JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2027, 28(1), 194–206
https://doi.org/10.12911/22998993/241753
ISSN 2299–8993, License CC-BY 4.0

Received: 2026.09.23
Accepted: 2026.11.16
Published: 2026.12.01

Sewage sludge composting in the arid Mangystau Region with thermophilic *Bacillus strains* and actinomycetes: Nutrient transformation and plant response

Akbota Begakhmet[1,2], Zhanar Tekebayeva[1*], Kamshat Kulzhanova[1], Aliya Temirbekova[1], Arman Abilkhadirov[1], Zhadyrassyn Nurbekova[1,3], Aslan Temirkhanov[1], Akhan Abzhalelov[1,2]

[1] Republican Collection of Microorganisms, Astana 010000, Kazakhstan
[2] Department of Management and Engineering in the field of environmental protection, L.N. Gumilyov Eurasian National University, Astana 010000, Kazakhstan
[3] Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, Astana 010000, Kazakhstan
* Corresponding author's e-mail: zanartekebaeva@gmail.com

ABSTRACT

The management and valorization of sewage sludge remain important environmental challenges, particularly in arid regions with low soil organic matter. This study addressed this issue by evaluating selected thermotolerant microorganisms during 90-day sewage sludge composting under the conditions of the Mangystau Region, Kazakhstan. Throughout the experiment, composting dynamics, compost maturity, nutrient transformation, and plant responses were assessed. The inoculated treatments showed more pronounced compost maturation than the uninoculated control. The combined *Bacillus*–actinomycete treatment was associated with more pronounced stabilization of organic matter and the most favorable plant response, with humus content reaching 20.0% by day 90. Compost maturation was also accompanied by reduced phytotoxicity and improved plant development, particularly in the inoculated treatments. Overall, the findings demonstrate the potential of selected thermotolerant *Bacillus* strains and actinomycetes for sewage sludge bioconversion and stabilization, supporting further evaluation of the resulting material as a potential organic soil amendment for use under arid conditions.

Keywords: sewage sludge, composting, thermophilic bacteria, *Bacillus*, actinomycetes, arid region.

INTRODUCTION

Wastewater treatment generates considerable quantities of sewage sludge containing organic carbon, nitrogen, phosphorus, potassium, and other nutrients that may be valuable for agricultural soils. Controlled stabilization and subsequent use of sewage sludge as an organic fertilizer or soil amendment can reduce the amount requiring disposal, return nutrients to biogeochemical cycles, and decrease dependence on mineral fertilizers. Therefore, sewage sludge recycling can be considered an important component of circular organic-waste and soil-resource management (Collivignarelli et al., 2019; Lu et al., 2012).

The application of organic materials is particularly relevant in arid and saline regions, where soil productivity is limited by low organic matter content, water scarcity, poor soil structure, high concentrations of soluble salts, and limited nutrient availability. Under these conditions, organic amendments can improve soil aggregation and water-holding capacity, stimulate microbial activity, and increase nutrient availability. However, the effectiveness of sewage sludge application depends on its degree of stabilization, application rate, soil properties, salinity level, climatic

conditions, and the characteristics of the cultivated plants (Sastre-Conde et al., 2015).

Research from arid and coastal regions has demonstrated the potential of sewage sludge to rehabilitate degraded and salt-affected soils. In Tunisia, its application improved soil chemical properties and stimulated several enzymes involved in nutrient cycling (Lakhdar et al., 2011). More broadly, sewage sludge amendments can increase soil organic matter and nutrient availability, improve biological activity, and support plant productivity in saline–alkaline soils, although their effectiveness depends strongly on the application rate and sludge quality (Jat Baloch et al., 2023). Studies conducted in China reported increased soil organic matter and nutrient contents, reduced salinity and alkalinity, and changes in the structure of the soil bacterial community (Bai et al., 2018; Li et al., 2021). In saline tidal-flat soils, sewage sludge application improved root development and increased rice yield; however, increasing the application rate could also increase soil electrical conductivity (Shan et al., 2021).

Aerobic composting is one of the principal methods used to biologically stabilize sewage sludge before its application. During this process, readily degradable organic compounds are oxidized, heat is generated, and the initial substrate is gradually converted into a more stable, humus-like material. Temperature, moisture, aeration, pH, substrate structure, and the initial C:N ratio determine the intensity of organic matter decomposition, the duration of the thermophilic phase, and the maturity of the final product (Onwosi et al., 2017; Wei et al., 2017). A rapid increase in temperature reflects microbial activity, whereas the maintenance of thermophilic conditions indicates intensive biochemical processes within the composting mass. Compost maturity should be confirmed by changes in pH, the C:N ratio, humus content, available and total nutrient contents, and the results of seed-germination and plant-growth tests (Bernal et al., 2009; Cesaro et al., 2015).

The use of specialized microorganisms can accelerate organic matter decomposition and nutrient transformation during composting. Thermophilic bacteria, including members of the genus *Bacillus*, tolerate elevated temperatures and produce thermostable hydrolytic enzymes involved in the degradation of proteins, polysaccharides, and other organic compounds (Finore et al., 2023). Actinomycetes, particularly *Streptomyces* spp., contribute to the decomposition of cellulose, lignin, and other resistant organic materials, especially during the cooling and maturation stages (Bhatti et al., 2017). However, the effectiveness of microbial inoculation depends on substrate properties, composting conditions, inoculum viability, and interactions between the introduced strains and indigenous microbial communities.

Sewage sludge recycling and soil restoration are particularly important for the Mangystau Region, located in the arid zone of western Kazakhstan. Limited precipitation, high evaporation rates, low soil organic matter, and the widespread occurrence of saline soils substantially restrict plant establishment. The production of a locally derived, stabilized organic amendment from sewage sludge could simultaneously support organic-waste recycling, return nutrients to the soil, and improve conditions for plant growth. Despite previous research on sewage sludge application to arid and saline soils, the composting of sewage sludge using thermophilic *Bacillus* strains and actinomycetes remains insufficiently investigated.

The objective of this study was to evaluate the effects of two microbial preparations based on thermophilic *Bacillus* strains, applied either alone or in combination with an actinomycete, on sewage sludge stabilization and compost maturation during a 90-day pilot-scale composting process under the arid conditions of the Mangystau Region, in comparison with an uninoculated control. We hypothesized that microbial inoculation, particularly the combined *Bacillus*–actinomycete treatment, would enhance organic matter stabilization, improve compost maturity indicators, and reduce phytotoxicity relative to the uninoculated control.

MATERIALS AND METHODS

Study materials and experimental design

A pilot-scale windrow composting experiment was conducted in Zhanaozen, Mangystau Region, Kazakhstan (43°20′20″ N, 52°51′20″ E), using dewatered sewage sludge as the principal organic substrate and dry stover as a bulking material. The initial physicochemical characteristics of the sewage sludge–dry stover mixture are presented in Table 1.

Each experimental treatment was established in three independent composting windrows (n = 3), with each windrow containing an initial total substrate mass of 1000 kg, comprising 800 kg of

Table 1. Initial physicochemical characteristics of the sewage sludge–dry stover mixture used for composting

Parameter	Sewage sludge–dry stover mixture
Moisture (%)	75
Total organic carbon (%)	46.43
Total N (%)	1.407
C:N ratio	33
pH	6.46

dewatered sewage sludge and 200 kg of dry stover. Thus, dry stover represented 20% (w/w) of the initial composting mixture and was incorporated to improve substrate structure, porosity, and aeration.

Each windrow was formed to approximately 4.0 m in length, 1.0 m in width, and 0.8 m in height. Three composting variants with the same initial substrate composition were established and differed only in the microbial preparation applied:

Variant I — 800 kg of dewatered sewage sludge + 200 kg of dry stover + 250 g of a microbial preparation containing a consortium of thermophilic *Bacillus* strains;

Variant II — 800 kg of dewatered sewage sludge + 200 kg of dry stover + 250 g of a microbial preparation containing the thermophilic *Bacillus* consortium supplemented with an actinomycete;

Variant III (control) — 800 kg of dewatered sewage sludge + 200 kg of dry stover, without microbial inoculation.

The microbial preparation used in Variant I consisted of five thermotolerant *Bacillus* isolates (1-1, 1-11, 2-12, W-NA-2t, and W-NA-4t) combined in equal proportions. The preparation used in Variant II contained the same five-*Bacillus* consortium supplemented with the actinomycete isolate W-NA-3t (*Streptomyces thermolineatus*), with all six strains combined in equal proportions. The viable-cell concentration of the preparations was approximately 1×10^8 CFU g^{-1}. Each inoculated windrow received 250 g of the corresponding preparation per 1000 kg of initial composting material, equivalent to 0.25 g kg^{-1} or 0.025% (w/w).

Composting was carried out under aerobic conditions for 90 days. Aeration was provided by mechanical turning of the windrows using a compost turner. Turning and moisture adjustment were performed on days 5, 15, 21, 26, and 30 of composting, particularly when a decline in pile temperature was observed. During each turning event, the composting mass was mechanically mixed to improve oxygen transfer and redistribute heat and moisture, while water was added when a decrease in pile moisture was detected using the portable moisture meter. Temperature was monitored during the active composting phase, and compost samples were collected after 30, 60, and 90 days for physicochemical and biological analyses. The internal temperature and moisture content of each compost pile were monitored daily throughout the composting process using a portable digital soil temperature and moisture meter. The probe was inserted into the central part of each pile at a depth of approximately 30–50 cm.

Microbial strains and molecular identification

Genomic DNA was extracted from pure cultures, and partial 16S rRNA gene sequences were amplified and sequenced following the general approach described by Weisburg et al. (1991). Consensus sequences were compared with nucleotide records in the National Center for Biotechnology Information (NCBI) database using BLAST (Altschul et al., 1990). Taxonomic assignments were evaluated based on sequence identity, query coverage, and consistency among the highest-scoring BLAST matches. For closely related *Bacillus* taxa, species-level identification based solely on partial 16S rRNA gene sequences was considered inconclusive when equivalent or near-equivalent matches were obtained for multiple species. The analyzed sequence lengths, BLAST query coverage, sequence identity, and taxonomic assignments are summarized in Table 3. In such cases, available genome-based evidence was used to resolve the taxonomic assignment. In particular, the taxonomic identity of isolate 2-12 was further evaluated using previously published whole-genome sequence data for the same isolate, designated RKM-2 (Zhanar, T. et. al., 2026).

Preparation and activation of inocula

Thermophilic bacterial and actinomycete isolates were initially recovered from sewage sludge samples collected at wastewater treatment facilities in the Mangystau Region, Kazakhstan. The samples were cultivated on nutrient agar at 50 °C to obtain thermotolerant microorganisms. From the resulting collection, six isolates were selected for further taxonomic identification

and formulation of the experimental microbial variants.

For biomass production, the selected isolates were cultivated in liquid culture media at 50 °C in a fermenter. *Bacillus* strains were grown in a modified liquid nutrient medium, whereas the actinomycete was cultivated in starch–ammonium broth. Following cultivation, the microbial biomass was harvested by centrifugation, and the resulting concentrated biomass paste was used to formulate the microbial preparations.

Prior to application, the microbial preparations were activated in 50 L of water supplemented with 200 g of dextrose and 200 g of wheat bran. The activation mixture was maintained for 18 h at room temperature and subsequently distributed uniformly over the corresponding composting windrows.

Physicochemical analyses

Compost pH was determined in an aqueous compost extract prepared at a fixed compost-to-water ratio. Total nitrogen (TN) was quantified by the Kjeldahl method (Bremner, 1996), while total organic carbon (TOC) was determined using the dichromate oxidation method described by Bremner and Jenkinson (Bremner and Jenkinson, 1960). The C:N ratio was subsequently calculated from the measured TOC and TN contents according to Equation 1:

$$C{:}N = \frac{TOC}{TN} \quad (1)$$

where: TOC is the organic carbon content and TN is the total nitrogen content, both expressed on a dry matter basis.

Humus was quantified gravimetrically or spectrophotometrically according to the laboratory protocol. Available N was extracted from fresh compost with 2 M KCl (1:5, w/v) and determined as the sum of NH_4^+–N and NO_3^-–N (Keeney and Nelson, 1982). Available P was extracted from air-dried compost with 0.5 M $NaHCO_3$ (pH 8.5; 1:20, w/v) and determined using the molybdenum-blue method (Murphy and Riley, 1962). Available K was extracted with 1 M NH_4OAc (pH 7.0; 1:10, w/v) and measured by flame photometry (ISO/TS 22171:2023). The results were expressed as mg kg^{-1} of compost dry matter. All concentrations refer to compost dry matter unless otherwise stated. Compost pH and temperature were measured using a portable digital soil pH and temperature meter.

Compost bioassays

Seed-germination bioassay

Phytotoxicity was assessed by a seed-germination bioassay using radish (*Raphanus sativus* L., cv. Dunganskii). Compost samples from Variants I–III collected after 30, 60, and 90 days were tested. Fifty grams of each sample were placed in a Petri dish and covered with filter paper, on which 25 seeds were evenly distributed. Each dish was moistened with 5 mL of distilled water and incubated for 72 h at 22–25 °C under natural light. Three independent Petri dishes were used for each treatment.

After 72 h, seeds with a primary root longer than 1 mm were considered germinated. Germination percentage was calculated for each Petri dish as follows (2):

$$G(\%) = \frac{N_g}{N_t} \times 100 \quad (2)$$

where: N_g is the number of germinated seeds and N_t is the total number of seeds.

Mean root length was calculated as:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} x_i \quad (3)$$

where: x_i is the length of each germinated root and n is the total number of germinated seeds.

A separate filter-paper control was used as the reference for phytotoxicity assessment. The control consisted of radish seeds placed on filter paper moistened with distilled water and was incubated under the same conditions and with the same number of replicates as the compost samples. Phytotoxicity was calculated as root-growth inhibition relative to the filter-paper control (Tiquia et al., 1996):

$$Phytotoxicity(\%) = \left(1 - \frac{\bar{X}_{sample}}{\bar{X}_{control}}\right) \times 100 \quad (4)$$

where: $\bar{X}_{sample}$ is the mean root length of seedlings grown in the tested compost sample *and* $\bar{X}_{control}$ is the mean root length of seedlings in the filter-paper control.

In this study, phytotoxicity was assessed based on root-growth inhibition relative to the filter-paper control rather than by using the classical Germination Index (GI).

Pot Experiment with Lepidium sativum L.

Cress (*Lepidium sativum* L.) was used as a rapid biological indicator of compost maturity and plant response (El Kajaf et al., 2026). For the pot experiment, only compost collected after 90 days of composting was used. Saline soil collected in Zhanaozen, Mangystau Region, Kazakhstan, was used as the base substrate. Twenty seeds were sown in 17-cm-diameter pots containing a total of 800 g of substrate.

Compost from the two microbially inoculated variants (Variants I and II) was incorporated separately into the saline soil at rates of 10%, 20%, and 30% (w/w). These rates corresponded to 80, 160, and 240 g of compost mixed with 720, 640, and 560 g of saline soil, respectively. The control consisted of 800 g of the same saline soil without compost addition. The composition of the potting substrates is presented in Table 2.

Statistical analysis

Data are presented as mean ± standard deviation (SD). For each experimental variant, n = 3 represents three independent composting windrows, which were treated as independent biological replicates. The reported mean values were calculated from these three independent replicates, and the error bars shown in the figures represent the corresponding SD.

Differences among Variants I, II, and III were evaluated separately at each sampling time point (30, 60, and 90 days) using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's multiple-comparison test was applied for pairwise comparisons among the variants. Differences were considered statistically significant at $p < 0.05$. Statistical analyses and graphical visualization were performed using GraphPad Prism 10.

Table 2. Composition of the substrates used in the pot experiment with *Lepidium sativum* L.

Compost rate (% w/w)	Saline soil (g)	Compost (g)
0 (control)	800	0
10	720	80
20	640	160
30	560	240

RESULTS

Identification of thermophilic isolates

Re-analysis of the partial 16S rRNA gene sequences confirmed that five isolates belonged to the genus *Bacillus*, whereas isolate W-NA-3t was assigned to *Streptomyces* (Table 3). The best BLAST matches for isolates 1-1 and 1-11 corresponded to *Bacillus subtilis*, with query coverage of 98% and sequence identities of 98.78% and 98.39%, respectively. Isolates W-NA-2t and W-NA-4t showed 100% query coverage and 100% sequence identity to *Bacillus* licheniformis, whereas W-NA-3t showed 100% query coverage and 100% identity to *Streptomyces thermolineatus*.

Re-analysis of the 766-bp partial 16S rRNA gene sequence of isolate 2-12 yielded 100% query coverage and 100% sequence identity. However, equivalent highest-scoring matches were obtained for several closely related *Bacillus* taxa, including records assigned to *B. rugosus*, *B. spizizenii*, and *B. subtilis*. Therefore, the partial 16S rRNA gene sequence alone was insufficient for an unambiguous species-level assignment of isolate 2-12. Whole-genome analysis of the same isolate, subsequently designated RKM-2, showed a maximum ANI of 99.76% with *Bacillus licheniformis*, exceeding the generally accepted 95–96% species-level ANI threshold. Phylogenomic

Table 3. Identification of the thermophilic isolates by 16S rRNA gene sequence comparison

Isolate	Sequence length (bp)	Best BLAST match	Accession	Query cover (%)	Identity (%)	Final identification
1-1	1080	*Bacillus subtilis*	PP767251.1	98	98.78	*Bacillus subtilis*
1-11	1077	*Bacillus subtilis*	OQ851992.1	98	98.39	*Bacillus subtilis*
2-12	766	Multiple equivalent *Bacillus* matches[1]	—	100	100.00	*Bacillus licheniformis*[2]
W-NA-2t	581	*Bacillus licheniformis*	OP316995.1	100	100.00	*Bacillus licheniformis*
W-NA-3t	626	*Streptomyces thermolineatus*	KJ571064.1	100	100.00	*Streptomyces thermolineatus*
W-NA-4t	783	*Bacillus licheniformis*	OZ346051.1	100	100.00	*Bacillus licheniformis*

analysis further placed RKM-2 within the *B. licheniformis* lineage, closest to *B. licheniformis* DSM 13 with 100% bootstrap support (Zhanar, T. et. al., 2026). Accordingly, isolate 2-12 is identified here as *Bacillus licheniformis*.

Physicochemical changes during composting

Temperature dynamics

The composting variants displayed distinct thermal trajectories during the first 30 days (Figure 1). Variants I and II heated rapidly from approximately 27 °C and reached maximum temperatures of about 60–61 °C on day 2. Temperatures then declined but showed secondary increases during the active phase, including peaks near days 9–10 and 17–18. Variant III exhibited a lower initial thermal response, with an early maximum of approximately 37 °C and subsequent values mainly below those of Variants I and II. By the end of monitoring, all variants approached approximately 25 °C, indicating transition toward the cooling and maturation phase. The early thermophilic response in Variants I and II was consistent with faster oxidation of readily degradable organic matter.

Evolution of pH and C:N ratio

The pH remained within the neutral-to-mildly alkaline range (Figure 2a). Between days 30 and 60, pH increased from 7.10 to 8.02 in Variant I,

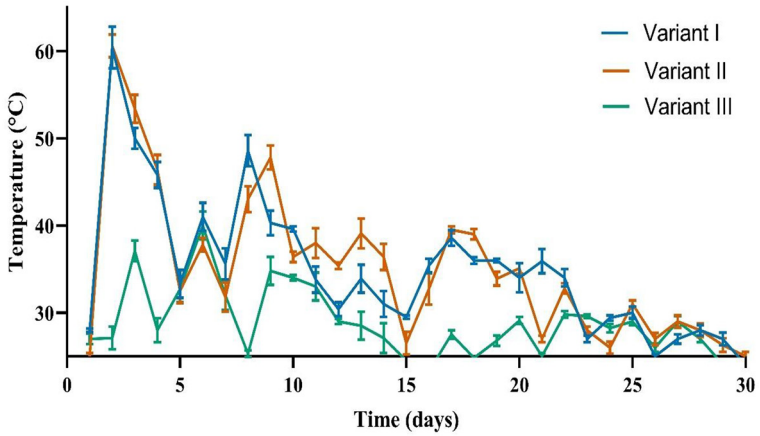

Figure 1. Temperature dynamics of the three composting variants over 30 days. Data are presented as mean ± SD (n = 3); error bars represent SD

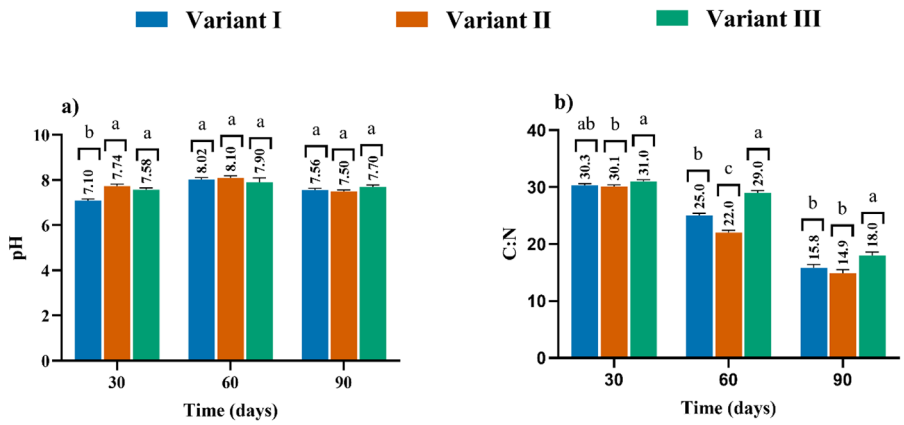

Figure 2. Changes in compost properties at 30, 60, and 90 days: (a) pH and (b) carbon-to-nitrogen ratio (C:N) in Variants I–III. Data are presented as mean ± SD (n = 3); error bars represent SD. Differences among Variants I–III at each sampling time point were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. Different lowercase letters above the bars indicate statistically significant differences among variants within the same sampling time point ($p < 0.05$), whereas bars sharing the same letter are not significantly different

from 7.74 to 8.10 in Variant II, and from 7.58 to 7.90 in Variant III. By day 90, the corresponding values had decreased to 7.56, 7.50, and 7.70.

In contrast, the C:N ratio decreased throughout the composting period (Figure 2b). In Variant I, it decreased from 30.0 on day 30 to 25.0 on day 60 and 15.8 on day 90. In Variant II, it declined from 30.1 to 22.0 and then to 14.5, whereas in Variant III it decreased from 31.0 to 29.0 and then to 18.0. Overall, the C:N ratio decreased by 47.3%, 51.8%, and 41.9% between days 30 and 90 in Variants I, II, and III, respectively. The increase in humus content, stabilization of pH, and reduction in the C:N ratio indicated progressive stabilization of organic matter.

Available nutrient dynamics

Available N decreased in all variants (Figure 3a). From day 30 to day 90, it declined by 69% in Variant I, 35% in Variant II, and 36% in Variant III. Variant III showed the highest final available-N content (2237.2 mg kg^{-1}).

Available P increased in all variants (Figure 3b), reaching 256, 200, and 234 mg kg^{-1} by day 90, respectively. Variant II maintained the highest available-K content throughout the experiment (4600–4850 mg kg^{-1}; Figure 3c). These differences indicate variation among the treatments in nutrient transformation and distribution during composting.

Total N, P, and K content

Total macronutrient contents showed non-monotonic patterns (Figure 4). Total N at day 30 ranged from 1.344% to 1.554%. At day 60, values were 1.456%, 1.218%, and 1.498% in Variants I, II, and III, respectively; by day 90, Variants I and II increased to 1.904% and 2.268%, while Variant

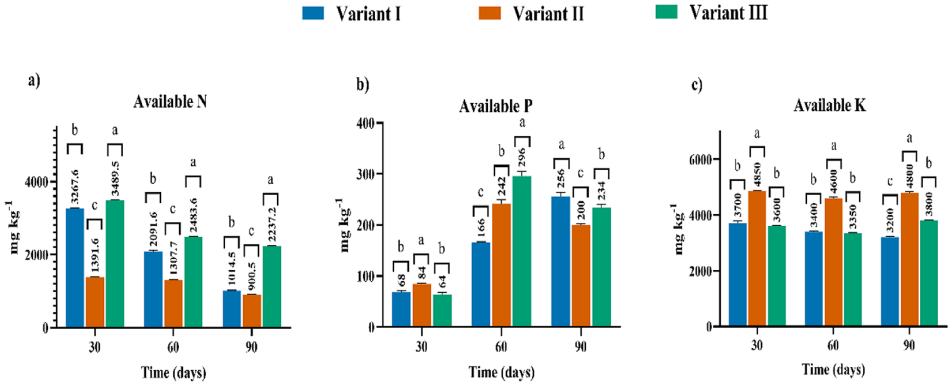

Figure 3. Available macronutrients during composting: (A) nitrogen, (B) phosphorus, and (C) potassium at 30, 60, and 90 days in Variants I–III. Concentrations are expressed as mg kg^{-1} on a dry matter basis. Data are presented as mean ± SD (n = 3); error bars represent SD. Differences among Variants I–III at each sampling time point were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. Different lowercase letters above the bars indicate statistically significant differences among variants within the same sampling time point ($p < 0.05$), whereas bars sharing the same letter are not significantly different

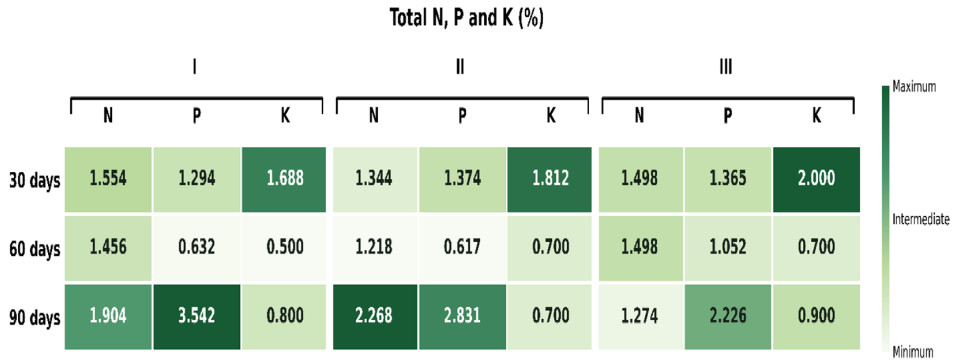

Figure 4. Heatmap of total nitrogen, phosphorus, and potassium (%) in Variants I–III after 30, 60, and 90 days. Color intensity is scaled to the values displayed in the figure

III decreased to 1.274%. Total P declined at day 60 in all variants and subsequently increased to 3.542%, 2.831%, and 2.226% at day 90. Total K also declined between days 30 and 60, reaching 0.500–0.700%, followed by modest recovery to 0.700–0.900% at day 90. Because composting changes both the mass and composition of the solid phase, these concentration shifts reflect the combined effects of mineralization, nutrient losses, redistribution, and dry-matter reduction.

Humus content

Humus content increased with composting time in all variants (Figure 5). In Variant I, it increased from 16.3% on day 30 to 18.3% on day 60 and 19.3% on day 90. In Variant II, humus content increased from 15.7% to 19.5% and then to 20.0%, reaching the highest final value. In Variant III, it increased from 16.0% to 17.8% and then to 18.0%. Thus, the net increases between days 30 and 90 were 3.0, 4.3, and 2.0 percentage points in Variants I, II, and III, respectively.

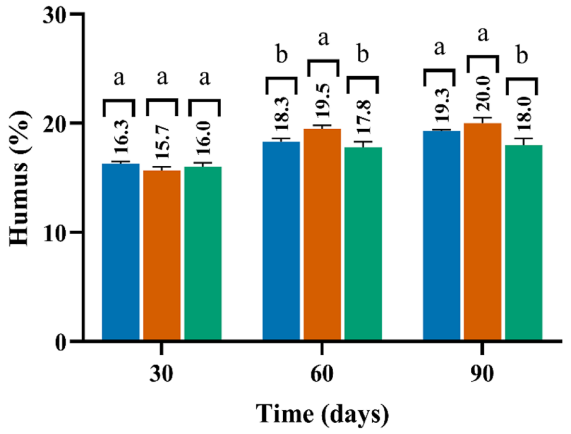

Figure 5. Humus content in Variants I–III at 30, 60, and 90 days of composting. Data are presented as mean ± SD (n = 3); error bars represent SD. Differences among Variants I–III at each sampling time point were analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. Different lowercase letters above the bars indicate statistically significant differences among variants within the same sampling time point ($p < 0.05$), whereas bars sharing the same letter are not significantly different

Seed germination bioassay and pot experiment

The phytotoxicity bioassay showed clear changes in radish germination and seedling development during compost maturation (Table 4; Figure 6). After 30 days, germination remained relatively low in all variants, ranging from 10.67 ± 2.31% in Variant III to 40.00 ± 4.00% in Variant II. At this stage, phytotoxicity was highest in Variant I (80.43%) and lowest in Variant II (69.25%).

By day 60, germination markedly increased in Variants I and II, reaching 92.00 ± 8.00% and 88.00 ± 8.00%, respectively, whereas Variant III showed only 34.67 ± 2.31% germination. Root development followed a similar pattern, with the greatest mean root length observed in Variant II (15.36 ± 1.54 mm). Phytotoxicity decreased to 62.93% and 57.05% in Variants I and II, respectively.

After 90 days, Variants I and II showed the highest germination rates, reaching 97.33 ± 2.31% and 98.67 ± 2.31%, respectively. Variant II also exhibited the greatest mean root length (22.65 ± 11.44 mm) and the lowest phytotoxicity (36.67%). In contrast, Variant III maintained substantially lower germination (36.00 ± 14.42%) and higher phytotoxicity (71.42%). The photographic observations were consistent with these results, showing more abundant and developed

Table 4. Radish seed germination, seedling growth, and phytotoxicity during composting

Variant	Composting time (days)	Germinated seeds (n/25)	Germination (%)	Shoot length (mm; mean ± SD)	Root length (mm; mean ± SD)	Phytotoxicity (%)
I	30	6.33 ± 0.58	25.33 ± 2.31	2.83 ± 0.07	7.00 ± 0.11	80.43
II	30	10.00 ± 1.00	40.00 ± 4.00	4.75 ± 1.09	11.00 ±1.55	69.25
III	30	2.67 ± 0.58	10.67 ± 2.31	4.33 ± 0.94	9.67 ± 1.05	72.97
I	60	23.00 ± 2.00	92.00 ± 8.00	9.39 ± 1.01	13.26 ± 0.71	62.93
II	60	22.00 ± 2.00	88.00 ± 8.00	9.41 ± 1.37	15.36 ± 1.54	57.05
III	60	8.67 ± 0.58	34.67 ± 2.31	5.62 ± 2.83	12.62 ± 0.44	64.70
I	90	24.33 ± 0.58	97.33 ± 2.31	7.33 ± 0.82	16.00 ± 1.14	55.27
II	90	24.67 ± 0.58	98.67 ± 2.31	16.12 ±10.64	22.65 ± 11.44	36.67
III	90	9.00 ± 3.61	36.00 ± 14.42	5.00 ± 0.54	10.22 ± 1.89	71.42

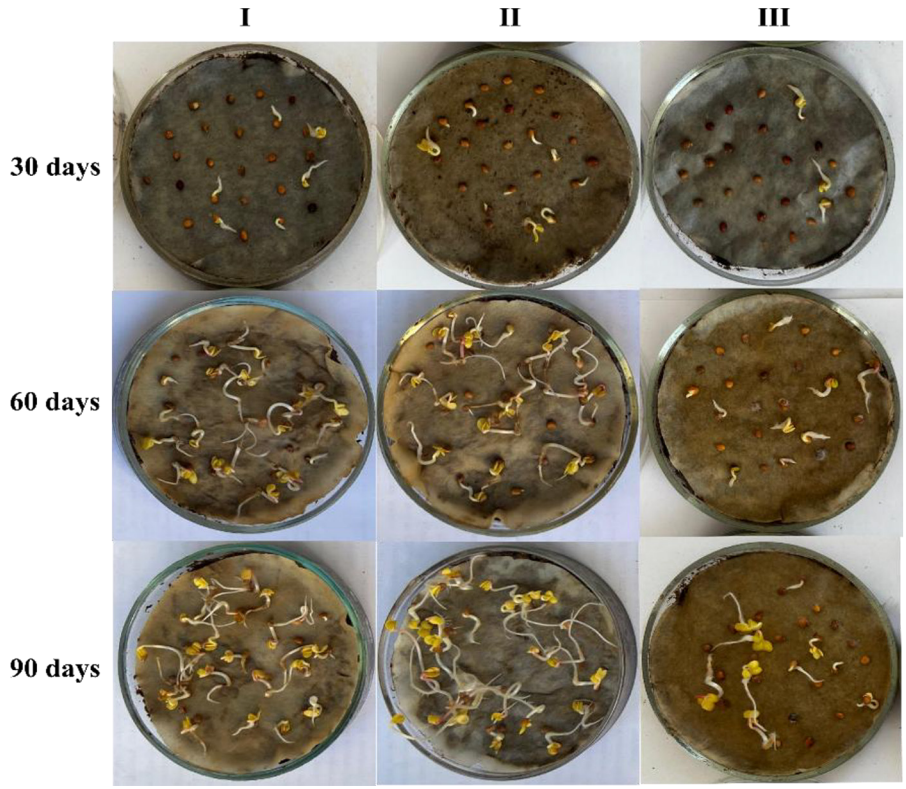

Figure 6. Radish germination response to compost material from Variants I–III sampled after 30, 60, and 90 days. Twenty-five seeds were used per Petri dish

seedlings in Variants I and II at the later stages of composting. Overall, the bioassay indicated a reduction in phytotoxicity during compost maturation, particularly in the inoculated variants, with Variant II showing the lowest phytotoxicity and the greatest mean root development after 90 days.

For the pot experiment, the two microbially inoculated compost variants (Variants I and II) were evaluated using the unamended substrate as the control (Table 5). In the control, mean root and shoot lengths were 2.10 ± 0.50 and 1.80 ± 0.89 cm, respectively. In Variant I, seedling growth generally increased with increasing compost proportion. At 10% compost, mean root and shoot lengths were 2.43 ± 1.30 and 2.23 ± 0.40 cm, respectively. At 30%, these values increased to 3.37 ± 0.78 cm for roots and 3.77 ± 0.55 cm for shoots.

Variant II showed a similar trend, with the strongest response at the higher compost rates. At 20% compost, mean root and shoot lengths reached 2.97 ± 1.08 and 3.20 ± 0.61 cm, respectively. The highest values were observed at 30%, where root length reached 4.43 ± 1.46 cm and shoot length 3.83 ± 0.61 cm. Overall, the most pronounced seedling development was observed at the 30% compost rate. At this concentration, Variant II produced the longest roots, whereas shoot length was similar between Variants I and II (Table 5).

Table 5. Root and shoot growth of *Lepidium sativum* in saline soil amended with compost from Variants I and II

Treatment	Compost rate (% w/w)	Root length (cm), mean ± SD	Shoot length (cm), mean ± SD
Control	0	2.10 ± 0.50	1.80 ± 0.89
Variant I	10	2.43 ± 1.30	2.23 ± 0.40
Variant I	20	2.67 ± 0.58	3.48 ± 0.70
Variant I	30	3.37 ± 0.78	3.77 ± 0.55
Variant II	10	2.00 ± 0.30	2.53 ± 0.63
Variant II	20	2.97 ± 1.08	3.20 ± 0.61
Variant II	30	4.43 ± 1.46	3.83 ± 0.61

DISCUSSION

The present study showed that inoculation with thermophilic *Bacillus* strains, particularly when combined with an actinomycete, was associated with more pronounced sewage sludge stabilization during the 90-day composting period. The rapid increase in temperature to approximately 60–61 °C in the inoculated treatments indicates intensive microbial metabolism during the initial phase of composting. This temperature range is characteristic of the thermophilic stage of sewage sludge composting, during which thermophilic bacteria, including *Bacillus* spp., and thermotolerant actinomycetes become important contributors to the degradation of proteins, carbohydrates, cellulose, and other organic compounds (Ivickytė and Povilaitis, 2026). Temperature development is one of the principal indicators of microbial activity during aerobic composting because oxidation of readily degradable organic substrates generates metabolic heat and facilitates transition to the thermophilic phase (Onwosi et al., 2017; Wei et al., 2017). The substantially weaker initial heating observed in the uninoculated treatment therefore supports the contribution of microbial inoculation to more intensive early decomposition, although the absence of microbial population monitoring does not allow the activity of introduced strains to be separated from that of the indigenous compost microbiota. However, temperature alone cannot demonstrate that the introduced microorganisms were directly responsible for the observed response, because the abundance and persistence of the inoculated strains were not monitored. Thus, the stronger thermal response should be interpreted as being associated with inoculation rather than as definitive evidence of a direct causal effect.

The increase in humus content and simultaneous decline in the C:N ratio provide further evidence of progressive organic matter stabilization. The combined *Bacillus*–actinomycete treatment reached the highest humus content (20.0%) and the lowest C:N ratio (14.5) after 90 days. A decline in the C:N ratio is generally associated with compost maturation because organic carbon is progressively mineralized and lost mainly as CO_2, whereas nitrogen is comparatively retained or redistributed among organic and inorganic pools (Bernal et al., 2009; Onwosi et al., 2017). These findings are consistent with recent experimental evidence demonstrating that microbial inoculation can directly promote humification. Chang et al., (2023) reported that inoculation with *Bacillus licheniformis* enhanced the degradation of readily biodegradable carbohydrates during the heating phase and lignocellulosic components during the cooling phase, resulting in approximately 20% higher humic acid content than in the uninoculated control. Similarly, Chen et al. (2022) found that actinomycete inoculation increased organic substrate degradation and promoted the conversion of fulvic-acid-like compounds into more complex humic substances. Thus, the greater humus accumulation observed in the treatment combining *Bacillus* with the actinomycete may reflect complementary microbial functions involved in both decomposition and subsequent humification. Nevertheless, because the present study did not directly characterize individual humic fractions or microbial succession, this interpretation should be considered a plausible mechanism rather than a directly demonstrated pathway.

The nutrient dynamics observed during composting also indicate active biochemical transformation rather than simple nutrient accumulation. Available N decreased in all treatments, while available P generally increased, and the combined microbial treatment maintained comparatively high available K concentrations. Nitrogen changes during composting may result from simultaneous mineralization, microbial immobilization, ammonia volatilization, nitrification, and incorporation into newly formed organic matter. In a composting system inoculated with a multifunctional microbial consortium containing *Bacillus* and *Streptomyces*, He et al. (2022) demonstrated enhanced organic matter degradation together with improved nitrogen retention, linking these changes to shifts in microbial carbon and nitrogen metabolism. Microbial inoculants have also been shown to alter phosphorus transformation among organic and inorganic pools during aerobic composting, indicating that changes in available P can result from both microbial mineralization and redistribution among different phosphorus fractions.

Importantly, increases in the measured concentrations of total N, P, or other nutrients should not be interpreted as an absolute increase in their total quantity within the composting system. During composting, carbon mineralization, water loss, and degradation of organic matter reduce the total dry mass of the material. Consequently, an apparent increase in nutrient concentration may

partly represent a concentration effect associated with dry-matter loss rather than net nutrient accumulation. Because a complete mass balance was not performed in the present study, the nutrient data should therefore be interpreted as changes in concentration and distribution rather than direct evidence of nutrient retention or gain.

An important finding was that the physicochemical changes were accompanied by a clear improvement in plant response. After 90 days, radish germination reached 97.33% and 98.67% in the two inoculated treatments, whereas the uninoculated treatment showed substantially lower germination and higher phytotoxicity. The combined *Bacillus*–actinomycete treatment also produced the greatest root elongation and the lowest phytotoxicity. This relationship is particularly important because chemical stabilization alone does not necessarily indicate biological maturity. A recent review by Tsabedze et al. (2025) emphasized that immature sewage-sludge compost may inhibit germination and root growth due to ammonium, soluble salts, organic acids, phenolic compounds, and other phytotoxic intermediates, and therefore biological assays should complement physicochemical maturity indicators. Kong et al. (2023) further demonstrated that compost phytotoxicity and germination response are strongly related to dissolved organic carbon and nitrogen, NH_4^+ concentrations, electrical conductivity, and volatile fatty acids. Consequently, the marked improvement in radish germination during the present experiment provides biological evidence that potentially inhibitory compounds were progressively transformed or reduced during maturation.

However, the phytotoxicity parameter used in the present study was calculated as root-growth inhibition relative to the filter-paper control and is not identical to the classical Germination Index (GI). Germination-based assays are useful indicators of compost maturity and phytotoxicity, but they represent only one component of compost quality assessment (Kong et al., 2023; Tsabedze et al., 2025). Moreover, improved germination and root elongation do not by themselves confirm the overall agricultural safety of sewage-sludge-derived compost, because its suitability for land application also depends on factors such as potentially toxic elements, salinity, and other contaminants (Cesaro et al., 2015; Jat Baloch et al., 2023). Therefore, heavy metals, sanitary parameters, electrical conductivity, salinity, and other relevant safety indicators should be assessed before unrestricted agricultural application is recommended.

The plant response is also relevant to the intended use of the compost under the arid and saline conditions of the Mangystau Region. Previous studies have shown that sewage-sludge-derived amendments can improve soil organic matter, nutrient availability, and plant performance in saline and salt-affected soils (Bai et al., 2018; Li et al., 2021; Shan et al., 2021). However, increasing sewage sludge application rates may also increase soil electrical conductivity and salt loading, thereby offsetting some of the agronomic benefits (Shan et al., 2021; Jat Baloch et al., 2023). Therefore, although the highest plant response in the present pot experiment was observed at the 30% compost rate, this should not be interpreted as evidence that 30% represents the optimal field application rate. Further research is required to determine the optimal compost application rate under saline soil conditions, taking into account electrical conductivity, salt accumulation, soil quality, plant response, and the economic feasibility of practical application.

An additional limitation of the pot experiment is that compost from Variant III, the uninoculated treatment, was not included. Therefore, the pot experiment demonstrates the effects of composts from Variants I and II relative to unamended saline soil, but it does not allow a direct conclusion that microbial inoculation improved plant growth compared with compost produced without inoculation. Future experiments should include the uninoculated compost as an additional treatment to enable a direct assessment of the effect of microbial inoculation on agronomic performance.

Overall, the concurrent thermophilic response, increased humus content, declining C:N ratio, nutrient transformation, and reduced phytotoxicity indicate progressive stabilization of sewage sludge during the 90-day process. The stronger overall response obtained when thermophilic *Bacillus* strains were combined with an actinomycete is consistent with previous studies showing complementary roles of *Bacillus* in rapid substrate degradation and actinomycetes in the transformation of more resistant organic fractions and humus formation. However, the interpretation of treatment effects should remain appropriately cautious. The experiment included three independent composting replicates for each treatment ($n = 3$), which allowed treatment-associated differences to be statistically evaluated. Nevertheless, further validation

under larger-scale and field conditions would be valuable to confirm the reproducibility and practical applicability of the observed responses.

CONCLUSIONS

The results of the 90-day pilot-scale composting experiment indicate that sewage sludge mixed with dry stover can undergo substantial stabilization under the arid conditions of the Mangystau Region. Among the tested treatments, the combination of thermophilic *Bacillus* strains with an actinomycete was associated with the most favorable overall response, including higher humus content, a lower C:N ratio, and improved plant response.

From a practical perspective, the results suggest that a 90-day composting period may be sufficient to achieve substantial stabilization of the sewage sludge–stover mixture under the tested conditions. The combined assessment of temperature, pH, C:N ratio, humus content, nutrient dynamics, and biological response can provide a useful framework for evaluating compost maturity.

Before practical field application, however, the resulting compost should be further evaluated for electrical conductivity, salinity, heavy metals, sanitary indicators, and other environmental safety parameters. These data are necessary to support the safe and effective use of the compost under arid and saline soil conditions.

Acknowledgments

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (AP23483494).

REFERENCES

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S00222836(05)80360-2
2. Bai, Y., Zuo, W., Shao, H., Mei, L., Tang, B., Gu, C., Wang, X., Guan, Y. (2018). Eastern China coastal mudflats: Salt-soil amendment with sewage sludge. *Land Degradation & Development*, *29*(10), 3803–3811. https://doi.org/10.1002/ldr.3092
3. Bernal, M. P., Alburquerque, J. A., Moral, R. (2009). Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology*, *100*(22), 5444–5453. https://doi.org/10.1016/j.biortech.2008.11.027
4. Bhatti, A. A., Haq, S., Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis*, *111*, 458–467. https://doi.org/10.1016/j.micpath.2017.09.036
5. Bremner, J. M. (1996). Nitrogen-Total. In D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, M. E. Sumner (Eds), *SSSA Book Series* (1st edn, Vol. 5, pp. 1085–1121). Wiley. https://doi.org/10.2136/sssabookser5.3.c37
6. Bremner, J. M., Jenkinson, D. S. (1960). Determination of organic carbon in soil: I. Oxidation by dichromate of organic matter in soil and plant materials. *Journal of Soil Science*, *11*(2), 394–402. https://doi.org/10.1111/j.1365-2389.1960.tb01093.x
7. Cesaro, A., Belgiorno, V., Guida, M. (2015). Compost from organic solid waste: Quality assessment and European regulations for its sustainable use. *Resources, Conservation and Recycling*, *94*, 72–79. https://doi.org/10.1016/j.resconrec.2014.11.003
8. Chang, Y., Zhou, K., Yang, T., Zhao, X., Li, R., Li, J., Xu, S., Feng, Z., Ding, X., Zhang, L., Shi, X., Su, J., Li, J., Wei, Y. (2023). Bacillus licheniformis inoculation promoted humification process for kitchen waste composting: Organic components transformation and bacterial metabolic mechanism. *Environmental Research*, *237*, 117016. https://doi.org/10.1016/j.envres.2023.117016
9. Chen, Y., Zhao, R., Jia, L., Wang, L., Pan, C., Zhang, R., Wei, Z. (2022). Microbial inoculants reshape structural distribution of complex components of humic acid based on spectroscopy during straw waste composting. *Bioresource Technology*, *359*, 127472. https://doi.org/10.1016/j.biortech.2022.127472
10. Collivignarelli, M., Abbà, A., Frattarola, A., Carnevale Miino, M., Padovani, S., Katsoyiannis, I., Torretta, V. (2019). Legislation for the Reuse of Biosolids on Agricultural Land in Europe: Overview. *Sustainability*, *11*(21), 6015. https://doi.org/10.3390/su11216015
11. El Kajaf, B., El Mejahed, K., Hafidi, M., Ezzariai, A., El Mesnaoui, E. H., El Gharous, M., An-Nori, A. (2026). Heavy metal behavior and phytotoxicity attenuate during sewage sludge composting from multiple sources in Morocco. *Frontiers in Environmental Science*, *14*, 1874957. https://doi.org/10.3389/fenvs.2026.1874957
12. Finore, I., Feola, A., Russo, L., Cattaneo, A., Di Donato, P., Nicolaus, B., Poli, A., Romano, I. (2023). Thermophilic bacteria and their thermozymes in composting processes: A review. *Chemical and Biological Technologies in Agriculture*, *10*(1), 7. https://doi.org/10.1186/s40538-023-00381-z

13. He, Y., Zhang, Y., Huang, X., Xu, J., Zhang, H., Dai, X., Xie, L. (2022). Deciphering the internal driving mechanism of microbial community for carbon conversion and nitrogen fixation during food waste composting with multifunctional microbial inoculation. *Bioresource Technology*, *360*, 127623. https://doi.org/10.1016/j.biortech.2022.127623
14. Ivickytė, V., Povilaitis, A. (2026). Sewage sludge composting: Strategies for process optimization and environmental impact reduction—A review. *Sustainability*, *18*(11), 5518. https://doi.org/10.3390/su18115518
15. Jat Baloch, M. Y., Zhang, W., Sultana, T., Akram, M., Shoumik, B. A. A., Khan, Md. Z., Farooq, M. A. (2023). Utilization of sewage sludge to manage saline–alkali soil and increase crop production: Is it safe or not? *Environmental Technology & Innovation*, *32*, 103266. https://doi.org/10.1016/j.eti.2023.103266
16. Keeney, D. R., Nelson, D. W. (1982). Nitrogen—Inorganic Forms. In A. L. Page (Ed.), *Agronomy Monographs* (1st edn, Vol. 9, pp. 643–698). Wiley. https://doi.org/10.2134/agronmonogr9.2.2ed.c33
17. Kong, Y., Zhang, J., Yang, Y., Liu, Y., Zhang, L., Wang, G., Liu, G., Dang, R., Li, G., Yuan, J. (2023). Determining the extraction conditions and phytotoxicity threshold for compost maturity evaluation using the seed germination index method. *Waste Management*, *171*, 502–511. https://doi.org/10.1016/j.wasman.2023.09.040
18. Lakhdar, A., Scelza, R., Ben Achiba, W., Scotti, R., Rao, M. A., Jedidi, N., Abdelly, C., Gianfreda, L. (2011). Effect of municipal solid waste compost and sewage sludge on enzymatic activities and wheat yield in a clayey-loamy soil. *Soil Science*, *176*(1), 15–21. https://doi.org/10.1097/SS.0b013e3182028d8a
19. Li, Y., Wang, Y., Shen, C., Xu, L., Yi, S., Zhao, Y., Zuo, W., Gu, C., Shan, Y., Bai, Y. (2021). Structural and predicted functional diversities of bacterial microbiome in response to sewage sludge amendment in coastal mudflat soil. *Biology*, *10*(12), 1302. https://doi.org/10.3390/biology10121302
20. Lu, Q., He, Z. L., Stoffella, P. J. (2012). Land Application of Biosolids in the USA: A Review. *Applied and Environmental Soil Science*, *2012*, 1–11. https://doi.org/10.1155/2012/201462
21. Murphy, J., Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5
22. Onwosi, C. O., Igbokwe, V. C., Odimba, J. N., Eke, I. E., Nwankwoala, M. O., Iroh, I. N., Ezeogu, L. I. (2017). Composting technology in waste stabilization: On the methods, challenges and future prospects. *Journal of Environmental Management*, *190*, 140–157. https://doi.org/10.1016/j.jenvman.2016.12.051
23. Sastre-Conde, I., Carmen Lobo, M., Icela Beltrán-Hernández, R., Poggi-Varaldo, H. M. (2015). Remediation of saline soils by a two-step process: Washing and amendment with sludge. *Geoderma*, *247–248*, 140–150. https://doi.org/10.1016/j.geoderma.2014.12.002
24. Shan, Y., Lv, M., Zuo, W., Tang, Z., Ding, C., Yu, Z., Shen, Z., Gu, C., Bai, Y. (2021). Sewage sludge application enhances soil properties and rice growth in a salt-affected mudflat soil. *Scientific Reports*, *11*(1), 1402. https://doi.org/10.1038/s41598-020-80358-2
25. Tiquia, S. M., Tam, N. F. Y., Hodgkiss, I. J. (1996). Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environmental Pollution*, *93*(3), 249–256. https://doi.org/10.1016/S0269-7491(96)00052-8
26. Tsabedze, S. A., Otieno, B., Thomas, A. R., Getahun, S. T. (2025). Phytotoxicity and quality in compost: A concise review of sewage sludge and green waste applications. *Journal of Material Cycles and Waste Management*, *27*(6), 4041–4058. https://doi.org/10.1007/s10163-025-02334-0
27. Wei, Y., Li, J., Shi, D., Liu, G., Zhao, Y., Shimaoka, T. (2017). Environmental challenges impeding the composting of biodegradable municipal solid waste: A critical review. *Resources, Conservation and Recycling*, *122*, 51–65. https://doi.org/10.1016/j.resconrec.2017.01.024
28. Weisburg, W. G., Barns, S. M., Pelletier, D. A., Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991
29. Zhanar, T., Arman, A., Akbota, B., Kamshat, K., Aliya, T., Zhadyrassyn, N., Temirkhanov, A., Zhandarbek, B., Akhan, A. (2026). Complementary genomic and functional potential of Bacillus subtilis RKM-1 and Bacillus licheniformis RKM-2 for organic waste bioconversion and composting. *Genes, 17*(10), 1222. https://doi.org/10.3390/genes17101222