



Potential Usage of Granite Waste as Eco-Friendly and Non-Toxic Coagulant for Removal of Pathogenic Bacteria and Toxigenic Fungi from Untreated Wastewater

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Abstract The accumulation of the industrial granitic waste represents an environmental problem so in last decades some studies carried out to manage and utilize this waste in a different innovative application. One of the most valuable innovative applications for using such granitic waste is preliminary using it in the field of water and wastewater treatment as non-toxic coagulant for removal of pathogenic bacteria (*Total coliform* and *Escherichia Coli*), Toxigenic fungi (*Aspergillus flavous*, *Aspergillus niger*, *Aspergillus tubingensis* and *penicillium notatum*) and pathogenic fungi (*Candida galibrata*, *Candida albicans* and *saccharomyces cerviacae*) from untreated wastewater. Some laboratory techniques were carried out to investigate the characterization of the studied granite waste (XRD, XRF, FTIR and laser particle size). The microbiological analyses were conducted to prove the ability of the studied granite waste as an effective and non toxic coagulant in wastewater treatment. The obtained results ensure the capability of the granite waste in removing the studied pathogens that reached up to 99.99 %. The different concentrations of the used granite waste were applied to ensure its non toxic effect. Finally the used granite waste giving favorable results to be used as a natural, ecofriendly and non-toxic coagulant for removing pathogens from wastewater.

Keywords Granite wastes, wastewater treatment, Coagulation, Bacteria, Fungi

1. Introduction

In the last decades, vast amount of the stone waste particularly dimensional stone waste such as marble and granite were increased and caused great environmental pollution which may be leads to huge disaster. Granite waste generated by the stone crushing industry has accumulated over years and the reduction in waste generation by manufacturing value-added products from the granite stone waste will boost up the economy of the granite stone industry [1]. Additionally, many searches have been carried out for the purpose of using the validity of different stone cutting wastes in the application of wastewater treatment. Furthermore, in the current search an innovative application for using granitic waste as low cost eco-friendly and non-toxic coagulant for removal of pathogenic bacteria and toxigenic fungi from untreated wastewater.

Coagulation and flocculation are two important processes in water and wastewater treatment. In brief coagulation can be defined as the process whereby destabilization of a given colloidal suspension. In a colloidal suspension, particles will settle very slowly or not at all because the colloidal particles carry surface electrical charges that mutually repel each other. This surface charge is most commonly evaluated in terms of zeta potential, the electrical potential at the slipping plane. To induce coagulation, a coagulant (typically a metallic salt) with the opposite charge is added to the water to overcome the repulsive charge and "destabilize" the suspension. For



example, the colloidal particles are negatively charged and alum is added as a coagulant to create positively charged ions. Once the repulsive charges have been neutralized (since opposite charges attract), van der Waals force will cause the particles to cling together (agglomerate) and form micro floc. [2] The second sub-process, termed flocculation, refers to the induction of destabilized particles in order to come together, to make contact and thereby, to form large agglomerates, which can be separated easier usually through gravity settling [3].

Domestic and industrial wastewater discharges contain material may comprise suspended, dissolved organic and inorganic matter, as well as several biological organisms, such as bacteria, algae, fungi or viruses. This material has to be removed, as it causes deterioration of water quality by reducing the clarity (e.g. causing turbidity or colour), causing infection and eventually carrying toxic compounds, adsorbed on their surfaces. In addition, organic matter is the main precursor to the formation of disinfection by-products, when chlorine is applied as disinfection agent. Coagulation usually used to solve this problem in a very short period of time (e.g. about 10 s), whereas flocculation occurs usually over a period of 20 to 45 min [4].

Coagulation process is very important stage for the maintenance of acceptable treated water quality and economic plant operation. Coagulation is not only effective in precipitation of particles, but also it has an important objective of removing pathogens [5].

Coagulants materials should have high efficiency in reducing turbidity, total dissolved salts, biodegradable organic carbon and microbiological content of surface waters used for drinking. The coagulant's effect on the organic content of the raw water is closely related to the microbiological concentration. Due to the increasing quality requirements for drinking water produced from surface water sources, it is necessary to increase the efficiency of coagulation-flocculation processes, in order to ensure the biostability of drinking water through advanced removal of the organic matter. The risk of the by-product formation during the reaction with the disinfectants and the humic and fulvic acids is also decreased; moreover, the growth of pathogenic bacteria in distribution systems is inhibited growth that has an impact on humans' health [6, 7]. The natural coagulants are locally sourced, are available in large amounts, produce less sludge, generate biodegradable sludge (which can be reused) and may be based on waste materials (such as banana peels, etc.) [8].

Furthermore the development of simple coagulants seems no longer to be sufficient enough. The need for more effective coagulants has led to the development of new coagulant categories such as the use of silica in the form of polysilicates for such a purposes [9]. The coagulants with complex structures containing silicon had been tested [10]. In the current study, the selection of the studied granite waste based mainly on its mineralogical and its chemical composition whereas it has enrichment in silica and aluminum such as polisilicates so, it can be used as a new and innovative coagulant.

The aim of the recent research is to monitoring the valuable and sustainable role of granitic waste in removal of pathogenic bacteria (total coliform and *E. coli*) and toxogenic fungi through coagulation-flocculation process. Moreover its application in wastewater treatment.

2. Materials

2.1. Materials

Two main categories of used materials for achieving the previous mentioned aim involve:

Firstly, the studied granitic powder waste used as a coagulant material in the current research has been collected from air-dried powder residual from the stone cutting landfill at Shaq El-Thoban area, east of Auto Strad road, Cairo Governate.

Secondly, Some chemicals and reagents were also involved in the recent research including: Potato dextrose (PDA) agar (Difco, USA) and Rose Bengal (RB) agar Base (Himedia, India) were used for detection and enumeration of filamentous fungi and total yeasts, respectively. Lauryl Tryptose broth is the media used in determination of total coliform media and *E. coli* by the MPN (most probable number) method. Reference substances used in toxicity test were 3,5-Dichlorophenol, Zinc sulfate heptahydrate and Potassium dichromate.

2.2. Sampling

Wastewater samples used in the current study were collected from Abo Rawash plant at Giza governorates, Egypt, in clean and sterilized plastic bottles, kept in iceboxes till reached the lab and stored at refrigerator at 4°C until used for several investigations in the current study.



3. Methods and Techniques

3.1. Raw Granitic Waste Characterization

Some laboratories techniques have been carried out within the current research to evaluate the ability of studied granitic waste on removal of total bacterial count and E Coli from wastewater. Some of these techniques focused on the different characterization of the granitic wastes include: (X-ray diffraction (XRD), X-ray Fluorescence (XRF), Particle Size Distribution (PSD) and Fourier transform infra-red spectroscopy (FT-IR).

As (XRD model X'Pert ProPhillips MPD PW 3050/60 X-ray diffractometer used for detailed mineralogical granitic waste while XRF (Phillips PW 1400 Spectrometer), Holland used to determine the major oxides of granitic waste. Granitic powder waste was identified using a Jasco-6100 Fourier transformed infrared spectrometer (FTIR; Varian model, Excalibur FTS 3000MX, Paolo Alto, CA, USA). The tested samples were prepared using the KBr pressed disc technique. The analysis was carried out between 400- 4000 cm^{-1} . The particle size distribution of the used granitic powder waste was determined using a laser diffraction analyzer (manufactured by Horiba LA-950, France).

3.2. Experimental Setup

The current study was set up to elucidate the effects of different granite waste concentrations in removal or reduction of Total Coliform, Escherichia coli and toxogenic fungi from wastewater during treatment process (coagulation and flocculation). The experimental flasks were designed using different concentrations of granitic waste doses (1, 2, 3, 4, 5, 7, 9 and 10 gm) added on 100 ml of untreated wastewater, then shaken for 60 min at rpm 150, The wastewater was allowed to retain in the flasks for 1h retention time (RT) then filtrated to be used for further analyses [11]. To achieve the reduction in enteric pathogens and fungi, the concentration of respective pollutants and organisms through the flasks of the experiment was measured to determine percent removal in each treatment.

3.2.1. Total Coliform

Total coliform media populations were determined by the MPN (most probable number) method, the media used tryptone broth and incubation at 35°C for 24 to 48 h. The confirmatory test using brilliant green lactose bile broth and incubation at 35°C for 24 to 48 h for total coliforms [12].

3.2.2. Escherichia coli (E. coli)

The filtrate of different concentrations were examined by MPN method. using lauryl broth tubes which arranged in three rows, each row contained 5 tubes. Serial dilution of each concentration was prepared and used to inoculate the broth tubes, then incubated at 35 °C for 48 h, positive tubes (gas production-acidic reaction-changing in colour from purple to yellow) used to inoculate tryptone water, then add 0.2-0.3 ml of Kovac's reagent, positive tubes give deep red colour on the upper layer. Calculate MPN from the number of indole positive tubes [12].

The percentage of removal of bacteria is calculated as follows:

$$\% \text{ of removal} = [(C_0 - C_t) / C_0] * 100$$

Where: C_0 is the bacterial count in the untreated sample

C_t is the bacterial count in the treated samples [13].

3.2.3. Filamentous fungi (FF) and total yeasts (Toxigenic fungi and pathogenic fungi)

Media were prepared as mentioned before and autoclaved at 121°C for 20 min. transfer 1ml of the prepared concentrations of treated wastewater to petridishes then pour media onto each sample separately and let to solidify. incubated at 25°C for 5-7 days. Filamentous fungi were identified on the basis of macroscopic and microscopic features. Served characteristics were recorded, (a) morphological characteristics such as septation of hypha; (b) formation and morphology of fruiting-bodies and conidiophores; (c) branching patterns and frequency of branching; and (d) size, form,

color and ornamentation of the conidia [14-16] were identified using Biolog GEN III Microplates (Biolog, USA) [12] sec 9610BG.



3.2.4. Toxicity Test

Microtox was applied to evaluate the acute toxicity and the feasibility to be a fast prescreening tool. It was applied in reused wastewater [17]. 1ml of each examined solutions which represent different concentrations of granite waste was added to 1L of autoclaved distilled water and the test carried and results recorded according to [18- 20] by using Microtox and *Vibrio fischeri* bacteria as tested organism. The reduction in intensity of light emitted from the bacteria was measured along with standard solutions and control samples. The results were normalized and the EC₅₀ (concentration producing a 50% reduction in light) is calculated using the software for Microtox Omni Azur. Extracts were placed in borosilicate glass vials and stored at 4°C for microtox analysis. Microtox Model 500 analyzer (SDIx, USA) was used for analysis and all detailed procedures were followed in the Microtox Users Manual. Freeze-dried luminescent bacteria, *Vibrio fischeri* were reconstituted and exposed in duplicate to a series of diluted samples resulting decrease in bioluminescence was measured after 5 and 15 minutes at a constant temperature of 15°C. All Microtox data were recorded and analyzed by on-line software and results are expressed as the Effective Concentration 50% (EC₅₀) in percent of extract to determine the degree of toxicity as in table (1).

Table 1: Toxicity degrees and EC₅₀ for the examined solutions

Degree of toxicity	Extremely Toxic	Very Toxic	Toxic	Moderate Toxic	Non-Toxic
EC ₅₀ Value %	0-19	20-39	40-59	60-79	80≥100

EC₅₀: The effective concentration causing 50% luminescence inhibition.

Bacteria Storage temperature: -25°C

4. Results and Discussion

4.1. Granitic Waste Characterization

It involves mineralogical, chemical and particle size of the granite waste. Mineralogically, XRD has been conducted for representative studied granitic waste as shown in XRD diffractogram figure (1). It can be detected that the mineralogical composition of studied granitic waste consisted essentially of quartz, albite and microcline with minor biotite as shown in figure (1).

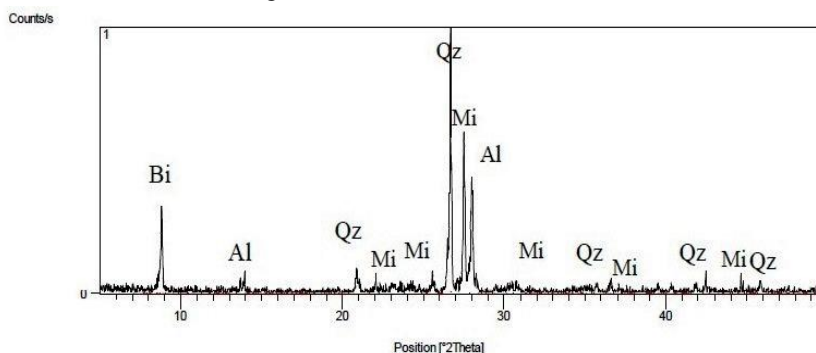


Figure 1: XRD diffractogram of the studied granite waste

Chemically, the studied granite waste sample has been analyzed by XRF and the obtained results are listed in table (2).

Based on the obtained results it can be revealed that, the chemical composition of the studied sample appeared SiO₂ % is the main major oxide by percentage followed by Al₂O₃% which represents the second major oxide. Moreover, it appears relatively enrichment in alkalis (K₂O%+Na₂O %) as the used granite waste most likely to be Alkali-feldspars granite derivatives.

Table 2: chemical analysis of the studied granitic waste by XRF

Sample Name	SiO ₂	Al ₂ O ₃	K ₂ O	Fe ₂ O ₃	Na ₂ O	CaO	MgO	TiO ₂	P ₂ O ₅	Cr ₂ O ₃	BaO	SO ₃	MnO	Cl-	L.O.I	Total
Raw granitic waste	67.40	14.00	5.15	4.12	2.95	2.48	0.94	0.58	0.17	0.17	0.15	0.13	0.06	0.05	1.57	99.99



The particle size distribution of granite waste illustrated in bimodal peaks figure (2). Moreover, it can be noticed that in the granitic waste the biggest quantitative percentage that are found at 4.3% and 2.9% with mean size 27.7 μm .

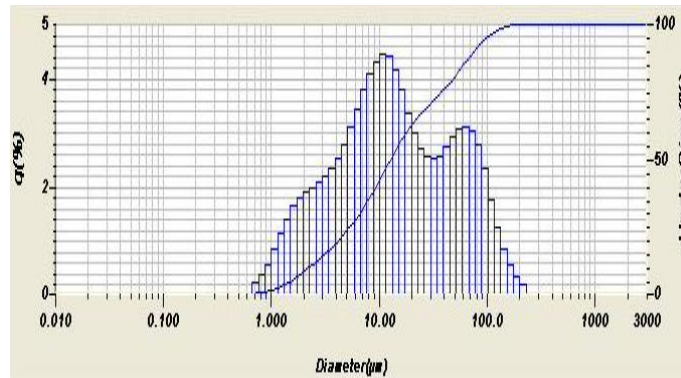


Figure 2: Laser particle size distribution pattern of the used granitic waste

FTIR spectra of granitic waste powder are represented in figure (3). There are different transmittance bands related to symmetric stretching vibration of Si-O-Si (Al) (at 1010 cm^{-1}), asymmetric stretching vibration of Si-O-Al (at 685 cm^{-1}), stretching vibration of S-O (within sulphate group at 1147 cm^{-1}). There is a stretching vibration of H OH at 1627 cm^{-1} and bending vibration of OH group at (3428 cm^{-1}).

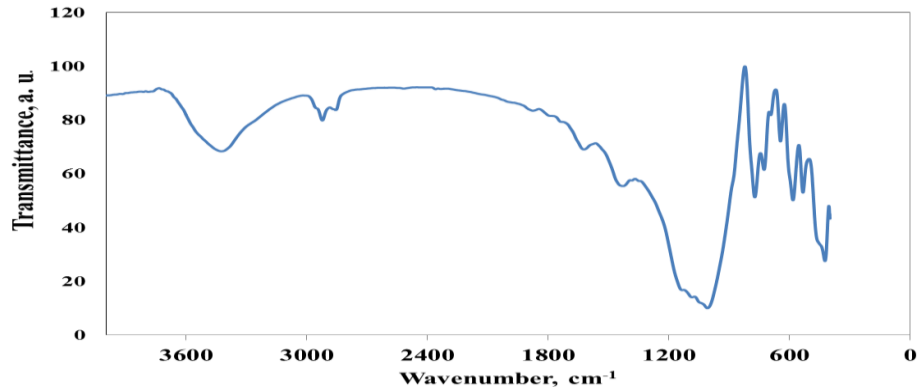


Figure 3: FTIR spectra of granite waste Powder

4.2. Utilization of granite waste as microbiological coagulant

It refers to the impact of the studied granite waste on the removal of toxogenic fungi and some types of bacteria. The following obtained results exhibit the role of granite waste and its ability for removing all of previously mentioned microorganisms. Definite doses of granite waste were examined on the following examinations (1, 2, 3, 4, 5, 7, 9, 10 gm/100ml).

4.2.1. Efficiency of granite waste in the removal of E Coli & Total Coliform from wastewater

Results of the removal % of bacterial load from wastewater by adding different doses (concentrations) of granite waste on untreated wastewater were listed in table (3) and illustrated graphically in figure (4) and the total bacterial count in figure (5). It was noticed that in the highest removal % of total coliform was obtained at 9 and 10g/100 ml treated wastewater with values of 2×10^2 and 1.8×10^2 respectively with removal % of 99.90% for each compared with value of untreated wastewater (control) which recorded 94×10^8 .

On the other hand the highest removal % for *E. Coli* was obtained at 9 and 10 g/100 ml treated wastewater with values $\leq 1.8 \times 10^2$ and 1.8×10^2 respectively with removal % of 99.90 % for each compared with value of untreated wastewater (control) which recorded 79×10^8 .

The obtained results of removal percentage are agreed with [21] who recorded that more than 99 % removal efficiency was obtained for *E. coli* and total coliforms using normal coagulants.



Table 3: the removal % of Total Coliform (TC) and *E. coli* from wastewater

Dose of tested material (g/100ml)	Total Coliform TC (CFU/100 ml)	TC Removal %	<i>E. coli</i> (CFU/100 ml)	<i>E. coli</i> Removal %
untreated wastewater	94×10^8		79×10^8	
1	84×10^7	91.06	58×10^7	92.65
2	63×10^7	93.29	49×10^7	93.79
3	46×10^7	95.1	33×10^7	95.99
4	38×10^5	99.95	26×10^5	99.96
5	14×10^4	99.99	10×10^4	99.99
7	11×10^3	99.99	6.8×10^3	99.99
9	2×10^2	99.99	1.8×10^2	99.99
10	1.8×10	99.99	$\leq 1.8 \times 10^2$	99.99

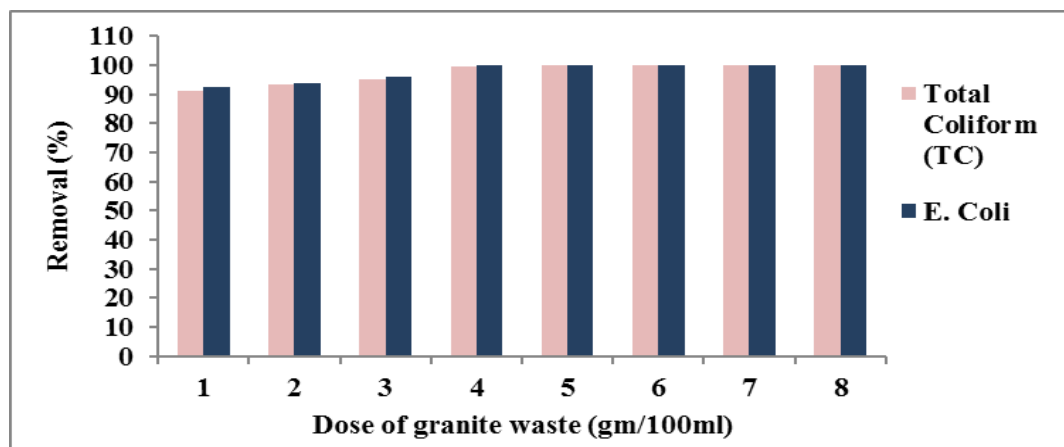
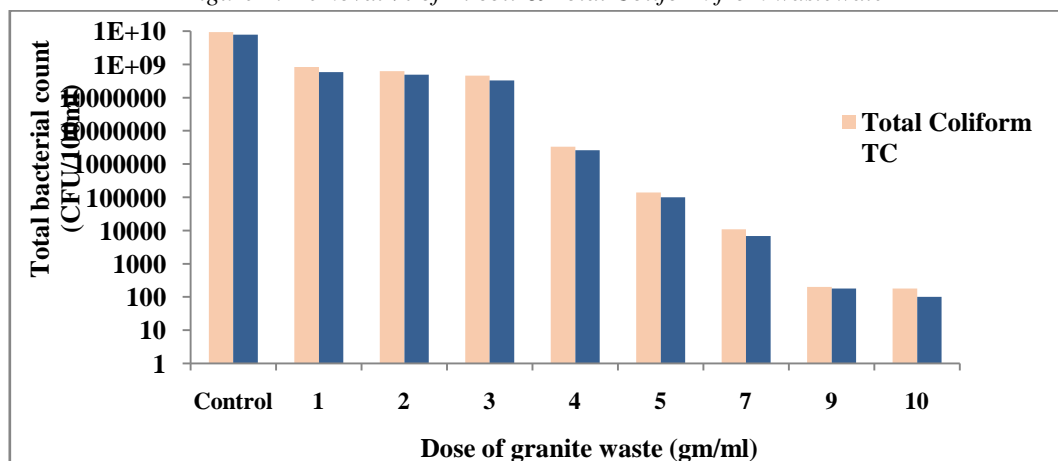
Figure 4: Removal % of *E. coli* & Total Coliform from wastewater

Figure 5: Total bacterial count CFU/100 ml after removal from wastewater

4.2.2. Efficiency of different concentrations of granite waste on removal of toxigenic and pathogenic fungi from wastewater

The studied untreated wastewater included many fungal isolates some of them are toxigenic *Aspergillus flavus* (4.2%), *Aspergillus niger* (5.6%), *Aspergillus tubingensis* (23.7%) and *penicillium notatum* (14.2%), other are pathogenic as *Candida galibrata* (23.6%), *Candida albicans* (9.5) and *saccharomyces cerviacae* (19%). It was noticed that, by adding different concentrations of granitic waste, as the concentration increased the isolates % decrease until disappeared as 10 gm/100ml as in figure (6).



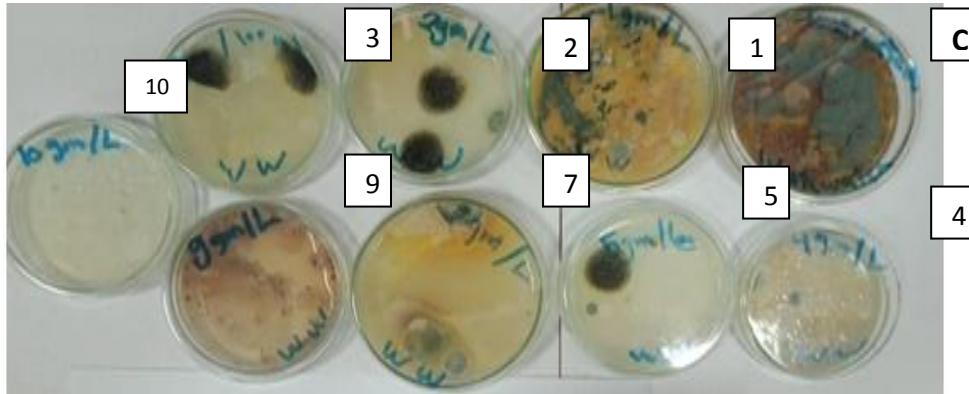


Figure 6: Photo showed the effect of different concentrations of granitic wastes on toxigenic and pathogenic fungi from wastewater

Results of the removal % of fungi from wastewater by adding different doses (concentrations) of granite waste on untreated wastewater were listed in table (4) and illustrated graphically in figure (7) and the fungal count in figure (8). The addition of different concentrations of granite waste on untreated water containing total fungal count 211 CFU/ 100 ml, it was noticed that the highest removal percentage was obtained at dose of 9 gm/100ml (82.9 %) with total fungal count 36 CFU/ 100 ml, whereas the lowest removal percentage was 24.6% at 1 gm/100ml with total fungal count 159 CFU/ 100 ml and disappeared at 10 gm/100ml (100 %) with total fungal count 0 CFU/ 100 ml.

Table 4: The effect of different concentrations of granite waste on toxigenic and pathogenic fungi from wastewater

Granite Dose (g/100ml)	Untreated wastewater (no add)	1	2	3	4	5	7	9	10
Fungi (CFU /100 ml)									
<i>Aspergillus tubingensis</i>	50	30	15	3	0	0	0	0	0
<i>Asperigiliuus niger</i>	12	8	4	2	2	1	0	0	0
<i>Aspergiliuus flavus</i>	9	6	4	2	1	1	1	0	0
<i>Penicillium notatum</i>	30	20	11	8	3	3	1	0	0
<i>Candida galibrata</i>	50	45	40	32	30	25	19	15	0
<i>Candida albicans</i>	20	20	20	18	18	16	14	11	0
<i>Saccharomyces cerviaca</i>	40	30	30	20	20	20	18	10	0
Total fugal count	211	159	124	85	71	66	53	36	0
Removal %	0	24.6	41.2	59.7	66.4	68.7	74.9	82.9	100

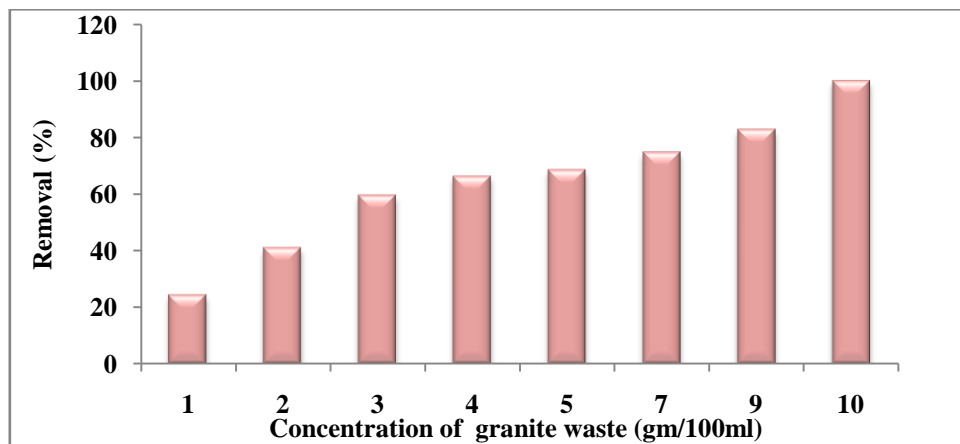


Figure 7: Removal % of toxigenic and pathogenic fungi from wastewater by different concentrations of granite waste



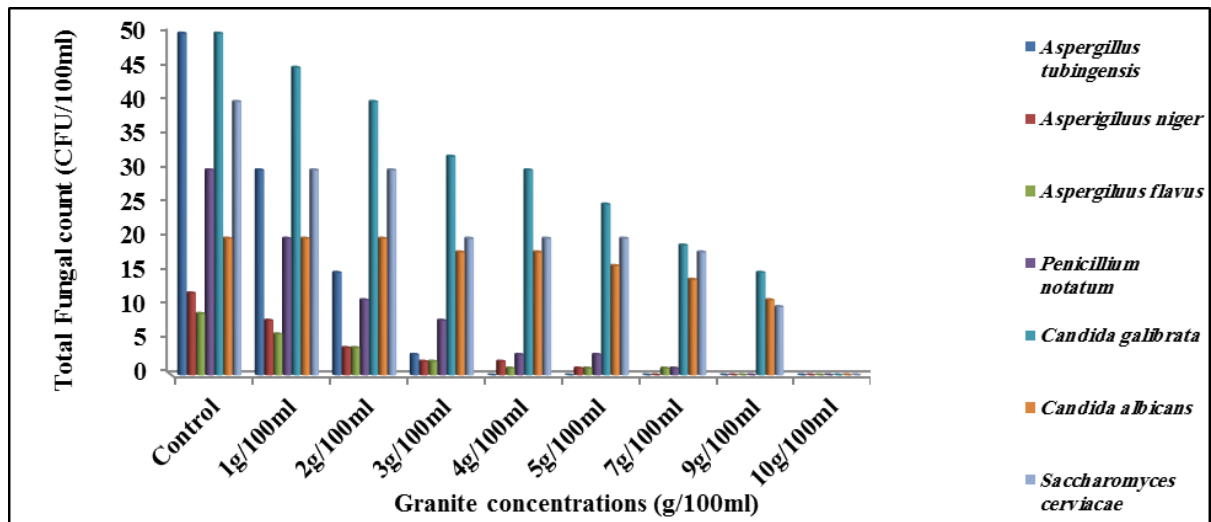


Figure 8: Total fungal count CFU/100 ml after removal from wastewater

4.3. Determination of granite waste toxicity

The Microtox test was carried out to determine the level of toxicity hence, it can be measured from samples whose pH is between 6.0- 8.0 [22]. To study the effect of toxicity of the studied granite waste, different concentrations were examined to measure the effective concentrations (EC_{20} and EC_{50}) inhibition in Bioluminescence of *Vibro Fischeri* (EC_{50} & EC_{20}) values were calculated after 30 min exposure time at 25 °C as listed in table (5). The obtained results of EC_{50} % indicated that all concentrations used in the current research were classified as non-toxic according to EC_{50} as listed in table (1). It can be concluded that, with increasing the concentration of granite waste, there is no effect on toxicity degree.

Table 5: Effective concentrations and toxicity degrees for different granite waste concentrations.

Dose of granite waste added to wastewater (gm)	EC_{50} (%)	EC_{20} (%)	Toxicity degree	pH before adjustment	pH after adjustment
1	98	39.4	Non-toxic	9	8
2	97	38.8	Non-toxic	9.4	8
3	95	38	Non-toxic	10	8
4	96	38.4	Non-toxic	10.5	8.5
5	91	36.4	Non-toxic	10.9	8,4
7	92	36.8	Non-toxic	11	8
9	90	36	Non-toxic	13	8.5
10	93	37.2	Non-toxic	13.7	8.5

EC_{20} : The effective concentration causing 20% luminescence inhibition.

EC_{50} : The effective concentration causing 50% luminescence inhibition.

4.4. Discussion

Generally, the granite waste achieved the most requirements of natural coagulant material. In the current study, the used granite waste chemically contains a high content of silica and alumina with positive charge. The wastewater containing high amounts of contaminants and pathogens (bacteria, fungi) always with negative charge [23] forming repulsive forces with each other. The granite waste with different concentrations was added to the water to overcome the repulsive charge and destabilize the suspension. Once the repulsive charges have been neutralized (since opposite charges attract), van der Waals force will cause the particles to agglomerate and form micro flocculations [2]. The flocculations after a period of time sedimented and filtered producing clear water. Further analyses to the filtrate confirmed that the granite waste have high tendency to treat the wastewater and achieve the requirements as a natural coagulant.



5. Conclusion

The used granite waste exhibited chemically enriched with silica and alumina. The granite waste was selected as natural coagulant based on its chemical and mineralogical composition. The positive effect for usage of granite waste as a natural and non-toxic coagulant can be reflected from the following obtained results. The different studied doses conducted to the current study were (1, 2, 3, 4, 5, 7, 9, 10 gm/ 100 ml).

- The highest removal % of total coliform was obtained at 9 and 10 g/100 ml treated wastewater with % of 99.90%.
- The highest removal % for *E. coli* was obtained at 9 and 10 g/100 ml treated wastewater with removal % of 99.90 %.
- The highest removal % for total fungal count was obtained at dose of 9 gm/100ml (82.9 %) whereas the lowest removal percentage was 24.6% at 1 gm/100ml and the total fungal count disappeared at 10 gm/100ml (100 %).
- The obtained results from toxicity test indicated that all concentrations used in the current research were classified as non-toxic according to EC₅₀.

The success of using the granite waste as a coagulant based on the main mechanism by which its ability to destabilize the suspension and neutralize the negative charge of the pathogens in wastewater and causing the particles to agglomerate and forming micro flocculations (coagulation).

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References

- [1]. Kala, F. (2013). Effect of granite powder on strength properties of concrete. *Int. J. Eng. Sci.*, 2(12): 36-50.
- [2]. Koohestanian, A., Hosseini, M. & Abbasian, Z. (2008). The Separation Method for Removing of Colloidal Particles from Raw Water. *American-Eurasian J. Agric. & Environ. Sci.*, 4(2): 266-273.
- [3]. Bratby, J. (2006). *Coagulation and Flocculation in Water and Wastewater Treatment*. IWA Publishing, London, 2nd Ed., 50-68.
- [4]. Duan, J. & Gregory, J. (2003). Coagulation by hydrolysing metal salts. *Advances in Colloid & Interface Science*, 100-102: 475- 502.
- [5]. Mohammadtabar, F., Khorshidi, B., Hayatbakhsh, A. & Sadrzadeh, M. (2019). Integrated coagulation-membrane processes with zero liquid discharge (ZLD) configuration for the treatment of oil sands produced water. *Water*, 11(7): 1348-1360.
- [6]. National Primary Drinking Water Regulations EPA-USA (2009).
- [7]. Geldreich, E. E. (1996). *Microbial Quality of Water Supply in Distribution Systems*. Boca Raton: CRC Lewis Publishers.
- [8]. Ang, W.L. & Mohammad, A.W. (2020). State of the art and sustainability of natural coagulants in water and wastewater treatment. *J. Clean. Prod.*, 262: 121267.
- [9]. Tzoupanos N. D. & Zouboulis, A. I. (2009). Coagulation flocculation processes in water/wastewater application of new generation of chemical reagents. 6th IASME/WSEAS International Conference on Heat transfer, thermal engineering and environment (HTE'08), Rhodes, Greece.
- [10]. C. Exley. (2012). A silicon - rich mineral water therapy for Alzheimer's disease. *Journal of Alzheimer's Disease*, 33(2):423-30.
- [11]. Kadaverugu. R., Shingare, R. P., Raghunathan, K., Juwarkar, A. A. & Thawale P.R., Singh, S.K. (2016). The role of sand, marble chips and *Typhalatifolia* indomestic wastewater treatment—A column study on constructed wetlands. *Environ Technol.*, 37(19):1–26.
- [12]. APHA (2017). *Standard Methods for the examination of water and wastewater*, American Public Health Association, 23th Ed., Washington DC.



- [13]. Reddy, D. H. K., Seshaiaha, K., Reddyb, A. V. R. & Leec, S. M. (2012). Optimization of Cd (II), Cu (II) and Ni (II) biosorption by chemically modified *Moringa oleifera* leaves powder. *Carbohydrate Polymers.*, 88: 1077-1086.
- [14]. Zycha, H. (1963). *Mucorineae Von Kryptogamenflora der mark Brandenburg*. Band Via: Pilze IIV erlag Von J. Cramer. Weinheim. Johnson Reprint Corporation, New York.
- [15]. Ainsworth, G.C. (1971). *Ainsworth and Bisbys Dictionary of the fungi*. Commonwealth Mycological Institute, Kew, Surrey, England.
- [16]. Booth, C. (1977). *Laboratory guide to the identification of majorspecies. Fusarium*, Commonwealth Mycological Institute, Kew, Surrey, England.
- [17]. Choi, K. & Meier, P.G. (2001). Toxicity evaluation of metal plating wastewater employing the microtox assay: A Comparison with Cladocerans and Fish. *Environ. Toxicol.*, 16: 136–141.
- [18]. Water quality – Determination of the inhibitory effect of water samples on the light emission of vibrio fisheri (Luminescent bacteria test) – Part 3: method using freez-dried bacteria ISO 11348-3:2007/Amd.1:2018(en).
- [19]. Mohamed, S. A. (2018). Application of Green Synthesis Ag-NPs from *Lawsoniainermis* (Henna) Plant as Antifungal & Non Toxic Nonmaterial in Drinking Water Purification. *Chemistry Research Journal*. 3(1):64-73.
- [20]. Sobhy, N. A., Razek, S. A. & El-Tayieb, M. M. (2017). Green Synthesis of Silver Nanoparticles using *LawsoniaalbaLam* (Henna) Plant Extract and Its Antibacterial Activity and Toxicity *Chemistry Research Journal*, 2(6):225-235.
- [21]. Meri, S., Guida, M., Anselmo, A., Mattei, M. L., Melluso, G. & Pagano, G. (2002). Microbial and COD removal in a municipal wastewater treatment plant using coagulation flocculation process. *Journal of Environmental Science andHealth, Part A—Toxic/Hazardous Substances & Environmental Engineering*, A37 (8):1483-1494.
- [22]. Okragla, E., Chraniuk, M. & Wolska, L. (2017). Microtox test as a tool to assess antimicrobial properties of herbal infusions used in urinary tract infections. *Acta Poloniae Pharmaceutica ñ Drug Research*, 74(3):895-901.
- [23]. Olitzki, L. (1932). Electric charge of bacterial antigens. *J Immunol.*, 22(4):251–256.

