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Sustainable Wastewater Purification and Microbial Decontamination for Aquaculture and Agriculture Using Eggshell Waste

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ABSTRACT

This study was conducted to assess the effectiveness of eggshell powder (ESP) adsorption to lessen the microbiological and heavy metal pollution brought on by wastewater, which is relevant for water reuseThe analysis of physicochemical parameters was conducted, and the results were recorded within the standard limits set by the Food and Agriculture Organization (FAO). For instance, the pH levels of RW1 (raw wastewater) and PW2 were recorded at 8.70 and 8.25, respectively. In contrast, the pH levels of WESP1 and WESP2 (wastewater treated with ESP) were 7.7. Additionally, the conductivity of WESP1 and WESP2 was measured at 340 and 320 µS/cm, respectively. The total dissolved solids (TDS) values of WESP1 and WESP2 ranged from 217 to 204mg/L. Soluble anions HCO^{3-}, Cl^-, and SO_4^- recorded 41.48, 79.52, and 7.92mg/L, respectively. Cations of Ca^{2+}, Mg^{2+}, and Na^+ were recorded at 9.50, 6.72, 44.85, and 8.58mg/L, respectively. Pathogenic microorganisms decreased at WESP1 and WESP2 more than at RW1 and RW2. The morphology of ESP was characterized using the spectroscopic method before and after using it as an adsorbent, including SEM (scanning electron microscopy) with SEM-EDX (Energy dispersive X-ray). Both the raw wastewater streams (RW1 & RW2) and the ESP-treated wastewater streams (WESP1 & WESP2) contained the following metals in the ESP-metal complex: oxygen percentages were 73.40, 40.91, and 29.27%; carbon percentages were 64.82, 62.60, and 41.31%; calcium percentages were 14.48, 4.57, and 0.00%; magnesium percentages were 0.56, 0.25, and 0.00%; aluminum percentages were 0.47, 0.28, and 0.00%; silicon percentages were 0.23, 0.10, and 0.00%; phosphorus percentages were 0.23, 0.22, and 0.00%; sulfur percentages were 2.0, 0.09, and 0.00%, respectively, in ESP (control), raw wastewater, and ESP-treated wastewater samples.

INTRODUCTION

Water contamination is a major problem in resource-poor and industrial countries **(Badrealam *et al.*, 2018).** Only 2.5 percent of the world's freshwater sources are available, and only 0.5 percent of that is suitable for human use **(Beth *et al.*, 2018)**. Water contamination is an emerging problem caused by domestic, commercial, industrial, and agricultural wastes **(Crini, 2019)**. Heavy metals (zinc, copper, lead, mercury, and cadmium) are released from a variety of sources, including battery facilities, pharmaceutical companies, metal processors, hospitals, agrochemicals, and pesticides. These sources either directly or indirectly discharge these heavy metals into surface water **(Kanyal & Bhatt, 2015; Sajjanar *et al*., 2018)**. Since heavy metals are nonbiodegradable and harmful to human health, these pollutants have a detrimental influence on the ecology **(Xu *et al*., 2019; Ogunlaja *et al*., 2019)**. Various studies of heavy metal's effects revealed that they cause kidney and liver problems, memory problems, infertility, hepatotoxicity, hemolysis, and cramps (**Fu & Wang, 2011)**.

The process of adsorption has garnered significant attention in the realm of heavy metal removal. Various affordable and effective materials, such as activated carbon, have been utilized as adsorbents in wastewater management due to their substantial specific surface area **(Senthilkumaar *et al*., 2006)**. However, the high cost and low generation efficiency of activated carbon have necessitated the development of new adsorbents. Lignocellulosic biomass wastes such as wheat straw **(You *et al*., 2016)**, rice husk and cocnut peels **(Franco *et al*., 2017)**, de-oiled soya and banana peel **(Van *et al*., 2017)**, and orange peel **(Munagapati *et al*., 2019)** have been widely studied as effective bio-sorbents in the industrial wastewater management due to their wide availability, abundant quantity, sustainability, and renewability. In fact, the proper utilization of waste resources for contaminant removal provides the twin benefits of ensuring a more economical and environmental solution instead of r waste disposal **(Van *et al*., 2017)**.

It was reported by **Politaeva *et al.* (2020)** that the adsorption of different pollutants by the aforementioned wastes during wastewater treatment is greater than 80% effective. Additionally, other elements, including structure, the precise surface area of the adsorbent, and the chemical makeup of pollutants, all affect how well a contaminant may be removed. Nowadays, many newly discovered natural bio-sorbents are abundantly used, such as eggshells, which can be used effectively to minimize contamination of wastewater **(Siti *et al*., 2013; Candido *et al*., 2019; Martins *et al*., 2019)**.

Kanani *et al*. (2020) reported that worldwide egg consumption is rising, and in 2016, the world's yearly egg output rose from 15.0 to 81.0 million tons. The International Egg Commission (IEC) has a goal known as Vision 365, which is a 10-year plan developed by the IEC to maximize the nutritional benefits of eggs and establish the egg's nutritional reputation on a worldwide scale. With the whole industry's support, this initiative will enable us to build the reputation of the egg-based on scientific facts, positioning eggs as an essential food for health. This vision emphasizes the daily consumption of one egg per person. However, every year, 76.7 million tons of eggs are produced, so the IEC envisions global egg consumption by 2032.

Accordingly, this large amounts of eggshell waste roughly 250,000 tons, are produced during the processing of eggs in hatcheries, fast-food restaurants, and homes. The production of eggshell trash worldwide is 5.92 million tons per year, and because of its abrasiveness, stench, and accumulation, it pollutes the environment **(Alsohaimi *et al*., 2020)**. A biomineralized composite of calcite crystals encased in an organic framework of protein fibers makes up the eggshell, which weighs roughly 11% of the entire egg. **(Oliveira *et al*., 2013; Quina *et al*., 2017)**. The main chemical components of eggshell waste (ESW), as demonstrated by **Bashir *et al*. (2015)**, **Soares *et al*. (2015)** and **Quina *et al*. (2017)** are calcium carbonate and calcium oxide. In addition to its surface macroporosity, this structure also features a total open void volume of 0.006 cm^3/g. The Brunauer-Emmett-Teller surface area (BET) ranges from 0.84 to 1.3 m^2/g **(Gao & Xu, 2012; Bashir *et al*., 2015).**

It is illustrated by **Ahmed *et al.* (2021)** that ESW has many biotechnological applications for biomedical, chemical, engineering, and environmental technologies. Adsorption of heavy metals, organics, total nitrogen, fluoride, soil contaminants, and radioactive substances was used for biotechnological and environmental approaches. Since eggshell waste (ESW) has natural pore channels and contains significant amounts of calcium carbonate, it is more applicable for use in these applications **(Al-Ghouti & Khan, 2018)**. Additionally, due to the eggshell's fibrous structure, which results in a great affinity for adsorbates, it is thought to be a potential bio-sorbent for heavy metals like as Pb, Zn, Fe, Cr, Mn, Cd, and Cu **(Liu *et al*., 2013; Daraei *et al*., 2014)**.

The main reason for choosing eggshell waste for wastewater treatment is that it has many benefits, including being cost-effective, eco-friendly, abundant, and massively produce **(Sankaran *et al*., 2020)**. Recently, researchers **(Mittal *et al*., 2016; Dayanidhi *et al.*, 2020)** have noted the adsorption capacity of eggshell waste (ESW) for removing both organic and inorganic contaminants from water, such as 3D ESM-supported titanium dioxide ($TiO2$) and aromatic dyes. With an average yearly growth of 3.2%, aquaculture is the world's largest food producing business. It was noted that during the year 2014, wild fish output outnumbered the consumption of farmed fish **(FAO, 2016)**. Aquaculture became very important in the seafood sector in the recent times owing to the depletion of the wild stock **(Allen *et al*., 2017)**. Moreover, it is recognized as the major contributor to seafood for human consumption. The correct implementation of aquaculture systems, particularly in developing nations, ensures food security. The percentage of fish produced for human consumption that comes from aquaculture is anticipated to rise from 52% (average in the period 2016–2018) to 58% in 2028 **(OECD/FAO, 2018)**. With a contribution to the GDP of 1.07%, India ranks second in the world for producing fish through aquaculture. According to a recent assessment by the National Fisheries Development Board, the fishing industry in India has amassed a capital of Rs 334.41 billion in the aftermath of the Economic Revolution through Blue Revolution in the nation. The main issues facing the globe today, especially the emerging nations, are water scarcity and the loss of natural water resources. Seafood security is related to important resources such as electricity and water, and environmental quality must be maintained to ensure an efficient food supply. Aquaculture production is expected to decline in the future due to a number of issues, one of which is the availability and accessibility of high-quality water. **(OECD/FAO, 2018).**

This study aimed to assess the effectiveness of eggshell waste (ESW) as a straightforward, successful, low-cost, and environmentally acceptable solution to remove water-polluting chemicals. removing *E. coli*, *Enterobacter* sp., *Salmonella* sp.,and *Shigella* sp. from wastewater as well as heavy metals (aluminum, fluoride, manganese, zinc, and iron).

MATERIALS AND METHODS

2. Eggshell waste (ESW) collection source

ESW can be collected from various sources, depending on the context and purpose. In the currrent study, ESW samples were collected from the local shop in Cairo, Egypt. There may be guidelines and legal requirements regarding egg collection, storage, and handling. These regulations are in place to ensure food safety, animal welfare, and environmental sustainability. It is advisable to adhere to local laws and guidelines when collecting ESW from any source.

2.1 Wastewater samples collection

Wastewater samples were collected from the water treatment plant in El-khanka, Qalyubia, Egypt, in the winter of 2022. The wastewater samples were collected from raw wastewater and primary treated wastewater. Samples were collected from various points within the wastewater stream to capture any spatial variations. Collecting samples were from different locations and mixed samples were taken at different spots to create a representative composite sample (Consortium). Five different samples were collected from the raw wastewater influent and made a consortia called RWW1, also took five different samples from primary treated wastewater effluent and made a consortia from them called PWW2. Storage of the samples were at a temperature of 4°C in sterilized glass or/and plastic bottles.

2.2 Media used for microorganisms cultivation and enumeration in wastewater

There are various types of media used for the detection and enumeration of microorganisms in wastewater samples. In this study, specific selective media for microorganisms were used as indicators of the unsuitability of water for agricultural purposes. The media utilized, as per DIFCO (1984), were as follows: Med. (1): Nutrient Agar (OXOID CM0003B), which is a general-purpose medium supporting bacterial growth, maintaining a pH of 7.0±0.2 at 25ºC. This medium was used for the enumeration of the total bacterial count. Med. (2) Yeast Extract Glucose Chloramphenicol Agar

(OXOID PO0183) pH 6.6±0.2 at 25°C, was employed for the enumeration of yeasts and molds. Med. (3) MacConkey sorbitol agar medium (OXOID CM0981) pH 7.1±0.2 at 25°C, was utilized for the detection of *E. coli*. Med. (4) *Salmonella Shigella (S.S)* agar medium (OXOID CM469) pH 7.0±0.2 at 25°C, was used for the detection of *Salmonella and Shigella spp*. Med. (5) *Bacillus cereus agar* base medium (OXOID CM0617) was maintained at pH 7.2±0.2 at 25ºC. Antimicrobic Vial B (Oxoid, Basingstoke, England), Approximately 30,000 units of polymyxin B per vial. One vial of Antimicrobic Vial Polymyxin B was dissolved in 5ml of distilled water, then added to 500 ml of *Bacillus cereus* agar base. *Bacillus cereus* agar base is used with Polymyxin B and Egg Yolk Emulsion for the isolation and presumptive identification of *Bacillus cereus*.

2.3 Preparation of eggshell powder (ESP)

ESP was washed with cold tap water to remove any residual egg or debris, and oven dried at 70±5°C for 15 minutes according to **(Rajoriya *et al*., 2021)**. Subsequently, it was grounded with an electrical blinder (Fresh ST-999 /350 wat-50Hertz-220volt) and saved in sterilized Jars, as shown in Fig. (1a, b, c, d). The chemical composition of ESP as monitored in Table (1) indicated that the calcium oxide was the most abundant component.

Table 1. Chemical composition of ESP, according to **Soares *et al*. (2015)**

Physical parameter	**Value**
Moisture (%)	1.00
pH	5.50
Electrical conductivity (ds/m)	0.45
Equivalent $CaCO_3$ (g $CaCO_3$/100 air-dried)	88.0
Organic matter (%)	6.30
Total organic carbon/total nitrogen	2.10
Germination index (%)	53.6
Respiration rate (mg C-CO_2/g C/d)	24.0
Chemical composition ESP (Wt. %)	
C	21.13
Na_2O	0.105
MgO	0.926
P_2O_5	0.415
SO_3	0.326
K_2O	0.054
CaO	76.99

Fig. 1. ESP preparation steps. a) Washing eggshell, b) Drying process for eggshell at 70°C for 10 min, c) Grinding dried eggshell with an electric grinder, and d) ESP preparation

2.4 Measurement of physicochemical parameters of wastewater

Wastewater samples were estimated for Physicochemical parameters including temperature, Biological oxygen demand (BOD) **(Tang & Chung, 2011)**, Chemical Oxygen Demand (COD) **(Arshad *et al*., 2021)**, Total Dissolved Solids (TDS) **(Gasim *et al*., 2012)**, Electric Conductivity (EC) **(Peavy *et al*., 1985)**, pH and Soluble Cations **(Bhatnagar & Sillanpaa, 2011)**, and Soluble anions **(Yeddou & Bensmaili, 2007)** parameters before and after treatment wastewater treatment. The total dissolved solid (TDS), conductivity, salinity, and heavy metals content of the wastewater samples were analyzed and recorded. Both the treated and non-treated effluent wastewater samples were analyzed periodically using a pH meter (BT600, Boeco, Germany), handy TDS meter (HI98301, Hanna, Italy), water conductivity tester (COND502, Taiwan), digital pen salinity meter (AZ8371, China) and atomic absorption spectrometer (AAS, Shimadzu, Japan) in line with the recommendations of the manufacturer.

2.5 Microbiological analysis of wastewater

Microbiological analysis was performed in two stages, the first was for RWW1 and PWW2 samples, and the second was for WESP1 and WESP2 by controlling dilutions, inoculating the nutrition, and cultivating media **(Malcheva & Veneta, 2022)** nonpathogenic microflora: Nutrient Agar for non-spore-forming bacteria and bacilli, Malt agar medium for molds, pathogenic microflora: (S-S) Desoxycholate Citrate Agar for *Salmonella sp.*, *Bacillus cereus* Agar for *Bacillus cereus*, MacConkey agar for *Escherichia coli* and coliforms (oxidase confirmatory test). Colony-forming units (CFU) computed per 1g of substrate are used to display the results given the amount of inoculation and dilution used **(Nustorova & Malcheva, 2020)**. The microbiological analysis was performed in the microbiological laboratory of the University of Ain Shams, Agriculture faculty, Cairo, Egypt.

2.6 Scanning Electron Microscope-Energy dispersive (SEM-EDX) analysis

Surface images and elemental content of ESW were recorded using SEM-EDX (Quanta FEG 250, FEI Company, Hillsboro, Oregon-USA) at EDRC, DRC, Cairo, Egypt. The ESP was examined under SEM conditions, utilizing a 10.1 mm working distance and an in-lens detector with an excitation voltage of 20 kV. Both the stub and the sample were coated with gold before being analyzed, as described by **Kaewmanee *et al.* (2009)**.

2.7 Statistical analysis

All collected data were statistically analyzed and expressed as means using IBM® SPSS® Statistics software (2017). Duncan's test **(Duncan, 1955)** at a P-value of 0.05 was applied.

RESULTS

3. Physicochemical characterization of wastewater effluents

The physicochemical quality parameters, including pH, TDS, conductivity, salinity, and other parameters of control wastewater samples and ESP treated samples were recorded and presented in Table (2).

Table 2. Chemical composition of wastewater before and after ESP treatment at 30°C for 72h at 150rpm

ESP Treatment	Sample code	EC (µS/cm	TDS (ppm)	pH	Soluble anion (mg/L)				Soluble cation (mg/L)			
					CO_3^{-}	HCO^{-3}	Cl^{-}	SO^{4-}	Ca^{++}	Mg^{++}	Na^{+}	K^{+}
Before	RW1	635±0.2^{a}	404.5±0.03^{a}	8.7±0.25^{a}	N.d	49.41±0.47^{a}	152.65±0.44^{a}	29.2±0.15^{a}	22.65±0.47^{a}	13.88±0.99^{a}	88.67±0.22^{a}	3.30±0.33^{b}
	PW2	585±0.11^{b}	376.0±0.25^{b}	8.3±0.58^{a}	N.d	45.75±0.17^{b}	141.82±0.17^{b}	27.1±0.44^{b}	22.71±0.05^{b}	12.93±0.47^{b}	82.46±0.28^{b}	3.10±0.97^{c}
After	WESP1	340±0.58^{c}	217.0±0.21^{c}	7.7±0.01^{b}	N.d	44.23±0.36^{c}	82.01±0.13^{c}	8.52±0.25^{c}	10.1±0.36^{c}	7.1±0.54^{c}	47.73±0.01^{c}	8.00±0.37^{a,b}
	WESP2	320±0.24^{d}	204.0±0.36^{d}	7.7±0.36^{b}	N.d	41.48±0.77^{d}	79.52±0.11^{d}	7.92±0.33^{d}	9.50±069^{d}	6.72±0.11^{d}	44.85±0.36^{d}	8.58±0.47^{a}

*N.d= not detected. Values in the same column followed by the same superscript letter don't significantly differ from each other at $P \leq 0.05$ **(Duncan, 1955)**

3. 1 Microbial analysis of wastewater (WW)

The microbiological analysis results of pathogenic microorganisms detection and total count (bacteria and fungi) enumeration of wastewater samples were presented in Fig. (2) and Table (3, 4). The maximum reduction in the total count of bacteria and fungi was observed in samples WESP1 and WESP2, as opposed to RW1 and PW2. In the same line the observation of *E. coli*, and *B. cereus*, in RW1 and PW2, while absence in WESP1 and WESP2 samples. *Salmonella* sp. , and *Shigella* sp. were absent in both raw and EPS treated wastewater samples.

Table 3. Detection of the presence of pathogenic microorganisms in wastewater before and after ESP treatment

.ESP Treatment	Sample code	Pathogenic indicator bacteria			
		E. coli	***Bacillus cereus***	***Shigella sp.***	***Salmonella sp.***
Before	RW1	+	+	-	-
	PW2	+	+	-	-
After	WESP1	-	-	-	-
	WESP2	-	-	-	-

- = Not detected, + = detected.

-

Table 4. Enumeration of total bacteria and fungi in wastewater before and after filtration ESP treatment

ESP Treatment	Sample code	Total fungi count (Spore/ml)						Total bacteria count (CFU/ml)					
		$x10^{1}$	$x10^{2}$	$x10^{3}$	$x10^{4}$	$x10^{5}$	$x10^{6}$	$x10^{1}$	$x10^{2}$	$x10^{3}$	$x10^{4}$	$x10^{5}$	$x10^{6}$
Before	**RW1**	17±0.2^{a}	13±0.1^{a}	11±0.11^{a}	9±0.05^{a}	7±0.05^{a}	5±0.47^{a}	241±0.08^{a}	216±0.28^{a}	188±0.33 ^{a}	103±0.08^{a}	88±0.97^{a}	72±0.9^{a}
	PW2	14±0.05^{b}	11±0.9^{b}	9±0.25^{b}	6±0.09^{b}	4±0.02^{b}	3±0.96^{b}	195±0.48^{c}	172±0.44^{b}	155±0.96^{b}	87±0.05^{c}	53±0.24^{b}	55±0.11^{b}
After	**WESP1**	11±0.12^{c}	8±012^{c}	7±0.17^{c}	5±0.14^{c}	2±0.16^{c}	1±0.45^{c}	231±0.36^{b}	184±0.58^{c}	147±0.24^{c}	97±0.69^{b}	51±0.03^{c}	37±0.63^{c}
	WESP2	9±0.1^{d}	8±0.47^{c}	2±0.11^{d}	0.00	0.00	0.00	120±0.77^{d}	97±0.63^{d}	83±0.02^{d}	69±0.11^{d}	42±0.97^{d}	36±0.14^{c}

Values in the same column followed by the same superscript letter don't significantly differ from each other at $P \leq 0.05$ **(Duncan, 1955)**

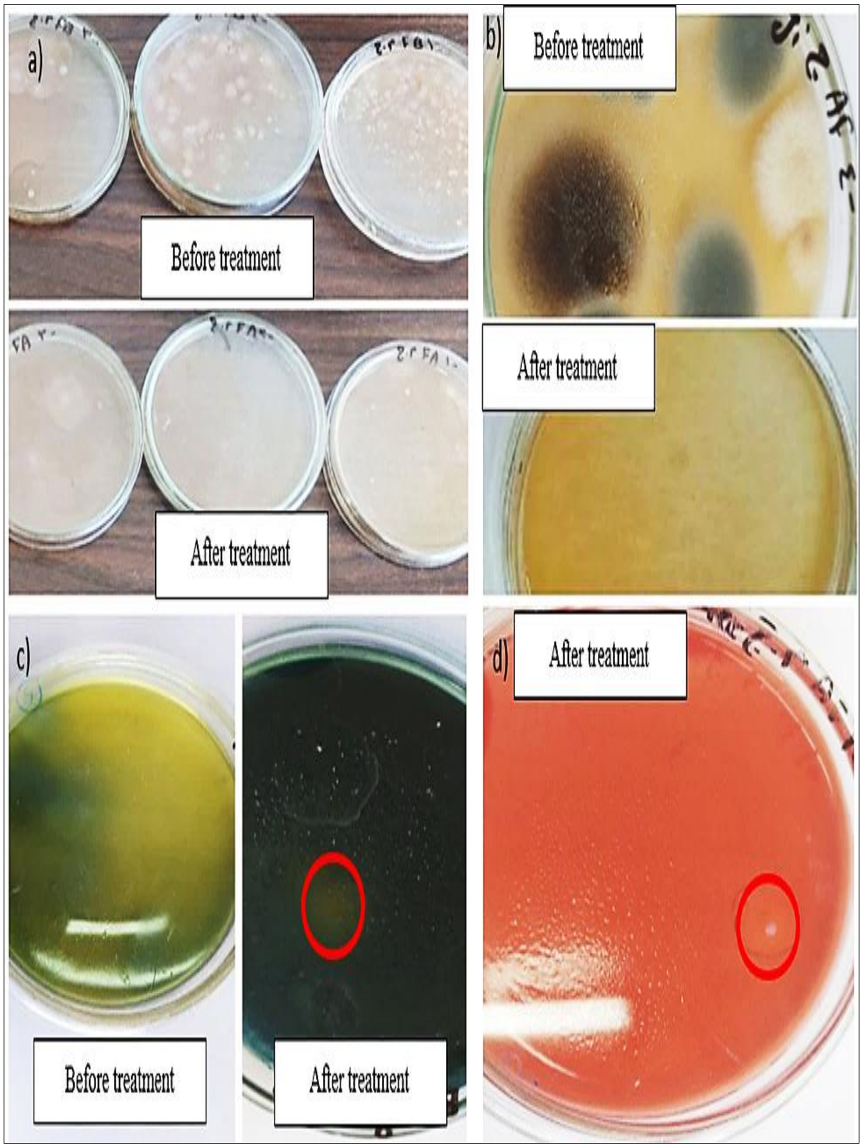

Fig. 2. The pathogenic microbes detection and total bacteria and fungi count of wastewater samples. (a) Enumeration of total bacteria before and after ESP treatment, (b) Enumeration of total fungi before and after ESP treatment, (c) Detection of *Bacillus cereus* before and after ESP treatment, (d) Detection of *E.coli* before and after ESP treatment

3.2 Scanning electron microscope (SEM) investigation

SEM characterization of ESP before treatment showed that it has a porous structure with interconnected pores of varying sizes Fig. (3a). It was obsereved in Fig. (3b, c) the pollutant molecules were adsorbed on the surface of the eggshell particles. The data described showed clusters or larger aggregates of eggshell particles.

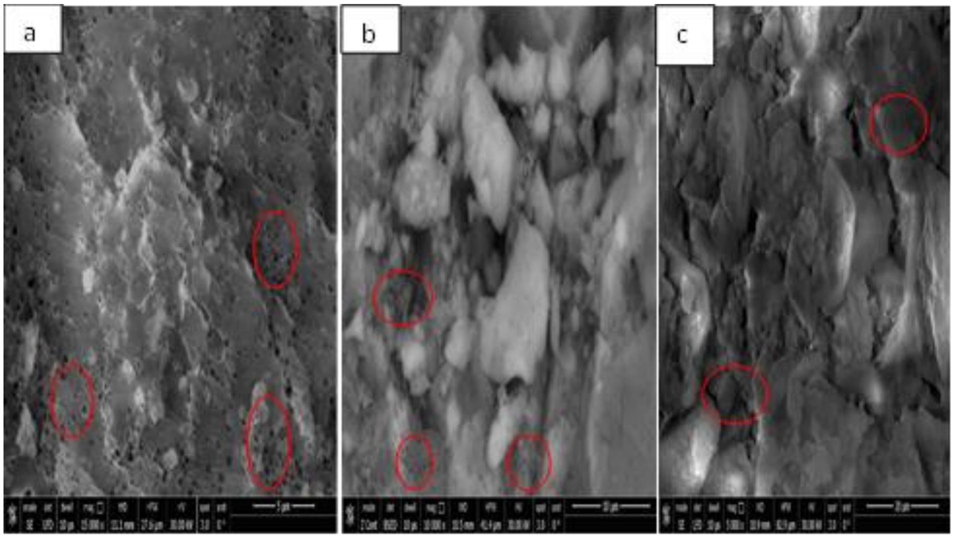

Fig. 3. Scanning electron microscope (SEM) investigation of ESP before and after wastewater treatment: (a) SEM for Eggshell waste powder (ESWP) as a control, (b) SEM for WESP1 (raw wastewater with ESP), (c) SEM for WESP2 (Pre-treated wastewater with ESP)

3.3 Energy Dispersive X-Ray analysis (EDX) analysis

EDX is used to analyze the elemental composition of samples. By utilizing X-ray spectroscopy, it identifies and quantifies the elements present on the surface of the ESP control sample and after wastewater samples treatment. As observed in Table (5), the oxygen (29.47%), and calcium (41.31%) were the only elements observed at ESP surface (control).

Table 5. Energy Dispersive X-Ray Analysis (EDX) of elements on the surface of eggshell powder (ESP)

Element	ESP (control)			Raw wastewater			ESP treated wastewater		
	W%	A%	E %	W%	A%	E %	W%	A%	E %
O	29.47	47.46	11.93	73.40	59.95	11.35	40.91	52.13	11.10
Ca	41.31	21.02	1.640	64.82	41.67	1.770	62.60	40.05	1.690
C	-	-	-	14.48	24.59	9.970	4.570	9.810	8.870
Mg	-	-	-	0.560	0.470	31.42	0.250	0.270	16.11
Al	-	-	-	0.470	0.450	22.83	0.280	0.210	16.15
Si	-	-	-	0.230	0.170	68.44	0.100	0.090	27.53
P	-	-	-	0.230	0.180	40.61	0.220	0.150	36.68
S	-	-	-	2.000	1.270	64.53	0.090	0.070	6.300

- = Not detected, W=Weight%, A=Atomic% and E=Error %.

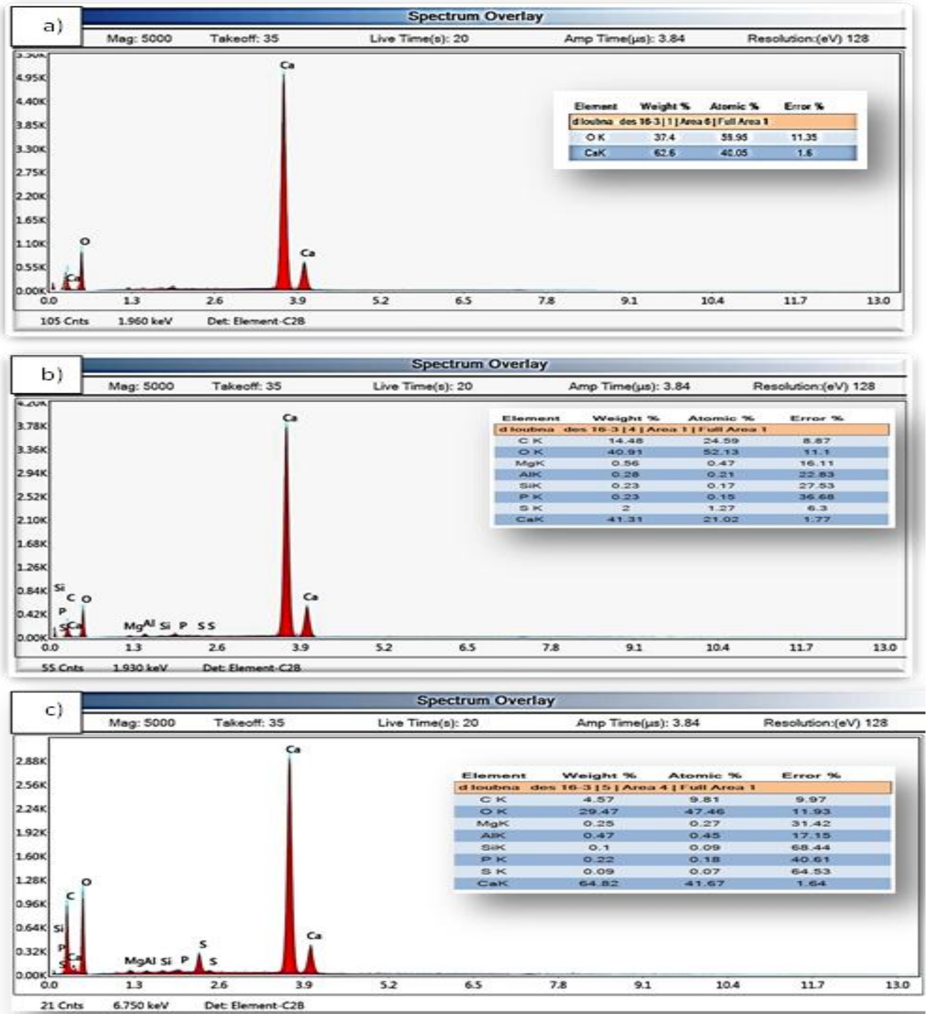

Fig. 4. Energy Dispersive X-Ray Analysis (EDX) of elements on the surface of Egg Shell (ESP). a) Control sample b) raw wastewater sample c) treated wastewater sample

DISCUSSION

In this study, we explored the potential of utilizing eggshell powder (ESP) as an abundant, cost-effective, and environmentally friendly bio-filter for wastewater treatment. The focus of our investigation was on evaluating ESP's efficacy in reducing microbial loads and heavy metal contaminants, which are critical concerns in wastewater reuse. The physicochemical parameters, including pH, total dissolved solids (TDS), conductivity, salinity, and other factors influencing wastewater quality, were carefully recorded. Specifically, the initial pH levels of raw wastewater samples (RW1 and PW2) were measured at 8.70 and 8.25, respectively, while those of the treated samples (WESP1 and WESP2) were reduced to 7.7. This notable decrease in pH by approximately 12.9% highlights a significant shift, likely attributed to the addition of chemicals during the treatment process, which can exhibit acidic properties. However, in the case of WESP1 and WESP2, the decrease in pH value occurs due to the neutralization reaction between the alkaline calcium carbonate and acidic components in the wastewater. Calcium carbonate reacts with acids, forming water, carbon dioxide, and soluble calcium salt. As previously illustrated by **Shayegan *et al.* (2015)**, the release of carbon dioxide gas and the formation of soluble calcium salts can contribute to the decrease in pH levels. It's worth noting that the observed pH range, which falls within the permissible limits of 6.0–8.7, aligns with the recommendations of the Food and Agriculture Organization **(FAO, 1992)**.

The electric conductivity (EC) of water is a measure of the ability of a solution to conduct an electric current; this ability depends on the dissolved solids, the presence of ions concentration, mobility, and temperature of the water. Water's conductivity is one of the crucial factors used to calculate the suitability of water for irrigation **(Gaur *et al.*, 2022)**. The conductivity of the case of WESP1 and WESP2 was recorded as 340 and 320µS/cm, respectively, which did not exceeded the limit prescribed by the FAO organization recommendations of 1200µS/cm **(FAO, 1992)**. In addition, it was found that the conductivity was eliminated by ~ 47% decrease from the mentioned universal levels.

The total dissolved solids (TDS) values of WESP1 and WESP2 ranged from 217 to 204 mg/L, showing a decrease of approximately 46-47%. These values remain well below the standard limit of 600 mg/L recommended by the Food and Agriculture Organization (FAO) **(FAO, 1992)**. The TDS is a very effective factor that affects the effluent discharge and doesn't cause damage to soil flora and fauna and leads to changes in soil porosity, soil texture, and water. The reduction in TDS levels was achieved through the process of co-precipitation and adsorption. The calcium carbonate in the ESP reacts with various dissolved ions in the wastewater, such as calcium, magnesium, sulfate, and carbonate ions. These reactions can lead to the formation of insoluble calcium salts, which then precipitate out of the solution. As a result, the concentration of dissolved solids decreases, leading to a reduction in TDS **(Fadia *et al*., 2021)**.

After ESP treatment, soluble anions HCO3-, Cl-, and SO4- decreased by 10.84%, 46.2%, and 70.8%, respectively. Conversely, cations Ca2+, Mg2+, and Na+ decreased by 55.23%, 48.8%, and 46.17%, while potassium cations increased by 58.75%. The decrease in anions can be attributed to the composition of ESP, primarily consisting of calcium carbonate (CaCO3), which increases the concentration of calcium (Ca2+) ions in the wastewater. Calcium ions can form insoluble precipitates with certain anions, such as phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}), decreasing their presence in the treated wastewater. Increasing cations: ESP can release calcium ions (Ca^{2+}) into the wastewater, which can increase the concentration of cations. This is particularly relevant when the original wastewater has a low concentration of calcium ions. The increase in calcium ions may affect the overall cation balance in the treated wastewater **(Li *et al*., 2018)**.

The result of microbiological tests at wastewater (WESP1 and WESP2) recorded the maximum effect more than the effect of reducing the total count of microorganisms at RW1 and PW2 as the presence of pathogenic microorganisms decreased at WESP1 and WESP2 compared to RW1 and PW2. The use of ESP in wastewater treatment led to a decrease in microbial enumeration present in the treated wastewater. ESP has been reported to possess antimicrobial properties attributed to substances such as lysozyme and calcium ions that can inhibit the growth and activity of pathogenic microorganisms **(Ahmed *et al*., 2021)**.

SEM characterization of control ESP revealed that ESP has a porous structure with interconnected pores of varying sizes. These pores can be observed as irregularly shaped voids or channels on the surface of the particles. The presence of these pores contributes to the high surface area and adsorption capacity of the ESP **(Pavlenko *et al*., 2022)**. It was observed that the pollutant molecules were adsorbed on the surface of the eggshell particles. These pollutants appeared as small clusters, coatings, or irregularly distributed spots on the surface. However, the adsorption process can lead to the aggregation or agglomeration of eggshell particles. This can occur when the adsorbed pollutants act as bridges between the particles, causing them to stick together and the formation of clusters or larger aggregates of eggshell particles **(Qianli *et al*., 2023)**.

EDX is used to analyze the elemental composition of samples. By utilizing X-ray spectroscopy, it identifies and quantifies the elements present on the surface of the ESP after adsorption and a control sample. In the observation, oxygen and calcium were the only elements detected in the ESP (control). Conversely, more elements could be noticed on the surface of ESP after water treatment because of the adsorption ability of the metals on the surface of ESP. The presence of the heavy metals in the ESP-metal complex for raw wastewater (WESP1) and pretreated wastewater (WESP2) were in the following sequence: oxygen was 73.40%, 40.91%; calcium 64.82%, 62.60%; carbon 14.48%, 4.57%; magnesium 0.56%, 0.25%; aluminum 0.47%, 0.28%; silicon 0.23%,0.1%, phosphorus 0.23%, 0.22%, sulfur 2.0%, 0.09%, respectively. Observing the increase in calcium percentage during the treatment process, it is attributed to the alkaline nature of ESP. The alkalinity promotes the dissolution of calcium carbonate, releasing calcium ions into the ESP-treated wastewater. These calcium ions can then become part of the treated effluent, increasing the concentration of calcium. Calcium is an essential nutrient for certain biological processes and can contribute to the growth of plants and fish. Additionally, the increased calcium concentration may enhance the flocculation and settling of suspended solids during wastewater treatment **(Reta *et al*., 2021)**. It was found that there is little difference in the proportions of the basic elements of ESP between the EDX before and after wastewater treatment. Raw wastewater contains a higher concentration of heavy metals and soluble minerals compared to primary treated wastewater. Consequently, the absorption of metals by ESP increased significantly in raw wastewater. In conclusion, it is recommended to consider ESP as an alternative method for the initial filtration of wastewater in the primary step of wastewater treatment plants. This study suggests that treating raw wastewater with ESP can potentially replace the use of numerous chemicals, thereby reducing the costs associated with wastewater decontamination and heavy metals removal during the primary treatment process.

CONCLUSION

The aim of this study was to assess the efficacy of eggshell waste powder (ESWP) in removing water-polluting substances such as heavy metals and microbial contaminants. ESWP demonstrated significant removal efficiency for aluminum, sulfur, and silicon, comparable to primary filtration treatments for wastewater. Additionally, after 72 hours of contact time at 30°C, *E. coli*, *Enterobacter* sp., *Salmonella* sp., and *Shigella* sp. were effectively eliminated. The research encompassed an analysis of the physical characteristics of eggshell waste using scanning electron microscopy (SEM-EDX). Furthermore, the study evaluated the chemical characteristics of wastewater before and after treatment, including parameters like Total Dissolved Solids (TDS), soluble cations, soluble anions, Electrical Conductivity (EC), and pH levels. The findings highlighted that eggshell waste offers a straightforward, cost-effective, and environmentally friendly solution for removing water pollutants. Based on the results, the study recommends ESWP as a bio-sorbent to efficiently remove toxic elements and microbial contaminants from wastewater. Notably, the elemental composition of the treated wastewater met the standards outlined in the FAO guidelines for water irrigation. Consequently, utilizing ESWP-treated water for irrigation purposes is feasible. Additionally, the presence of soluble calcium in the treated wastewater makes it a valuable nutritional source for certain plant species.

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