



Phytotoxic assessment of textile sludge extracts and sludge based vermicompost on the growth of mung bean (*Vigna radiata*)

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Abstract

Conventional non-biodegradable pollutants make up a substantial portion of industrial contaminants present in natural habitats, and the negative consequences of ingestion or entanglement on terrestrial and living species. This study was conducted to determine the phytotoxicity of textile solid waste and vermicompost extract in terms of root elongation and the physiological performance of *Vigna radiata* seeds. To assess the phytotoxicity of waste and industrial runoff streams, sensitive and basic ecotoxicological bioassays such as seed germination and root elongation tests are commonly used. Various criteria such as the number of seeds per dish, the test size, and the type of assistance used remain unpredictable, despite the experiments being conducted according to national and international guidelines. The effects of leaching, or the release of contaminants from sludge into natural habitats, on plants are little understood. Some plant species are very vulnerable to a wide range of chemicals, with seedling development being the stage of life which was most affected. Leachates from textile sludge do affect seed germination and a significant number of seedlings showed developmental abnormalities or reduced seedling growth.

Keywords: *Vigna radiata*, phytotoxicity, germination, *Eisenia fetida*, biomass

Introduction

One of the most basic methods of environmental bio monitoring is the seed germination and root elongation test [1]. To test the toxicity of contaminated liquid samples on seedling growth and root elongation, a seed germination method was developed [2]. In most cases, municipal solid waste/sludge is combined with MSW and dumped in landfills, amplifying the toxicity of MSW due to different contaminants. Excess rainwater percolates into the waste layers in a landfill, resulting in landfill leachate [3]. Heavy metal contamination has an effect on not only agricultural productivity and efficiency, but also on the health of the environment and watersheds, as well as on the health and lives of animals and humans when it enters food chains [4]. Plant bioassays are widely used to assess the genotoxicity of heavy metal polluted soils and water [5]. The fact that this kind of contamination is invisible, long-term and irreversible is the most serious aspect [6].

Toxicity of solid wastes leachate was reported using multiple bioassays [7, 8]. One of the most feared consequences of leachates for future generations is genotoxicity. Heavy metals and chemical contaminants in textile sludge have the ability to damage crops and soil microorganisms. Contaminated crops degrade the feed and food consistency [9]. For environmental biomonitoring, *Allium cepa* is a responsive, accurate, and cost-effective test method [10]. *Eisenia fetida* has been used to make vermicompost from sludge and platinum mining waste [11]. The Coprophagic groups of the earthworms consume high organic waste and are feasible even for waste control (i.e. *Eisenia fetida*) [12]. According to Tahir *et al* [13] plants such as *Helianthus annuus*, *Thlaspi caerulescens*, and *Brassica juncea* may live in polluted soil and uptake a variety of toxic heavy metals. Plant-based approaches are mostly used to

reduce harmful heavy metal biocompatibility in degraded soils [14]. Due to a severe scarcity of good quality water in the coming decades, wastewater treatment systems, especially biological ones, have become hotspots for environmental sustainability, whereas the use of treated industrial wastewater for irrigation of crop plants has become a necessity [15].

The present study shows that vermiculture can be used to stabilize industrial sludge. Textile sludge (TS) was mixed with cow dung in different ratios to produce three different feed mixtures for earthworm *Eisenia fetida*. In the present communication, *Vigna radiata* was used to evaluate the toxicity of the extracts of textile sludge and vermicompost sludge. This study results represents the sensitivity of the seed germination and it is clear that the root elongation test can be done by filter paper method. The seeds of *Vigna radiata* were used to test the toxicity of four extracts namely; textile sludge extracts (TSE), Vermicompost extracts (Test A, Test B, Test C).

Materials and Methods

Textile solid waste

In this study, discharge sludge wastes were collected in a textile industrial region, near SIPCOT, Perundurai, Erode district, Tamil Nadu, India. Sludge samples were collected at the outlet of the sanitization area. Collected sludge samples were air dried and powdered for further experiments.

Vigna radiata seeds

Seeds of *Vigna radiata* were purchased from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Among them ten *Vigna radiata* seeds were selected and inoculated in each experimental petridishes. Seeds were

stored at room temperature and in the dark. Selected seeds were surface sterilized with 0.1% mercuric chloride for 1 to 2 min followed by repeated rinsing with distilled water. The morphological parameters like seedling length and fresh seedling weight in gram were recorded after 5th day. All the experiment was done in triplicate and mean values are reported.

Earthworms

The Epigeic (or) surface dwelling earthworm *Eisenia fetida* was obtained from Periyar Maniyammai University, Vallam, Thanjavur, Tamil Nadu, India. The earthworm *Eisenia fetida* hatchling and clitellated adults were randomly picked for experiment from several stock cultures containing 500- 2000 earthworms maintained in the laboratory with cow dung and leaf litter as a culturing medium.

Preparation of experimental media

The circular plastic tubs were filled with the feed mixture as given below and were subjected for vermicomposting process. The mixtures were mixed manually to facilitate precompost up to 15 days. At the end of 15 days, 50 earthworms with an average weight of 1.23 ± 0.2 g earthworm were introduced into each feed mixture. The moisture content was maintained at 60–80 % throughout the study period by sprinkling adequate quantity of water. The whole assay was performed under room temperature in triplicates [16].

Treatment specifics

Test A (1.0:1.0 ratio): 1000 g of textile solid wastes+ 1000 g of cow dung powder

Test B (1.5:0.5ratio): 1500 g of textile solid wastes+ 500 g of cow dung powder

Test C (0.5:1.5ratio): 500 g of textile solid wastes+ 1500 g of cow dung powder

Phytotoxicity test

To assess the effects of textile solid waste and vermicompost extract were prepared in laboratory and chemical/ physical analyses of the extract were performed. Both extracts were previously exposed to natural environmental conditions. Then the phytotoxicity of the extracts was evaluated in a separate seed germination and seedling growth experiment by using *Vigna radiata*.

Seed germination and growth bioassays of *Vigna radiata* seeds were used to assess the toxicity and maturity of vermicompost obtained with each treatment. Aqueous extracts were prepared from textile solid waste and vermicompost with distilled water (1:10w/v). The seed germination bioassay was evaluated according to Kumar *et al* [17]. Two pieces of sterile tissue paper were placed in a 15x100mm sterilized petridishes and wetted with the Textile sludge and Vermicompost extracts. Ten seeds of the *Vigna radiata* were placed on top of the filter paper and incubated for 5 days in a room temperature [18].

Germination rate for all tested seeds was over 90% which guaranteed the viability of the seed. Air-dried sludge and vermicompost extract were prepared. Subsequently, all dishes were placed on the lab workbench cupboard at room temperature and the darkness for germination. According to

the EPA guideline, germination means the resumption of active growth by an embryo. Moreover, as defined by Finkelstein *et al* [19], germination is the initial emergence of the radicle from the seed coat. The primary root should attain a length of 5 mm for the seed to be counted as having germinated. When at least 65% of seeds of control have germinated and developed roots that are at least 20 mm long, germination experiment concludes, and germination rate could be calculated. In our case, all of them *Vigna radiata* seeds germinated within 48 h. Three replicates of each treatment were tested. After the initial 48 h, in each dish, the number of acceptable seedlings was counted and divided by the total number of seeds added (10) to calculate the germination rate. At the end of the experiment (5 days later), all seedlings were pulled out and the root elongation was measured which is defined as the length from the tip to radicle. After 5 days of the experiment, the number of germinated seeds in the sample (GSS) and the number of germinated seeds in the control (GSC) were counted for each experiment [20].

$$GI = RLS \times GSS / RLC \times GSC$$

Statistical studies

Data were statistically evaluated by one-way analysis of variance using Graph pad prism 6.0 software. To determine any significant difference among the parameters analyzed at ($P > 0.05$) level of significance. Tukey's multiple comparison tests was used to compare treatment means between each groups.

Results and Discussion

Physicochemical characterization of textile sludge and vermicompost extracts

Physicochemical properties of aqueous textile sludge and vermicompost extracts were analyzed before starting the treatment process and compared with environmental quality standards. In this research, the non-granular, dark-colored, and odorous physicochemical characteristics of the analyzed sludge were discovered. Since the sludge from secondary garment waste water disposal systems is rich in organic carbon and plant nutrients, it can be used as a raw material for organic fertilizers and building materials [21]. The effect of treatments on different Physico-chemical properties of textile sludge extract and the impact of vermicompost extract on green gram (*Vigna Radiata*) growth are given below. The physical and chemical properties of all treatments were completely transformed by earthworms after 45 days of vermicomposting, suggesting that the biomass was matured. In all treatments where epigeic earthworm *Eisenia fetida* was introduced, the vermicompost produced was darker than the original content.

Table 1: Physicochemical characterization of textile sludge and vermicompost extracts

| Parameters | Aqueous extract of TS | Aqueous extract of VC |
|-------------|-----------------------|-----------------------|
| pH | 5.7 ± 0.02 | 7.8 ± 0.02 |
| EC | 49.53 ± 0.76 | 4.174 ± 0.2 |
| Temperature | 44°C | 28°C |
| Carbon | 121.60 ± 0.46 | 29.79 ± 0.6 |
| Nitrogen | 0.89 ± 0.02 | 12.28 ± 0.02 |
| Odour | foul-smelling | Good aroma |
| Shape | Non granular | Cube (even) |

Table 2: List of general parameters that remains variable in seed germination

| Parameters | Sample details |
|--------------------|---------------------------|
| Species | <i>Vigna radiata</i> |
| Support | Sterile filter paper |
| Seed pre-treatment | Yes, 10% mercury chloride |
| Temperature | Room temperature |
| Dish | Sterile petridishes |
| Number of seeds | 10 |
| Amount of sample | 10-15ml |
| Duration | 120hours |

Effects of seed germination test in both extracts

The phytotoxic effects of TS and VC extracts on seed germination and seedling growth of *Vigna radiata* were tested under laboratory conditions for five days. The highest seed germination and seedling growth were observed with the vermicompost C (Fig 1). It might be due to the presence of available forms of nutrients in vermicompost C aqueous extract after going through mineralization. Tukey's multiple comparisons test also supported these findings, as the confidence intervals were found: TSE vs. VC A (-9.601 to -0.5989), TSE vs. VC B (-6.765 to 1.165), TSE vs. VC C (-17.31 to 0.7065) and ($R^2 = 0.8044$), VC A vs. VC B (-0.9551 to 5.555), VC A vs. VC C (-10.63 to 4.234), VC B vs. VC C (-15.37 to 4.368) and ($R^2 = 0.2724$) (Table 3). Lower treated wastewater concentrations had a favorable effect on agronomic parameters, while higher treated wastewater concentrations had a substantial negative impact [15]. The seed germination test is a widely accepted protocol for evaluating the compost phyto-toxicity as well as the compost stability [22, 23]. The effect on green gram plants was dependent on its physico-chemical characteristics and doses. The minimum plant height was recorded in TSE and maximum was observed in vermicompost sample. The extent of photo-toxicity caused by insufficient composting can also be taken as an indicator of the compost chemical instability [24]. However, due to the selective toxicity of different composting materials towards seeds of different species, it will be necessary to select species that are sensitive to the specific composting materials before this test can be used for the evaluating of compost stability [25]. The seed germination of *Vigna radiata* seeds was not affected by vermicompost extracts from the final products of all different treatments. Among the four treatments, the relative seed germination of Type C extract seedlings recorded the highest range of (99%), while TSE extract seedling had the very lowest range of (35-40%) (Fig: 1). The germination index was not harmed by vermicompost extracts, and there was no evidence of a phytotoxic effect. In compost made from spent garbage, found a germination index of more than 80% [22]. Phytotoxicity levels below 50% may suggest that the compost has not reached full maturity. This may be attributed to unmetabolized phytotoxic compounds like acetic, propionic, butyric, and isobutyric acids, which hinder the germination level Contreras [26]. In conclusion, when plants were exposed to textile sludge extracts, they showed toxicity signs, behaviour, and growth.

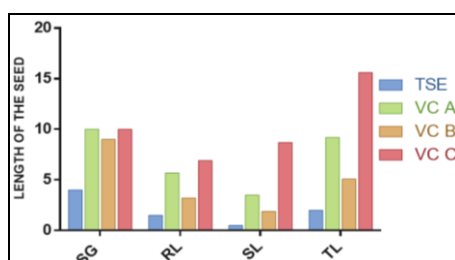


Fig 1: Effects of seed germination test in textile sludge and vermicompost extracts (TSE, VC A, VC B, and VC C)

Table 3: Tukey's multiple comparisons test seed germination test in textile sludge and vermicompost extracts (TSE, VC A, VC B, and VC C)

| Tukey's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant | Summary | q |
|-----------------------------------|------------|------------------|-------------|---------|-------|
| TSE vs. VC A | -5.100 | -9.601 to 0.598 | Yes | * | 7.733 |
| TSE vs. VC B | -2.800 | -6.765 to 1.165 | No | ns | 4.820 |
| TSE vs. VC C | -8.300 | -17.31 to 0.7065 | No | ns | 6.289 |
| VC A vs. VC B | 2.300 | -0.9551 to 5.555 | No | ns | 4.822 |
| VC A vs. VC C | -3.200 | -10.63 to 4.234 | No | ns | 2.938 |
| VC B vs. VC C | -5.500 | -15.37 to 4.368 | No | ns | 3.804 |

Total biomass parameters of eisenia fetida

Table 4 indicates the values achieved in *E. fetida* over the experimental duration with various growth and reproduction parameters. The survival and growth of *E. fetida* got reduced when the proportion of textile sludge in the feed mixtures was increased. Mortality was recorded in test B (1500 g of textile solid wastes+ 500 g of cow dung powder) treatments. Test A and test B had the highest number of total earthworms and clitellated earthworms. Untreated pig manure caused worms to die within a few hours, according to Chan and Griffiths [27]. This meant that higher proportion of textile sludge in the feed mixture causes *E. fetida* to take time to recover sexual maturity and reproduce. Elvira *et al* [28] found that worms could not live in paper-pulp mill sludge, but that feed mixtures of paper-pulp mill sludge with pig and poultry slurry could be used for vermicomposting. The authors have reported that the mortality was due to deterioration mechanisms that result changes in environmental characteristics.

Table 4: Reproduction parameters measured in earthworms exposed to textile activated sludge

| Treatment | Total biomass of earthworms (g) | Cocoons per Eisenia fetida | Hatchlings per cocoons | Cocoon weight (mg) | Hatchability (%) |
|-----------|---------------------------------|----------------------------|------------------------|--------------------|------------------|
| Type A | 15.29±0.40 | 6.03±0.15 | 4.17±0.01 | 11.6±0.01 | 69.5±0.16 |
| Type B | 15.08±0.78 | 3.1±0.1 | 2.26±0.37 | 8.04±0.57 | - |
| Type C | 15.57±0.21 | 7.64±0.49 | 11.6±0.84 | 11.6±0.84 | 68.3±12.9 |

Conclusion

Vermicomposting, which utilizes earthworms and their associated microbes to convert textile sludge waste into nutrient rich manure, is shown to be an efficient and environmentally friendly technology. The activity of epigeic organisms was significantly increased in vermicompost products produced from textile sludge waste mixtures. The study shows the textile sludge extract induce phytotoxic effects, as evidenced by seedling growth inhibition based on root and shoot length. On the other hand, vermicompost extract quality and seed density had no impact on *Vigna radiata* GR, total lengths, among multiple variable germination and elongation test proceeding parameters.

Likewise, vermitechnology may be a feasible technology for turning agricultural sludge to vermifertilizer for long-term land regeneration. According to the findings of this report, the earthworm *E. fetida* can digest textile sludge waste mixtures with enzyme activity to generate nutrient-rich manure.

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Conflict of Interest

The authors have no conflict of interest.

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