



Evaluation of Phytotoxicity of Greenly Treated Domestic Wastewater Using Spent Plant Leaf Powders / Original article

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تقييم السمية النباتية لمياه الصرف الصحي المنزلية المعالجة
بطريقة خضراء باستخدام مساحيق أوراق النباتات المستهلكة

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Abstract

Health and ecosystems face widespread, impactful problems. Untreated wastewater is discharged into waterways and lands in most developing countries, containing harmful substances and pathogens. WHO estimates that nearly 1.7 billion people drink contaminated water in developing countries, and that contamination leads to nearly 500,000 diarrheal deaths each year. However, existing treatment methods are insufficient to remove dissolved substances and specific pollutants; they are very expensive, energy-intensive, require advanced technologies, and high operational skills. The process of removing pollutants from wastewater using plant waste powders involves a combination of mechanisms, including adsorption, complexation, and ion exchange. These processes are characterized by the presence of functional groups, active chemical compounds, and surface structures, which underscores their importance in treatment. This research aims to evaluate the toxicity of water treated with plant powders and the toxicity of spent plant powders based on germination.

Using powder of Pine (*Pinus brutia*) and olive (*Olea europaea*) leaves, wastewater treatment, and analysis experiments were conducted. Phytotoxicity experiments were also conducted on cress (*Lepidium sativum*), monitoring the rate of germination, its delay, and any harmful effects on plants. Results were compared with raw wastewater and drinking water, and with the maximum permissible values for wastewater used for various purposes. The results showed that the use of unmodified olive and pine leaf powder improves the efficiency of the water treatment process. The spent powders can be used for two consecutive treatment cycles with an acceptable reduction in removal efficiency, and contaminant removal. The treatment method or the spent powder proved safe, compared to treatment plant or stability pond methods, which require longer time. The odor of the treated water was acceptable. This is an environmentally friendly method that achieves the goals of environmental, health, and sustainability development. It is available in large quantities, at a low cost, and with good efficiency.

Keywords: phytotoxicity, domestic wastewater, plant leaves, powders, greenly treatment.



المستخلص

تواجه الصحة والنظم البيئية مشكلات مؤثرة على نطاق واسع؛ فمياه الصرف الصحي تُصرف دون معالجة إلى المجاري المائية والأراضي في معظم البلدان النامية، وهي تحتوي على المواد الضارة ومسببات الأمراض. تشير منظمة الصحة العالمية WHO إلى أن نحو 1.7 مليار شخص يشربون مياه ملوثة في البلدان النامية وأن تلوثها يؤدي إلى وفاة نحو 500 ألف شخص بالإسهال كل عام. لكن طرائق المعالجة المطبّقة غير كافية لإزالة المواد الذائبة والملوثات النوعية، مكلفة جداً وبحاجة للطاقة، وتتطلب تقنيات متطورة ومهارة عالية في التشغيل. تنطوي عملية إزالة الملوثات من مياه الصرف الصحي بمساحيق المخلفات النباتية على مجموعة من الآليات؛ بما في ذلك الامتزاز والتعقيد complexation والتبادل الشاردي والترسيب على سطح المسحوق، فهذا يتميز بوجود المجموعات الوظيفية والمركبات الكيميائية النشطة والبنية السطحية؛ ما يؤكّد أهمية استعمالها في المعالجة.

يهدف هذا البحث research aims إلى تقييم سُمية المياه المعالجة بالمسحوق النباتي وكذلك سُمية المسحوق النباتي المستهلك اعتماداً على الإنبات. فقد جُمعت أوراق نباتات الصنوبر *Pinus brutia* والزيتون *Olea europaea*، وأُجريت تجارب معالجة مياه الصرف الصحي وتحاليلها، وتجارب السمية النباتية على الرشاد (*Lepidium sativum*) بمراقبة معدّل الإنبات أو تأخيره أو أي تأثير ضار على النباتات، وأُجريت مقارنة بنتائج مياه الصرف الخام وماء الشرب وبالقيم القصوى المسموح بها في مياه الصرف المستعملة لأغراض مختلفة. أظهرت النتائج أن استعمال مسحوق أوراق الزيتون والصنوبر غير المعدّلة يفيد في تحسين كفاءة عملية معالجة المياه، ويمكن استعمال المساحيق المستهلكة دورتين متتاليتين في المعالجة مع انخفاض مقبول في كفاءة الإزالة وإزالة الملوثات، وتبيّن أن طريقة المعالجة أو المسحوق المستهلك آمنة، مقارنة بطرائق محطات المعالجة أو برك الاستقرار التي تحتاج إلى وقت أطول، وكانت رائحة المياه المعالجة مقبولة. وهذه طريقة صديقة للبيئة تحقق أهداف التنمية البيئية والصحية والاستدامة، متوافرة بكميات كبيرة، الكلفة المنخفضة والكفاءة الجيدة.

الكلمات المفتاحية: السمية النباتية، مياه الصرف المنزلي، أوراق النباتات، المساحيق، المعالجة الخضراء.



Introduction

Health and ecosystems face significant pollution problems on a wide scale. Wastewater pollution is of particular concern due to the presence of pathogens such as viruses, bacteria, fungi, protozoa, and helminths (Cabral 2010, Rose *et al.*, 2015, Al-Gheethi *et al.*, 2018). Untreated wastewater is often discharged directly into waterways, freshwater, and agricultural lands in most developing countries, causing serious health and environmental problems. More than two billion people live in countries with water scarcity, and the situation is expected to worsen in some regions due to climate change and population growth. An estimated 1.7 billion people drank contaminated water in 2022, in addition to chemical contamination. Contaminated water causes approximately 500,000 diarrheal deaths each year (WHO 2023). The water quality of the Tigris and Euphrates rivers in Iraq is deteriorating due to the low discharges from upstream countries, return water from drains and wastewater, and nature of the lands through which the rivers pass (Obaid 2024).

There are many technologies applied, such as oxidation, ozonation, coagulation, chemical precipitation, electrochemical treatment, ion exchange, and activated sludge, to remove organic and inorganic pollutants, chemical toxins, and pathogens from wastewater. However, they have disadvantages, the most important of which are: high cost, especially when treating large quantities of water, need for energy, and high operating skill. Process of removing pollutants from wastewater using plant waste powder involves a set of mechanisms; including physical and chemical adsorption, complexation, ion exchange, and precipitation on the surface of the plant powder, in addition to containing numerous functional groups such as hydroxyl, carboxyl, carbonyl,



amino, sulfhydryl, phosphoryl, and phenols, presence of a rough and heterogeneous surface structure, and polar chemical compounds such as phenols, tannins, flavonoids, and saponins that are characterized by bioactivity (Omojate *et al.*, 2014). Plant waste consists of materials of organic origin such as cellulose, hemicellulose, lignin, pectin, and extractives, which are useful in removing various pollutants (Malik *et al.*, 2017, Escudero *et al.*, 2019).

Several researchs have been conducted to evaluate the efficiency of different plant powders in removing pollutants from water (Chandran *et al.*, 2015, Ahmadi and Mostafapour, 2017, Rehman *et al.*, 2019), as well as to use plant waste powder in multiple cycles in removing pollutants (Adeniyi and Ighalo 2019, Tran *et al.*, 2019). This method is a promising alternative to wastewater treatment due to its low cost and high efficiency. It is widely available and has a low economic value. Furthermore, it can be regenerated and reused in treatment. It is environmentally friendly and achieves environmental, health, and sustainability development goals. The research aims to evaluate the toxicity of treated wastewater to the germination of cress, as well as the toxicity of the spent plant powder to germination.

Methodology

$$\text{removal efficiency (R \%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where, C_i and C_f : initial and final concentrations of pollutants in domestic wastewater, respectively.

The leaves of olive and pine used in experiments were collected, in different sites in Damascus, according to the applied methods (Sharma and Bhattacharyya 2005). Domestic wastewater samples were collected and analyzed, in Damascus city, according to standard methods (APHA 2017),



and treatment experiments were carried out according to the applied methods (Chiban *et al.*, 2012, Mokif *et al.*, 2018, Haghizadeh *et al.*, 2020). 250 ml flasks containing 100 ml of domestic wastewater were used; according to the following steps (Figure 1): Plant powder dose 1 g/L, at 25 °C, pH 6.5, treatment time 60 minutes, and shaking speed 150 rpm. After treatment, the water was filtered with Whatman filter paper (No. 45) for the purpose of conducting pre- and post-treatment tests. As for the turbidity experiments, the treated water was left for 5 minutes to settle, then 10 ml was withdrawn from the upper layer of the water; for turbidity analysis. All experiments were conducted in three replicates. A control was used by adding 100 ml of domestic wastewater without plant powder. The pH values of the domestic wastewater were adjusted using HCL (0.1 M) and NaOH (0.1 M) after adding the plant leaf powder. The removal efficiency was calculated using the following equation:

$$\text{removal efficiency (R \%)} = (C_i - C_f) / C_i \times 100$$

where, C_i and C_f are the initial and final concentrations of pollutants in the domestic wastewater, respectively.

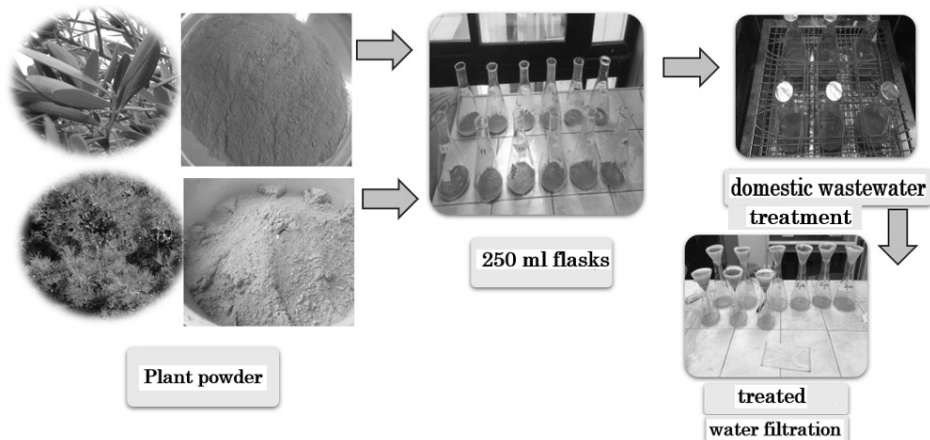


Figure 1. Stages of domestic wastewater treatment using plant powders.



Preparation of plant extract

Aqueous extracts of plants powder were prepared separately. 50 g of leaf powder was placed in a Soxhlet apparatus and 500 ml of solvent was used at a ratio of 1:10 (v/w) for 7 hrs. The extracts were then filtered and evaporated using a rotary evaporator under reduced pressure at 40°C., they were stored in a refrigerator at 4°C until use (Bissa and Bohra 2008). All extracts were sterilized before by using 0.45 µm membrane filters.

Phytotoxicity tests

The germination percentage of cress seeds was calculated to determine the phytotoxicity of treated wastewater, and of the splet plant powders after the treatment process, as follows: (1) To determine the phytotoxicity of treated water: 40 g of sterile, moistened soil was placed in 50 g plastic containers with 30 ml of treated wastewater. 30 seeds were planted in each container. Drinking water was used as positive control, and crude wastewater was used as a negative control. The containers were placed at 25°C, alternating between light and darkness (10 hrs. of darkness versus 14 hrs. of light) for 10 days. (2) To determine the toxicity of splet plant powders after treatment: Plant powders were dried in the shade for 14 days until weight was relatively stable. Then, 20 g of the dried powders were placed in 50 g plastic containers, where 30 seeds were planted and watered with 30 ml of distilled water. The soil served as positive control. The plastic containers were then placed for 3 days at 25°C, alternating between dark and light (10 hrs of darkness versus 14 hrs of light). All previous experiments were conducted with three replicates each, and the germination percentage for all experiments was calculated (Darvishi 2014, Robalds *et al.*, 2016) using the following equation:

$$\text{Germination rate (\%)} = \text{No of germinated seeds} / \text{total No of seeds} \times 100$$



Results and Discussion

1. Physical and chemical analysis of domestic wastewater

Table 1 shows the results of physical and chemical analyses of domestic wastewater.

Table 1. Physical and chemical properties of domestic wastewater.

Testes		1	2	3
Temperature, C°	24.0 ± 0.3	-	-	-
pH	8.38 ± 0.07	6 - 9		
TDS, mg/L	570 ± 4.0	15000	1500	-
EC, S/cmμ	824 ± 3.5	-	-	-
Turbidity, NTU	174 ± 4.7	-	-	-
Nitrate, mg/L	26.50 ± 0.34	20	25	25
Phosphate, mg/L	28.3 ± 0.15	20	20	20
BOD, mg/L	146.4 ± 11.30	30	100	150

1: Maximum permissible level of wastewater used for irrigation of cooked vegetables, parks, and urban roadsides. 2: For fruit trees, lawns, cereals, and forage crops. 3: For industrial crops and forest trees. 1, 2, and 3, accordint to Syrian pstandards.

Table 1 shows that the pH value of domestic wastewater was alkaline, reaching 8.38 ± 0.07 . Water pH affects the status of nutrients, nitrifying bacteria, and microelements. Some substances in water may be toxic due to their interaction with high or low pH levels. Water temperature was 24.0 ± 0.3 °C. Temperature significantly affects the rates of biochemical reactions, treatment processes, and sedimentation efficiency (Mohammed *et al.*, 2012). Electrical conductivity (EC) value was 824 ± 3.5 S/cmμ, which is consistent with the total dissolved solids (TDS) value of 570 ± 4.0 mg/L (Noorjahan 2014). TDS values were within the maximum permissible limits



depending on crop type. Turbidity value was 174 ± 4.7 NTU, and BOD value was 146.4 ± 11.3 mg/L, which represents the amount of oxygen consumed by bacteria aerobically at 20°C over five days. It often exceeded the maximum permissible limit. High values lead to depletion of dissolved oxygen (DO), making conditions anaerobic and increasing the toxicity of the medium.

2. Results of domestic wastewater treatment using plant leaves powder

Table 2 shows the results of water treatment using olive and pine leaf powder, respectively.

Table 2. Results of domestic wastewater treatment using plant leaf powder, 1 g/L.

Testes	Crude WW	treated WW using	
		<i>Olive</i>	<i>Pine</i>
Temperature, C°	23.7	25.0	25.0
pH	7.45	6.5	6.5
TDS, mg/L	568	501.83	448.83
EC, S/cm μ	820	730.78	687.73
Turbidity, NTU	176	82.09	41.82
NO ₃ -N, mg/L	21.53	12.92	15.47
Phosphate, mg/L	27.0	19.67	21.85
BOD, mg/L	149.3	204	191.6

The results of table 2 show a decrease in the TDS value when treated with olive leaf powder by 11.65%, and with pine leaf powder by 20.98%, and a decrease in EC value with olive and pine powders by 10.88% and 16.13%, respectively. The TDS values after treatment are within the maximum permissible limit in wastewater. The turbidity value of domestic wastewater treated with olive powder also decreased by 53.36% and 76.24% in the case of pine. Dissolved solids are directly proportional to electrical conductivity



(Vunain et al., 2019). The effectiveness in reducing the concentration of dissolved solids is attributed to the active functional groups that contribute to the formation of organometallic complexes (Assaffii et al., 2012). The active functional groups in the plant powder, such as carboxyl, hydroxyl and amino, work to neutralize the charges on the outer surfaces of suspended materials and collect and precipitate the suspended materials (Lee *et al.*, 2014, Choy *et al.*, 2015).

The results of table 2 show that the nitrate and phosphate values decreased by 39.97% and 27.13% in olive powder, and by 28.16% and 19.09% in pine powder, respectively. This is consistent with the results of other studies (Ismail 2012, Qiao *et al.*, 2019). The increase in the value of BOD is due to the release of soluble organic compounds present in the plant powder (Kushwaha et al., 2013, Edogbanya et al., 2016); which is consistent with the results of other studies (Anastasakis 2009, Shan et al., 2017). In the acidic range, the powder surfaces acquire new active sites; due to the increased H⁺ ions and increased removal efficiency due to the electrostatic attraction between nitrate and phosphate ions and the active surface sites of the powder (Krishnan and Haridas 2008, Ismail 2012, Robalds et al., 2016).

3. Regeneration of plant leaves powder in wastewater treatment

The results show that the percentage of phosphate and nitrate removal using olive powder decreased by 7–21%, respectively, after two consecutive treatment cycles compared to the initial value (cycle 0). The percentage of removal decreased by more than 38% after the third treatment cycle (Table 3). Similarly, the percentage of removing by pine powder decreased by 8–21% and 7–29%, respectively, after two consecutive treatment cycles



compared to the initial value (cycle 0), and reached more than 73% after the third treatment cycle.

These results were not consistent with other studies; the phosphate removal efficiency from aqueous solutions decreased by 13.63% and 14.25%, respectively, after the third cycle using NaOH (0.2 M) as a regeneration solution for plant powders modified with layered double oxides (Mg-Al) (Zhang *et al.*, 2019). Amine-modified bagasse powders can be used for at least three consecutive cycles with a slight decrease in nitrate and phosphate removal efficiency (Ren *et al.*, 2016).

Table 3. Efficiency of plant leaf powder in removing nitrate and phosphate after three consecutive cycles.

testes	Crude WW	(Olive powder) treatment cycles			
		0	1	2	3
NO ₃ -N, mg/L	26.3	15.23	15.99	18.75	19.46
Uptake %		42.1	39.2	34.5	26.0
Phosphate, mg/L	29.2	18.63	19.41	20.82	25.14
Uptake %		36.4	33.4	28.7	13.9

testes	Crude WW	(Pine powder) treatment cycles			
		0	1	2	3
NO ₃ -N, mg/L	24.7	11.99	12.87	15.68	21.29
Uptake %		51.47	47.9	36.5	13.8
Phosphate, mg/L	26.5	15.93	16.77	18.10	23.93
Uptake %		39.9	36.7	31.7	9.7

The decreased removal efficiency after several cycles may be attributed to partial degradation of cellulose and hemicellulose under acidic or alkaline conditions, the decomposition of some surface functional groups or active adsorption sites (Ren *et al.*, 2016), the decrease in the amount of active



compounds at each treatment cycle, as well as external environmental factors (Zhang *et al.*, 2019), the accumulation of ions at the adsorption sites (Mohan and Sumitha 2008), and the disruption and damage to the structure of the plant powder (Kong *et al.*, 2015, Nag *et al.*, 2016).

4. Phytotoxicity

4.1 Phytotoxicity tests of treated domestic wastewater

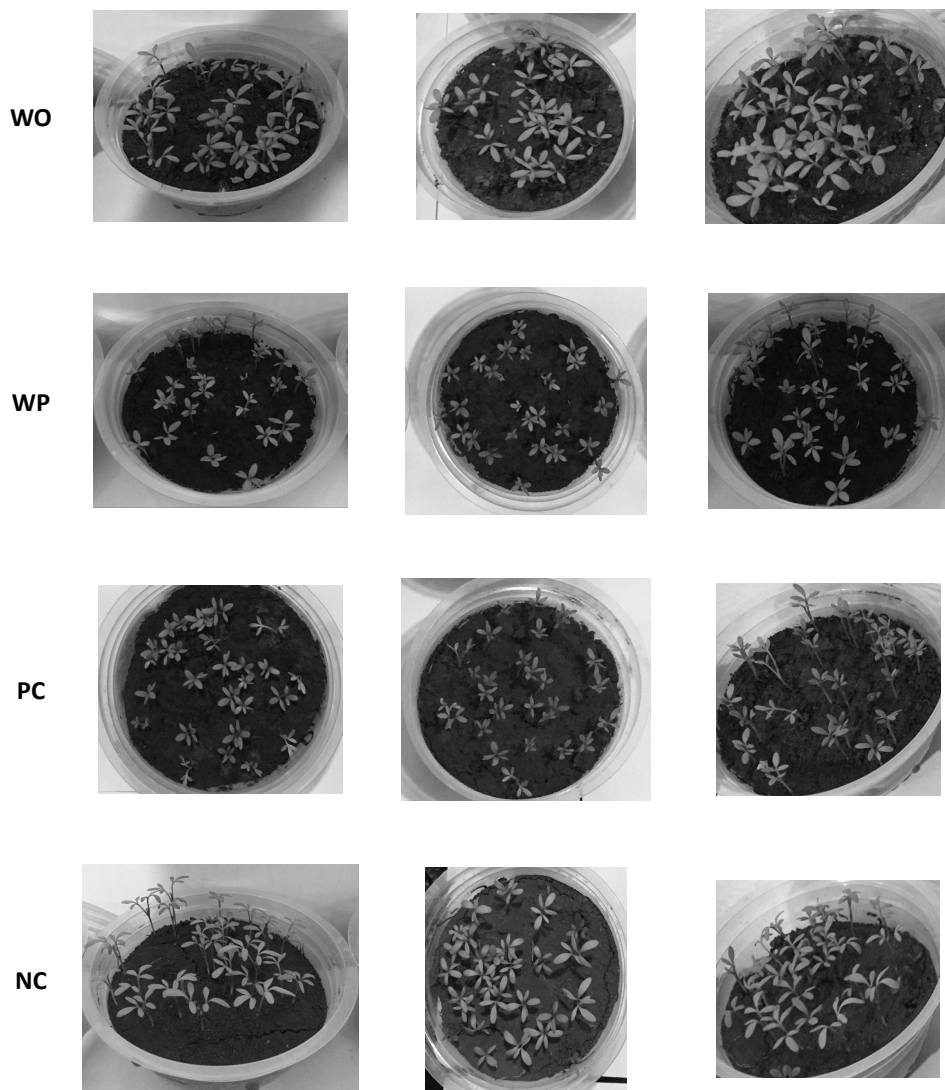
The results of Table 4 show that the germination rate of cress seeds irrigated with domestic wastewater treated with olive and pine leaf powder was 96.67% on the tenth day. This is more than 6% higher than irrigation with raw wastewater, but is mostly like that of drinking water (Figure 2).

Table 4. Phytotoxicity testing of treated wastewater and used plant powders.

Phytotoxicity of treated domestic wastewater						
treated water	After 4 days		After 7 days		after 10 days	
	Seed №	GR%	Seed №	GR%	Seed №	GR%
<i>Olea</i> powder	22	73.33	26	86.67	29	96.67
<i>Pinus</i> powder	22	73.33	28	93.33	29	96.67
Row wastewater	20	66.67	26	86.67	27	90.00
Drinking water	26	86.67	28	93.33	29	96.67
Phytotoxicity of spent plant powders after 3 days						
GR%	<i>Olea</i>	<i>Pinus</i>	Soil			
	86.67	80.00	96.67			

Seed №: Number of germinated seeds/ GR%: germination rate %

The delayed seed germination in treated water may be due to the presence of plant polyphenols. Higher concentrations inhibit or delay seed germination, unlike lower concentrations.



WO: wastewater treated by Olive powder// WP: wastewater treated by Pine powder //
PC: Positive Control (drinking water)// NC: Nigative Control (wastewater nontreated)

Figure 2. Phytotoxicity tests of treated domestic wastewater.



A temperature between 20-30°C is one of the most important external factors affecting germination. The germination rate of cress irrigated with water from different sources exceeded 90% (Bansal 2015). Research has shown that the best rate is achieved when irrigated with drinking water of moderate concentrations. Raw domestic wastewater has a negative effect due to its salinity and harmful substances (Nana *et al.*, 2019).

4.2 Phytotoxicity tests of spent plant powders

The results confirm that the use of spent plant powders is safe, as they are non-toxic due to their biodegradable nature (Zucconi and Bertoldi 1981). The germination rate using olive powder and pine powder reached 86.67% and 80%, respectively, after 3 days (Table 4), which is more than what others reported (Robalds *et al.*, 2016). Spent powders can be added as a soil quality improver; they increase permeability and water holding capacity, organic matter content, and are a source of nitrate and phosphate (Buenaño *et al.*, 2019).

Accordingly, the importance of using unmodified plant leaf powder in treating domestic wastewater can be emphasized. It is also environmentally friendly, low-cost, and reusable, which increases economic feasibility and achieves sustainable environmental development goals when it is needed.

Conclusions

1. The process of removing pollutants from water occurs quickly, depending on the type of pollutant, compared to other treatment methods such as plant treatment units or stability ponds, which require longer processing times. The odor of the treated wastewater is acceptable. Even if the BOD values of the treated wastewater do not decrease, it is considered non-toxic.



2. Spent olive and pine leaf powders can be used for two consecutive cycles to remove nitrate and phosphate from domestic wastewater with an acceptable reduction in removal efficiency. The plant powder remains free from damage, tearing, or destruction after two reused cycles.
3. Wastewater treated with leaf powders can be reused for irrigation without any toxic effects or inhibition of germination rates, based on phytotoxicity tests.

Recommendations

1. Develop the wastewater sector, find solutions to mitigate environmental and health damage, treatment of pollution, conserve water and agricultural land, and raise farmers' awareness of the dangers of irrigating crops with wastewater.
2. Improve the performance and efficiency of plant leaf powder in treating domestic wastewater, and develop low-cost methods for regenerating plant powders and increasing the economic feasibility of the treatment process.
3. Determine the toxicity of the plant leaf powder sludge produced after treatment and its potential for various uses.
4. Determine safe or long-term disposal methods.
5. Determine the efficiency of plant powders in removing heavy metal ions, chemical dyes, pharmaceutical compounds, and chemical pesticides from industrial and agricultural wastewater.
6. Determine the optimal conditions for achieving maximum efficiency in treating various types of wastewater for each plant species.



7. Use carefully studied mixtures of local plant waste powders in water treatment, eliminating specific pollutants, isolating its natural products (chemical compounds), and developing medical and pharmaceutical products.

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