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A Comparative Study on the Bioadsorption of *Punica granatum* Peel and its Activated Charcoal

Debanjali Shome, Priyanka Pradhan and U Shivagama Sundari*
Department of Life Sciences, Kristu Jayanti College, Bengaluru, Karnataka, India

ABSTRACT

Agricultural waste act as promising adsorbents for heavy metals, while their characteristics and mechanism needs to be studied. In this paper, the adsorption capacity of a select agricultural waste and its derivative was compared. One major issue with industrial waste water treatment is the removal of heavy metals, which are poisonous components. Bio-sorbents can be used to remove heavy metals from industrial waste. The bio-sorbent used in the present study is pomegranate peel (PGP) and its activated charcoal for removal of Cadmium (Cd^{2+}) and Chromium (Cr^{2+}) metals from aqueous solutions. Physical and chemical characterizations of the bio-sorbents have been done. Adsorption experiments have been performed to determine and compare the adsorption capacities of the two biosorbents. The results of this study suggest that activated charcoal biosorbent is highly efficient in the removal of heavy metals however; PGP can also be used for this purpose for lower efficiency.

KEYWORDS: Bioadsorption, Bioadsorbents, Heavy metals.

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1. INTRODUCTION

Heavy metals are a group of trace elements i.e., metals and metalloids with an atomic density greater than 4 ± 1g / cm^3. The presence of heavy metals from the transition series, viz. Cu, Pb, Fe, Ni etc. in the environment is of major concern due to their toxicity to many life forms *(El-Ashtoukhy et al., 2008)*. *(Acharya et al., 2018)* remarked that certain heavy metals are cumulative poison. There are two main sources of heavy metals in wastewater, viz. human and natural. These metals are characterized by their significant persistence, toxicity and accumulation in the environment especially in water, for it can distribute these metals along the food chain *(Ben-Ali et al., 2017)*. These metal ions are non-degradable and are constant in the environment. Therefore, the elimination of heavy metal ions from wastewater is important to protect public health *(Salman H. Abbas, 2014)*.Agricultural waste has been an ecological burden for society but have promising prospective for the production of low-cost and sustainable adsorbents for several kinds of water treatment applications *(Bhatnagar, 2015)*. Many agricultural wastes are often rich in cellulose *(Chao Hu, 2020)*.

Adsorption is accepted as an effective and economic method for wastewater treatment. Because adsorption is sometimes reversible, adsorbents can be regenerated by various suitable desorption process *(Fu and Wang, 2011)*. The biosorption process has shown many desirable features including its rapid kinetics of adsorption and desorption, the selective removal of metals over a wide range of pH and temperatures in addition to the low capital and operation cost is a major advantage of this process *(Migahed, 2017)*. Different types of biomasses are used as biosorbent like waste agriculture materials, to remove heavy metals through biosorption. The surface modifications of biomass by acids or alkalis aids in removing most of the non-cellulosic components in order to increase the efficiency of the metal biosorption *(Abdic S et al., 2018)*. Adsorbents based on activated carbons (ACs) are widely used to remove noxious heavy metal contaminants due to their well-developed porous structure and a high specific surface area, as well as due to the presence of different surface functional groups.

Pomegranate is a fruit-bearing, deciduous shrub that belongs to the family Punicaceae. Pomegranate (*Punica granatum*) fruits are widely consumed fresh and in pro-

* Author for correspondence: U Shivagama Sundari | Email: sundariutharanan@gmail.com

cessed forms as juice, jams and wine. Pomegranate peels, a by-product of pomegranate juice industry is inexpensive and are characterized by substantial amounts of phenolic compounds, including hydrolysable tannins. Hence, pomegranate peel constitutes an efficient, low cost and environment friendly source of bio-adsorbent *(Ben-Ali et al., 2017)*.

Scanning Electron Microscopy (SEM) has been used extensively for measuring microstructural feature sizes and their distribution/density, quantifying the 3D morphology of objects, measuring particles, examining porosity etc. *(B. J. Inkson, 2016)*. Fourier transform infrared (FTIR) spectroscopy probes the vibrational properties of amino acids and cofactors, that are sensitive even to minute structural changes *(Berthomieu, 2009)*. The purpose of this work was to assess the heavy metals (Lead and Cadmium) adsorption capacity of raw pomegranate peel and its activated charcoal from aqueous solution through Atomic Absorption Spectrophotometry (AAS).

2. MATERIALS AND METHODS

2.1. Sample Procurement

6 kg of hybrid pomegranate fruit was bought from K.R market, Bangalore.

2.2. Preparation of peel extract

The pomegranate peel was washed with preheated double distilled water several times. It was air dried for 24 hours then at 100˚C for one hour in hot air oven. The peel was then weighed which was found to be 514.36g. The peel was ground and sieved to particle size >250 mm. The powder was then weighed, which was found to be 513.53g. The powder obtained was divided into two parts of 200g each for the preparation of biosorbents.

2.3. Preparation of the bio-sorbents

The first part containing 200g of the prepared powder was acidified with 20% phosphoric acid and heated in a muffled furnace at 500˚C for one hour. The charcoal was powdered and weighed, which was found to be 94.47g. This was labelled as “Activated charcoal” (Figure 1). The second part was used without any modification hence it was labelled as “Raw”. The yield from prepared biosorbents was calculated from the following equation.

$$\% \text{ yield} = W_O - W_C / W_o \times 100 \qquad (1)$$

Where, W_O is the mass of material before carbonisation, W_C is the mass of material after carbonisation. The biosorbents was then characterized with pH, Boehm titration, Iodine number, FTIR spectrum analysis.

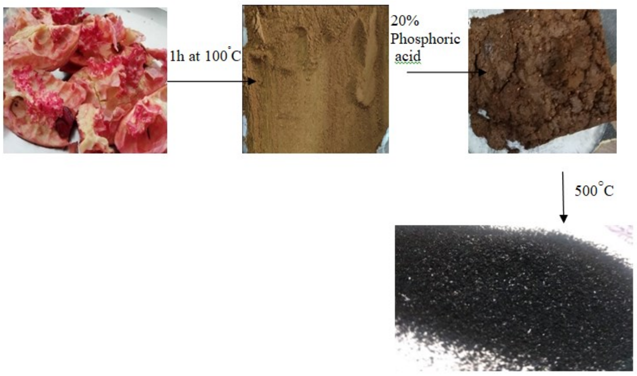


Figure 1: Steps for preparation of activated charcoal from pomegranate peel

2.4. Characterization of the biosorbents

2.4.1. pH of the bio sorbents

The equilibrium pH of the bio-sorbents was determined by placing 1g of the pomegranate peel (PGP) in 100ml of double distilled water. The whole was shaken for 24 hours. The pH of the suspension was measured using a pH meter *(Ben-Ali et al., 2017)***.**

2.4.2. Boehm titration

1.5 g of the bio-sorbents were added to 50 ml of the three reaction bases of 0.05 M concentration of $NaHCO_3$, Na_2CO_3 and NaOH. The samples were agitated by shaking for 72 hours and then filtered .10 ml aliquots of samples was pipetted. To the aliquot of $NaHCO_3$, 20ml of 0.05 M HCl was added. To the aliquot of Na_2CO_3, 30ml of 0.05M HCl was added. This acidified solution was back titrated with 0.05 M NaOH. The aliquots of the NaOH was titrated directly with 0.05M HCl *(Goertzen et al., 2010).*

2.4.3. Iodine number

The iodine adsorption is determined by sodium-thiosulphate volumetric method. The biosorbents was stirred with 0.1N iodine solution and filtered. The resulting filtrate was titrated with sodium thiosulphate solution (0.1N) using starch as an indicator *(American Society for Testing and Materials, 2014)*.

2.4.4. SEM analysis

The structural image such as SEM (scanning electron microscope) was an important tool that was used to examine the key changes in the surface of the biosorbents *(Mohammed et al., 2018)*.

2.4. 5. Point of zero charge

The pH_{pzc} (point of zero charge) of the sample was determined by mixing 0.15 g with 50ml 0.01N solution of NaCl, pH had been adjusted between 2 to 12 with NaOH and HCl. The containers were shaken for 48 hours. After filtration the pH was measured as described by Samia *Ben Ali et al. (2017).* The intersection pH_{final} versus $pH_{initial}$ curve and the bisector gives the pH_{pzc} value.

2.4.6. FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis had been carried out to identify active sites for adsorption mechanism *(Ben-Ali et al., 2017).*

2.4.7. Chemical characterization of the biosorbents

2.4.7.1. Test for carbohydrates

2.4.7.1.1. Molisch's test

1 ml of test solution was treated with 1% alpha napthol and 2-3 ml of conc. H_2SO_4. Appearance of reddish violet or purple colour at the junction of the two liquids indicated the presence of carbohydrates.

2.4.7.1.2. Seliwanoff's test

3 ml of Seliwanoff's reagent was added to 1ml of the test sample. It was heated on a water bath for 1 minute. Appearance of rose red precipitate indicated the presence of carbohydrates.

2.4.7.1.3. Fehling's test

2 ml of the test solution was heated with Fehling's solution. Appearance of brick red precipitate confirmed the presence of carbohydrates.

2.4.7.2. Test for fats and oils

5 drops sample was mixed with 1ml of 1% copper sulphate and a few drops of 10% NaOH. Appearance of clear blue solution confirmed the test.

2.4.7. 3.Test for tannins

2.4.7. 3.1. Ferric chloride test

2 ml of test solution was mixed with a few drops of ferric chloride solution. Appearance of blue colour confirmed the test.

2.4.7.3.2. Gelatin test

1 ml of test solution was mixed with few drops of 1% gelatin containing 10% NaCl. Appearance of white precipitate confirmed the test.

2.4.7.4. Test for Alkaloids, phytates, oxalates and total phenolics

Tests for alkaloids and oxalates were performed following the procedure of *Romelle et al., 2016*. The tests for phytates and total phenolics were performed according to the procedures of *Reddy et al., 1982* and *Dewantoo et al., 2002,* repectively.

2.4.7.5. Test for flavonoids

2.4.7.5.1. Alkaline reagent test

5 drops of 5% NaOH was added to 1 mL of test solutions that resulted in an increase in intensity of yellow colour upon addition of a few drops of 2M HCL indicated the presence of flavonoids.

2.4.7.5.2. Lead acetate test

10 drops of lead acetate was added to 1 mL of the test solutions. Appearance of yellow precipitate confirmed the presence of flavonoids.

2.5. Preparation of metal solution

1000 ppm of metal solution was prepared using double distilled water *(Sobhy et al., 2018)***.**

2.6. Adsorption Experiments

Adsorption experiment was carried out for Chromium and Cadmium at constant concentration and temperature using the two biosorbents (PGP and AC). The test solutions of Cadmium contained 2 ppm concentration and that of Chromium contained 5 ppm concentration. 0.25 g of biosorbents were added to each of the tubes separately and were incubated for 2 hours then filtered using Whatmann filter paper. Adsorption Spectroscopy was used to measure the clear solution. The test was done in triplets. The percentage removal of heavy metal from solution was calculated by following equation:

$$\% \text{ removal} = C_o - C_i / C \times 100 \qquad (2)$$

Where, C_0 was initial concentration of heavy metals, C_i was the final concentrations of heavy metals. The amount of metals adsorbed onto pomegranate peel, q_e (mg/g), was calculated using equation

$$q_e = (C_o - C_e)V/W \qquad (3)$$

Where, C_o and C_e are the initial and final concentration of the heavy metals respectively (mg/L). q_e is the adsorption capacity, V is the volume of the solution (L) and W is the mass of the adsorbate (g) *(El-Ashtoukhy et al., 2007 and Sobhy et al., 2018).*

3. Results and Discussion

3.1. Biosorbents preparation

The yield from each prepared biosorbents was found to be 52.77%

3.2. Characteristics of the adsorbing material

3.2.1. pH of the adsorbents

The pH of the adsorbent has a significant impact on the uptake of heavy metal since it determines the surface charge of the adsorbents and the degree of ionization. The pH of the prepared pomegranate peel was observed to be 5.42 and that of the activated charcoal is found to be 1.99.

3.2.2. Boehm titration

The surface functional group was determined by Boehm titration methods as shown in Table 1. The concentration of acidic sites present on the adsorbent was calculated by understanding that $NaHCO_3$ neutralizes carboxylic groups, Na_2CO_3 neutralizes carboxylic and lactonic groups, NaOH neutralizes all surface groups hence $NaHCO_3$ is used for calculating carboxylic group, Na_2CO_3 for lactonic group and NaOH for phenolic group **(**Table 2**)**. Ɗ$_{csf}$ depicts the presence of surface functional groups on the surface of the adsorbents

Table 1: Functional Groups for Different Biosorbents

Titration	**Functional groups (mmol/g)**		
	Carboxylic	**Lactonic**	**Phenolic**
PGP	19.6	22.4	34.63
AC	27.2	16.8	12.8

Table 2: Carbon Surface Functionalities for Different Adsorbents

Samples	**Ɗ$_{CSF}$ (mmol/g)**		
	$NaHCO_3$	**Na_2Co_3**	**$NaOH_{direct}$**
PGP	-1.45	-1.1	1.85
AC	-1.3	-3.45	1.3

3.2.3. Point of zero charge (pH_{pzc})

The point of zero charge is the pH at which the surface of your adsorbent is globally neutral, i.e., contains as much positively charged as negatively charged surface functions. The point of zero charge of the bioadsorbents was determined to be 1.49 (Table 3, Figure 1)

Table 3: pH of the Adsorbents for Point of Zero Charge

Samples	**Initial**	**Final**
Pomegranate peel (PGP)	2	2. 8
Activated Charcoal (AC)	6	5. 14

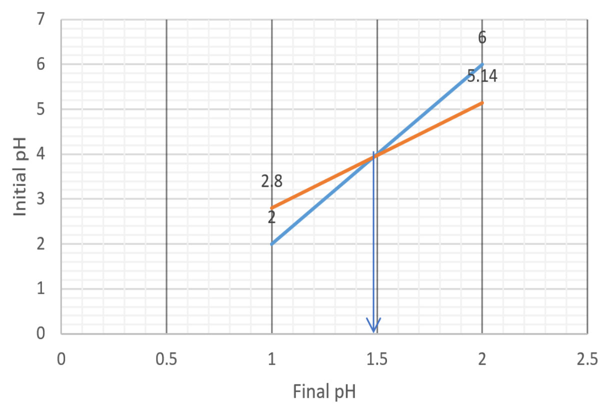


Figure 1: Point of Zero Charge of the Bioadsorbents

3.2.4. Iodine number

The iodine adsorption number determines the pore structure of the adsorbent. Activated charcoal prepared from the pomegranate peel has a higher surface area and porosity, its iodine number is found to be 414.65. However, PGP has a relatively lower iodine number of 89.16.

3.2.5. Chemical characteristics of the adsorbent

The results of the chemical characteristic of the adsorbing material is given in Table 4.

Table 4: Chemical Characteristics of the Adsorbent

Chemical test	Pomegranate Peel (PGP)	Activated Charcoal (AC)
Carbohydrates		
Molisch's test	+	+
Seliwanoff's test	+	+
Fehling's test	+	-
Fats and oils	-	-
Tannins		
$FeCl_3$	+	-
Gelatin	+	-
Phytates	+	+
Alkaloids	-	-
Oxalates	-	-
Phenolics	+	+
Flavonoids	+	-

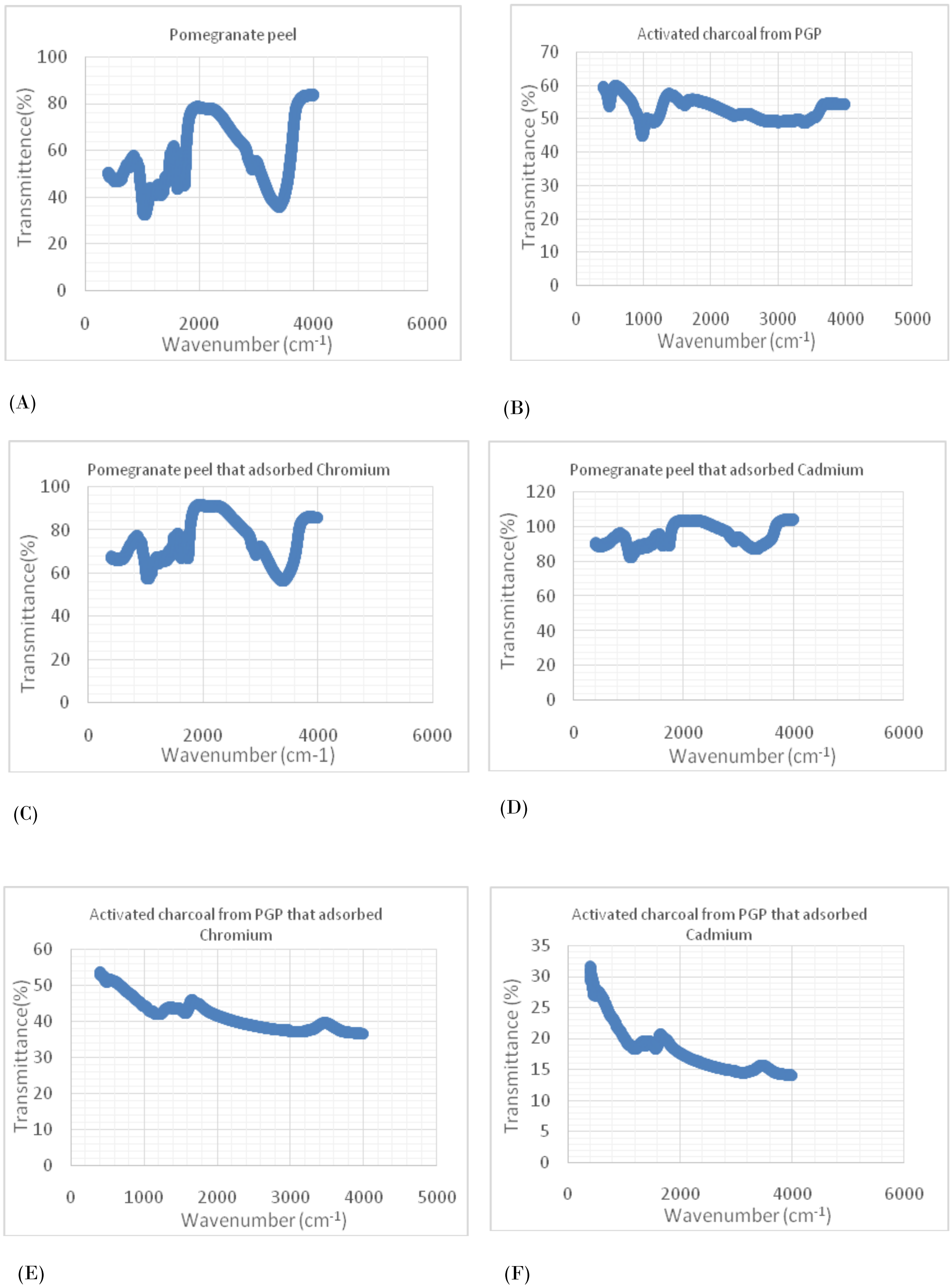
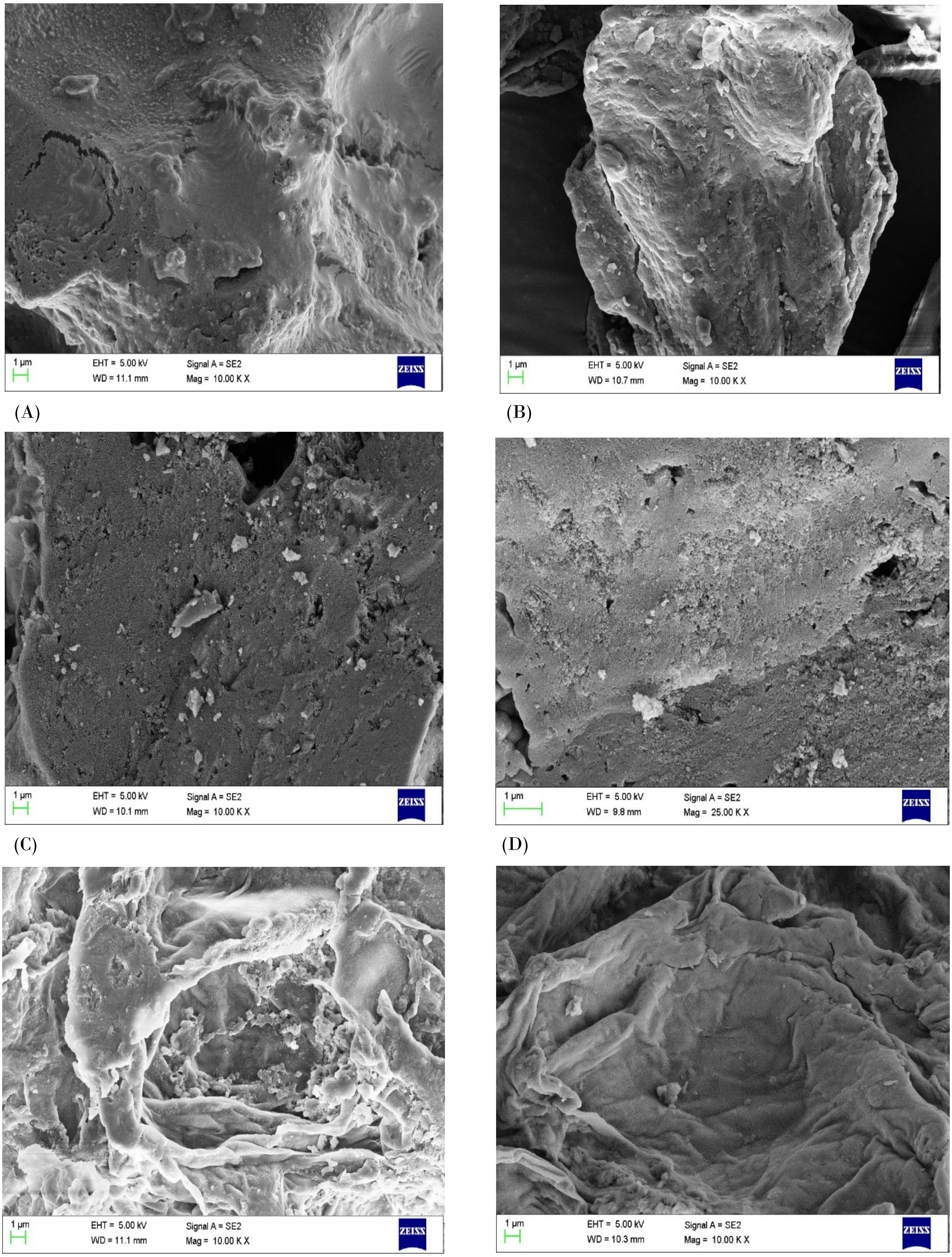


Figure 2: FTIR Spectra of (A) Pomegranate Peel (B) Activated Charcoal Prepared from the Peel before Adsorption, (C, D) Pomegranate Peel that Adsorbed Chromium and Cadmium Metals and (E, F) Activated Charcoal Prepared from the Peel that Adsorbed Chromium and Cadmium Metals.

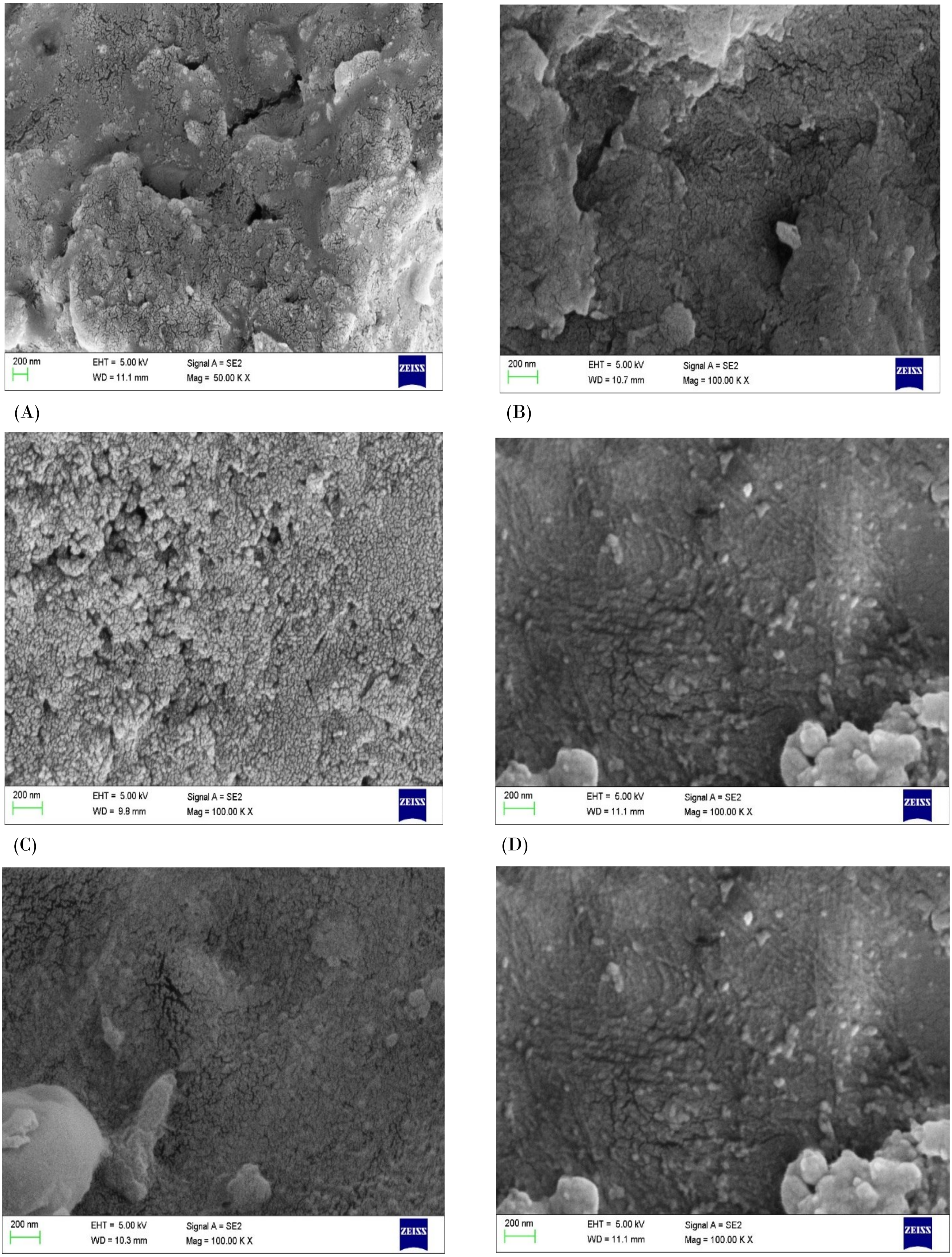


(A) (B)

(C) (D)

(E) (F)

Figure 3: Scanning Electron Micrographs (at 1µm resolution) of (A) Pomegranate peel (B) Activated charcoal prepared from the Pomegranate peel (C) Activated charcoal that adsorbed cadmium (D) Activated charcoal that adsorbed Chromium (E) Pomegranate peel that adsorbed Chromium (F) Pomegranate peel that adsorbed cadmium



(A) (B)

(C) (D)

(E) (F)

Figure 4: Scanning Electron Micrographs (at 200 nm resolution) of (A) Pomegranate peel (B) Activated charcoal prepared from the Pomegranate peel (C) Activated charcoal that adsorbed cadmium (D) Activated charcoal that adsorbed Chromium (E) Pomegranate peel that adsorbed Chromium (F) Pomegranate peel that adsorbed Cadmium

3.3. FTIR (Fourier Transform infrared) spectroscopy

The spectra of PGP and its activated charcoal before and after adsorption of heavy metals are shown in Figure 2. In the spectra the absorption peak at about 3200-3400 cm-1 corresponds to the OH stretching groups. The rise in the **peak shifting** from 2950-2970 cm-1 was observed after Cadmium adsorption onto the surface of PGP shows methyl C-H asymmetric/ symmetric stretch. The peak 1590-1650 cm-1 shows the presence of primary amine group which depict the functional group attached to the backbone. The C-F bond stretch is due to association of halogen with carbon atom. The minor band shift of the C-O stretching of alcoholic hydroxyl group to 1050 cm-1 which proves that this groups are not involved in the adsorption process.

3.4. Scanning Electron Microscope (SEM) analysis

SEM analysis results are depicted in Figure 3 and 4. Elemental analysis result of AC showed that carbonization of dried pomegranate peel caused increase in elemental carbon contents which was verified with SEM analysis. SEM results verifies that the pore size of activated charcoal is larger than PGP. As a result, AC can adsorb a larger amount of heavy metal than PGP which makes AC a more efficient bioadsorbent.

3.5. Adsorption experiment

The percentage removal of Cadmium by pomegranate peel (PGP) is 0.05 and by activated charcoal (AC) is 0.1875 and that of Chromium by pomegranate peel is 0.06 and by activated charcoal is 0.08. The adsorption capacity of PGP for Cadmium is 20 mg/L and for Chromium is 60 mg/L. AC shows higher adsorption capacity by adsorbing Cadmium at 60 mg/L and Chromium at 80 mg/L.

4. CONCLUSION

Adsorption of heavy metals has been proved to be efficient method. The present study has been carried out to determine the efficiency of pomegranate peel and its modification. They were physically and chemically characterized. The percentage removal of heavy metals was calculated and activated charcoal was highly efficient compared to the pomegranate peel.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

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