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Application of *Pistacia atlantica* Leaves Powder as Natural Material To Remove Nitrate and Phosphate Ions From Domestic Wastewater by Characterization, Bio-removal, and Phytotoxicity Studies

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Abstract: Description of *Pistacia* leaves powder using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), Energy-dispersive X-ray spectroscopy (EDX), specific surface area according to nitrogen adsorption (S_{BET}) and methylene Blue (MB), and point of zero charge determination (pHpzc). A series of batch adsorption tests were conducted to study effect of various factors (plant powders dose, contact time, temperature, pH) on the percentage of nitrate and phosphate removal from domestic wastewater. The adsorption kinetics, regeneration ability test of plant powder, and phytotoxicity tests for treated water and spent powder on germination were studied. Results of S_{BET} analysis showed that *Pistacia* leaves powders have a low surface area and microscopic pores, SEM images revealed rough surfaces with uneven cavities, EDX analysis showed that there are high percentages of carbon and oxygen, good percentages for nitrogen, and few percentages of potassium, calcium, magnesium, phosphorous, sulfur and chlorine, and FTIR analysis showed that there are more than five distinct absorption peaks. The maximum value of nitrate and phosphate removal was 76.47% and 52.20%, respectively, at powder dose of 2 g/L, temperature 25 °C, and pH 5, and the percentage of nitrate and phosphate removal increased with increasing contact time until equilibrium was reached after 120 min for nitrate and 180 min for phosphate, and It was found that adsorption of ions follows kinetics of reaction from pseudo-second-order model, and powders can be Regeneration and used for two successive cycles with a slight decrease in removal efficiency. Germination tests on *Lepidium sativum* indicate no phytotoxicity. That is, *Pistacia* leaves powder is one of the natural products that are effective in removing nitrate and phosphate from domestic wastewater.

Keywords: Domestic Wastewater, *Pistacia* Leaves, Nitrate, Phosphate, Adsorption Kinetics, Regeneration Study, Phytotoxicity.

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INTRODUCTION

Unquantifiable quantities of domestic, agricultural and industrial wastewater are discharged untreated into natural water systems; Which causes serious environmental problems in many countries of the world. These wastes contain heavy metals, dyes, inorganic anions and harmful chemicals, some of which are stable and indissoluble, in addition to environmental movement and bioaccumulation in the food chain, beside their toxicity and health risks to humans and animals at very low concentrations (1). Nitrate and phosphate concentrations above 0.5-1 mg/L in water bodies lead to the emergence of eutrophication phenomenon, excessive growth of algae and consumption of dissolved oxygen, which leads to damage in water systems and deterioration of water quality (2), and leaching of nitrate and phosphate lead to pollution of groundwater and water bodies. Increased nitrate cause miscarriages and reduced milk production, and a high concentrations (> 300 mg/L) in water or plants irrigated with wastewater lead to an acute toxicity to livestock and the occurrence of diseases, and cause Methemoglobinemia syndrome and cancer.

Several chemical, physical and biological techniques have been used to remove nitrate and phosphate, such as biological de-nitrification, electrocoagulation, reverse osmosis, electro-dialysis, ion exchange, and chemical precipitation by aluminium sulfate and iron sulfate, and membrane technology (7), but these technologies are expensive, requires additional operation and maintenance costs, and produces large amounts of toxic sludge. Therefore, use of plant waste as an adsorbent in wastewater treatment is an effective and inexpensive alternative, as it is highly efficient, environmentally friendly and renewable (3), such as removing heavy metals (4), and chemical ions (5, 6) from water. More than 90% of phosphate was removed from aqueous solutions using *Phoenix dactylifera* powder at 120 min, 18 °C, pH 5, a shaking rate of 200 rpm, and an initial phosphate concentration of 50 mg/L (8), 80% removal of nitrate using Banana peels powder during 30 minutes, and an initial concentration of nitrate 200 mg/L (9), and removing nitrate between 96-100% and phosphates between 56-77% using leaves powders of three plant species (10).

Biosorption mechanisms include by plant waste powders different mechanisms such as Electrostatic Attraction, Van der Waals Attraction, Ion Exchange, Complexation, and Covalent Binding (11). The effectiveness of biosorption depends on the physical and chemical properties of plant waste, in addition to the initial concentration of the contaminated, plant powder dose, contact time, pH, and temperature (12).

The aim of this study was to characterize *Pistacia atlantica* leaves powder using different techniques (SEM, EDX, FT-IR, SBET, and pHpzc), and to evaluate its efficiency as low-cost, available and environmentally friendly materials in removing nitrate and phosphate from domestic wastewater by studying the effect of various factors (plant powders dose, contact time, temperature, and pH), and test its regeneration ability, and to test the reusability of this water through phytotoxicity studies in order to reduce negative impacts on the environment and achieve sustainable development and economic feasibility.

MATERIALS AND METHODS

Preparation of Plant Powder

Leaves of *Pistacia atlantica* were collected from Sweida city in Syria, and washed with distilled water 2-3 times, and dried in shade for two weeks until the weight was stable, then they were ground and sieved with 250 µm mesh, and stored in sterilized glassware in the refrigerator at 4 °C until use without any treatment. The symbol *Pa*-LP is used to indicate *Pistacia atlantica* leaves powder.

Collection of Domestic Wastewater Samples

Samples were collected from the end point in Jaramana city (east of Damascus) in polyethylene glass bottles with a capacity of 1000 mL. The glassware was washed with hydrochloric acid and then distilled water 3 times to remove acid residues (13). The values of some physical, chemical and microbiological characteristics of domestic wastewater samples used in the experiments are shown in Table 1.

Table 1: Minimum and maximum values for the characteristics of domestic wastewater samples used in experiments.

Tests	Untreated domestic wastewater	Units
Temperature	21.32 – 23.43	°C
pH	7.20 – 7.62	-
Total Dissolved Solids	571 - 606	mg/L
Electrical Conductivity	823 – 949.66	µS/cm
Turbidity	169 – 189	NTU
Nitrate	30.5 – 40	mg/L
Phosphate	42 - 50	mg/L
Biochemical Oxygen Demand	195 – 250	mg/L
Viable Count Bacteria	276×10^5 - 322×10^5	CFU/mL
Total Coliform	224×10^4 – 295×10^4	CFU/mL

Characterization of Prepared Plant Powder

Point of zero charge determination (pHpzc)

Mass titrations method was adopted by taking 0.02-6 g of powder and adding 20 mL of 0.03 M KNO_3 solution to it for 24 hours at a temperature of 25 °C and a shaking speed of 250 rpm (14). The pHpzc value is the balanced pH versus amount of plant powder.

Determination of specific surface area (S_{BET}) of plant powders by nitrogen adsorption

The specific surface area and porosity of the plant powders used in experiments were measured by Micromeritics Gemini 3 device according to Brunauer, Emmett and Teller (BET) for adsorption of nitrogen (N2).

Determination of surface area of plant powders by methylene blue adsorption

Methylene blue dye adsorption was studied by preparing a series of concentrations from its aqueous solution 0 to 5.82 mg/L (Figure 1), 0.05 g of powder was taken and 10 mL of dye solution was added at a concentration of 93.75 mg/L at room temperature for 150 minutes at a shaking speed of 160 cycle/min and pH 8, then the solutions were centrifuged at 4500 rpm for 15 minutes. The absorbance was measured by a UV/VIS spectrophotometer (Model: Optizen 2120UV PLUS) at the maximum wavelength λmax = 665 nm. The concentration of each solution at equilibrium C_e (mg/L) was calculated from the calibration curve, adsorbate amount of the dye was calculated according to the following relationship (15):

$$a = V(C_0\text{-}C_e)/W \text{ .... (Eq. 1)}$$

Where C_0 is the initial concentration of the dye (mg/L), C_e is the equilibrium concentration of the solution (mg/L), V is the total volume of solution (L), W is the weight of *Pa*-LP (g).

The percentage removal R (%) was calculated from the following relationship (15):

$$R = C_0\text{-}C_e/\ C_0 \times 100 \text{ .... (Eq. 2)}$$

The surface area of the powders was calculated by applying the relationship:

$$S = qe \times Na \times 130\times10^{-20} \text{ .... (Eq. 3)}$$

Note that the cross-sectional area of methylene blue is 130×10^{-20}, $Na = 6.022\times10^{23}$.

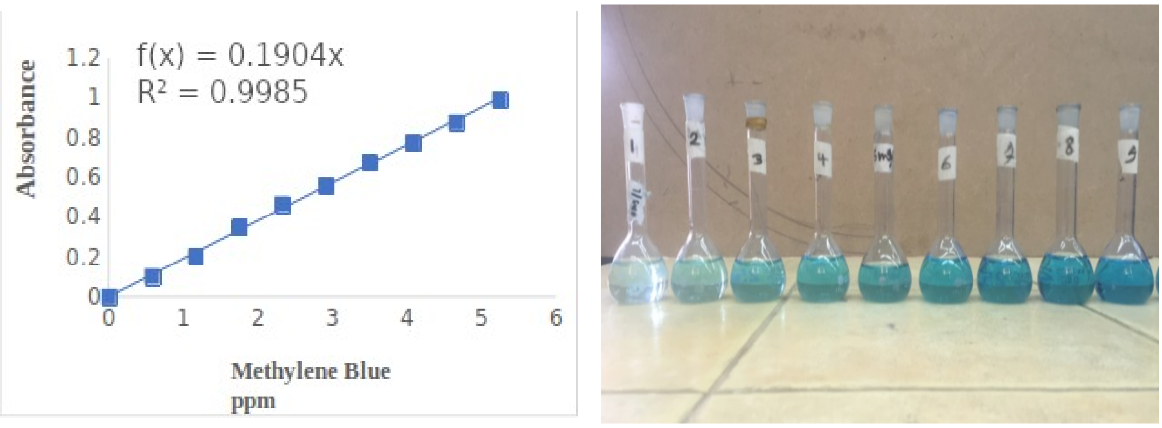


Figure 1: Absorbance of a methylene blue solution as a function of concentration.

Characterization of the prepared plant powder using a scanning electron microscopy (SEM)

A VEGA\\XMU electron microscope was used, which is connected to an X-ray analysis unit (EDAX-AMETEX), the sample was dried and then placed on a piece of special carbon adhesive, and attached to an aluminum holder, then inserted into the sample chamber of the microscope, and a high vacuum system was applied to it 10^{-3} mbar and 30 KV high voltage for chemical composition analysis of samples and determining pore dimensions (8).

Fourier transform infrared (FT-IR) analysis

The FT-IR spectrum was recorded with a Nicolet 6700 device to determine the effective functional groups of plant powders by taking 1 ± 0.1 mg of the sample with 100 ± 0.1 mg of dry potassium bromide (KBr) by placing the mixture between two metal discs and by pressing the material turned into a coherent transparent disc within a special holder, which was placed in FT-IR spectroscopy device disk (16). The conditions used were: Number of sample scans: 32, Number of scan points: 8480, spectral range: 400 - 4000 cm^{-1}, and Laser frequency: 15798.3 cm^{-1}.

Experiments of Domestic Wastewater Treatment by *Pistacia atlantica* Leaves Powder

A series of experiments were conducted to study the effect of plant powders dose, contact time, temperature, and pH to removing nitrate and phosphate from domestic wastewater (Table 2) by batch adsorption method with some modification (6, 17)**.** 250 mL Erlenmeyer flasks each with 100 mL of domestic wastewater with a laboratory shaker at 150 rpm, the water was filtered after treatment with Whatman (No.45). The concentrations of nitrate and phosphate were measured after treatment according to standard methods using a HACH DR 5000 spectrophotometer (13). The wastewater pH values were adjusted using HCl (0.1 M) and NaOH (0.1 M), and all experiments were performed with triplicates, and the removal efficiency was calculated by the following equation (17):

$$\% \text{ Removal} = C_i\text{–}C_f/C_i \times 100 \text{ .... (Eq. 4)}$$

Where C_i, C_f are the initial and final concentration of nitrate and phosphate in domestic wastewater.

Regeneration Ability Test of Plants Powder

This experiment was conducted to evaluate the efficiency of plant powder prepared for reuse three successive cycles after the first cycle for removing nitrate and phosphate from domestic wastewater**.** It was placed according to the optimal conditions (Table 2) in 100 mL of domestic wastewater, then the efficiency of removing nitrate and phosphate from the water was determined (Cycle 0), the samples were dried in shade at room temperature and placed in 100 mL of NaOH solution (0.5 M) for 180 minutes at a temperature of 25 °C depending on the ion exchange mechanism, then washed with distilled water, the dried samples were applied in the next treatment process and the removal efficiency was calculated from the water (cycle 1), the treatment process was repeated through three successive cycles, and all experiments were performed with three replications (17, 18).

Table 2: A series of experiments for removing nitrate and phosphate from domestic wastewater by using *Pa*-LP.

Series of Experiments	Powder Dose (g/L)	Contact Time (min)	Temperature (°C)	pH	Nitrate concentration in wastewater (mg/L)	Phosphate concentration in wastewater (mg/L)
Effect of Powder Dose	0.5-4	300	27	6.5	32.56	42
Effect of Contact Time	2	30-240	27	6.5	31.53	44
Effect of Temperature	2	120 (NO_3) 180 (PO_4^{-3})	15-35	6.5	30.5	42.5
Effect of pH	2	120 (NO_3) 180 (PO_4^{-3})	25	5-9	36.56	49.8
Powder Regeneration	2	120 (NO_3) 180 (PO_4^{-3})	25	6	40	50

Phytotoxicity Tests For Treated Domestic Wastewater and Spent Plant Powders

The germination percentage of *Lepidium sativum* seeds was studied to determine the phytotoxicity of the treated domestic wastewater and spent plant powders after the treatment process as follows (19):

(1) Determination of the phytotoxicity of treated water: 40 g of sterile soil moistened with treated domestic wastewater (30 mL) was placed in 50 g plastic plates and 30 seeds of *Lepidium sativum* were planted in each plate. Drinking water was used as a positive control and untreated domestic wastewater as a negative control, then the plates were placed at 25°C and alternated between dark and light (10 h dark vs 14 h light) for 10 days.

(2) Determination of the phytotoxicity of spent plant powders after the treatment process: the plant powders were dried in the shade for 14 days until the weight was relatively stable. 20 g of the dried powders were placed in 50 g plastic plates, 30 seeds of *Lepidium sativum* were planted in them and irrigated with distilled water (30 mL), and soil was used as a positive control, then the plastic plates were placed for 3 days at a temperature of 25°C alternated between dark and light (10 h dark vs 14 h light). All experiments were performed with three replications and the percentages of germination for all experiments were calculated using the following equation (19):

Germination percentage of *Lepidium sativum* seeds (%) = number of germinated seeds / total number of seeds x 100 .... (Eq. 5).

Study of Adsorption Kinetics

The adsorption kinetics of nitrate and phosphate ions were studied according to the pseudo-first order and pseudo-second-order model, which are known in batch systems.

The pseudo-first-order model is expressed by the following equation (20):

$$\ln (a_e - a) = \ln a_{1e} - k_1 t \text{ .... (Eq. 6)}$$

Where a_1: the reaction rate constant of pseudo-first-order (min^{-1}), a_{1e}: the adsorbate amount (mg/g); [calculated by graph from the slope and secant value when plotting ln (a_e-a) versus t], a_e and a (mg/g) are the amount of nitrate and phosphate sorbed at equilibrium and time, respectively.

The pseudo-second-order model is expressed by the following equation (20):

$$t/a = 1/k_2(a_{2e})2 + (1/a_{2e})\, t \text{ .... (Eq. 7)}$$

Where k_2: the reaction rate constant of pseudo-second-order (g/mg min), a_{2e}: the adsorbate amount (mg/g); [calculated by graph from the slope and secant value when plotting t/a versus t], a: the amount of nitrate and phosphate sorbed during time.

The adsorbate amount of nitrate and phosphate was calculated according to the following relationship (20):

$$a = V(C_0 - C_e)/W \text{ .... (Eq. 8)}$$

Where C_0 is the initial concentration of the dye (mg/L), Ce is the equilibrium concentration of the solution (mg/L), V is the total volume of solution (L), W is the weight of the *Pa*-LP (g).

Statistical Study

Correlation relationships were studied based on Pearson's correlation coefficients using Correlation

Matrix (SPSS22 software), and one-way analysis of variance (ANOVA) was adopted to test the presence of significant differences. Data was considered statistically significant at minimum level of $P < 0.05$.

RESULTS AND DISCUSSION

Point of zero charge determination (pHpzc) of *Pa*-LP

The pHpzc of the used powder was set at 6.97 (Figure. 2); which indicates that the charge is negative on the surface of the powder at pH higher than 6.97 and positive at pH less than 6.97. The pHpzc value of the used powder corresponded to that of *Salix babylonica* and *Platanus* leaves powder 6.98 and 7.00, respectively (21, 22), and was less than that of coconut pulp fiber 8.2 (23), and higher than olive stones and Grape Stalks residues 4.2 and 5.2, respectively (14). The pHpzc is an essential property in determining the electrical neutrality of materials used in treatment at a certain pH value, in addition to its effect on the ionization of surface functional groups and understanding the mechanism of the effect of pH of solution.

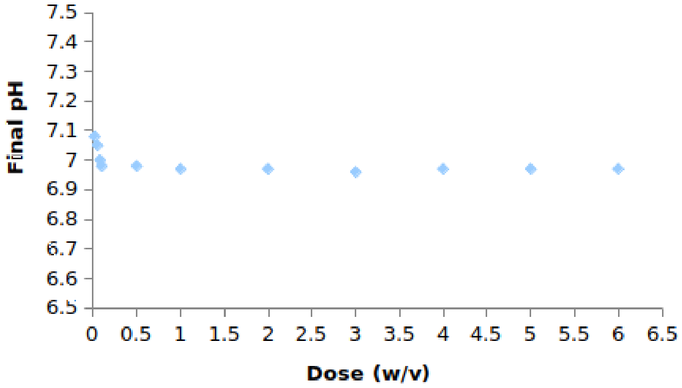


Figure 2: The pHpzc of *Pa*-LP.

Determination of the surface area of plant powder according to nitrogen adsorption (S_{BET}) and methylene blue (MB)

The specific surface area of *Pa*-LP was 1.1 ± 0.05 m^2/g according to BET, and total pore volume was $0.030\times10^{-2}\pm0.02$ cm^3/g, and the average pore radius was 0.543 ±0.27 nm; That is, the plant powder has micropores (24). This is consistent with several studies that indicated a decrease in the specific surface area and pore size of plant powder that was not treated physically or chemically. The specific surface area ranged between 0.48-10.9 m^2/g (25, 26, 27, 28, 29), and the total pore size ranged between 0.0002-0.11 cm^3/g (30, 31, 32, 33, 34), and the average pore diameter of *Pistacia vera* shell powder was 0.77 nm (30) and *Prunus dulcis* leaves 3.713 nm (33) and *Arundo donaxn* plant 4.320 nm (18).

The surface area of the studied powders according to adsorption of methylene blue was 40.283 ± 0.09 m^2/g, which confirms the adsorption efficiency (Figure 3); The percentage removal of methylene blue was 97.695% ± 0.23 and the adsorption capacity (qe) was 18.317 ± 0.04 mg/g; this may be attributed to the electrostatic attraction forces between dye (+) and sites (-) on the surface of the powder because the specific surface area of the leave powder is low and the ability of the surface functional groups is greater in removing contaminants (35).

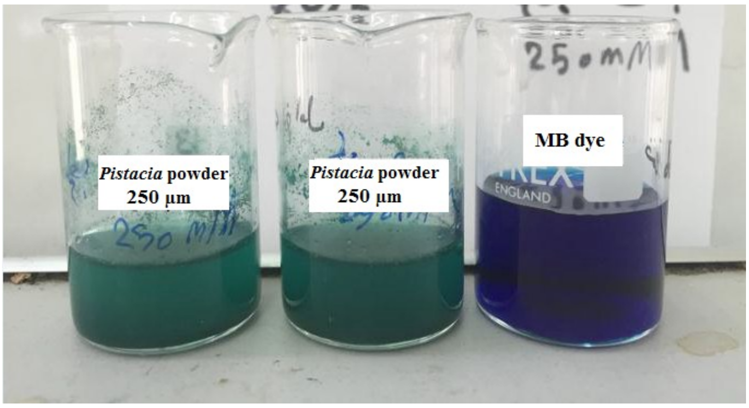


Figure 3: Efficiency of *Pa*-LP in removing methylene blue dye.

The adsorption capacity of methylene blue for oak leaves powder was 33.5 mg/g (15), and for orange and banana peels 18.6 and 20.8 mg/g, respectively (36). The surface area according to adsorption of methylene blue to chemically modified tea leaves was 174.8 m^2/g, and *Aegle marmelos* trees leaves powder 110.21 m^2/g (37). It is noticed that the recorded surface area of the powders prepared according to the adsorption of methylene blue is higher than the surface area according to nitrogen adsorption.

Characterization of samples prepared using scanning electron microscopy (SEM)

The results showed that studied samples contain irregularly shaped structure with uneven cavities, heterogeneous rough surfaces and pores of different dimensions (Figure. 4). Researches has indicated that the irregular surface structure of the plant powder is effective in removing anions, cations and heavy metals from domestic wastewater (10, 38, 39).

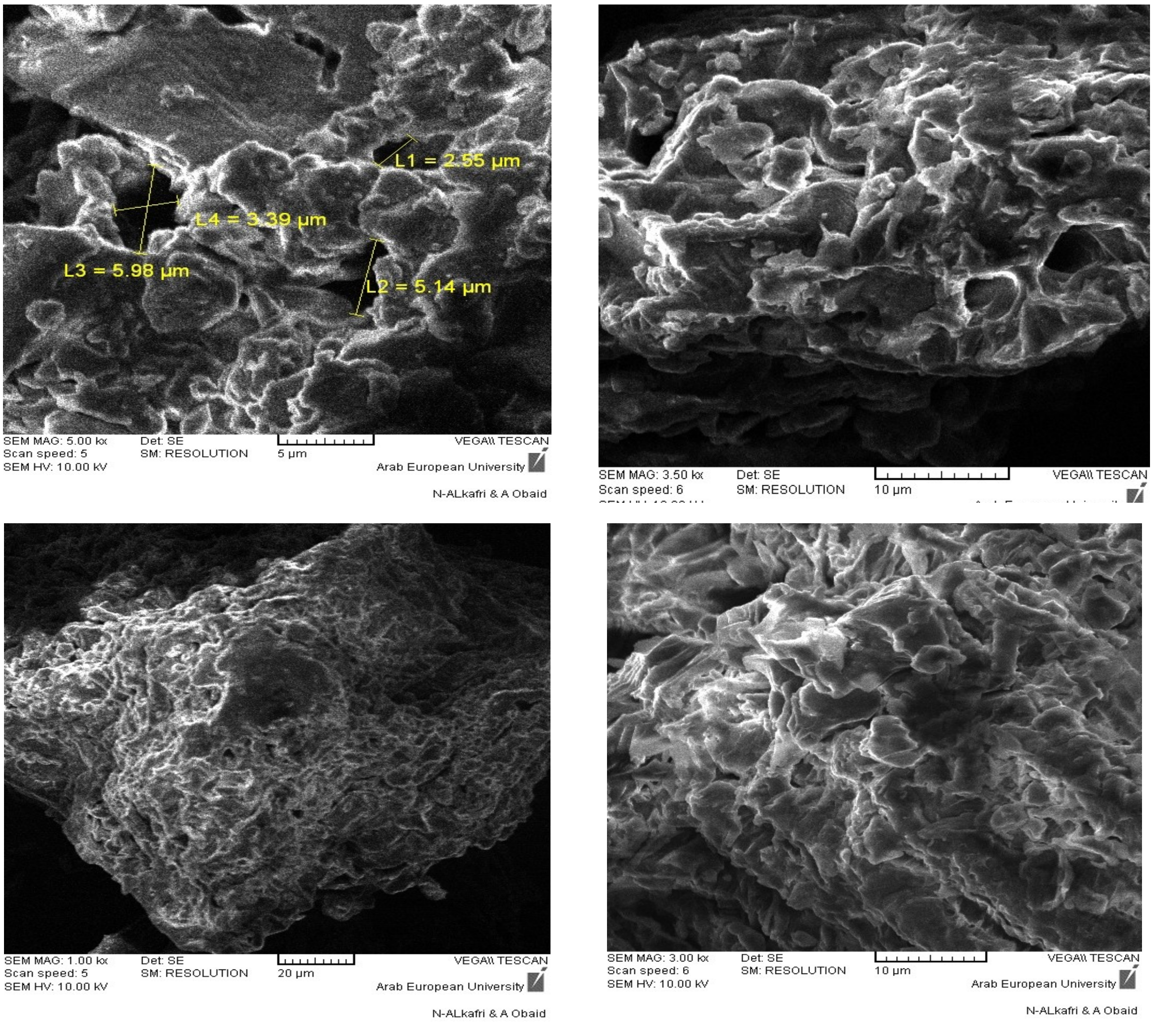


Figure 4: The surface structure of *Pa*-LP using SEM.

Characterization of samples prepared by Energy-dispersive X-ray spectroscopy (EDX)

EDX analysis showed the presence of high percentages of carbon and oxygen and good percentages for nitrogen. In addition to the presence of few percentages of potassium, calcium, magnesium, phosphorous, sulfur and chlorine (Figure 5), as all the previous elements are essential in *Pistacia atlantica*, and the appearance of silicon is due to the sample holder made of aluminum and dotted with silicon. It is noted that the weight percentages of same elements differ in several areas of the surface of samples (Table. 3). This indicates that the surfaces of samples are heterogeneous. These results are consistent with the researches that confirmed that plant powders contain high percentages of carbon and oxygen between 29.10-38.52% and 52.25-59.51%, respectively, and low rates between 0.22-3.72% for the rest of the elements such as potassium, calcium, magnesium, sodium, chlorine, sulfur and iron (10). Plant leaves powders contain high percentages of carbon and oxygen between 31-51% and 31.4-64.1%, respectively, and nitrogen between 0.34-3.61%, this varies according to characteristics and composition of leaves for each plant species (40).

Table 3: The weight percentages % of elements analyzed by EDX in the studied areas on the surface of *Pa*-LP.

Elements (K)	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Average Percentages%**
C (K)	59.09	59.14	61.4	61.13	60.19
O (K)	30.64	34.88	27.7	27.55	30.1925
N (K)	5.77	3.88	10.62	6.77	6.76
K (K)	2.24	0.39	-	1.0	0.9075
Ca (K)	1.85	-	0.03	1.05	0.7325
P (K)	0.41	0.35	-	0.48	0.31
Mg (K)	-	0.84	0.08	0.56	0.37
S (K)	-	0.15	-	0.45	0.15
Cl (K)	-	0.15	-	0.4	0.1375
Si (K)	-	0.22	0.01	0.62	0.2125

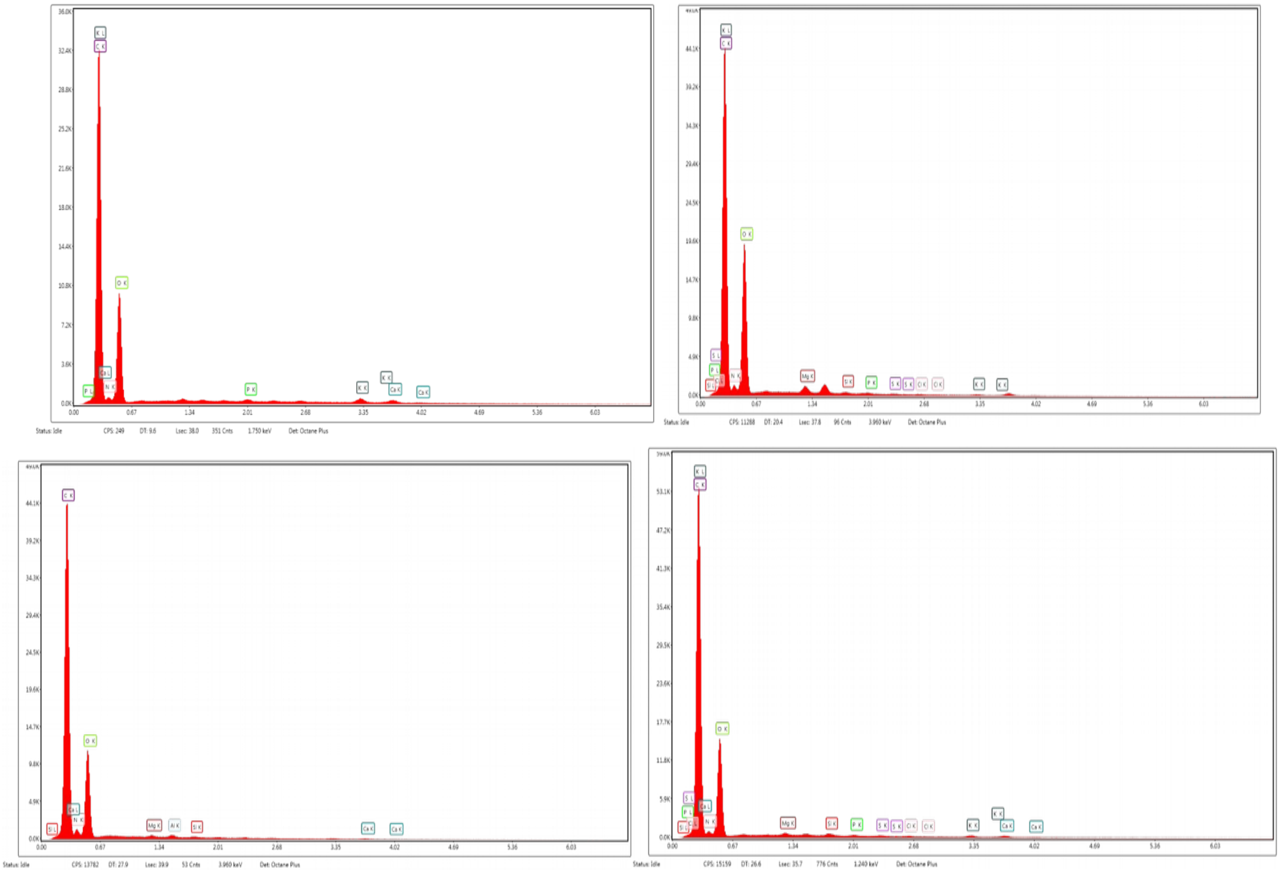


Figure 5: EDX spectra of studied areas on the surface of *Pa*-LP.

Fourier transform infrared (FT-IR) Spectroscopy of *Pa*-LP

More than five distinct absorption peaks are observed (Figure. 6); This confirms the complex nature of the studied samples (41). The broad and strong peak observed between 3000-3700 cm^{-1} is du to the stretching vibration of the hydroxyl (-OH) group of of alcoholic and phenolic compounds, and stretching vibration of the amine (-NH) group (42, 43). The peaks at 2920 and 2842 cm^{-1} are attributed to the asymmetric and symmetric stretching of the methylene (CH$_2$) and methyl (CH$_3$) groups (44, 45), and the peak corresponding to stretching vibrations –NH$_2^+$, –NH$^+$, and –NH groups appears in the range 2300-2800 cm^{-1} usually (46). The observed peak at 1700-1725 cm^{-1} is attributed to stretching of carbonyl (C=O) group of carboxylic acid or ketones (43, 47), and the peak at 1620 cm^{-1} appears for C=O bond stretch of the amide group, or N-H bond bend, or carbonyl group stretch of -COOH (41, 44, 48). The peak 1536 cm^{-1} belongs to carboxyl groups (44), and the peak 1454 cm^{-1} corresponds to the bending of C-H group that appears in the range 1445-1485 cm^{-1} (41), and the peak 1370 cm^{-1} is attributed to the bending of CH3 group or C–N stretching vibration, which corresponds to the amine groups (8, 47, 49). The peak corresponding to the stretching of P-O-C group appears in the range between 1190-1240 cm^{-1} (41), and the peak 1070 cm^{-1} is attributed to the stretching of C-O or C-N group of the primary amine group (41, 42, 48), while the peaks at 763 and 831 cm^{-1} are due to the bending of C-H group in the aromatic ring (47).

Some changes were observed on *Pistacia atlantica* powdered leaves after removal of nitrate and phosphate, such as a decrease in the intensity of the broad peak between 3000-3700 cm^{-1}, This is consistent with the research that indicated that changes in the intensity of the peak between 3400-3600 cm^{-1} after adsorption may be attributed to the complexation of ions with hydroxyl groups -OH, which confirms the important role of acidic groups (hydroxyl and carboxyl ions) in the complexation of ions and ion exchange process (44). The changes observed at the peak 2920 cm^{-1} indicate ion exchange between symmetric or asymmetric C-H protons of aliphatic acids (44), and the peak disappears at 2355 cm^{-1} indicating the possibility of adsorption of nitrate and phosphate onto the surface of the powder by the electrostatic attraction between NO_3^-, PO_4^{-3} and positively charged amine sites (50). It was observed that the intensity of the peak 1620 cm^{-1} became less extreme, and this agrees with many studies that confirmed the changes at the peak of 1620 cm^{-1} after adsorption of phosphate, lead (Pb^{+2}) and cadmium (Cd^{+2}) on lemon peel powders and mistletoe leaves from polluted water (48).

Changes in the range between 1500-1660 cm^{-1} may be due to electrostatic attraction forces between adsorption sites and adsorbate ions (44), it was observed that the peak intensity of 1225 cm^{-1} decreased, which may be attributed to an important role in the stretch of P-O-C group in adsorption of phosphate; The P-O-C group has a major role in adsorption of phosphate from aqueous solutions on *Phoenix dactylifera* fiber powders (8). FTIR spectroscopy analysis shows that the former surface functional groups present in *Pa*-LP are involved in adsorption and removal of contaminants from domestic wastewater by various mechanisms (eg. ion exchange, electrostatic attraction, surface complexity).

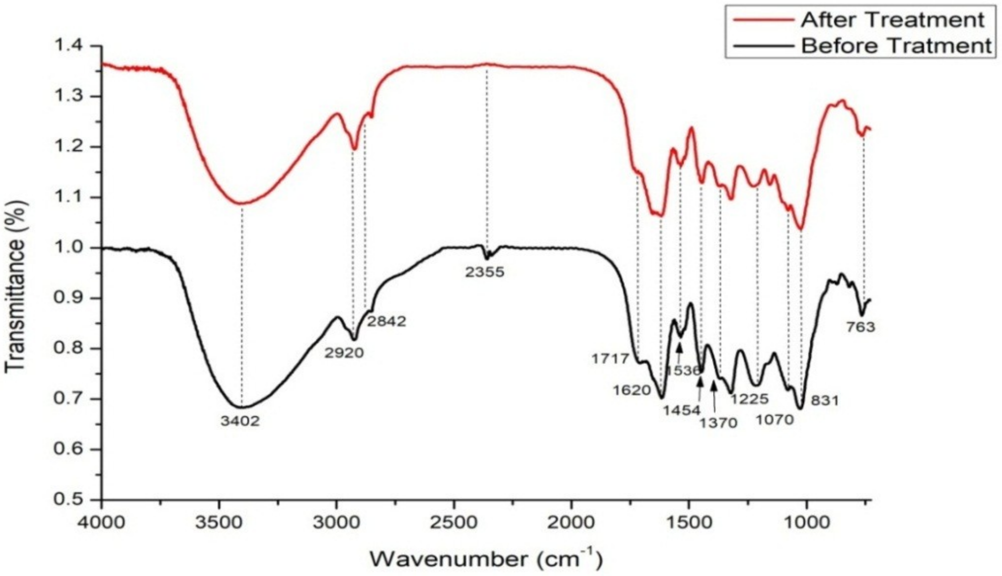


Figure 6: FT-IR spectroscopy of *Pa*-LP before and after domestic wastewater treatment.

Removing nitrate and phosphate from domestic wastewater using *Pa*-LP

Effect of Plant Powder Dose

Figure 7 shows that increasing *Pa*-LP dose from 0.5 to 3 g/L led to an increase in removal efficiency of nitrate from 26.28 to 64.37% and phosphate from 17.85 to 47.61%; This confirms the existence of a direct correlation between powder dose and efficiency of removal to a certain extent, and the adsorption capacity at 2 g/L was 10.28 mg/g for nitrate and 9.55 mg/g for phosphate which decreased to 6.98 mg/g for nitrate and 6.66 mg/g for phosphate when using 3 g/L, so 2 g/L of powder was used to complete the experiments. This is due to the fact that increase in powder dose leads to an increase in the surface area and available adsorption sites (50, 51), but the increase in powder dose over the optimum value leads to a decrease in removal efficiency due to the accumulation and agglomeration of powder particles (52). This is consistent with the results of researches on *Phoenix dactylifera* fibers and stones (8, 53), rice husk and citrus limetta residues (2), and tea leaves treated with N,N-dimethylformamide (50).

Effect of Contact Time

Figure 8 shows the increase in the efficiency of removing nitrate and phosphate with an increase in contact time to a certain extent until stability. The process of removing ions from water is carried out in two stages. The first is fast during 60 min at a rate of 60.67% for nitrate and 120 min at a rate of 42.72% for phosphate, then followed by a gradual increase in removal, but became slow until 120 min at a rate of 63.52% for nitrate and 180 min at a rate of 44.54% for phosphate and after that the removal is relatively constant, so the equilibrium time was adopted at 120 min for nitrate and 180 min for phosphate to complete the experiments, and the rapid removal is initially attributed to the abundance of active sites on the surface of powder, and the result of their gradual occupancy due to presence of repulsive forces between adsorbate ions on the surface of powder and other ions in the water, thus the adsorption becomes less efficient and slower until it reaches equilibrium (10). It has been observed that the removal efficiency of prepared powders depends on the type of ions because the removal of nitrate is higher than that of phosphate; This may be attributed to the specificity of the interactions between the ions and the active sites on the surface of the powder (39), in addition to the presence of many ions in domestic wastewater that may compete with phosphate ions for adsorption sites (54).

The equilibrium time required to remove nitrate and phosphate from water varied with the species of plant powder; As it was 120 min for plant powders with a removal efficiency of 96, 98, 100% when using 1 g/L (10), 25-30 min for wheat straw powder modified with amino groups (55), and the percentage of phosphate removal from wastewater was 89.1% after 120 min using 2 g/L of chemically modified rice husk powder (56). It was possible to remove about 80% of phosphate after 60 min of treatment using 1.5 g/L of lemon peel powder and pH 5.5 (48), and it was 94.68% after 120 min of treatment using 6 g/L of *Phoenix dactylifera* fiber powders, temperature of 18 °C, shaking speed of 200 rpm, and pH 5 (8), and this differs from the results of this research due to the difference in the species of plant powder and the conditions of the experiment.

Effect of Temperature

Figure 9 shows a gradual increase in the efficiency of removing nitrate from 54.75 to 63.50% and phosphate from 36.47 to 44.47% with increasing temperature from 15 to 25 °C. Several studies have indicated an increase in the removal efficiency of nitrate and phosphate with increasing temperature (16, 48, 57). Decreased removal efficiency is observed when the temperature is increased above 25°C; The efficiency of removing nitrate and phosphate decreased to 57.86% and 34.58%, respectively, when the temperature was increased to 35 °C, and this is in agreement with one of the studies that indicated a decrease in the phosphate removal efficiency from 75.5% to 55.2% when the temperature was increased from 30 to 40 °C (56). This is attributed to the exothermic adsorption process or to the weak adsorption forces between the active sites of the plant powders and the adsorbate ions. The adsorption capacity of various pollutants such as dyes, heavy metals, anions and cations increases with the increase in temperature, due to the rapid increase in the mobility of ions; This facilitates its diffusion from the solution to the active surface adsorption sites, in addition to a decrease in the viscosity of the solution, an increase in the number of active sites for the sample, and a decrease in the thickness of the boundary layer surrounding to the adsorbent material (sample); Which leads to a decrease in the mass transfer resistance of the adsorbates (ions) in the boundary layer at high temperatures and increases the adsorption efficiency, and this indicates that the adsorption process is endothermic (6, 58, 59). Other studies have indicated a decrease in the adsorption efficiency when the temperature is increased, due to the weak bonds between the adsorbate molecules and the active binding sites of the adsorbent, which indicates that the adsorption process is exothermic (60, 61). The adsorption capacity increases with an increase in temperature to a certain extent and then decreases with any additional increase in temperature (35), and a slight increase in the adsorption capacity was observed with increasing temperature and the removal was independent of temperature (62, 63).

Effect of pH

Figure 10 shows that the removal efficiency of nitrate gradually increases from 20.67 to 76.47% and phosphate from 13.05 to 52.20% with decreasing pH values from 9 to 5. This is consistent with several studies that indicated the best removal of nitrate and phosphate at low pH values (acidic medium); This is due to the fact that in the range of high pH values, the surfaces of the powders acquire more negative charges due to the increase of OH^-, which leads to a decrease in the removal efficiency due to the increase in the repulsive force between the nitrate and phosphate ions and the negatively charged OH^- ions, in addition to the possibility of competing OH^- ions with nitrate and phosphate for active adsorption sites, while at lower pH values, the surfaces of the powders acquire more positive charges due to the increase in H^+ ions leads to the increase in the removal efficiency due to the electrostatic attraction between the nitrate and phosphate ions and the active surface sites of the powder (19, 23, 52, 53, 56). It has been observed that adsorption of anionic pollutants often occurs at low pH values of the solution due to the large contribution of electrostatic attraction forces (40).

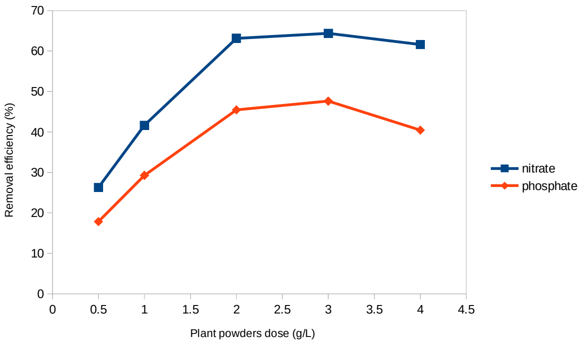


Figure 7: Effect of *Pa*-LP dose in removing nitrate and phosphate from domestic wastewater (Contact Time: 300 minutes, Temperature: 27 °C, pH 6.5, and Shaking Speed: 150 rpm.

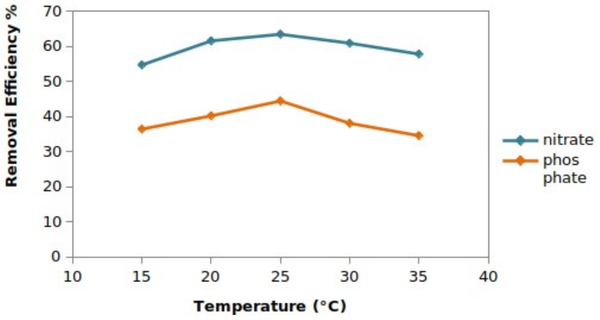


Figure 9: Effect of temperature in removing nitrate and phosphate from domestic wastewater (Powder Dose: 2 g/L, Contact Time: 120 minutes For Nitrate, 180 minutes For Phosphate, pH 6.5, and Shaking Speed: 150 rpm).

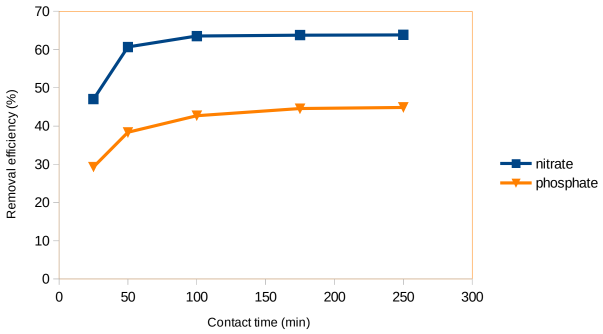


Figure 8: Effect of contact time in removing nitrate and phosphate from domestic wastewater (Powder Dose: 2 g/L, Temperature: 27 °C, pH 6.5, and Shaking Speed: 150 rpm).

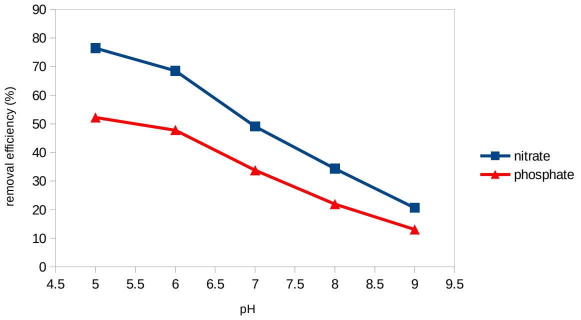


Figure 10: Effect of pH in removing nitrate and phosphate from domestic wastewater (Powder Dose: 2 g/L, Contact Time: 120 minutes For Nitrate, 180 minutes For Phosphate, Temperature: 25 °C, and Shaking Speed: 150 rpm).

Studying of regeneration of *Pa*-LP

The results showed that the percentage of nitrate and phosphate removal decreased by about 11% after two successive cycles of treatment compared to the initial value (cycle 0). While the percentage of removal was observed to decrease more than 30% after the third cycle of treatment (Table 4) compared to the initial value (cycle 0), so it is possible to use the powder of the spent leaves for two successive cycles in removing nitrate and phosphates from domestic wastewater with a slight decrease in removal efficiency. The results of this research did not agree with other research; The efficiency of removing phosphate 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 *Brassica pekinensis* and *Brassia campestris* L powders modified with layered double Mg-Al oxides (17). Amine cross-linked reed powders can be used for at least three successive cycles with a slight decrease in the efficiency of removing nitrate and phosphate (18).

The decrease efficiency of removing nitrate and phosphate after several successive cycles of treatment may be attributed to the following reasons: Loss of plant powder weight each cycle, partially due to the destruction of cellulose and hemicellulose at acidic or alkaline conditions (1), and Loss of some surface functional groups or active adsorption sites (18, 64), or due to the accumulation of ions in the adsorption sites (65) and the difficulty of their desorption at regeneration, in addition to the rupture and damage to the plant powder structure as a result of its regeneration by NaOH solution that was used In this research.

Several studies indicated a gradual decrease in the adsorption efficiency of various pollutants such as dyes and heavy metals with an increase in the number of regeneration cycles (22, 66), and other studies indicated increase in the adsorption efficiency after the first cycle (67) as a result of using HNO_3 solution in regeneration, it is an activator of adsorption sites.

Table 4: Average percentage of removal of nitrate and phosphate from domestic wastewater after three successive cycles using *Pa*-LP.

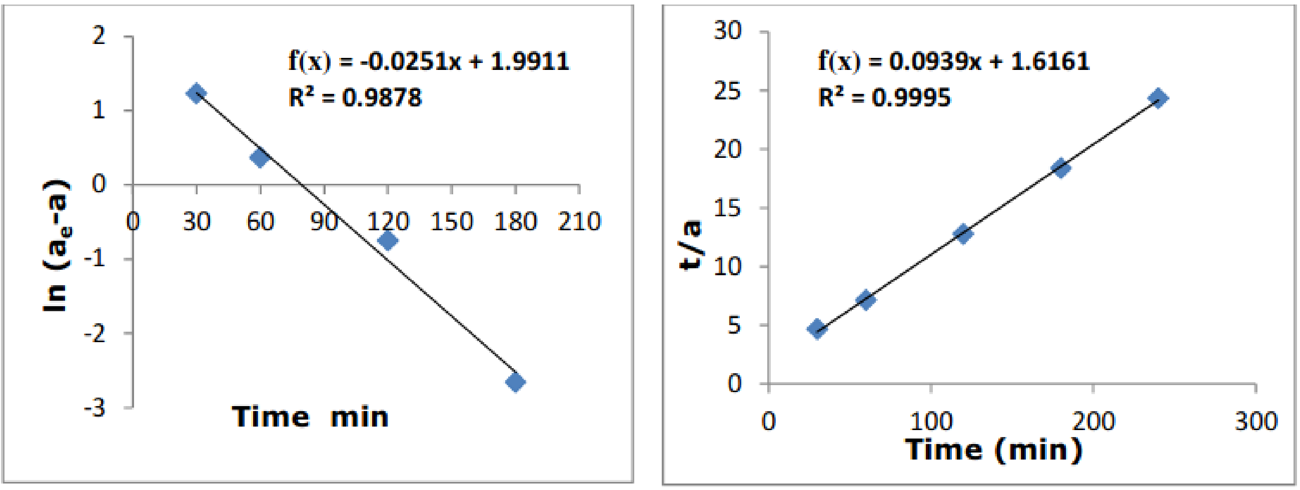
Tests	**Treatment Cycles (Adsorption Cycles)**			
	0	**1**	**2**	**3**
Nitrate	68.57%	66.50%	57.50%	38%
Phosphate	47.80%	43.74%	39%	15.4%

Study of adsorption kinetics

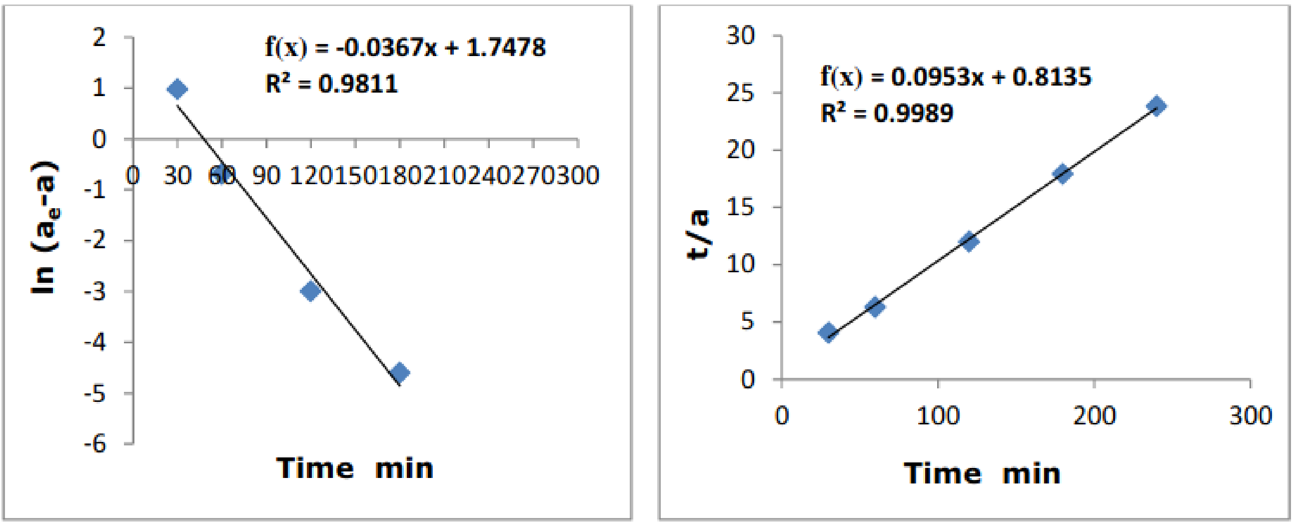
Table 5 and Figure 11 shows the adsorption kinetics parameters for the nitrate and phosphate ions according to the pseudo-first-order and pseudo-second-order equations. It was observed that a good agreement of the experimental data with the pseudo-second-order equation model, due to the convergence of the value of a_{2e} = 10.53 mg/g calculated graphically from its experimental value of a_e = 10.06 mg/g for nitrate, and the convergence of the value of a_{2e} = 10.75 mg/g calculated graphically from its experimental value of a_e = 9.87 mg/g for phosphate. In addition to that R^2 of pseudo-second-order equation (R^2 = 0.9989) for nitrate and (R^2 = 0.9995) for phosphate is higher than its value for the pseudo-first-order equation (R^2 = 0.9811) for nitrate and (R^2 = 0.9878) for phosphate. Therefore, the adsorption process of nitrate and phosphate on the *Pa*-LP follows a pseudo-second-order equation, this is in agreement with several studies that confirmed that adsorption of nitrate and phosphate on raw or modified plant powders follows a pseudo-second-order equation (17, 19, 50, 55, 68).

Table 5: Adsorption kinetics parameters for nitrate and phosphate ions.

Tests	**a_e** **(experimental)**	**Pseudo-first-order**			**Pseudo-second-order**		
		a_{1e} (***calculated***)	**K_1 1/min**	**R^2**	**a_{2e}** (***calculated***)	**K_2 g/(mg·min)**	**R^2**
Nitrate	10.06	5.737	0.036	0.9811	10.53	0.0111	0.9989
Phosphate	9.87	7.323	0.025	0.9878	10.75	0.00535	0.9995



Phosphate adsorption kinetics: (left) Pseudo-first-order model, (right) Pseudo-second-order model.



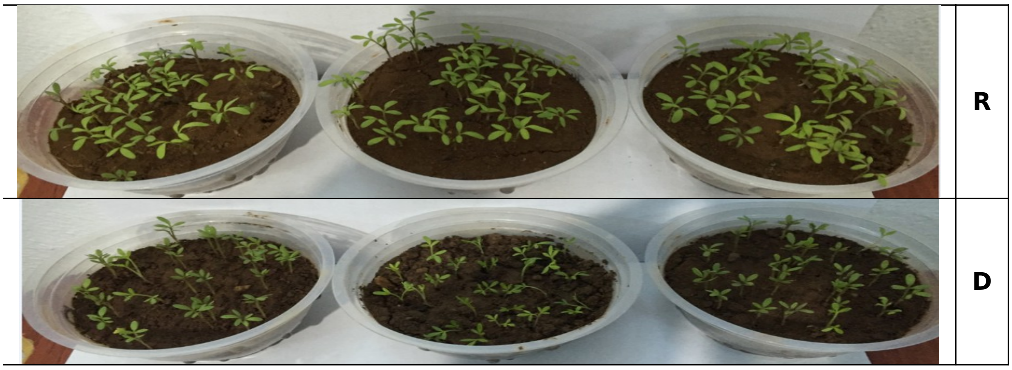
Nitrate adsorption kinetics: (left) Pseudo-first-order model, (right) Pseudo-second-order model.

Figure 11: Adsorption kinetics of nitrate and phosphate according to Pseudo-first-order model and Pseudo-second-order model.

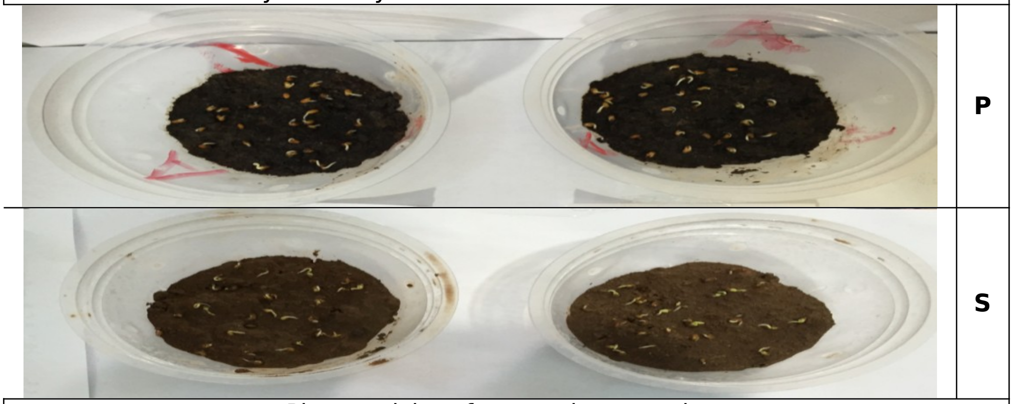
Phytotoxicity tests for treated domestic wastewater and spent plant powders

The test for the germination of *Lepidium sativum* seeds is an indicator for the rapid assessment of phytotoxicity. The percentage of germination of *Lepidium sativum* irrigated with treated domestic wastewater by Pistacia leaves powder after 10 days was 93.33%, higher than the percentage of germination of *Lepidium sativum* irrigated with untreated domestic wastewater, which was 88.86%, and reached 97.76% when irrigating seeds with drinking water. It was observed that the germination of seeds irrigated with untreated domestic wastewater and drinking water began to germinate after 3 days to reach stability after 7 days, but the germination percentage increased from 90% for seeds irrigated with treated wastewater after 7 days to 93.33% after 10 days (Figure 12). The delay in seed growth in treated water may be due to the presence of polyphenols released from *Pistacia* leaves; High concentrations lead to inhibition or delay of seed germination, unlike low concentrations (69). The temperature between 20-30 °C is one of the most important external factors affecting germination; As the percentage of *Lepidium sativum* germination irrigated with water from different sources (dams, wells, distilled, sewage) exceeded 90% (70), this is consistent with the results of the experiments of this research conducted at 25 °C, and the best percentage was when irrigated with drinking water; This may be due to the fact that it contains mineral elements and salts in moderate concentrations that can be easily absorbed by the seeds (71). The effect of raw domestic wastewater was bad due to the high concentration of salts, harmful substances and heavy metal ions such as chromium and cadmium, which have an inhibitory effect on seed germination (71, 72). These results confirm the possibility of reusing treated domestic wastewater for irrigation.

The spent *Pistacia* powders were non-toxic to *Lepidium sativum* plant due to their biodegradable nature and the germination percentage was 82.2% after three days. Spent plant powders can be added as a soil quality enhancer as it increases permeability, water holding capacity, organic matter content and is a source of soil nitrate and phosphate supply (19, 73, 74).



Phytotoxicity of treated domestic wastewater



Phytotoxicity of spent plant powders

R: untreated Domestic Wastewater (Negative Control), **D**: Drinking Water (Positive Control), **T**: Treated Domestic Wastewater, **P**: Spent *Pistacia* Powders, **S**: Soil (Control).

Figure 12. Phytotoxicity tests of treated domestic wastewater and spent plant powders.

Statistical Study

The statistical study confirmed the presence of a strong positive correlation between nitrate and phosphate $R^2 = 0.984$ for the effect of plant powder dose, $R^2 = 0.989$ for the effect of contact time, $R^2 = 0.995$ for the effect of temperature, and $R^2 = 0.998$ for the effect of pH value. The one-way analysis (ANOVA) test showed that there were significant differences between all the average values recorded for the tests after wastewater treatment at a significant $P < 0.05$.

CONCLUSION

This study indicated the possibility of using *Pa*-LP without prior treatment or modification as an environmentally friendly, low cost and good effective in removing nitrate and phosphate ions from domestic wastewater. The results of the SEM analysis which is connected to EDAX-AMETEX unit showed a heterogeneous, irregular, and rough surface structure with pores of different dimensions, in addition to containing a group of elements such as carbon, oxygen and nitrogen, potassium, calcium, magnesium, phosphorous, sulfur and chlorine. FTIR analysis showed the presence of hydroxyl, carboxylate, carbonyl, amine, and methylene groups.

The maximum removal of nitrate and phosphate was at powder dose of 2 g/L and temperature 25 °C. The equilibrium time was reached within 120 min for nitrate and 180 min for phosphate from exposure time, and the removal efficiency decreased significantly with increasing pH values from 5 to 9. The nitrate and phosphate adsorption kinetics follows the pseudo-second-order equation model. The regeneration studies showed the possibility of using *Pa*-LP in two successive cycles to remove nitrate and phosphate with a slight decrease in the removal efficiency compared to the initial value.

The results of the phytotoxicity study showed the possibility of using wastewater treated by *Pa*-LP in irrigating crops without any toxic effects on germination rates. The spent *Pa*-LP were non-toxic to plants and due to their biodegradable nature they could be added as soil enhancer in agricultural land.

The results of the present study show that the low cost and good removal efficiency of non-modified, non-toxic, and reusable *Pa*-LP make them potentially attractive natural materials for removing nitrate and phosphate from domestic wastewater. Further experiments should be conducted in future studies to test the adsorption dynamics of nitrate and phosphate onto *Pa*-LP in fixed-bed column experiments, and determining isotherms and thermodynamics of adsorption. In addition to expanding the scope of research and studying the possibility of removing heavy metals from wastewater using *Pa*-LP.

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