> values for MSL leachate exposed to *B. oleracea*, *L. esculentum*, *C. sativus*, and *Z. mays* were 2.66%, 3.12%, 4.27%, and 5.22%, respectively. These values indicate that *B. oleracea* was the most sensitive bioassay, whereas *Z. mays* was the least sensitive. All tested bioassays showed severe phytotoxic responses to the exposed higher leachate concentrations, represented by complete inhibition for RSG, RRE, and GI. Lower leachate concentrations exhibited stimulatory effects on RSG, whereas RRE and GI were hindered, even at these lower concentrations. The results revealed that although RSG and RRE were effective and promising parameters in phytotoxicity evaluation, GI was the most responsive parameter for phytotoxicity assessment. The high levels of organic and inorganic compounds in the leachate are likely the primary cause of the phytotoxicity observed in the bioassays. The results of this study highlight the pollution potential of landfill leachate in Saudi Arabia and will furnish supplementary reference information for hazard assessment and future leachate management.

**Keywords:** landfill leachate, physicochemical properties, plant bioassays, phytotoxicity, probit analysis.

### INTRODUCTION

Over the past four decades, Saudi Arabia has undergone significant transformation, driven by rapid population growth, urbanization, industrialization, and socioeconomic advancement. The population has expanded to 29 million, with an annual growth rate of 3.4% (Radwan et al., 2021). This growth, combined with changes in consumption patterns and increased per capita income,

has led to a notable rise in municipal solid waste (MSW) production, with estimated ranges between 1.5 and 1.8 kg/day per capita and over 15 million tons of solid waste annually (Radwan et al., 2021). Moreover, the escalation in MSW generation is closely tied to the rapid urban expansion, rural-to-urban migration, the influx of international migrant workers, and the growth of trade and industry in urban centers (Ouda and Cekirge, 2013). These developments, combined with the

expanding population, have compounded the challenges of solid waste management in the country.

Although waste management programs focus on reducing waste generation and maximizing recycling, landfilling remains a prevalent approach for managing municipal solid waste in developing countries, including Saudi Arabia (Mallick, 2020). The primary environmental concern associated with solid waste landfilling is related to the potential generation of leachate and its migration to the surrounding environment, posing a contamination risk to downstream surface waters and groundwater (Kjeldsen and Christophersen, 2001). Leachate is produced when municipal solid waste in a landfill endures a complex series of biological, chemical, and physical degradation processes, as well as the excess rainwater percolating through the waste layers, resulting in pollutants transferring from the waste material to the formed leachate (Christensen et al., 2001). Moreover, even after landfill closure, there is a potential for landfill sites to generate leachate for many decades, especially for sites that were operated without engineered construction (Bloor et al., 2006).

Leachate is characterized by substantial concentrations of dissolved organic materials, xenobiotic organic compounds (XOCs), inorganic salts, ammonia, heavy metals, and many other toxic substances. Ammonia in landfill leachate may pose an environmental threat for many years, even after landfill closure, while the risk of other most persistent pollutants may last for hundreds of years (Kjeldsen et al., 2002). Various physicochemical parameters are usually determined in landfill leachate, for instance, pH, electrical conductivity (EC), color, total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), BOD/COD ratio, ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) and others. These parameters vary widely depending on the landfilling method, the nature of the buried waste, landfill age, prevailing climate conditions, moisture availability, decomposition stage, and many other factors (Orescanin et al., 2011). In a previous study, the generated leachate from a landfill in Alriyadh City had a substantial concentration of organic matter and inorganic ions, including heavy metals, compared with other global landfill leachate (Al-Wabel et al., 2011).

Chemical analyses can identify specific dangerous compounds, but the identification is not enough to assess the overall toxic effects (Cheung et al., 1993; Baun et al., 1999). While chemical

analyses provide valuable perception into the composition of leachate, it does not reveal the toxic impacts that may arise from the chemicals leachate out from the landfill (Clement et al., 1996). Toxicity tests represent an essential methodological approach to identifying, characterizing, and assessing such toxic risks (Assmuth and Penttila, 1995). Moreover, unlike chemical analyses, bioassays directly evaluate the leachate constituent's bioavailability, synergistic, antagonistic, or additive effects. This approach allows for a more comprehensive biological impact assessment without relying on assumptions or extrapolations typically associated with chemical analyses alone (Kjeldsen et al., 2002; Pivato and Gaspari, 2006). Therefore, chemical analyses are best combined with bioassays to ensure an integrated evaluation of potential environmental toxicity (Bloor et al., 2006; Byrne et al., 2008).

The toxicity of leachate has been well investigated in many countries around the world, including the utilization of various testing organisms from different trophic levels and the involvement of landfills with varying characteristics in terms of age, operating conditions, and waste diversity. The reported toxicity results from these studies revealed that leachate was highly toxic to various tested organisms such as bacteria (Clement et al., 1996; Fan et al., 2006), fish (Sisinno et al., 2000; Alkassasbeh et al., 2009), invertebrates (Verbel et al., 2008; Suliasih et al., 2010), algae (Martinen et al., 2002; Ward et al., 2002), and higher plants (Chung et al., 2007; Hoss et al., 2022; Hashemi et al., 2023).

Plant bioassays, valued for their sensitivity and ease of use compared to animal bioassays, have been validated through international studies under the guidelines of the United Nations Environment Program (UNEP), the World Health Organization (WHO), US Environmental Protection Agency (USEPA), and the Organization for Economic Cooperation and Development (OECD) (Sang and Li 2004; Chung et al., 2007). The toxicity and hazard posed by pollutants to the growth and survival of plants are identified as phytotoxicity (Kapanen and Itavaara, 2001). Wang and Keturi (1990) summarized several advantages of phytotoxicity tests: First, dry seeds often remain viable over long periods with minimal maintenance and low cost, requiring little effort to use. Second, these tests are simple, affordable, and need only essential equipment. Third, the small sample sizes used in these tests help minimize exposure to and disposal of hazardous materials.

Phytotoxicity in terrestrial systems is commonly assessed using seed germination tests, which measure endpoints such as relative germination rate, root elongation, and early seedling growth (Tam and Tiquia, 1994; Kapanen and Itavaara, 2001). These measurements are widely used to assess the impacts of pollutants or phytotoxins on plant development. Many plant species have been recommended for phytotoxicity tests using seed germination and root elongation methods, such as cabbage, lettuce, oats, carrot, cucumber, tomato, wheat, rice, and others (USEPA, 1982).

Several studies were conducted to evaluate the phytotoxicity of landfill leachate on numerous species of plants. These studies demonstrated that leachate can trigger beneficial and adverse plant responses in terms of growth, biomass production, and genetic stability (Sang et al., 2010; Mor et al., 2013). Complex interactions between plant species, leachate composition, and concentration levels influence plant responses to leachate exposure, including its toxic impacts. Sang et al. (2010) investigated the physiological response of *Z. mays* to landfill leachate toxicity. They observed that low concentrations (2.5% and 5%) promoted root and bud growth, while 10% showed growth inhibition that increased with extended exposure time. At 50% and 100%, growth was nearly entirely suppressed. A seed germination bioassay using *Cicer arietinum* (Bengal gram) seeds was conducted on old and fresh landfill leachate samples from a municipal landfill site in Mumbai, India (Quraishi et al., 2019). The study results revealed that the germination rates for seeds exposed to untreated leachate samples, both old and fresh leachate, were 11.1 and 22.2%, respectively. In a study by Kalousek et al. (2020), the effects of landfill leachate on two hemp plant (*Cannabis sativa*) varieties were assessed. Leachate application negatively impacted growth parameters, including plant height, root length, dry weight, total leaf area, and photosynthetic pigment content. While 10% and 15% concentrations caused minor reductions, 20% significantly affected all measured parameters, and 40% led to severe stunting or plant death.

Palm et al. (2022) investigated the tolerance of *Sinapis alba* (mustard) and *Triticum aestivum* (wheat) to varying concentrations of leachate from an MSW landfill in the Czech Republic. The study results revealed that 20% to 50% of leachate concentrations enhanced shoot biomass, leaf expansion, primary root growth, and carbon

assimilation. Higher leachate concentrations above 50% decreased these physiological parameters, significantly affecting seedling root elongation. Research using *Sorghum bicolor* seeds showed that raw leachate, with high concentrations of metals and organic compounds, significantly inhibited seed germination and root development, indicating acute phytotoxicity (Hashemi et al., 2023). With exposure to a raw leachate concentration of 75%, only a germination rate of 0.1 for *Sorghum bicolor* was obtained.

This study was conducted to characterize the physicochemical properties and to assess the phytotoxicity of landfill leachate sampled from the Al-Mufarrihat Sanitary Landfill (MSL) in Al-Medinah Al-Munawwarah (MM) province, Saudi Arabia. Various plant species bioassays comprised of cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* L.), cabbage (*Brassica oleracea* var. *capitata* L.), and corn (*Zea mays* L.) were utilized for the phytotoxicity assessment. To the best of our knowledge, no previous study has been conducted to assess landfill leachate phytotoxicity in Saudi Arabia.

## MATERIALS AND METHODS

### Study area description and site characteristics

The site of MSL is located in MM province, Saudi Arabia (latitude 24°19'28.3" N, longitude 39°27'52.4" E) (Figure 1a). The location is approximately 20 kilometers from the highway connecting the MM province with Yanbu city. The landfill commenced operations in 2006, covering an estimated area of 68,750 m<sup>2</sup>, and it is planned to close in 2018. To address future needs, the general municipality of MM province proposed a plan to extend the landfill's operational cells. In 2023, the municipality committed to maintaining and reclaiming the closed cells and expanding the landfill area to 10,580,557 m<sup>2</sup>.

The MSL was developed using controlled operation and disposal techniques. Each layer is compacted and covered with an appropriate layer of soil before depositing the next. These covering layers are designed to prevent access by vectors and scavenging birds, reduce odor, and limit rain infiltration. Additionally, the MSL site construction process also included lining the bottom and sides with impermeable materials like a geosynthetic clay liner, high-density polyethylene



**Figure 1.** (a) Descriptive map for MSL landfill site, and (b) leachate collection pond

geomembrane, and geotextile to prevent infiltration and contamination into the underlying soil and groundwater. The landfill is equipped with multiple gas wells, gas power generation plants, and leachate drainage pipes for collecting and discharging leachate to a collection pond. Additionally, it has a leachate biological treatment plant consisting of three aeration ponds. The landfill handles a daily accumulation of solid waste, primarily MSW, estimated between 1,000 and 1,500 tons, with an average of 1,300 tons collected from nearly all areas of MM province. At the time of sampling, the landfill was actively operating, producing young leachate mixed with older mature leachate.

### Leachate sampling

Raw leachate sampling followed the standard methods for examining water and wastewater (APHA 2012). Leachate samples were collected from the collection pond at the MSL site (Figure 1b) over three months to estimate the leachate decomposition stage. At each sampling time, the samples were collected from various points within the collection pond and mixed together to represent a homogenous sample. Leachate sampling from the biological treatment ponds could not be performed due to their malfunction and the ongoing maintenance program at the time of sampling. The one liter (1L) high-density polypropylene sampling bottles were thoroughly rinsed with distilled water in the lab and then with leachate at the sampling site before filling the final, thoroughly

mixed samples. The bottles were filled with leachate, leaving no air space to prevent aerobic degradation during transfer and storage. All collected samples were labeled and placed in cool sampling boxes filled with ice to maintain a low temperature during immediate transfer to the laboratory. Upon arrival at the laboratory, leachate samples were refrigerated at 4 °C to preserve their physicochemical properties for immediate assessment. For heavy metal analyses, samples were acidified with concentrated nitric acid to a pH of 2.0 to inhibit microbial activity and prevent the precipitation of heavy metals. Aliquots of leachate samples intended for phytotoxicity tests were frozen at -20 °C for later evaluation. In this respect, Muna et al. (1995) found that storing leachate at deep freezing temperatures does not significantly alter its toxicity toward *Daphnia magna*.

### Physicochemical analyses

Leachate samples were analyzed at the analytical laboratory of the Faculty of Sciences, Taibah University. In the field, hydrogen ion concentration (pH), electrical conductivity (EC), and total dissolved solids (TDS) were measured using a portable multi-meter probe (HI-9811-51 Hanna Instruments). Inductively coupled plasma mass spectrometry (ICP-MS) was utilized after preliminary filtration through a 0.45 μm micro-porous cellulose acetate membrane filter for heavy metal analyses. The remaining physicochemical parameters: total suspended solids

(TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), and sulphate ( $\text{SO}_4^{2-}$ ) were analyzed following the recommended methods described in the standard methods for the examination of water and wastewater (Table 1) (APHA 2012). Leachate samples were diluted as necessary for specific parameters. All chemicals employed in the analytical determination were of analytical grade purity. Each analysis was performed in triplicate, and the results represent the average values. The quality of leachate was evaluated against the permissible values for treated wastewater (TWW) discharge into the environment pronounced by the Ministry of Environment, Water and Agriculture (MEWA) in the Executive Regulations for the Protection of Aqueous Media from Pollution (MEWA 2020). Additionally, standards for raw sewage water entering treatment plants (SWTP) (Alkudhiri et al., 2019) were used for comparative purposes. To our knowledge, there are no specific regulations or standards for landfill leachate discharge in Saudi Arabia.

### Seed material preparation and phytotoxicity tests

Based on the physicochemical analyses results, leachate samples with the highest concentrations of most parameters were selected for toxicity testing. Phytotoxicity tests were conducted following the guidelines of USEPA (2012) and Tiquia et al. (1996). Seeds of *C. sativus*, *L. esculentum*, *B. oleracea*, and *Z. mays* were sourced from local seed retailers. Twenty healthy seeds of each tested species were selected and surface sterilized by soaking and shaking in a 1% sodium hypochlorite solution to eliminate any potential bacterial and fungal contaminants. Following

sterilization, the seeds were rinsed several times with sterile deionized water to ensure the removal of any residual disinfectant. Subsequently, the seeds were introduced evenly into the Petri dishes of uniform size (150 mm diameter) lined with two filter paper discs (Whatman No.1, 150mm diameter). Range finding tests (RFT) were conducted using varying leachate concentrations of 1%, 5%, 10%, 20%, 50%, and 100%. Based on the RFT results, two sets of five definitive leachate concentrations in a logarithmic series were prepared with distilled water (DW) as a diluent. The first selected set of 1.5%, 2.2%, 3.2%, 4.6%, and 6.8% was used for *B. oleracea* and *L. esculentum*. An expansive range of 1.9%, 2.7%, 3.7%, 5.2%, and 7.5% was applied for *C. sativus* and *Z. mays*. The filter discs were then moistened with 10 mL of DW for control groups and with the same quantity of the prepared various leachate diluted concentrations. Each concentration in the dilution series was tested in triplicate. The prepared Petri dishes were capped properly with their lids, rapped with flexible film (Parafilm), and incubated in a culture chamber (24 °C/80% humidity) for five days in the dark. After the incubation period, physical observations were conducted to assess the phytotoxicity endpoint parameters. A germination rate of above 90% was an essential quality requirement for the control samples used in the study. Seeds were considered to have germinated when the radicle visibly emerged from the seed coat (Cheng and Chu, 2007). Root length measurements in each tested concentration were conducted using a standard centimeter scale for randomly selected representative seedlings. The measurement was from the root tip to the readicle with 1mm precision. The phytotoxicity endpoints percentages of relative seed germination (*RSG*), relative root elongation (*RRE*), and germination index (*GI*), a factor of relative seed germination

**Table 1.** Testing methods for leachate quality parameters

Parameter	Method name	Reference No.
TSS	TSS dried at 103–105 °C	SM' 2540 D
COD	Open reflux method	SM' 5220 B
BOD	5-day BOD test	SM' 5210 B
$\text{NH}_3\text{-N}$	Titrimetric method	SM' 4500 $\text{NH}_3\text{C}$
$\text{NO}_3^-$	Cadmium reduction method	SM' 4500- $\text{NO}_3^- \text{E}$
$\text{PO}_4^{3-}$	Ascorbic acid method	SM' 4500-P E
$\text{SO}_4^{2-}$	Turbidimetric method	SM' 4500- $\text{SO}_4^{2-} \text{E}$
Heavy metals	(ICP-MS) (model 7500) of Agilent, USA	SM' 3125 B

**Note:** \*SM: standard methods for the examination of water and wastewater.

and relative root elongation, were assessed using Equations 1, 2, and 3 (Tiquia and Tam, 1998).

$$RSG\% = (SGL/SGC) \times 100 \quad (1)$$

where: *SGL* and *SGC* are the mean values of the number of seeds germinated in landfill leachate and negative control, respectively.

$$RRE\% = (REL/REC) \times 100 \quad (2)$$

where: *REL* and *REC* are the mean measured root elongation in landfill leachate and negative control, respectively.

$$GI\% = (RSG \times RRE) / 100 \quad (3)$$

where: *RSG* is the relative seed germination (%), and *RRE* is the relative root elongation (%) which are calculated using Equations 1 and 2, respectively.

### Statistical analysis

Physicochemical and phytotoxicity analyses for all tested parameters were conducted in triplicate. The results for physicochemical analyses were evaluated by descriptive statistical analyses (min, max, mean, and std dev). The effective

concentration ( $EC_{50}$ ), defined as the concentration at which 50% of the population exhibits a specified response or achieves 50% of the maximal effect of the toxicant after a given exposure duration, along with its 95% confidence limits, was calculated using USEPA toxicity data analysis software (version 1.5) based on Finney's probit analysis (FPA) method (Finney 1971).

## RESULTS AND DISCUSSION

### Physicochemical characterization

The physicochemical characteristics of MSL leachate analyses are shown in Table 2. These results are expressed as min, max, mean, and std dev. Moreover, MEWA standards for TWW discharge to soil, land, and surface water (MEWA, 2020) and the standards for raw sewage water entering the treatment plants (SWTP) (Alkudhiri et al., 2019) were listed. For a more realistic comparison, the physicochemical results from this study were evaluated against other recorded data from local, regional, and global landfills (Table 3). All leachate samples exhibited a strong, unpleasant odor. The measured pH values ranged between 8.05 and

**Table 2.** Physicochemical characteristics of MSL leachate

Parameter (mg/L)	Min	Max	Mean	Std Dev	TWW discharge MEWA standard	SWTP standards
PH	8.05	8.47	8.28	0.21	6-8.4	6-9
EC	17.27	28.94	22.79	5.86	–	–
TDS	9684	16128	13165.33	3253.16	2000	–
TSS	1825	2658	2168	435.52	40-50	600
COD	8840	12930.67	10527.11	2137.37	20-50	1000
BOD	963	1592	1296.33	316.18	20-40	500
BOD/COD	0.11	0.14	0.12	0.01	–	–
NH <sub>3</sub> -N	1041.66	1434.29	1210	202.2	1.9-5.0	80
NO <sup>3-</sup>	44.92	73.68	60.94	14.66	10-15	-
PO <sub>4</sub> <sup>3-</sup>	9.75	24.58	16.06	7.66	20-30	25
SO <sub>4</sub> <sup>2-</sup>	26.75	107.12	75.21	42.67	–	1000
Cu	0.21	0.85	0.47	0.33	0.2-0.4	1.2
Cr	0.62	1.74	1.18	0.56	0.1	0.2
Cd	0.005	0.03	0.015	0.013	0.01-0.1	0.02
Ni	0.43	1.37	0.92	0.47	0.2	2
Pb	0.03	0.29	0.16	0.13	0.1	1
Zn	2.17	5.56	3.69	1.72	2-4	2.6
As	0.07	0.43	0.22	0.19	0.1	0.1
Fe	9.81	15.67	12.27	3.04	5	–
Mn	0.13	1.04	0.56	0.45	0.2	5

**Note:** \*all parameters in mg/L except pH, temp (°C), and EC (mS/cm).

**Table 3.** Variations in leachate quality from MSL with other local, regional, and global landfills

Parameter (mg/L) <sup>a</sup>	Current study	Saudi Arabia <sup>1</sup>	Kuwait <sup>2</sup>	Oman <sup>3</sup>	Tunisia <sup>4</sup>	Morocco <sup>5</sup>	Malaysia <sup>6</sup>	India <sup>7</sup>	Greece <sup>8</sup>	Poland <sup>9</sup>
PH	8.28	5.94–6.32	6.9–8.2	8.26-8.54	8.0–8.3	8 ± 0.1	8.1	8.06-8.89	8	7.4-8.7
EC	22.98	42.5–58.3	5.4–16.9	54.1-60.86	54.6–82.2	41.3	26	51.24–68.88	–	–
TDS	13165.33	–	600–8430	24,210-36,247	-	22,350 <sup>a</sup>	14,680	23.95–33.96 <sup>a</sup>	11850	3795-7830
TSS	2168	2280–8912	104–1460	1213-1270	–	14000	1391.11	–	220	–
COD	10527.11	13900–22350	158–9440	28000–33600	5100-5800	4276	3583	5000–25,000	3510	2260–4110
BOD	1296.33	–	30-600	11700-13200	–	340	834.27	350.09–2100	295	–
NH <sub>3</sub> -N	1210	–	–	4173-4655	711.5–892	2792	1693.33	395.21–684.21	1099.8	132–460.9
NO <sub>3</sub> <sup>-</sup>	60.94	–	–	34.92	250-1540	9.5	53.75	–	13.3	–
PO <sub>4</sub> <sup>3-</sup>	16.06	–	–	16.63-39.8	<0.42	132.3	62.33	0.83–5.43	20.4	–
SO <sub>4</sub> <sup>2-</sup>	75.21	980.6–1944.1	55–3650	36.19-129.8	5570–7540	322	112.5	–	155	–
Cu	0.47	0.124–0.246	0-0.2	1.83–2.43	0.05-0.1	0.05	0.01	0.27–0.56	–	0.07–0.49
Cr	1.18	0.21–0.336	-0.01	10.42	1.7–1.8	1.63	0.09	0.00–0.35	–	0.023–0.58
Cd	0.015	<0.002	–	–	<0.0018	<0.01	0.001	–	–	–
Ni	0.92	0.384–0.718	0.4–0.6	BDL <sup>b</sup>	0.5	0.16	0.07	0.58–0.98	–	0.05–0.165
Pb	0.16	<0.04	0–0.2	BDL	<0.036	<0.01	0.01	0.00–1.07	–	0.00–0.076
Zn	3.69	0.11–0.226	0.2–4.8	BDL	0.7-0.9	1.16	0.1	0.46–0.79	–	0.75–2.56
As	0.22	1.09–1.682	–	BDL	–	–	0.16	0.00	–	–
Fe	12.27	134.4–190.2	0.3–54.1	16.69–26.47	3.5–5.1	2.07	1.2	7.55–12.11	–	2.6–38.73

**Note:** \*all parameters in mg/L except pH and EC (mS/cm), a: (g/L), b: (below detection limit), 1: Al-Wabel et al. (2011), 2: Al-Yaqout and Hamoda (2003), 3: Siddiqi et al. (2022), 4: Frikha et al. (2017), 5: Bellouk et al. (2023), 6: Budi et al. (2016), 7: Anand and Palani (2022), 8: Grilla et al. (2024), 9: Wdowczyk and Pulikowska (2021).

8.47 with a mean value of 8.28, which is reliable for old landfill leachate of MSL and within the permissible limit of TWW discharge by MEWA. Moreover, this pH result indicates leachates in the methanogenic decomposition phase. According to Pohland and Harper (1985), pH is a crucial indicator for landfill stabilization phases. It plays a significant role in properly evaluating the buried refuse's acid formation and methane fermentation stages. El-Fadel et al., (2002) reported that alkaline pH (>7) is commonly observed at landfills aged ten years after first waste disposal. In addition to that, stabilized leachate usually shows relatively constant pH with minor variations that may range between 7.5 and 9 (Umar et al., 2010). Among the factors that contribute to the high value of pH is the decrease in the concentration of accumulated short-chain fatty acids due to anaerobic consumption and the reduction in the concentration of partially ionized free volatile fatty acids due to its consumption by methanogenic bacteria (Chu et al., 1994). The recorded pH values in this study were in good agreement

with the values reported by many previous studies for stabilized leachates from regional and global landfills (Umar et al., 2010; Abunama and Othman 2021; Siddiqi et al., 2022).

The mean EC value of 22.79 mS/cm for MSL leachate (Table 2) suggests high dissolved inorganic materials and significant cations content, indicating that a broad spectrum of solid waste types was buried in the studied landfill. Khattabi et al. (2002) compared EC from young and aged municipal solid waste leachate and observed an increase in EC with the aging of the landfill. Al-Wabel et al. (2011) reported EC values ranging from 42.5 to 58.3 dS/m for landfill leachate sampled from a landfill located in Riyadh city (Table 3), which may be attributed to the elevated measured dissolved inorganic species in their study, such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>. The obtained EC values for MSL leachate in this study were in good agreement with the previously reported ranges by many researchers for leachates that have undergone methanogenic conditions (Fatta et al., 1999; Fan et al., 2006; Hussein et al., 2021). However,

other researchers obtained higher EC values of 82.2 mS/cm (Frikha et al., 2017) and 68.88 mS/cm (Anand and Palani, 2022).

The measured values for TDS and TSS ranged from 9684 mg/L to 16128 mg/L and from 1825 mg/L to 2658 mg/L, respectively (Table 2). These values were much higher than the allowable discharge limits for TWW by MEWA, which were limited to 2000 mg/L and 40–50 mg/L, respectively. Similar to EC, the high values of TDS reflect the prominent existence of soluble inorganic materials, demonstrating their closely related patterns (Tatsi and Zouboulis, 2002). On the other hand, high values of TSS in leachate could indicate the presence of organic and inorganic solids, which can provide adsorptive sites for certain undesired chemicals and pathogenic biological agents (Aluko et al., 2003). Al-Yaqout and Hamoda (2003) reported a high TDS value (8430 mg/L) for a Kuwait landfill with similar climate conditions (Table 3). They emphasized the extensive mineralization due to the active anaerobic breakdown of waste in the landfill. The increase in TDS concentration was observed to increase the salinity of the receiving water, elevating the toxicity level by changing the ionic composition (Umar et al., 2010).

The TDS levels observed in this study align with those reported in the literature for landfill leachates under methanogenic conditions, with values of 14,680 mg/L reported by Budi et al. (2016) and 11,850 mg/L reported by Grilla et al. (2024). A greater TDS range of 24,100–34,800 mg/L and 23.95–33.96 g/L was obtained by Gupta and Rajamani (2015) and by Anand and Palani (2022), respectively for leachate collected from an MSW old landfills in India. In general, the values of TSS obtained in this study are much lower than those of TDS values, which can be explained by primarily water-soluble substances in the solid components of leachate (Visvanathan et al., 2004). Consistent with this study result, TSS concentrations of 2250 mg/L and 2180 mg/L were reported by other researchers (Gupta and Rajamani 2015; Abunama and Othman 2021, respectively). Lower values of 120 mg/L (Arliyani et al., 2023) and 220 mg/L (Grilla et al., 2024) were recorded. In contrast, higher concentrations of 14000 mg/L and 14860 mg/L were found in the literature by Bellouk et al. (2023) and Quraishi et al. (2019), respectively.

The bulk measures of leachate dissolved organic content include BOD and COD, whereas the ratio of both BOD/COD is commonly used as

an indicator for the stage of waste decomposition and revealing the changes in its biodegradability (Lo 1996; Barlaz et al., 2002). The mean values for these parameters for MSL leachate were 1296.33 mg/L, 10527.11 mg/L, and 0.12, respectively, which exceeded the permissible standards for BOD and COD by MEWA/TWW and SWTP (Table 2). These high levels of BOD and COD reveal severe contamination in MSL leachate due to the decomposition of organic matter. Nevertheless, the obtained BOD and COD concentrations in this study fell within the typical ranges reported by many researchers for partially stabilized landfills at the intermediate long methanogenic stage. Anand and Palani. (2022) reported average COD and BOD values of 14286 mg/L and 1035.17 mg/L for closed and active landfills in Hyderabad, India. Likewise, Arliyani et al. (2023) achieved 9216 mg/L and 1140 mg/L, respectively, for the Griyomulyo landfill in Indonesia. Other researchers obtained higher values for similar stabilized old landfill leachate, such as Abunama and Othman (2021) and Siddiqi et al. (2022), who recorded COD values of 51840 mg/L and 33600 mg/L, respectively, and BOD values of 5110 and 13200, respectively.

The BOD/COD was known to indicate the maturity of landfill leachate, which usually decreases with landfill aging (Lo, 1996). BOD level usually decreases faster than COD levels as the landfill age increases, resulting from the prompt degradation of biodegradable landfilled waste (Abunama et al., 2021). Generally, landfill leachate from young landfills usually maintains a higher BOD/COD ratio, whereas leachate from older or stable landfills exhibits a lower BOD/COD ratio (Fan et al., 2006). Tatsi and Zouboulis. (2002) identified BOD/COD ratios ranging from 0.5 for a relatively fresh leachate to 0.2 for an older stabilized leachate. Another figure was mentioned by Lindamulla et al. (2022); a BOD/COD ratio from new landfills usually falls in the range between 0.5 and 1.0, whereas for old landfills, the ratio is below 0.1. Thus, based on these figures, the calculated BOD/COD ratios ranged from 0.11 to 0.14 for MSL (Table 2), indicating a moderately stabilized leachate, low biodegradable, and had not reached the final stable methanogenic phase. These ratios coincided with many other researchers reported results of 0.16–0.3, 0.17, and 0.18 by Alkassasbeh et al. (2009), Bialowiec (2015), and Welter et al. (2018), respectively.

Ammonia ( $\text{NH}_3\text{-N}$ ) is landfill leachate's most significant long-term contaminant (Christensen et

al., 2001; Abdel-Shafy et al., 2024). Ammonia is typically generated through the anaerobic biological degradation of available proteins in plant and animal buried waste, along with industrial waste containing ammonia or ammonium compounds, such as fertilizers, synthetic rubber, plastics, and food preservatives (Pivato and Caspari, 2006). El-Fadel et al. (2003) highlighted  $\text{NH}_3\text{-N}$  as the ideal parameter for assessing residual pollution potential in landfills and the required post-closure care period. The high ammonia concentrations will threaten the vital role of microorganisms in the anaerobic decomposition processes. Subsequently, it inhibits their growth and activity (Fatta et al., 1999). In addition to their toxic effect on many organisms, elevated levels of ammonia result in eutrophication and acidification (Lee et al., 2006). In this study, the measured  $\text{NH}_3\text{-N}$  concentrations ranged between 1041.66 mg/L and 1434.29 mg/L, which is significantly beyond the allowable limit of 1.9–5.0 mg/L and 80 mg/L for MEWA/TWW and SWTP standards, respectively (Table 2). This high level of  $\text{NH}_3\text{-N}$  in the current study of aged landfill is expected since there is no identified mechanism for ammonia degradation under methanogenic conditions. Consequently, leaching is the only process for decreasing its concentration during refuse decomposition (Kjeldsen et al., 2002; Barlaz et al., 2002). Table 3 shows various levels of ammonia from different landfills in many countries, regional and global. Similar values of 1099.8 mg/L and 1693.33 were reported by Grilla et al. (2024) in Greece and Budi et al. (2016) in Malaysia, respectively. Whereas much higher levels of 4173–4655 mg/L and 2792 mg/L were obtained by Siddiqi et al. (2022) in Oman and Bellouk et al. (2023) in Morocco, respectively.

The assessed inorganic macro-components in MSL leachate were  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{SO}_4^{2-}$ . Table 2 illustrates the values obtained for these parameters, which ranged from 44.92 mg/L to 73.68 mg/L, 9.75 mg/L to 24.58 mg/L, and 26.75 mg/L to 107.12 mg/L, respectively. The mean values for these parameters were 60.94 mg/L, 16.06 mg/L, and 75.21 mg/L, respectively. These values were within the permissible limits by MEWA/TWW and SWTP standards, except  $\text{NO}_3^-$  exceeded them (Table 2). Naveen et al. (2017) stated that nitrate in landfill leachate is predominantly formed by the nitrification process that follows the oxidation of ammonium to nitrite. The current study levels for  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and  $\text{SO}_4^{2-}$  coincided with the

reported values of 53.75 mg/L (Budi et al., 2016), 20.4 mg/L (Grilla et al., 2024), and 36.19–129.8 mg/L (Siddiqi et al., 2022), respectively. Considerably elevated levels of 250–1540 mg/L (Frikha et al., 2017), 184–342 mg/L (Abunama and Othman, 2021), and 1250–7250 mg/L (Gupta and Rajamani 2015), respectively, were also reported by other researchers.

Heavy metals (Cd, Cr, Cu, Fe, Ni, Pb, Zn, Al, and Mg) analyses for MSL leachate revealed various concentrations (Table 2). The overall average concentrations were generally moderate to low, displaying the following descending sequence in terms of abundance:  $\text{Fe} > \text{Zn} > \text{Cr} > \text{Cu} > \text{Mn} > \text{Pb} > \text{Ni} > \text{As} > \text{Cd}$ . The maximum detected values for most MSL heavy metals exceeded the permissible limits by Saudi's MEWA/TWW standards except for Cd (0.03 mg/L). Nevertheless, these maximum values were generally below the allowable limits by Saudi's SWTP standards except for Zn (5.56 mg/L) and As (0.43 mg/L). The results showed relatively high levels of Fe, with a mean value of 12.27 mg/L, while no discharge limits are included in SWTP standards. The low levels of heavy metals in MSL are reasonable due to the alkaline pH that dominated the leachate assessment ( $>8$ ). It was reported that the lower levels of heavy metals are dominant with the increase in landfill age. Besides, at the alkaline conditions prevailing in the methanogenic phase, the concentration of heavy metals will decrease due to subsequent immobilization resulting from adsorption and precipitation processes (Tatsi and Zouboulis, 2002; Oman and Junestedt, 2008).

Fe high concentrations in leachate suggest that iron-based materials, construction materials, tin-based materials, paints, pigments, polishing agents, metal scrap, and electrical materials were dumped in the landfill (Aziz et al., 2004; Abunama et al., 2021). The high recorded concentrations of Zn indicate that the landfill possibly received plenty of batteries, fluorescent lamps, zinc-plated materials, fertilizers, and cement-loaded waste (Mor et al., 2006; Singh et al., 2008). Elevated levels of Fe in this study were found to be consistent with previous levels of 16.69–26.47 mg/L (Siddiqi et al., 2022) and 7.06–14.45 mg/L (Abunama and Othman, 2021). Much higher levels of 190.22 mg/L and 54.1 mg/L for landfills in Saudi Arabia (Al-Wabel et al., 2011) and Kuwait (Al-Yaqout and Hamoda 2003) were recorded (Table 3.2). Similarly, the reported Zn values in this study were in agreement with other values of 2.56

mg/L and 3.0 mg/L by Wdowczyk and Pulikowska. (2021) and Naveen et al. (2017), respectively, for old leachate in the methanogenic phase.

### Phytotoxicity assessment

#### Leachate median effective concentration ( $EC_{50}$ )

In this study, various plant species bioassays comprised of *C. sativus*, *L. esculentum*, *B. oleracea*, and *Z. mays* were utilized to evaluate the phytotoxicity of MSL landfill leachate. The results of the preliminary range-finding tests indicated that the tested MSL leachate caused complete inhibition of seed germination at exposure concentrations of 20%, 50%, and 100% for all tested plant species. At an exposure concentration of 10%, all tested plant species showed positive signs of seed germination, with higher germination percentages observed for *C. sativus* and *Z. mays* seeds compared to *B. oleracea* and *L. esculentum*. The range finding tests also demonstrated that the lowest concentration, which caused no inhibition effect for seed germination, was 1.0% for all tested plants. Based on that, each tested plant was exposed to 5 definitive concentrations for phytotoxicity assessment. These definitive concentrations were grouped into a broad range of 1.9%, 2.7%, 3.7%, 5.2%, and 7.5% for *C. sativus* and *Z. mays* bioassays and a narrower range of 1.5%, 2.2%, 3.2%, 4.6%, and 6.8% for *B. oleracea* and *L. esculentum* bioassays. Based on the FPA method using the USEPA software program, the estimated seed germination effective concentrations ( $EC_{50}$ ) and the 95% confidence limits that resulted from MSL landfill leachate phytotoxicity on the various tested plants are listed in Table 4.

These results revealed that the tested MSL leachate was highly toxic to all tested plant species, with variable inhibition for seed germination rates in response to the different leachate dilutions. *B. oleracea* exhibited the highest sensitivity, with the lowest  $EC_{50}$  value of 2.67%, followed by *L.*

*esculentum* with an  $EC_{50}$  of 3.2%. On the other hand, *C. sativus* showed lower sensitivity, with an  $EC_{50}$  of 4.33%, while *Z. mays* was the least sensitive, with an  $EC_{50}$  of 5.51%. In agreement with these results, other researchers reported a comparable  $EC_{50}$  value of 3.51% for *L. esculentum* exposed to old mature landfill leachate (Suliasih et al., 2010). Cheng and Chu. (2007) evaluated the phytotoxicity of landfill leachate from different landfills in Hong Kong using *Brassica chinensis* (Chinese white cabbage) and *Lolium perenne* (perennial ryegrass). They reported that  $EC_{50}$  values ranged from 2.95% to 45.9%, which increased with leachate strength. The phytotoxic effects of landfill leachate in Tunisia were evaluated using *L. esculentum*, *Lolium perenne*, *Helianthus annuus*, and *Medicago sativa* seeds (Turki and Bouzid, 2017). The study reported  $EC_{50}$  values for the raw leachate ranging from 4% to 5% across the four species tested. Variation in phytotoxicity responses across different studies is expected due to the significant differences in landfill leachate toxicity, which are influenced by the specific plant species tested and the distinct physicochemical properties of the leachate. Therefore, performing a targeted phytotoxicity assessment for each unique combination of leachate and plant species is crucial (Bialowiec, 2015).

#### Phytotoxicity assessment parameters (RSG, RRE, and GI)

The toxic effect of MSL leachate on the various tested bioassays in terms of RSG, RRE, and GI was assessed, and the results are shown in Figures 2–5. These results were expressed as tested leachate concentrations versus RSG, RRE, and GI percentages. All seeds in the control groups were guaranteed to germinate at a minimum rate of 90% in each replicate. A higher germination rate of test species in the negative control indicates lower uncertainty in the test results (Wang and Keturi 1990). The results showed that the

**Table 4.** Estimated effective concentration ( $EC_{50}$ ) values and its confidence limits for MSL leachate phytotoxicity on tested plant species

$EC_{50}$	Exposure conc.	95% Confidence limits lower–upper
<i>B. oleracea</i>	2.667	2.373–2.986
<i>L. esculentum</i>	3.202	2.789–3.688
<i>C. sativus</i>	3.202	3.623–5.261
<i>Z. mays</i>	5.514	4.539–6.846

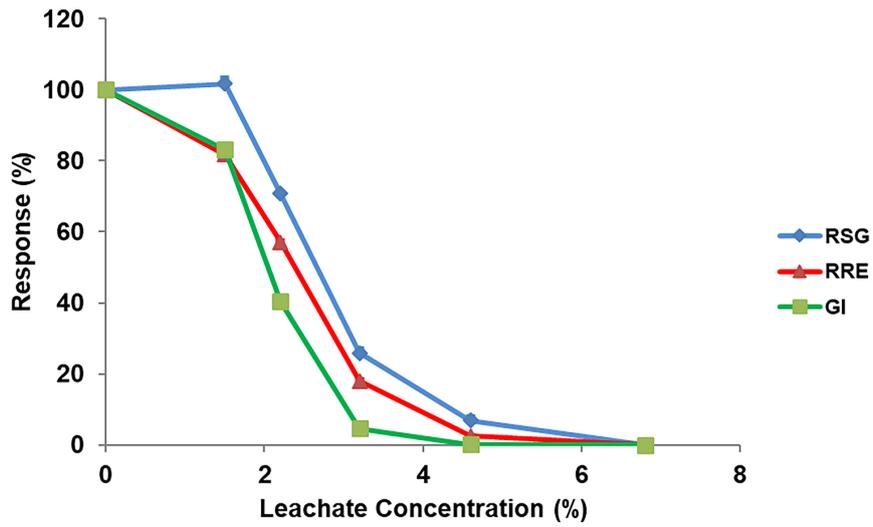


Figure 2. RSG, RRE, and GI of *B. oleracea* exposed to MSL leachate

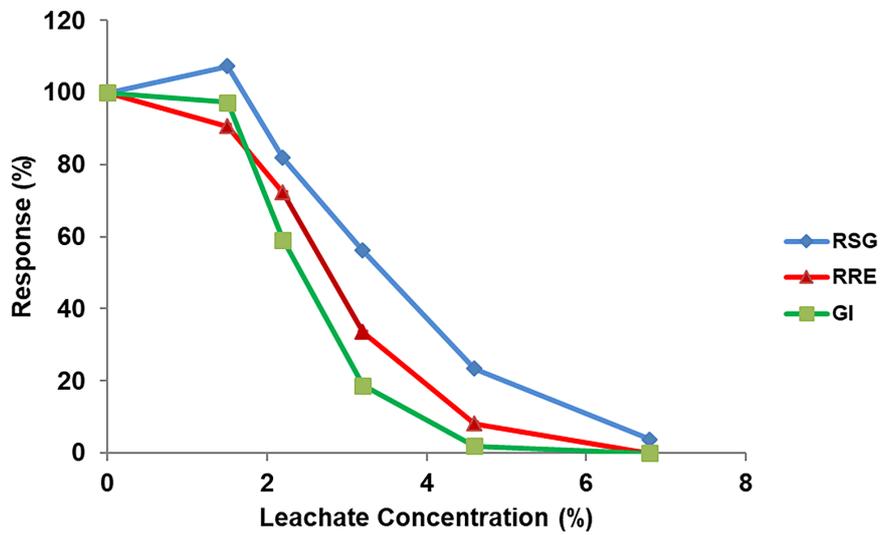


Figure 3. RSG, RRE, and GI of *L. esculentum* exposed to MSL leachate.

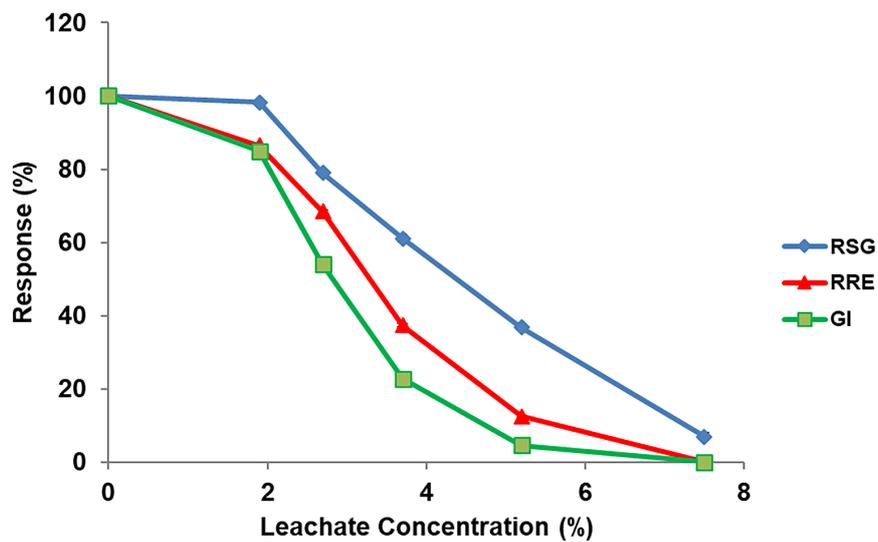


Figure 4. RSG, RRE, and GI of *C. sativus* exposed to MSL leachate

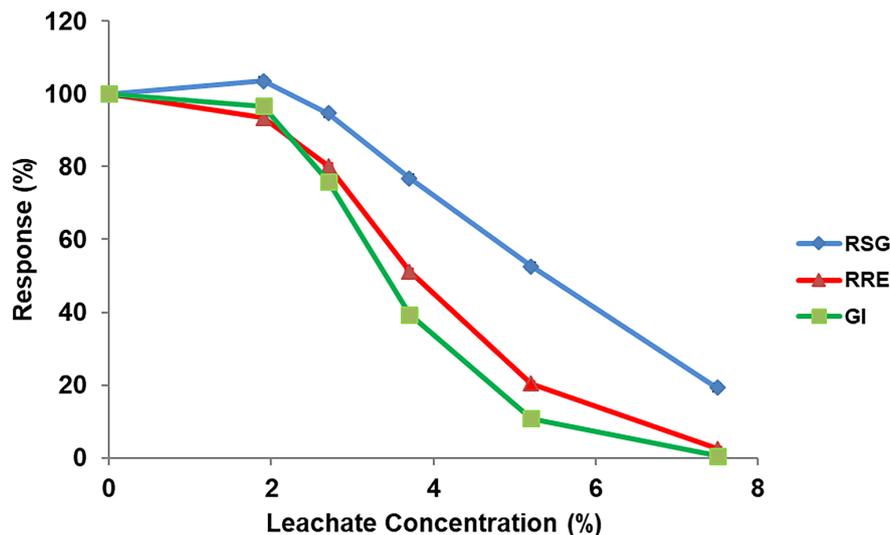


Figure 5. RSG, RRE, and GI of *Z. mays* exposed to MSL leachate

RSG values decreased proportionally with increased leachate concentration. However, the lowest exposure leachate concentration of 1.5% for *B. oleracea* and *L. esculentum* and 1.9% for *C. sativus* and *Z. mays* plants showed ideal seed germination rates of 101.76%, 107.31%, 98.26%, and 103.53%, respectively with respect to the control groups. Such promotion in seed germination may be attributed to the presence of appropriate quantities of inorganic salts and essential plant nutrients such as N, K, P, Ca, and Mg in the highly diluted leachate. Similar assumptions for seed germination enhancement by highly diluted leachate were reported in many previous studies (Zaltauskaite and Cypaite 2008; Gupta and Paulraj 2014; Li et al., 2008; Li et al., 2017).

Seed germination showed a considerable reduction of 70.72%, 81.83%, 78.95.34%, and 89.47% in terms of RSG for *B. oleracea*, *L. esculentum*, *C. sativus*, and *Z. mays*, respectively, when leachate concentrations were increased to 2.2% for *B. oleracea* and *L. esculentum*, and 3.2% for *C. sativus* and *Z. mays* (Figure 2–5). A significant germination inhibition was observed at the highest leachate exposure concentration of 6.8% for *B. oleracea* and *L. esculentum* bioassays when RSG rates of 0.0% and 3.8%, respectively, were obtained. However, the highest exposure concentration of 7.5% for *C. sativus* and *Z. mays* showed lower responses to leachate toxic effects with recorded RSG of 8.79% and 17.53%, respectively. These results conclude that seed germination rates for all tested plants were greatly influenced by exposure to higher leachate concentrations,

with observed variable responses among different tested plant species.

The impact of toxic substances on seed germination varies based on multiple factors, including the specific plant species and interrelated variations in seed structure, diverse anatomical forms of the seed coat and its subsequent permeability, differential uptakes, seed size, and metabolism (Cutillo et al., 2003; Suliasih et al., 2010; Vaverkova et al., 2017). The seed coat, particularly as an essential factor for seed germination, maintains variable anatomical forms among different species and differs from that in other plant tissues and structures (Wada et al., 2011). The poor germination rate observed at the highest tested concentrations could be explained by the extra quantities of inorganic salts and, consequently the elevated EC value. Ramana et al. (2002) reported that MSW with high total salts results in osmotic stress, substantially reducing germination rates. The excessive amounts of salts and  $\text{NH}_4^+$  were identified as primary causes of phytotoxicity by Cheng and Chu. (2007). Other researchers attributed the suppression of seed germination at elevated leachate concentrations to the effects of concentrated pollutants, which in turn damage the defense system, disrupt the enzyme activities, trigger membrane lipid peroxidation, and ultimately interfere with metabolic processes (Gupta and Rajamani 2015; Arunbabu et al., 2017). The overall obtained results in this study were consistent with the findings of other researchers who reported a marked decrease in seed germination with the increase in landfill

leachate exposed concentrations (Ramana et al., 2002; Zulkepli et al., 2019; Sourkova et al., 2020). In contrast to the findings of this study, Li et al. (2017) observed enhanced seed germination and increased pot yield in *Z. mays* when treated with a 10% leachate dilution. This discrepancy may be explained by the marked different characteristics of the leachate used in their study.

The results showed that for all tested leachate dilutions in this study, a more toxic effect on relative root elongation (RRE) was detected than RSG results (Figures 2–5). The lowest toxic effect for landfill leachate was observed at the minimum exposure concentration of 1.5% for *B. oleracea* and *L. esculentum* and 1.9% for *C. sativus* and *Z. mays* when RRE values of 81.71%, 90.64%, 86.47%, and 93.44%, respectively were obtained. At a higher exposure concentration of 2.2% for *B. oleracea* and *L. esculentum*, a perceptible decrease in RRE of 57.07% and 72.21% was obtained, respectively. A lower response was shown by *C. sativus* and *Z. mays* at a concentration of 2.7%, with obtained RRE of 68.43% and 80.14%, respectively. Increasing the exposure concentration to 4.6% for *B. oleracea* and *L. esculentum* showed significant toxic effects with reported RRE of 2.43% and 8.17%, respectively. Similar substantial responses of 12.56% and 20.39% were observed for *C. sativus* and *Z. mays* at an exposure concentration of 5.2%. The most significant sensitivity was observed at the highest exposure concentration of 6.8% for *B. oleracea* and *L. esculentum* and 7.5% for *C. sativus* and *Z. mays*. All tested plant species exhibited 0.0% RRE, except for *Z. mays*, which showed an RRE of 2.48%. The observed reduced RRE in this study is probably attributable to the deterioration of root meristematic tissue, which adversely affects cell permeability, the production of growth hormones, and cell differentiation processes (Wdowczyk and Pulikowska, 2021). These results indicate that root elongation is a more responsive parameter to leachate toxic effects than seed germination. The result of this study is consistent with many preceding studies. Tiquia et al., (1996) found that root elongation is a more sensitive indicator than seed germination when assessing the inhibitory effects of spent litter on various plant species. Zaltauskaite and Cypaite. (2008) concluded that root length is the most sensitive endpoint compared to other endpoints, such as shoot system growth and plant biomass. Anand and Palani. (2022) reported remarkable root growth inhibition of 97.81% and

84.21% for *L. esculentum* and *L. sativum* exposed to 10% concentration of active landfill leachate.

The germination index (GI) was determined according to the achieved RSG and RRE endpoints for all tested plants, and the results are shown in Figures (3.1-3.4). GI incorporates both endpoints, providing a comprehensive indicator for landfill leachate phytotoxicity. The results of GI revealed varied responses for all tested plants compared with the obtained values for RSG and RRE. At the lowest exposure concentrations of 1.5% and 1.9% from both tested sets of leachates, similar responses were observed for *B. oleracea* and *C. sativus* with GI values of 83.14% and 84.97%, respectively. Likewise, *L. esculentum* and *Z. mays* responded similarly, with GI values of 97.26% and 96.73%, respectively. These findings indicate that the low leachate concentration did not significantly impact the growth of all tested plant species. However, increasing leachate concentration from 2.2% to 4.6% for *B. oleracea* and *L. esculentum* significantly impacted the total plant growth, with GI values decreasing markedly from 40.36% to 0.17% and from 59.12% to 1.92%, respectively. A lower toxic effect was observed for *C. sativus* and *Z. mays* when the concentration increased from 2.7% to 5.2%, with GI values reducing from 54.03% to 4.62% and from 71.7% to 10.73%, respectively.

Severe growth inhibition was observed at the highest concentrations of 6.8% and 7.5% for both sets of exposed leachate. All tested plant species showed 0.0% rates of GI except for *Z. mays*, which exhibited a minor rate of 0.44%. These results suggest that GI is a more sensitive endpoint than RSG and RRE. The highest tested concentrations induced severe phytotoxic effects and radically inhibited the growth of all tested plant species. Furthermore, the GI results showed a lower response for *C. sativus* and *Z. mays* over *B. oleracea* and *L. esculentum*. Keeling et al., (1994) observed that GI values  $>100\%$  are associated with stimulating plant growth, while those  $<100\%$  are considered phytotoxic. Subsequently, Roig et al. (2012) classified GI responses into four categories: 1)  $GI \geq 100\%$ , indicating beneficial effects on plant growth; 2)  $GI \geq 80\%$ , reflecting no significant phytotoxic effects; 3) GI between 50–80%, signifying moderate phytotoxicity; and 4)  $GI \leq 50\%$ , indicating substantial phytotoxicity.

In agreement with the findings of this study, other researchers have reported lower sensitivity in *C. sativus* and higher sensitivity in *B. oleracea*

and *L. esculentum* compared to other tested plant seeds for leachate phytotoxicity assessment. However, no previous study was found in the literature to compare *Z. mays* with other testing plants. Hoss et al. (2022) reported 13.28% and 49.61% GI values for *L. sativa* (lettuce) and *C. sativus*, respectively, for tested raw leachate samples. Vieira and Droste. (2019) observed higher sensitivity for *L. esculentum* seeds compared to *L. sativa* seeds with germination inhibition rates of 100% and 13.4%, respectively, as the leachate exposure increased to 100% concentration. At an exposure concentration of 10% leachate, Anand and Palani. (2022) reported a higher response for *L. esculentum* over *L. sativum* with 36.67% and 100% seed germination rates, respectively. Two separate studies evaluated the impact of landfill leachate with consistent physicochemical characteristics from a sanitary landfill in Brazil using *C. sativus* and *B. oleracea* (Franco et al., 2017a; 2017b). Seed germination, radicle growth, and aerial part development were utilized as endpoint parameters. The findings revealed that *B. oleracea* exhibited higher sensitivity, showing a 61% seed germination inhibition at a 6.25% leachate concentration. In contrast, *C. sativus* demonstrated a lower response to landfill toxic impact, experiencing an 83% reduction in seed germination at a 50% leachate concentration.

Landfill leachate comprises various constituents of dissolved organic, inorganic, heavy metals, XOCs, aromatic hydrocarbons, pesticides, etc. This mixture of diverse contaminants exerts substantial toxic effects even with trace amounts in the leachate (Ghosh et al., 2017). In this study, the physicochemical analyses of leachate revealed significant levels of organic and inorganic components. Notably, parameters such as TDS, TSS, COD, BOD,  $\text{NH}_3\text{-N}$ , and  $\text{NO}_3^-$  were observed at concentrations exceeding the permissible limits by MEWA/TWW and SWTP, highlighting severe pollution risks. Many researchers studied the relationship between the physicochemical properties of leachate and its toxic impact on various species of testing organisms from different trophic levels. The close association between leachate organic load, indicated by COD, and posed toxic impacts on many organisms was well documented (Clement et al., 1997; Ward et al., 2002; Verbel et al., 2008).

Similarly, Sharma and Kumar (2021) linked the severe phytotoxicity observed in *Phaseolus aureus* (Mung bean) during seed germination tests to the presence of persistent organic compounds

in both fresh and old MSW dumping sites, leading to significant growth inhibition and impaired root development. Many other studies confirmed that ammonia is the primary pollutant responsible for the observed toxicity in diverse bioassays with various test organisms (Silva et al., 2004; Pivato and Gaspari, 2006; Byrne et al., 2008). A significant correlation was observed between high Leachate Pollution Index (LPI) values and increased phytotoxicity, as evidenced by the inhibition of root and shoot growth at higher leachate concentrations (Wdowczyk and Pulikowska, 2021). Other researchers have related phytotoxicity to the impact of heavy metals, explicitly linking the reduced germination rates and root growth in *L. sativa* and *L. esculentum* to elevated levels of Pb, Fe, and Zn in leachate. Additionally, these metals also increased micronuclei formation, indicating a significant genotoxic effect (Vieira and Droste, 2019). As Pablos et al. (2011) described, the relationship between the physicochemical parameters and the overall toxicity of the leachate should be interpreted as an association rather than a cause-effect relationship. Samples exhibiting elevated levels of specific parameters, such as ammonia, alkalinity, and COD, should be identified as potentially toxic and managed appropriately, including pre-treatment before discharge.

## CONCLUSIONS

The results of the physicochemical analyses showed substantial ranges of organic and inorganic fractions for the studied leachate. Most of the parameters analyzed, such as TDS, TSS, COD, BOD,  $\text{NH}_3\text{-N}$ , and  $\text{NO}_3^-$ , were far beyond the permissible standards of MEWA/TWW and SWTP. The low  $\text{BOD}_5/\text{COD}$  ratio indicates the landfill has entered its final decomposition phase, producing a moderately stabilized leachate with low biodegradability. Heavy metal concentrations obtained in this study were moderate to low with the following descending order of abundance:  $\text{Fe} > \text{Zn} > \text{Cr} > \text{Cu} > \text{Mn} > \text{Pb} > \text{Ni} > \text{As} > \text{Cd}$ . All tested plant species exhibited a strong phytotoxic response to landfill leachate potential toxicity. The tested leachate substantially impacted all phytotoxicity evaluated endpoints in this study. The lower exposed leachate concentrations showed stimulatory effects on RSG, while RRE and GI were retarded even at lower concentrations. The phytotoxic effect was more remarkable as the concentration of

the exposed leachate increased. This study's assessment of phytotoxicity endpoints demonstrated that RRE and GI were more reliable indicators, exhibiting higher responses than RSG across all tested plant species. In order of sensitivity, *B. oleracea* and *L. esculentum* showed higher sensitivity than *C. sativus* and *Z. mays*. All tested plant species were found suitable for landfill leachate phytotoxicity assessment. However, based on their higher sensitivity, *B. oleracea* and *L. esculentum* are more advantageous.

This study underscores the severe toxicity of the leachate examined, emphasizing the need for effective treatment, on-site or at wastewater facilities, before being discharged into the environment. The findings of this study are expected to raise awareness about the potential risks of landfill leachate in Saudi Arabia, inform future toxicity research, and support MSW management efforts. Further investigations using various toxicity testing methods and test species from different trophic levels, employing a suite of bioassays, will be crucial for a comprehensive evaluation of the ecological risks posed by landfill leachate.

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