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Assessing the Environmental Safety of Sewage Sludge: C. elegans as a Model for Toxicity Evaluation

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ABSTRACT

Background: Sewage sludge, a by-product of wastewater treatment plants (WWTPs), is frequently applied as an agricultural amendment due to its nutrient-rich composition. However, the presence of heavy metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs) raises concerns regarding ecological safety.

Objective: This study aimed to assess the environmental safety of wet and sun-dried sludge from the Bharwara WWTP, Lucknow, using *Caenorhabditis elegans* as a model organism.

Methods: Chemical analysis of wet and dry sludge samples included heavy metals, pesticide residues and PAHs. Ecotoxicological analysis was the determination of growth, recovery, and reproductive productivity of C. elegans in sludge.

Results: Sludge that was wet had a higher number of contaminants than dry, and this means that sun-drying is effective in decreasing the number of toxicants. C. elegans growth was not largely affected but wet sludge greatly affected recovery and reproduction but dry sludge was not so toxic.

Implication: Sludge processing has a great impact on ecological safety. Sludge dried in the sun is less harmful to soil organisms and C. elegans is a sensitive model to use in integrated toxicity evaluations. The results indicate the significance of post-treatment measures to achieve sustainable and safe repurposing of sewage sludge to agriculture.

Keywords: Sewage sludge, Ecotoxicity, *Caenorhabditis elegans*, Heavy metals, Wastewater treatment

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INTRODUCTION

Sewage sludge is a by-product of wastewater treatment plants (WWTPs), and its amounts grow regularly all over the world as a result of rising urbanization and industrialization (Di Giacomo and Romano, 2022; Hubeny et al., 2021; Achkir et al., 2023; Hubeny et al., 2021; Negi, Verma, Singh, Mahapatra, and Jatav, 2022). The sludge is a heterogeneous mix of organic content, nutrients, and potentially dangerous pollutants, such as heavy metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs) that becomes highly dangerous when not properly handled (Krishna, Sachan, and Jatav, 2022; Regitano et al., 2022; Mokhtar, Makhtar, and Mokhtar, 2021; Kamaruzaman, Jaafar, Mohideen, and Fatinathan, Due to the good nutrient/caloric content of treated sludge, it is also often reused as farm fertilizer, which is an opportunity to recycle nutrients and valorize wastes (Chojnacka, 2023; Chowdhury, Bandyopadhyay, and Bhunia, 2022; Di Costanzo, Cesaro, Di Capua, and Esposito, 2021). The fact that residual contaminants are still present, however, makes both vigorous monitoring and ecotoxicological evaluation indispensable in order to avoid unintended consequences on the ecology.

The traditional chemical analysis also offers essential clues on the concentration of single pollutants, but they fail to reflect the overall biological responses of complex mixtures found in sludge (Sigurnjak Bureš et al., 2021). Hology by using model organisms as ecotoxicological methods can facilitate study toxicity of sludge by filling this gap between chemical composition and ecological risk (Kar, Sanderson, Roy, Benfenati, & Leszczynski, 2020; Spurgeon, Lahive, Robinson, Short, and Kille, 2020). Among the diverse bioassays, Caenorhabditis elegans has proven itself as an effective system to examine environmental toxicity because of its fast life-cycle, genetic portability,[similar stress-response networks] and also responsive to a wide range of chemical pollutants. Notably, endpoints like growth, reproduction and recovery in C. elegans offer valid models of sublethal and reproductive toxicity which are essential in forecasting population level impacts in natural ecosystems (Boros & Ostafe, 2020).

It has been demonstrated that physicochemical properties of sludge, especially moisture content and processing procedures can have a substantial effect on the bioavailability of contaminants and the ecological risk (Feng, Burke, Chen, and Stewart, 2023; Nunes, Ragonezi, Gouveia, and Pinheiro de Carvalho, 2021; Tytla, Widziewicz-Rzońca, Kernert, Berna, and Sslaby, 2023). The wet sludge, which contains more water, has a higher ability to hold onto soluble toxicants, making the soil-dwelling organisms more likely to be bioaccumulated (Gonzalez-Alcaraz et al., 2020). On the other hand, the sludge dried in the sun or composted has less contamination mobility and ecological toxicity, so it is a relatively safer alternative to use in agriculture (Mabrouk et al., 2023). In spite of these considerations, there are very few systematic comparisons of the toxicological impacts of wet and dry sludge in terms of standardized bioassays, especially in developing areas where sludge management habits are not uniform (Bernegossi et al., 2022; Steele, Meng, Venkatesan, and Halden, 2022; da Silva Souza, Lacerda, Aguiar, Martins, and de Oliveira David, 2020).

Due to the ongoing demand to recover discarded resources in a sustainable way through wastewater, as well as the growing interest in using sludge as an amendment to agriculture, integrated ecotoxicological modeling uniting chemical profiling with biologically relevant outcomes is urgently required (Ammar, Maury, Morin, and Sghir, 2020; Kathi, Singh, Yadav, Singh, and Mahmoud, 2023). Not only does this approach enlighten risk management strategies, but it also directs any policies made in terms of sludge treatment, storage, and land application. Using C. elegans as model, researcher can obtain rapidity, sensitivity and ethically viable assessment of the sludge toxicity, providing a translational model of predicting environmental safety and ecological impact.

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It is against this backdrop that this research undertaking seeks to determine the safety of sewage sludge obtained in the Bharwara WWTP located at Lucknow, India, by examining the chemical constituents of wet and dry sewage sludge, and establishing the impacts of this on growth, recovery and reproduction in the C. elegans. This comprehensive methodology will aim to offer practical findings regarding the sludge management in practices, the possible advantages of the post-treatment measures post-sludge reuse (like sun-drying) in terms of mitigating environmental risks.

METHODOLOGY

The Bharwara Wastewater Treatment Plant (WWTP), located in Punjab, was used to sample sewage sludge over two consecutive years (20232024) of the wet and sun-dried sludge. Wet samples were kept in 4C and analyzed after 4850 hours, whereas the preparation of dry sludge was done by sun-drying over the course of 810 days. The chemical characterization of the sludge entailed the quantification of heavy metals in the sludge (e.g., lead, cadmium, chromium, and nickel) by atomic absorption spectrophotometry, pesticide residues by gas chromatography-mass spectrometry, and polycyclic aromatic hydrocarbons (PAHs) by conventional EPA techniques. As a model organism representative of ecotoxicity testing, Caenorhabditis elegans (wild-type N2 strain) were cultured and synchronized L1-larvae were collected through bleaching. About 50-60 larvae were incubated with 1 g of wet or dry sludge on nematode growth medium plates and control samples were left without sludge. Worms were incubated at 72-75 hours, and recovery, growth (body length), and reproduction (number of eggs per worm) recorded under the stereomicroscope at 40X, and all the experiments were triplicated. One-way ANOVA was conducted together with the use of the post-hoc test (Tukey) with p-values below 0.05 taken as statistically significant. This method made it possible to evaluate environmental safety of treated sewage sludge comprehensively and note that the wet and dry sludge were different in their impacts on C. elegans.

RESULTS

The chemical analysis of the collected sludge samples showed that both wet and dry sludge contained a number of heavy metals, pesticide residues and polycyclic aromatic hydrocarbons (PAHs). The contaminants were found in larger amounts in wet sludge than in dry sludge which indicated that the sun-drying process is effective in lowering contaminants of possibly harmful compounds. Particularly, the wet sludge had 48.5, 3.2, 22.8, and 15.1 mg/kg of lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni), respectively, but the same was reduced in the dry sludge to 42.1, 2.9, 19.7 and 13.8 mg/kg, respectively (Table 1). On the same note, pesticide residues such as organophosphates and carbamates were found in lesser amounts in dry sludge. Wet sludge total PAHs were 18.5 mg/kg and dry sludge was reduced at 14.8 mg/kg.

Sludge exposure did not cause any major impact on C. elegans growth with body length averages similar to control (Table 2). The type of sludge, however, affected recovery and reproduction endpoints. Worms in wet sludge exhibited a high degree of reduced recovery (82.3%), and reproduction (average 46 eggs per worm) in comparison with controls (98.5% recovery, 60 eggs per worm). Conversely, the worms subjected to dry sludge had a greater recovery (91.2) and reproduction rate (average 54 eggs per worm), which showed that dry sludge is relatively harmless to ecological functions (Table 3). The results indicated that the ecological toxicity of sewage sludge could be decreased by sun-drying treatment, and C. elegans could be used as a good model to evaluate the environmental safety.

Table 1: Heavy Metal Concentrations in Sludge Samples (mg/kg)

Metal	Wet Sludge	Dry Sludge
Lead (Pb)	48.5	42.1
Cadmium (Cd)	3.2	2.9
Chromium (Cr)	22.8	19.7
Nickel (Ni)	15.1	13.8

Figure: 1

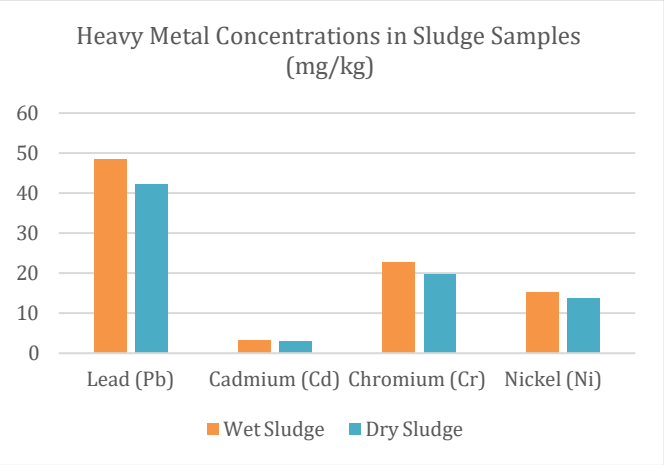


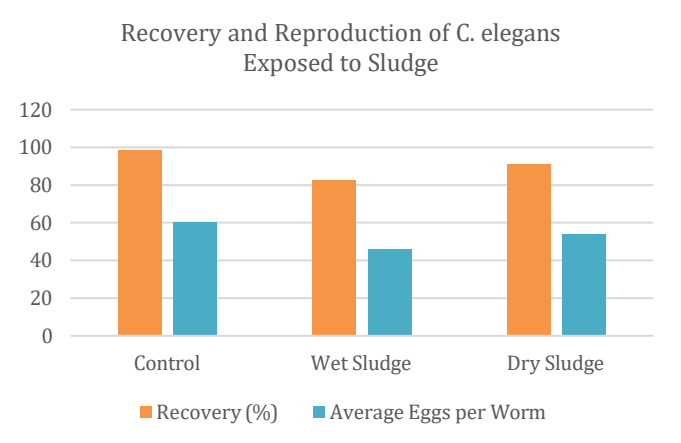
Table 2: Growth of *C. elegans* Exposed to Sludge (Average Body Length in mm)

Treatment	Average Body Length ± SD
Control	1.20 ± 0.05
Wet Sludge	1.18 ± 0.06
Dry Sludge	1.19 ± 0.05

Table 3: Recovery and Reproduction of *C. elegans* Exposed to Sludge

Treatment	Recovery (%)	Average Eggs per Worm
Control	98.5	60
Wet Sludge	82.3	46
Dry Sludge	91.2	54

Figure: 2



DISCUSSION

The current research gives important insights into ecotoxicology implication of wastewater treatment plant sludge with the different effects of wet and dry sludge on Caenorhabditis elegans.

In our chemical analyses, heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs) were present in large amounts in sludge, and these findings accompany the past evidence which identifies sewage sludge as a refuge of organic and inorganic pollutants (Košnář, Mercl, Pierdonà, Chane, Míchal, & Tlustoš, 2023). The greater contaminant burden in wet sludge supports previous authors who indicate that water retention in sludge promotes the survival of soluble toxicants but sun-drying or drying sludge greatly degrades their levels. This decrease in dry sludge highlights the importance of straightforward post-treatment methods in controlling the environmental risk before the agricultural use.

Assessment of ecotoxicity in C. elegans revealed that growth was not significantly different among treatments, and that body size could not be a sensitive endpoint of toxicity depending on sludge. Recovery, on the other hand, reproductive output varied greatly with the type of sludge, with wet sludge having strong negative impacts. These results are in agreement with the well known sensitivity of reproductive and recovery endpoints in nematodes to environmental contaminants especially heavy metals and PAHs. This observation of partial recovery in worms exposed to dry sludge supports the fact that processing methods are important factors in alleviating reproductive toxicity which is a very important determinant as far as population sustainability is concerned in the natural ecosystem.

The differences in impact of wet and dry sludge on reproduction highlight how intricate the connection between sludge physicochemical and biology contaminant bioavailability can be. The high water content in wet sludge can also make soluble toxicants easier to move, and they can be more readily taken up by the nematodes, but under sun exposure, the harmful substances could be either partially volatilized or immobilized, and therefore have a relatively lower ecotoxicity level. Such observations prove to be in accord with more sweeping environmental safety evaluations that recommend pre-treatment of sludge application as a major step towards sustainable land application.

Furthermore, the C. elegans model organism was very efficient in terms of high-throughput, sensitive and ethically viable ecotoxicology testing. The life cycle of the nematode is well-characterized, the organism reproduces quickly, and the stress-response pathways are conserved, which permits effective extrapolation of the toxicity endpoints to the ecological risk assessment processes. Our results support the emerging review that bioassays of nematodes can obtain supplementary components to traditional chemical analyses to give complete details regarding ecological danger that traditional chemical assessments could disregard.

CONCLUSION

The analysis reveals that sewage sludge processing technique is highly effective in determining the environmental safety, and wett sludge has elevated concentrations of heavy metals, pesticides, and PAHs, such that recovery and eligibility diminish in C. elegans. Conversely, sludge dried by the sun yielded less contaminants and relatively less impact on nematode reproduction and recovery, which demonstrates its more benign ecological work. The findings underscore the need to combine chemical analysis with biologically relevant endpoints to have a holistic ecotoxicological approach. C. elegans was demonstrated effective in assessing sublethal and reproductive toxicity, and provides a fast, sensitive, and ethically viable model that can be used to monitor the environment. Post-treatment measures, including sun-drying, can help to mitigate environmental risks considerably to justify the sustainable use of sludge in agriculture and protect the health of the soil and the overall ecosystem activity.

Data Availability

Available from corresponding author on request.

Author Contributions

Sehrish Younas: Conceptualization, Supervision, Writing – Review & Editing
Naveed Alam: Data Curation, Formal Analysis, Visualization
Kamran Rauf: Methodology, Investigation, Writing – Original Draft

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Conflict of Interest

None.

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