



Impact of Sewage on Seed Germination and Early Growth of Amaranthus: A Phytotoxicity Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JK and RMJ conceptualized. Authors RJ and RS did the methodology. Author SJ did the formal analysis and investigation. Authors JK, KP and RJ wrote original draft preparation. Author SS wrote, reviewed and edited the manuscript. Author RMJ did the resources. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the phytotoxic effects of sewage on the germination of red amaranthus as a preliminary step before field trials.

Study Design: Completely Randomized Design (CRD) with four replications.

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Methodology: The experiment utilized six treatment levels - control, 20%, 40%, 60%, 80%, and 100% raw sewage - employing a Completely Randomized Design (CRD) with four replications. The experimental data were collected and analyzed.

Results: The results showed that the physico-chemical parameters such as electrical conductivity (EC) and total dissolved solids (TDS) were relatively high in the sewage, causing toxicity to plant, severely affected seed germination and early seedling growth. The maximum toxicity (85-100%) was recorded at 100% sewage concentration, while the minimum relative toxicity percentage (1.5-3.1%) was observed at a 20% sewage. The highest phytotoxicity (100%) occurred at 7 days after sowing (DAS) in the 100% effluent.

Conclusion: The dilution with lower EC and TDS had the less adverse effect on the growth of roots and shoots as compared with the higher concentrations. This study indicates that raw sewage wastewater could be harmful, but diluting it may allow it to be safely used for irrigating amaranthus.

Keywords: Sewage irrigation; phytotoxicity; germination test; Amaranthus; Sewage dilution.

1. INTRODUCTION

Sewage wastewater, though rich in essential nutrients for plant growth, also contains heavy metals, pathogens, and pollutants. Freshwater accounts for only 2.5 to 3% of Earth's total water, with nearly 70% allocated to agriculture. Given this scarcity, sustainable development increasingly relies on sewage irrigation as an alternative resource [1]. In urban areas, a significant portion of water used for irrigation is dedicated to maintaining vegetation, aesthetic quality, and municipal amenities [2]. Some countries are planning to expand the use of treated wastewater for irrigation, with targets such as Saudi Arabia's goal to increase wastewater use to 65% by 2016 [3]. Recycling wastewater for agricultural irrigation offers benefits by adding essential plant nutrients and organic compounds to the soil, while also providing a means of waste disposal [4]. Studies have shown that wastewater is enriched with valuable resources, including macronutrients, micronutrients, and organic matter, which are crucial for soil productivity and fertility [5]. However, despite these agricultural benefits, wastewater and sewage runoff can contain heavy metals and other substances that pose health risks to humans [6]. According to Samarrai [7], the concentration of heavy metals, bacteria, fungi, and salts in sewage wastewater can vary depending on its treatment and source. Metals such as Cu, Fe, Zn, and Mn in sewage effluents can be phytotoxic, and their accumulation in crops can lead to health hazards [8].

Radosevich et al. [9] emphasized that seed germination is a critical process for plant reproduction and population control, and it plays

a significant role in determining crop yield. Seed germination and early plant development bioassays are commonly used to assess phytotoxicity [10]. Research in this area has been conducted sporadically across various countries. Dayama [11] found that even highly diluted industrial effluent (5%) could significantly reduce seed germination in *Cicer arietinum*, causing phytotoxicity due to the accumulation of toxic substances from the growth medium [12]. The impact of effluents on plants varies depending on the species, as well as the type and concentration of toxic materials in the effluent [13].

This underscores the need for thorough scientific studies before any specific waste can be used for irrigation on particular crops and under specific environmental conditions. Despite the recognized importance of seed germination in the context of sewage treatment, the mechanisms underlying phytotoxicity or beneficial effects in seeds remain poorly understood. This research aims to investigate the phytotoxic effects of sewage on the germination of amaranthus as a preliminary step before field trials and to recommend that relevant government agencies effectively monitor farmers' activities and discourage the use of sewage for irrigation without proper treatment.

2. EXPERIMENTAL METHODS

2.1 Sewage Wastewater

Effluent samples were collected in 2 L plastic containers from a sewage dumping site at the Agricultural College and Research Institute in Madurai in May 2024. After collection, the samples were promptly transported to the

laboratory for physio-chemical analysis. Parameters such as pH, electrical conductivity, biological oxygen demand (BOD), chemical oxygen demand (COD), carbonate, and bicarbonate were analysed according to standard methods [14]. To determine metal concentrations, a 100 mL portion of the effluent sample was digested with Aqua regia. The digest was then filtered using Whatman no. 42 filter paper, and the filtrate was diluted to 100 mL with distilled water before being analysed using Microwave Plasma-Atomic Emission Spectroscopy (MP-AES) [15].

2.2 Seed Germination Assay

The germination experiment was conducted with *Amaranthus* (*Amaranthus* sp. L.) CO 2 as test crop. Sterilized petri dishes, lined with a double layer of filter paper, were used for the experiment. These petri dishes were incubated at a controlled temperature of $26 \pm 2^\circ\text{C}$ to facilitate germination.

2.3 Germination Experiment

Uniformly sized *Amaranthus* seeds were selected and sterilized with 0.1% HgCl_2 before being thoroughly washed with distilled water to prevent surface contamination. Twenty sterilized seeds were placed in each petri dish. The experiment utilized six treatment levels - control, 20%, 40%, 60%, 80%, and 100% raw sewage - employing a Completely Randomized Design (CRD) with four replications. The treatments were as follows: Control: 100 mL distilled water (DW); 20% sewage: 20 mL raw sewage + 80 mL DW; 40% sewage: 40 mL raw sewage + 60 mL DW; 60% sewage: 60 mL raw sewage + 40 mL DW; 80% sewage: 80 mL raw sewage + 20 mL DW; 100% sewage: 100 mL raw sewage maintained at a time. Four replicates were maintained for each treatment, including the control. Germination percentages were recorded after 3 and 7 days. Growth parameters, such as shoot and root lengths, were measured after 7 days. Following this period, seedlings were harvested, and fresh weights were recorded. For dry weight determination, seedlings were incubated at 60°C for 24 hours.

2.3.1 Germination percentage

The germination percentage was determined using the formula described by Raun et al. [16].

$$G = \frac{Sg}{ST} \times 100$$

where, G = germination (%), Sg = number of seed germinated, and ST = total number of seed set for the test.

2.3.2 Germination energy and capacity

Germination energy is the percentage of seeds that germinate by 3 days after sowing (DAS), while germination capacity refers to the percentage of seeds that germinate by 7 DAS, as per Bam et al. [17].

2.3.3 Germination speed

Speed of germination was measured by using following formula [18].

$$G_s = \frac{Sg_{72}}{Sg_{168}} \times 100$$

where, G_s = speed of germination, Sg_{72} = number of seed germinated at 72th h, and Sg_{168} = number of seed germinated at 168th h.

2.3.4 Relative toxicity

Relative toxicity (% RT) of sewage on the seed germination of Amaranth over control was calculated by using the following formula of Chapagain [19].

$$R_T = \frac{x-y}{x} \times 100$$

where, RT = relative toxicity (%), x = germination percentage in control at particular hour of incubation, and y = germination percentage in the presence of effluent at the same hour of incubation.

2.3.5 Phytotoxicity

The phytotoxicity was calculated using the formula of Chou and Lin [20].

$$\text{Phytotoxicity (\%)} = \frac{R_c - R_t}{R_c} \times 100$$

R_c = radical length of control and R_t = radical length of test.

2.3.6 Vigour index

Vigour index of the seedlings was calculated using the formula proposed by Abdul-baki and Anderson [21].

$$\text{Vigour index} = G \times L$$

where, G = germination (%) and L = seedling length.

2.3.7 Fresh weight and dry weight

Ten seedlings from each treatment were collected, and their fresh weights were recorded. The weight per plant was subsequently calculated based on these measurements. The same seedlings used for fresh weight measurement were placed in a hot air oven at 60°C for 24 hours. After drying, the seedlings were removed from the oven and allowed to cool in desiccators. Their dry weights were then recorded.

2.4 Statistical Analyses

The physico-chemical analysis of elements present in the sewage was conducted three times, and the data collected from each repetition were compiled for statistical analysis. The data from the seed germination assay were also analyzed to assess the significance of variance ($P < 0.05$). To compare treatment means, standard errors were calculated using SPSS and Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Properties of SEWAGE

The physico-chemical characteristics of the sewage are presented in Table 1. The collected sewage appeared yellowish-brown in color. The pH of the sewage was 8.1, indicating a basic nature, which falls within the permissible pH range (5.5-9.0) for irrigation water [22]. This increase in pH is likely due to the presence of various soluble salts in the sewage water. Despite a 3.94% rise, the pH of the sewage remained within the acceptable limits for use on agricultural land [23]. The electrical conductivity (E.C.) of the sewage water was 3.3 dS/m. The total dissolved solids (TDS) were found to be 2115 mg/l, above the CPCB standard limit of 2100 mg/l for public sewers. The FAO standard range for TDS in irrigation water is 450 to 2000 mg/L [24]. The levels of calcium (51.31 mg/L) and magnesium (39.68 mg/L) were also noted. Among the metals, the iron content in the sewage was 23.5 mg/L, which is higher than the permissible limit set by the CPCB. Chitdeshwari et al. [25] reported an iron concentration of 36.75 mg/L in the sewage at Madurai, Tamil Nadu. The concentrations of copper and lead were above the permissible limits, with copper exceeding the

0.20 mg/L [26]. The lead concentration was 2.3 mg/L, which is below the maximum limit of 5 mg/L [27]. However, the concentrations of copper, chromium, nickel and iron exceed the prescribed limits.

Table 1. Physico-chemical properties of sewage

Parameters	unit	Sewage
Turbidity	NFU	45
pH		8.1
EC	ds/m	3.3
Total Dissolved Solids	mg/l	2115
Total suspended solids	mg/l	415
Carbonate	meq/l	0
Bicarbonate	meq/l	12.2
Calcium	mg/l	51.31
Magnesium	mg/l	39.68
Chloride	mg/l	187
Biological oxygen demand	mg/l	580
Chemical oxygen demand	mg/l	1060
Copper	mg/l	3.4
Chromium	mg/l	1.9
Nickel	mg/l	2.5
Lead	mg/l	2.3
Zinc	mg/l	1.04
Iron	mg/l	23.5

3.2 Seed Germination Assay

3.2.1 Germination energy

Seed germination and early seedling growth are crucial stages in the life cycle of plants and are highly susceptible to environmental stress. As germination is the initial physiological process, several growth parameters, including germination rates, as well as the subsequent growth and yield of crops, are used to evaluate the level of pollution [28]. In the case of red amaranth, the highest seed germination percentage at 3 days after sowing (DAS) was observed in the control group. But with sewage maximum germination energy (91.5%) was found with 20% sewage (Fig. 1a). At the same time, the minimum germination was recorded in 100% concentration of the sewage. Germination energy increased up to 60% sewage concentration and then slowly decreased to 62.3% at 100% sewage concentration. In stress conditions, the energy forming molecules may be disturbed and subsequently carbohydrates and protein metabolites of the membrane are altered [29], which might lead to reduction in absorption of water by the seeds/seedlings. Suppression of

germination at higher concentrations of sewage might be due to high levels of total dissolved solids which enhanced the salinity and conductivity of the solute absorbed by the seeds before germination. The promotion of seedling growth by lower concentration of sewage might be due to the presence of plant nutrients in the sewage.

3.2.2 Germination capacity

The germination capacity of red amaranth was significantly affected by increasing levels of sewage concentration. As shown in Fig. 1b, the highest seed germination rate of 98.25% at 7 days after sowing (DAS) was observed in the control group and at 20% sewage concentration. In contrast, a complete inhibition of germination (0%) was recorded at 100% sewage concentration. Within the sewage treatments, the maximum germination occurred at 20% and 40% concentrations, but it progressively declined as the effluent concentration increased. Across all dilutions, the germination capacity decreased with higher sewage concentrations. Some researchers attribute this delayed germination to the presence of elevated salt or metal concentrations [30], while others link it to reduced auxin production, also a consequence of higher metal ion concentrations.

The increased germination rates (96.8 & 95.10 %) at lower sewage concentrations (20% and 40%) compared to higher concentrations (over 40%) suggest that sewage treatment stimulated the germination of previously inactive seeds, Vinod [31]. This effect may be due to the creation of favourable environmental conditions by lower sewage concentrations, which supported seed germination and allowed the seeds to utilize the nutrients present in the sewage [32]. Additionally, the diluted sewage may have promoted plant growth at these lower concentrations [33]. However, higher sewage concentrations inhibited red amaranth germination, likely due to the negative impact of increased total solids and heavy metal stress on the germination process.

The salt content in the surrounding environment can limit water absorption by osmosis, further inhibiting seed germination [34].

3.2.3 Germination speed

The speed of germination varied across different sewage concentrations, as shown in Table 2. The highest germination speed (100%) was observed in the control group, while the lowest was recorded at 100% sewage concentration. Exposure to different sewage water solutions reduced germination speed by 5.1% to 100% compared to the control. Raw sewage significantly inhibited the growth of red amaranth, with root length in raw sewage being slightly shorter than the control, which affected germination speed. Germination speed increased up to 60% sewage concentration but decreased as the concentration increased beyond this level. Rashid et al. [35] also reported that seeds of *Bidens pilosa* and *Lolium perenne* exhibited slower germination speeds when exposed to toxic plant extracts.

3.2.4 Root and shoot lengths

The effect of sewage application on plant rooting and shooting varied depending on the concentration used in the experiment. Higher sewage concentrations significantly reduced the fresh and dry weight of seedlings. Growth parameters such as root and shoot length, measured at 7 days after sowing (DAS) (Table 2), decreased as sewage concentration increased. The maximum root length (3.5 cm) and shoot length (4.7 cm) were observed at 20% sewage concentration at 7 DAS. Conversely, the minimum root and shoot lengths were recorded at 100% sewage concentration. No radicle formation occurred at 80% and 100% sewage concentrations, and at 100% concentration, minimum shoot growth was observed. Similar inhibitory and stimulatory effects of various effluents on the germination of several plant species have been reported by [36].

Table 2. Effect of different concentration of sewage on Seedling parameters of amaranthus

Treatment	Germination speed (%)	Root length (cm)	Shoot length (cm)	Fresh weight (mg/seedling)	Dry weight (mg/seedling)
Control	100.0 ± 0.0	3.4 ± 1.2	4.5 ± 0.14	1.83 ± 0.23	0.26 ± 0.01
20% Sewage	91.5 ± 4.4	3.5 ± 0.6	4.7 ± 0.06	1.85 ± 0.21	0.28 ± 0.02
40% sewage	89.2 ± 3.8	3.2 ± 0.15	4.4 ± 0.21	1.80 ± 0.01	0.26 ± 0.00
60% sewage	90.6 ± 4.6	2.7 ± 0.20	4.0 ± 0.15	1.75 ± 1.71	0.25 ± 0.02
80% sewage	90.4 ± 3.8	2.2 ± 0.6	3.7 ± 0.15	1.69 ± 0.03	0.24 ± 0.02
100% sewage	62.2 ± 7.8	1.7 ± 0.09	3.0 ± 0.24	1.56 ± 0.03	0.22 ± 0.01

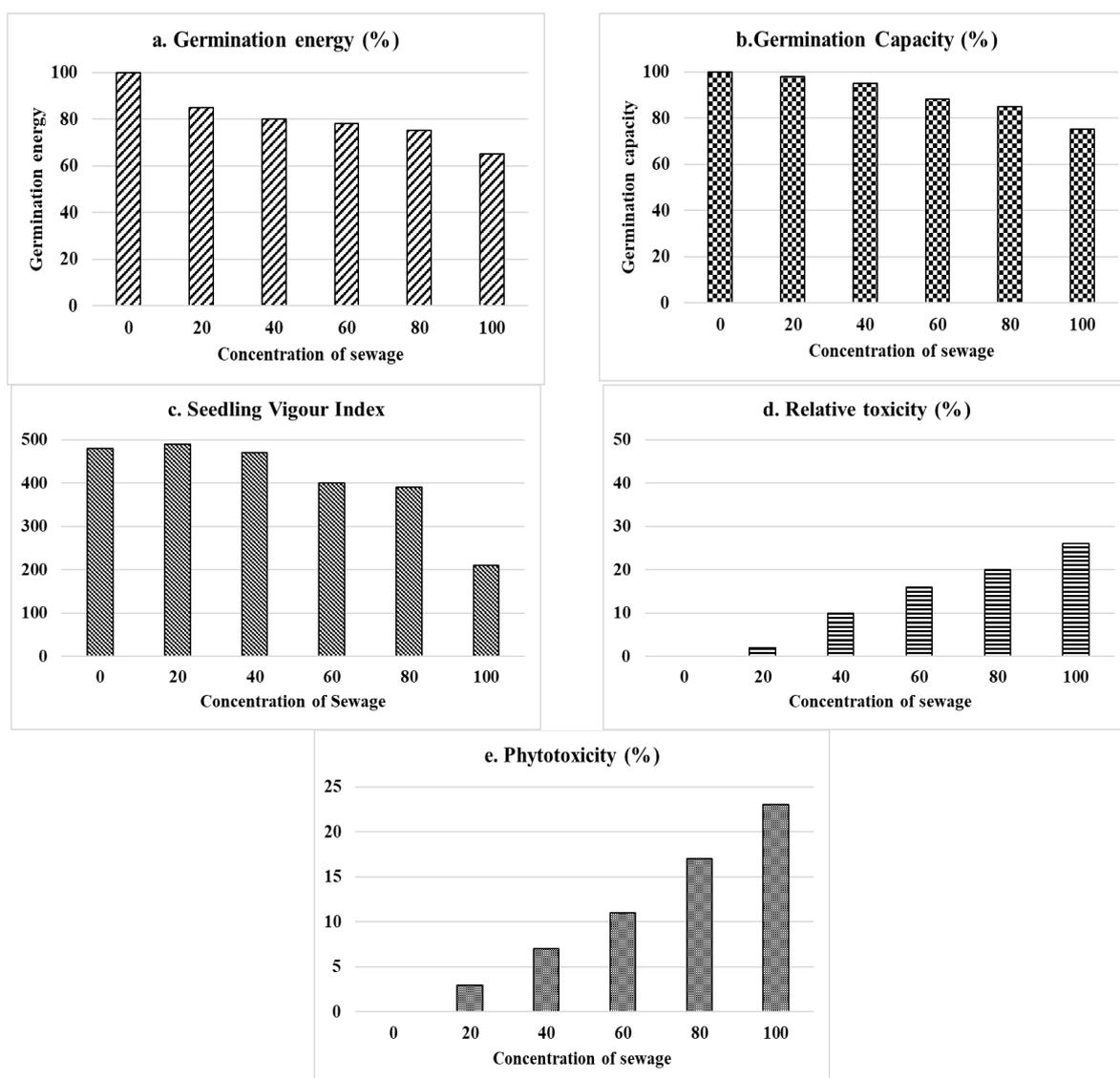


Fig. 1. Effect of sewage on a. Germination energy, b. Germination capacity, c. seedling vigour, d. relative toxicity, e. phytotoxicity of Amaranthus

The lack of oxygen for the germinated seeds restricted their energy supply through aerobic respiration, which is crucial for the growth and development of young seedlings. This led to limited growth of both the radicle and plumule, as noted by Hadas [37]. Singh et al. [38] investigated the effects of fertilizer factory wastewater on seed germination, seedling growth, and the shoot and root lengths of gram (*Cicer arietinum*), they found that a 25% concentration of effluent promoted growth, particularly in root and shoot length, at 21 days. The lower effluent concentrations likely provided essential nutrients like nitrogen and phosphorus, which may have enhanced plant growth, Augusthy and Mani [33].

Higher concentrations of heavy metals interfered with root and shoot length, likely due to their impact on physiological processes within the plant. This interference may include the inhibition of enzyme activities, disruption of nutrient absorption, water imbalance, and changes in hormonal balance, resulting in altered membrane permeability [39]. Sharma et al. [40] noted that chromium (Cr) phytotoxicity manifests as inhibited seed germination, stunted early seedling growth, reduced root development, leaf chlorosis, and decreased biomass production. Similarly, Vaithiyathan and Sundaramoorthy [41] observed that African marigold seedlings exhibited the greatest shoot length (29.10 cm) and root length (6.80 cm) at a 10% concentration

of sugar mill effluent, whereas the lowest shoot length (9.43 cm) and root length (1.83 cm) were recorded at a 100% concentration of the effluent.

3.2.5 Fresh weight and dry weight

The fresh and dry weights of seedlings increased at lower sewage concentrations and decreased at higher concentrations, as shown in Table 2. The highest fresh weight (1.85 mg per seedling) and dry weight (0.28 mg per seedling) were recorded at 7 days after sowing (DAS) in the 20% sewage concentration. In contrast, the lowest fresh and dry weights were observed in 100% sewage concentration across all dilutions. In all cases, fresh weight consistently decreased as sewage concentration increased. These findings align with previous reports on the effects of various industrial effluent treatments on different crops [42].

The presence of optimal nutrient levels in the lower sewage concentrations likely contributed to the increased fresh and dry weights of seedlings, due to the presence of various chemicals in the sewage [43]. Conversely, higher sewage concentrations led to a decrease in seedling weight, possibly due to poor growth under effluent stress. According to Chetan and Ami [44], copper had a significant impact on reducing the leaf fresh weight in *Amaranthus* seedlings. The roots, which are in direct contact with the effluent, may be particularly affected by higher concentrations, potentially inhibiting cell multiplication and root growth of *Vigna radiata*, *Cajanus cajan*, and *Sorghum bicolor* [29]. Vaithyanathan and Sundaramoorthy [41] also reported that African marigold exhibited the highest fresh weight (3.59 g per seedling) and dry weight (0.420 mg per seedling) at a lower concentration (10%) of sugar mill effluent, while the lowest fresh weight (0.760 mg per seedling) and dry weight (0.074 mg per seedling) were observed at the highest concentration (100%) of the effluent.

3.2.6 Seedling vigour index

A decrease in the seedling vigor index of amaranth was observed across all tested effluent solutions as their concentrations increases (Fig. 1c). The highest vigor index (430%) was recorded at 7 days after sowing (DAS) in the 20% sewage concentration, while the lowest was observed at 100% concentration at the same time point. The variation in seedling vigor may be attributed to genotypic differences and the

varying levels of sewage stress across different concentrations.

3.2.7 Relative toxicity of seed germination

The minimum relative toxicity percentage of red amaranth seed germination (1.5–3.1%) was observed at a 20% sewage concentration, while the maximum toxicity (85–100%) was recorded at 100% concentration (Fig. 1d). Toxic effects became evident at higher sewage concentrations. Regarding the relative toxicity percentage in red amaranth, the lowest toxicity was observed at 20% concentration of sewage, and the highest at 100%. Notably, relative toxicity significantly increased above 40% effluent concentration, reaching its peak at 100%. Similar results were reported by David and Rajan [45] and they found that the minimum relative toxicity percentage occurred at 60% effluent concentration, with a gradual increase as the concentration rose further.

3.2.8 Phytotoxicity

Phytotoxicity significantly increased with rising effluent concentrations. The highest phytotoxicity (100%) was recorded at 7 days after sowing (DAS) in the 100% sewage concentration, while the lowest was observed in the control group (Fig. 1e). The high level of phytotoxicity in amaranth exposed to sewage could be attributed to the sewage's elevated pH, electrical conductivity (EC), total dissolved solids (TDS), and metallic content. The lowest phytotoxicity was observed at a 20% sewage concentration, which was comparable to the phytotoxicity at 40% concentration. The highest phytotoxicity was recorded in the raw sewage. Similar toxic effects of dyeing industry effluent were reported by David and Rajan [45] in lady's finger.

4. CONCLUSION

Germination and early seedling growth of amaranthus were significantly affected by the different concentrations of sewage. Based on the experimental observations, it is concluded that the physico-chemical parameters such as electrical conductivity, total dissolved solids were relatively high in the sewage causing toxicity to the plant, severely affected seed germination and early seedling growth. Germination energy, germination capacity, germination speed, root length, shoot length and seedling vigour index were declined gradually with increasing effluent concentrations. Sewage dilution with lower EC

and TDS had less adverse effect on the germination and early growth of roots and shoots. But the dilution with high EC and TDS affected the germination and growth drastically. The exposure of seeds to the low concentration of sewage during germination had no or lower phytotoxicity to early seedling growth of amaranth as compared with the higher concentrations. As a result, the raw sewage effluent could possibly lead to soil deterioration and low productivity. The results of this study suggest that the physico-chemical properties of the sewage wastewater might be harmful for crops and human being through food chain by bioaccumulation. So, the use of sewage should be restricted in crop fields without proper treatment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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