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Green Synthesis of Zinc Oxide Nanoparticles using *Allium sativum* Plant Extract and Photocatalytic Activity of Rhodamine B

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ABSTRACT

The green approach is regarded as an economical and environmentally responsible way to create nanoparticles. In the field of dye degradation, the photocatalytic degradation mediated by nanoparticles is an effective method. Rhodamine B is a dye that, even at very low concentrations, is highly mutagenic and carcinogenic. Prior to discharge, Rhodamine B must be removed from wastewater. A low-cost plant employed in the manufacture of zinc oxide nanoparticles is *Allium sativum*. By using XRD and SEM, the synthesized nanoparticles were identified. Rhodamine B was eliminated using zinc oxide nanoparticles. The results demonstrated 99.99% Rhodamine B decolorization at reaction rates of 0.028 mg/L/min for 180 min at 5 ppm dye concentration, 1 g/L catalyst concentration, and pH 6. Analysis using FTIR, HPTLC and UV-Visible spectrophotometers proved Rhodamine degradation. *Vigna radiata* L, a key agricultural plant, was used to test the toxicity of Rhodamine B and treated effluent. The findings supported the treated effluent's lower toxicity than the original chemical by showing that the plant and soil had a less harmful reaction to being irrigated with treated water.

Keywords *Allium sativum*, Degradation, Rhodamine B, Toxicity, Zinc oxide nanoparticles.

INTRODUCTION

Rhodamine B (RhB) is a xanthene dye that is widely used in the color, pigment, food and cosmetic sectors because to its excellent water solubility and inexpensive cost (Vithalani and Bhatt 2022a). The natural water ecology is disrupted by higher solubility of RhB in water, which reduces light penetration and lowers the photosynthetic activity and growth of aquatic plants (Vithalani and Bhatt 2023). RhB removal from wastewater is a challenging task. Many physical and chemical techniques for dye remediation have been explored. The treatment based on nanoparticles is quicker, cheaper, and more efficient than other approaches. Rhodamine B is being treated in the current investigation using zinc oxide nanoparticles (ZnO NPs).

Zinc oxide (ZnO) has better stability and high

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photocatalytic activity thus, it is widely used in the treatment perspective. ZnO shows better antimicrobial activity. An extensive study indicates that controlled oxygen vacancies can manipulate ZnO's optical, surface, and electronic properties (Boon *et al.* 2018). ZnO has applications in different areas such as photo catalysis, gas sensor, biosensor, anti-bacterial agent, and supercapacitors. Synthesis of ZnO nanoparticles is carried out with chemical and physical approaches (Wang *et al.* 2018).

Now-a-days ZnO is utilized as nanostructures in photochemical and photo electrochemical treatment. For the catalysis, under visible light junction must be developed. The ionic bond strength of Zn is higher, which shows low electronegativity. Combined with low band metal oxides composites, a junction enhances the absorbance of visible light. During hetero junction's formations, the up-down movement of semiconductors produces electrical energy, which activates photocatalytic reaction activity (Vithalani and Bhatt 2022b). The co-precipitation method synthesised combine Ag/ZnO mediated photocatalyst. Photocatalysis activity was checked in a batch reactor; efficiency of ZnO combined with Ag was checked. The results show a decrease in BOD, COD of wastewater which concludes that the photocatalysis treatment efficiently degrades dyes present in wastewater (Mesa *et al.* 2018).

MATERIALS AND METHODS

Media, chemicals and reagents

All chemicals and media utilized in this study were analytical grade. RhB was purchased from the dye industry, Vatva, Ahmedabad, Gujarat. All other chemicals and media were purchased from SRL and Himedia laboratories, India.

Green synthesis of NPs and characterization

Synthesis of NPs was performed as described by Saxena *et al.* (2010). Two steps of the synthesis process were; the preparation of vegetable extracts and the synthesis of zinc oxide nanoparticles. The *Allium sativum* L. plant was used to produce vegetable extracts. To extract the compounds, 20 g of *Allium sativum* L. was chopped into small, accurate pieces in 100 mL distilled water and kept on magnetic stirring for 20 min at 75 to 80°C. The extract was cooled to room temperature and the solution was filtered through filter paper prior to being used to make nanoparticles. The remaining extracted solution was used for the next cycle of synthesis and was kept at a temperature 4-5 °C. In ways to construct ZnO NPs, 20 mL of extracted *Allium sativum* L. solution was combined with 2 mL of zinc nitrate, which was thoroughly mixed prior keeping in a water bath at 80°C for 8 h. The resultant paste was further calcinated for 2 h at 450°C in a furnace. The dried mass was produced and pulverized into powder by using mortar and pestle. XRD and SEM analysis were used to further characterize the ZnO NPs powder.

Photocatalytic decolorization of RhB

The synthetic ZnO was introduced to 1000 mL of wastewater that comprised 5 ppm RhB and pH 6.5 at a concentration of 1 g/L. Flasks with the mixture were mounted on magnetic stirrer and exposed to sun light. The photocatalytic reaction was carried out and samples were collected on a frequent basis at fixed time interval to check out decolorization. Decolorization and rate of decolorization were performed.

Optimization of physical and chemical parameters

Any chemical reaction depends critically on both physical and chemical factors. Irradiation time, nano-catalyst concentration, dye concentration and pH of the reaction system were taken into consideration to evaluate the impact of different parameters on decolorization of RhB. To improve the decolorization, process parameters were optimized using the one factor at a time (OFAT) method.

Effect of ZnO NPs concentration on RhB decolorization

RhB degradation can be impacted by zinc oxide dosage. Both positive and negative effects on the rate of photodegradation can be attributed to the catalyst quantity (Isai and Shrivastava 2019). By changing the concentration from 0.5 to 2 g/L, the impact of ZnO NPs concentration on the dye decolorization was investigated.

Effect of RhB concentration on decolorization

The rate of decolorization depends on the development of OH radicals which adsorb upper surface of catalyst and interact with dye molecules. Increased dye molecules are available for excitation and energy transfer as the original dye concentration increase (Parvaz *et al.* 2021). This dependence was related to the formation of several monolayers of absorbed dye on the nanoparticle surface, which is favored at high dye concentration (Chanu *et al.* 2019). Variation in dye concentration, 1 to 11 ppm with the increment of 2 ppm, was examined to determine maximum dye decolorization.

Effect of irradiation time on RhB decolorization

The length of exposure time grows as dye degradation accelerates. Decrease in dye degradation after certain time limit is mainly attributed to the difficulty in the reaction of short chain aliphatic compounds with •OH radicals and the short lifetime of photocatalyst, because of active sites deactivation by strong by-products deposition (Reza *et al.* 2017). The impact of solar light irradiation was investigated with 60, 120, 150 and 180 min irradiation time.

Effect of pH on RhB decolorization

The adsorption-desorption processes and the separation of the photogenerated electron pairs on the nanoparticles surface are both impacted by pH modification (Kazeminezhad and Sadollahkhani 2016). The pH of system was altered from 2 to 8 to investigate the role of pH in the degradation of dyes.

Recyclability and reusability of ZnO NPs

Reusability of the catalyst is crucial in determining treatment costs. The effectiveness of the catalyst was evaluated by using the same catalyst concentration throughout the number of cycles and observing RhB decolorization as described by Qian *et al.* (2019).

COD reduction

COD reduction was performed according to standard protocol of close reflux method (APHA 2012).

Degradation study

UV-Visible spectrophotometer analysis

UV-Visible spectrophotometer analysis was carried out after sample collection at every fix interval. The 5 mL samples were centrifuged at 10,000 rpm (12298 ×g) for 15 min three times. The UV-visible scanning of extracted dye and intermediates was carried out from 180 to 700 nm.

HPTLC analysis

HPTLC analysis was performed according to Dhingra (2015). Sample was applied on a silica gel plate (HPTLC Silica gel 60 F254, Merck, Germany) using micro syringe sample applicator and by spraying with nitrogen gas (Linomat V, CAMAG, Switzerland). Butanol: Acetic acid (glacial) : Water (4:1:5 v/v) mixture was used as the solvent system to resolve metabolites on TLC plate. The system was developed in pre-equilibrated twin through chamber and scanned by TLC scanner (CAMAG TLC scanner 4, Switzerland) at 366 nm.

FTIR analysis

The various functional groups present in RhB and test were studied through FTIR spectral analysis. Samples were prepared as control and test, 5 mL samples were centrifuged at 10,000 rpm (12298 ×g) for 15 min three times. The supernatant was collected as sample and the spectra were recorded in the region from 400 to 4000 cm^{-1}.

Phytotoxicity study on Vigna radiata

Vigna radiata L. seeds were used to check impact of the RhB (5 ppm) and treated effluent. Each petri plate received daily irrigations of 5 mL of RhB (5 ppm), distilled water and treated effluent. The petri dish was kept outside and the germination process was monitored.

RESULTS AND DISCUSSION

Characterization of ZnO NPs

The characterization of ZnO NPs was performed by

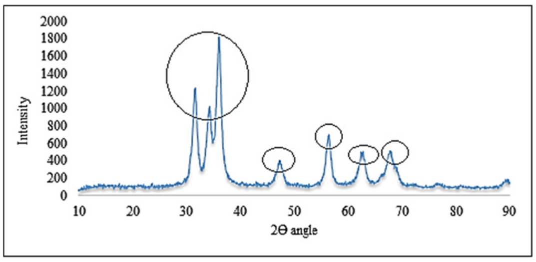


Fig. 1. XRD analysis of ZnO NPs.

XRD and SEM. The data were compared with standard ZnO and similar results were found by Stan *et al.* (2015). The XRD graph is shown in Fig. 1. The morphological study of synthesised ZnO NPs was performed through SEM analysis. The aggregated irregular shape particles were observed. Most of the NPs have high surface area (Fig. 2).

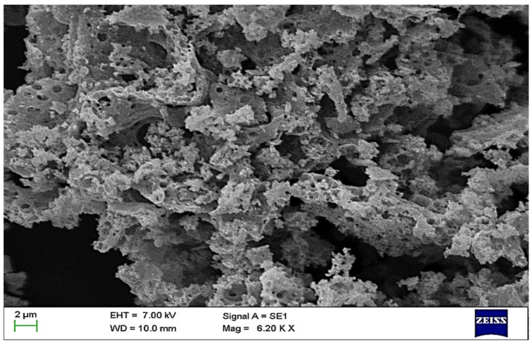
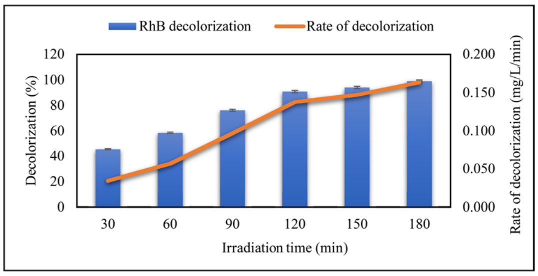


Fig. 2. SEM analysis of ZnO NPs.

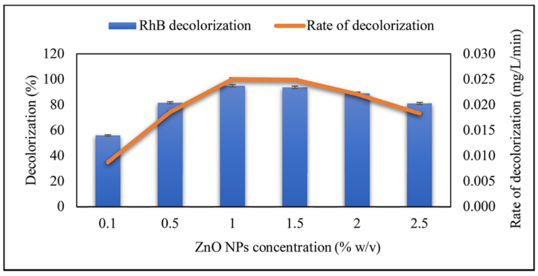
Optimization of physical and chemical parameters

Effect of ZnO NPs concentration on RhB decolorization

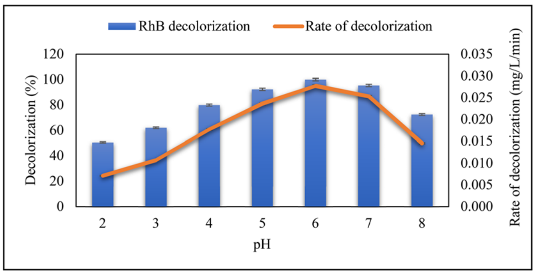
Nano catalysts are crucial component in the removal of organic molecules. Increased catalyst concentration to some extent accelerates decolorization before



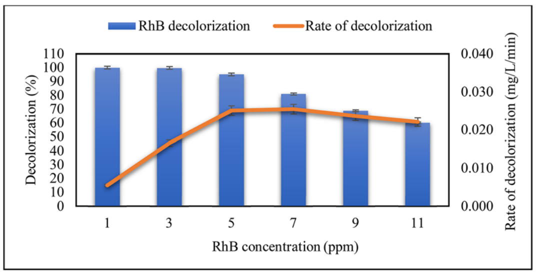
Graph 3. Effect of irradiation time on RhB decolorization.



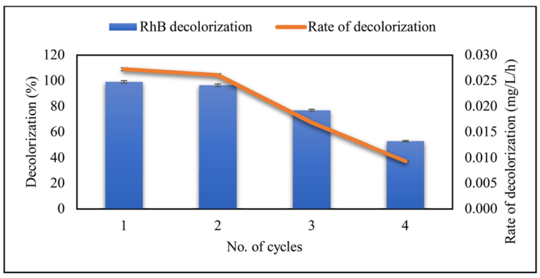
Graph 1. Effect of ZnO NPs concentration on RhB decolorization.



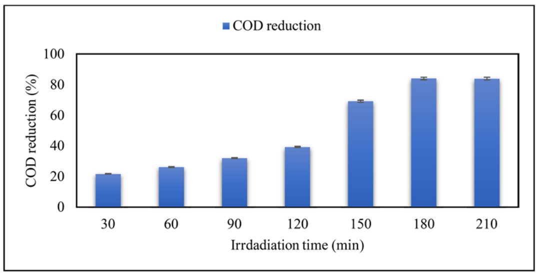
Graph 4. Effect of pH on RhB decolorization.



Graph 2. Effect of RhB concentration on decolorization.



Graph 5. Recyclability and reusability of ZnO NPs.



Graph 6. COD reduction with irradiation time.

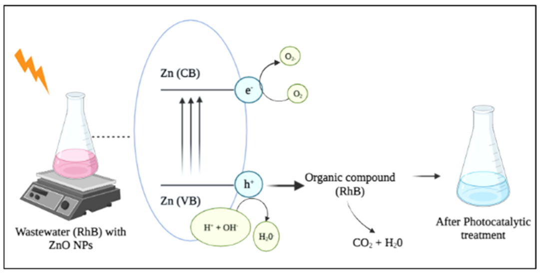


Fig. 3. Mechanism behind the degradation of RhB through ZnO NPs.

slowing it down (Velidandi *et al.* 2021). In the current study, a catalyst concentration of 1 g/L resulted into maximum decolorization of 95.11% at a decolorization rate of 0.025 mg/L/min (Graph 1). AgNPs-Fe_2O_3 was used in an investigation by Muraro *et al.* (2020) showed 58.36% decolorization in the presence of solar radiation.

Effect of RhB concentration on decolorization

The frequency of decolorization was decelerated by the increasing dye concentration when adsorbed onto the surface. The study of optimization of dye concentration in ranged from 1 to 11 ppm with increments of 2 ppm each. The highest decolorization of 95.11% and the rate of decolorization 0.025 mg/L/min were obtained as shown in (Graph 2). The highest 70% decolorization efficiency was obtained by Rana *et al.* (2016).

Effect of irradiation time on RhB decolorization

The photocatalytic decolorization of RhB dye was investigated in relation to the duration of the catalyst expose to light. RhB decolorization was observed to

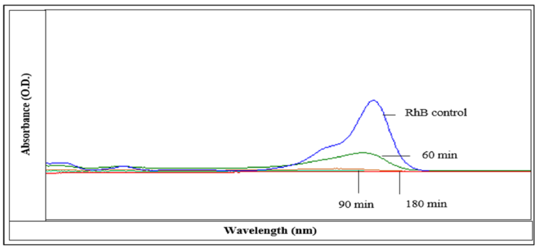


Fig. 4. UV-Visible spectrophotometer analysis of RhB control and test.

rise with catalyst irradiation period before becoming constant. Maximum RhB decolorization 99.11% was observed in 180 min reaction time at a rate of 0.027 mg/L/min (Graph 3). Al-Bedairy and Alshamsi (2018) showed RhB decolorization 71% after 240 min reaction time by using ZnO NPs.

Effect of pH on RhB decolorization

In the production of hydroxyl radicals, pH is crucial. Maximum 99.99% decolorization was observed at a rate of 0.028 mg/L/min by ZnO NPs with pH 6 (Graph 4). Selvam *et al*. (2020) observed maximum decolorization at pH 6 through SnO_2 NPs.

Recyclability and reusability of ZnO NPs

Nano-catalyst has practical applications in the decolorization of industrial dyes with its effectiveness and ability to be recycled (Sajjadi *et al.* 2021). Therefore, an experiment was conducted to determine the recyclability of ZnO nanoparticles and their effectiveness throughout cycles. After decolorization, 5 ppm of dye was repeatedly applied to assess the effectiveness. The decrease in decolorization from 99.11 to 52.74% was observed after completion of 4^{th}. The rate of decolorization was decreased from 0.026 to 0.009 mg/L/h (Graph 5). The study was correlated with Alhokbany *et al.* (2019).

COD reduction

Chemical oxygen demand is a typical organic material found in industrial effluent, its presence is dependent on the chemicals employed in the manufacturing process. The COD of synthetic wastewater was 3.8

kg. COD reduction 84.18% was observed after the 180 min reaction time (Graph 6). Selvin *et al.* (2017) were indicated 50% COD reduction in 180 min reaction time. The complete COD reduction was not get because the formations of intermediate that can be interfere according to Khan *et al.* (2021).

Mechanism of photocatalytic degradation of RhB

Photocatalytic degradation of RhB mediated due to ZnO NPs. When ZnO NPs were exposed to solar light, which triggers the molecules to be excited and move to high energy level. After certain time energy becomes reduced and molecules comes into lower energy state. This resulted into electrons (e^-) and holes (h^+) to happen. These free radicals were responsible for degradation of RhB and converted into CO_2 and H_2O according to Shaikh *et al.* (2020). Fig. 3 illustrated the mechanism behind the degradation of RhB.

The subsequent reactions are listed below :

$hv + ZnO\ NPs \rightarrow h^+ + e^- + ZnO\ NPs$

$ZnO\ NPs + O_2 \rightarrow O_2^{\cdot}$

$h^+ + RhB \rightarrow CO_2 + H_2O$

Degradation study

UV-Visible spectrophotometer analysis

The UV- Visible spectrophotometer analysis was performed to check the reduction in intensity of dye. Maximum absorption peak of RhB, which occurred at 554 nm and gradually reduced over time, was discovered. The degradation of RhB was confirmed by the lower intensity of peaks at 310 and 360 nm, which subsequently decreased after 180 min of reaction (Fig. 4).

HPTLC analysis

The degradation of RhB was verified by the HPTLC examination (Fig. 5). The degradation was confirmed by the variations in R_f values. RhB dye degradation was confirmed by the absence of an RhB peak after 180 min of reaction time.

FTIR analysis

FTIR analysis was subjected to identify functional groups and newly synthesised compounds in treated effluent. Comparison between RhB (Control) and test confirmed degradation of RhB into various metabolites. The addition of peaks in test confirms the

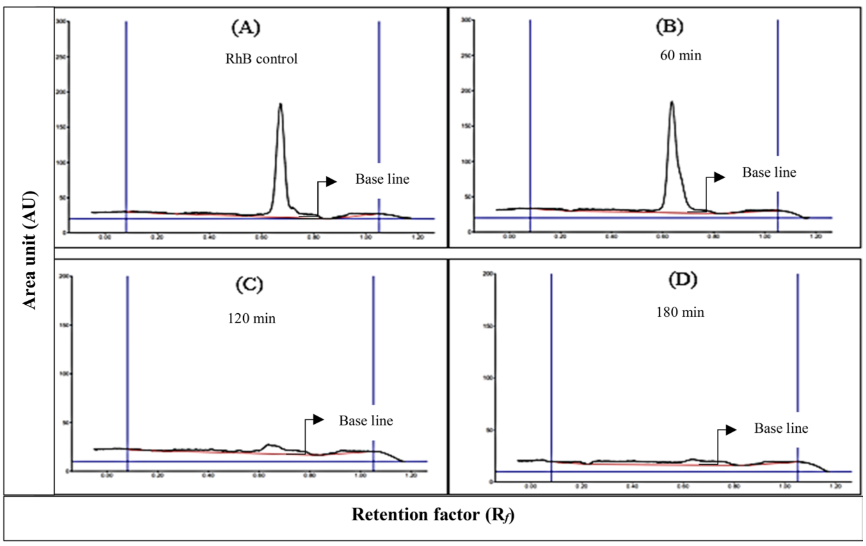


Fig. 5. HPTLC analysis of control and test.

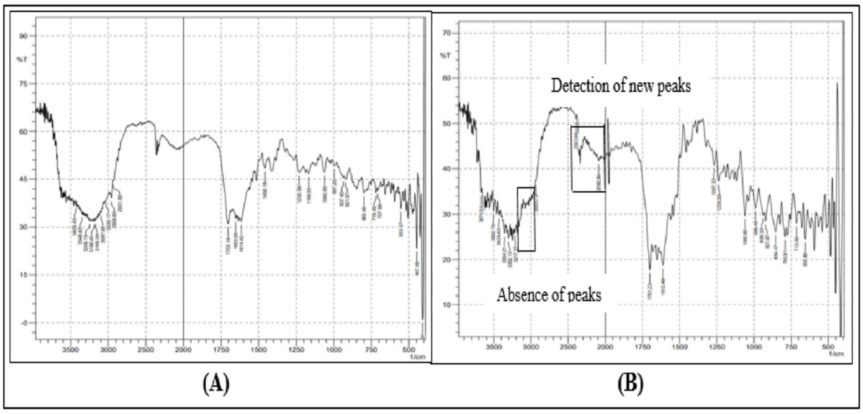


Fig. 6. FTIR analysis of (A) RhB control and (B) test.

Table 1. Effect of RhB and treated effluent on *Vigna radiata*.

Parameters	Distilled water	RhB (control)	Treated effluent
Germination (%)	100	40	100
Plumule length (cm)	6.2 ± 0.03	3.4 ± 0.2	6.1 ± 0.05
Root length (cm)	9.0 ± 0.05	1.7 ± 0.1	8.9 ± 0.4

formation of new function groups due to metabolites. The control shows different functional groups such, 416, 655, 713, 790 (C-Cl stretching), 921, 939, 989 (O-H loop formation), 1060 (C-O stretching), 1238, 1267 (C-O-C stretching), 1612 (Amide bending), 1701 (Ketone group), 2933 (CH_2 stretching), 3200, 3302 (O-H stretching) 3354, 3429, 3502, 3670 (NH_3 stretching) (Fig. 6 (A)). The treated effluent shows the functional group such as 408, 447, 553, 707, 719 (C-Cl stretching), 800, 921, 937, 997 (CO bending), 1060 (C=O stretching), 1166 (C-O-C stretching), 1230 (amide bond stretching), 1458 (C=O stretching), 1614 (C-C, C=C stretching), 1653 (C=O amide stretching), 1703 (ketone), 2931 (CH_2 stretching), 2958 (CH-CH_2 stretching), 3030, 3097, 3169, 3186 (CH stretching), 3248 (NH_2 stretching), 3348, 3429 (OH group stretching) (Fig. 6 (B)).

Toxicity study on Vigna radiata L.

Phytotoxicity is a crucial component of the soil pollution control process. The growth, germination, chlorophyll content, and other aspects of plants gets impacted by contaminated irrigation water (Soni *et al.* 2016). The significant plant *Vigna radiata* L., which is used in agriculture was studied in the present research. The growth of *Vigna radiata* L. was observed after 7 days of incubation and the germination (%), root, and shoot length were measured. The results are mentioned in (Table 1). The plants seeds were irrigated with RhB absorbed the color, which had an impact on the growth of the plants. Due to RhB's toxicity, the root and shoot did not develop adequately. Seeds nourished with treated wastewater grown properly. Findings were in accordance with Haq *et al.* (2018).

CONCLUSION

The environmental friendly, economically feasible, and productive approach for wastewater treatment is the green approach towards the synthesis of NPs. The *Allium cepa* plant extract was used to synthesize the ZnO NPs, which were characterized by XRD and SEM analysis. The finding indicated 99.99% RhB decolorization with 5 ppm dye concentration, 1 g/L catalyst concentration, 6 pH, and 180 min reaction time at reaction rate 0.028 mg/L/min. FTIR, HPTLC and UV-Visible spectrophotometer analysis was proven the degradation. *Vigna radiata* L., a significant agricultural plant was subjected to toxicity testing. The results revealed a lowered toxic effect on the plant and soil after irrigating it with treated water, supporting the treated effluent's lower toxicity than the original chemical.

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