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A Novel Method to use the Waste Products of Wood apple (shell and seed) for Phytoremediation and Nutritional Purpose

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Abstract : *Present paper deals with the study of shell of wood apple for remediation of Cr(VI) and Cd(II) from aqueous solution. It also deals with the evaluation of the nutritional contents of the wood apple seeds, which is quite often discarded in general. The study suggested that the shell can be used effectively in remediation of Cr and Cd as during study, the level of the stock solutions of these metals showed decline WAS could adsorbed about 88% Cr(VI) and about 92% Cd(II) in a stock of 90 mgL^{-1} under acidic conditions. The extent of removal varies with different process parameters like pH, agitation time, adsorbent dosage and initial concentration. On the other hand, the seeds of wood apple is an unconventional source of protein and the research was undertaken to study the seed protein content of the wood apple fruit in the form of Seed Protein Content (SPC) containing about 75g protein and several other nutritional components per 100g SPC. Hence the present research aims to utilize the waste biomass of wood apple (shell and seed) to human welfare instead of its free disposal that results in increased level of environmental pollutions.*

Key Words : *Wood Apple Shell (WAS), heavy metal removal, aqueous solution, seed protein content (SPC).*

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Introduction:

Water pollution by heavy metals has become a serious global concern because metals are non-biodegradable and are toxic to human beings. An increase in pollution has consequently led to increase in the effluent discharge into the aquatic ecosystem. Ground, surface, and processing waters frequently contain inadequate amount of dissolved heavy metals from sources like mines, factories, and other industries.

The term heavy metal refers to the element of atomic number 21 or higher (e.g. scandium and above).Any metal or metalloid may be considered as a “contaminant” if it occurs where it is unwanted or in a form or concentration that causes a detrimental effect on the environment or to the living organisms. At least 20 metals are classified as toxic and half of these are emitted into the environment in quantities that pose risks to human health. (Davis et.al.,2003)

Among the heavy metal ions Cadmium (Cd^{+2},atomic no.48) is one of the extremely toxic and carcinogenic metal ion due to its solubility and mobility in aqueous solutions. Even at low concentrations cadmium is highly toxic to living organisms. In case of humans cadmium toxicity can cause renal dysfunction, hypertension, hepatic injury, lung damage, and teratogenic effects.

Cd(II) enters into water bodies through various industrial discharges like smelting, metal plating, cadmium-nickel batteries, and alloy industries, and also it enters through sewage sludge. In view of its toxicity, cadmium is listed by the US Environmental Protection Agency (USAEPA) as one of the 129 priority pollutants and set 0.01mgL”1 as safety level in drinking water.

Whereas Chromium (Cr^{+6},atomic no.24) is considered as hazardous pollutant worldwide because it is mutagen (a substance that causes genetic mutation) and potential carcinogen (Suantak Kamsonlian et al., 2013). The tolerance limit for Cr (VI) for discharge into inland surface waters is 0.1 mg/L and in potable water is 0.05 mg/L according to Environmental Pollution Act (1990) standards for Cr discharge.

An adsorbent can be considered as low cost if it is abundant in nature, requires little processing and is a byproduct of waste material from waste industry. (Hossain et.al.,1999) Hence we used wood apple shell as an adsorbent for our research.

On the other hand Wood apple seeds are important unconventional sources of proteins which when incorporated in food products would improve the functional properties such as absorption of water or oil and they are also good nutritional supplements. Interestingly, though the seed is a rich source of protein and oil, very little work was reported on these aspects. The seed composition and fatty acid profile were reported as 28% protein and 34% oil (Halim et al.,2002). Utilization of this seed protein is very limited and hence, the present investigation was undertaken to study wood apple seed meal and seed protein concentrate of the wood apple.

Materials and Methods :

Biosorbent Preparation: The Wood apple shell (WAS) was prepared from the hard shell of wood apple fruit. The shells were broken down to smaller pieces and soaked in concentrated sulphuric acid at 1:1 (W:V) ratio for 24 hrs. The shells were made into small pieces, washed several times in deionized water, dried under sunlight, and grounded in a steel mill to get fine powder. The fine powder was washed to remove soluble material with deionized water and dried at 70‘“C temperature in hot air oven for about 3 hours.

Preparation of Stock Solutions of Cr (VI) and Cd (II) : Stock solution of Cr (VI) was prepared (1000 mg L^{-1}) by dissolving $K_2Cr_2O_7$ in double distilled water. Similarly, stock solution of Cd (II) was prepared (1000 mg L^{-1}) by dissolving $Cd(NO_3)_2.4H_2O$ in double distilled water.

Adsorption Process : 500 mg WAS along with 1000 mL stock solutions each of Cr (VI) and Cd (II) were placed in conical flasks separately and sealed properly using aluminium foil. Sealed flasks were placed in shaker incubator for 3 hrs at a speed of 300 rpm at 25 °C temperature. The contents of the flasks were filtered through whatman filter paper and the filtrates obtained were centrifuged and U.V. Spectrophotometer readings were taken at suitable wavelength. Concentration of un-adsorbed Cr (VI) in aqueous solution was determined by 1,5-diphenyl carbazide method (Hasanah et.al., 2012). A calibration graph of absorbance versus known concentration of Cr (VI) was obtained using spectrophotometer at 540 nm. Similarly, the amount of adsorbed Cd (II) was determined using spectrophotometer at 310 nm (Chaudhary et al., 2014). The amount of metal ion adsorbed was calculated using the formula given below:

$$Q_e = V/M\ (C_o - C_e)$$

Q_e = the amount of metal ion sorbed.

V = solution volume (1000 mL).

M = mass of WASAC (500 mg).

C_o = initial concentration.

C_e = final concentration.

The WAS materials were characterized by their high degree of porosity, incredible large surface areas and superficial surface containing functional groups and possess a high adsorption capacity (Muthusamy et al., 2012). Various studies revealed that the most abundant functional groups found onto the prepared activated carbon. Hence, various tests were performed in order to show the presence of functional groups like alcohols, amines, esters as well as estimation of protein (Lowry method), phenols (Folin Ciocalteu method) and organic acid (Titrimetric estimation) were done using the standard methods.

Preparation Seed Protein Content (SPC): Outer hard shell of wood apple fruit was broken and the seeds were separated from the pulp. The seeds were then washed with running water and the seeds were dried in hot air oven at 45°C for 4 hours. The seeds were then grinded in a porcelain mortar to separate the hulls and they were manually removed to obtained the cotyledon (de-hulled seeds). The de-hulled seeds were grinded in mixer. The de-hulled seed flour was soaked in hexane at room temperature with occasional stirring for a period of 6 hour to remove fat. The solvent was decanted and the fat free meal was air dried at room temperature (Mc.Grath et al., 2001). The final product was called as seed protein content (SPC). Proximate composition like moisture, protein (Lowry method), carbohydrate (Anthrone method), polyphenol (Folin Ciocalteu method) of SPC were determined.

Results and Discussion:

Extent of Remediation: Above study revealed that WAS adsorbed a considerable amount of Cr^{+6} and Cd^{+2} from their respective solutions and it was very clear from the experimentation that, there are various factors working with the rate of sorption, they are – pH, agitation time, adsorbent doages, initial-metal ion concentration.

Removal of Cr (VI): The concentration of un-adsorbed Cr(VI) in aqueous solution was determined by 1, 5-diphenyl carbazide method (Saikaew and Kaewsam, 2009). A calibration graph of absorbance versus known concentrations of Cr (VI) was obtained using spectrophotometer at 540 nm. This has been shown in Table 1.

Table 1. Table for adsorption of Cr^{+6} at different initial concentrations onto WAS.

Concentrations of Cr^{6+} (mg/L)	Absorbance (540 nm)	Concentration (mg/mL)
90	0.109	40
110	0.070	25
130	0.039	15

Removal of Cd (II): The concentration of metal ion sorbed onto WAS was determined spectrophotometerically (at 310 nm wavelength). This has been shown in Table 2.

Table 2. Table for adsorption of Cr^{+6} at different initial concentrations onto WAS.

Concentrations of Cr^{2+} (mg/L)	Absorbance (310 nm)	Concentration (mg/mL)
90	0.600	60
111	0.250	25
130	0.100	10

Hence, the amount of metal ions absorbed, (Qe) onto WAS was computed by the following equation:

$$Qe = V/M\ (C_o - C_e)$$

where C_o and C_e are the initial and equilibrium metal ion concentrations, respectively, whereas V and M are solutions volume and mass of adsorbent, respectively. Blank test was performed in the same experimental conditions without adsorbent WAS for both the metal ions.

Effects of parameters on the extent of Remediation :

1. **pH Effect :** At lower pH, WAS became negatively charged and the approach of metal cations towards the WAS increased due to strong electrostatic force of attraction between negatively charged WAS and metal cations. Hence, acidic medium increased the rate of adsorption. The amount of Cr-ion adsorbed onto WAS surface in different pH was 88% (in acidic medium) and 80% (in neutral medium). Cd-ion adsorbed on WAS was 92% (in acidic medium) and 48% (in neutral medium). Cd-ion adsorbed on WAS was 92% (in acidic medium) and 48% (in neutral medium). This has been shown in Fig. 1.

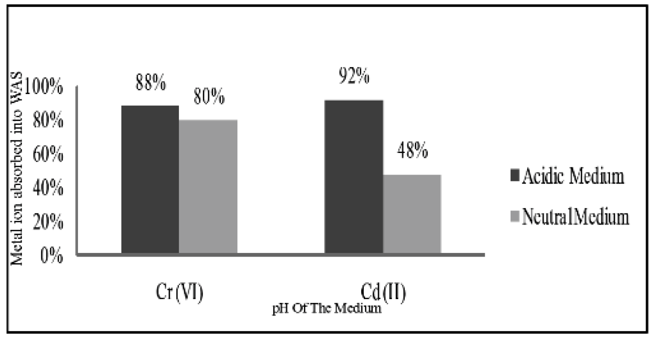

Fig. 1. Showing the effect of pH on remediation extent

2. **Agitation Time :** With increase in agitation time the rate of adsorption also increased as the metal cations got more time to be in contact of WAS surface. At the agitation time of 60 minutes, 78% Cr(VI) was removed while 50% Cd(II) was removed. At the 120 minutes, 83% Cr(VI) and 72% Cd(II) was removed. The most efficient time was found to be 180 minutes, as the maximum adsorption was found for both the metal ions, 87% of Cr(VI) and 90% Cd(II) was removed from the stock solutions using WAS. This has been shown in Fig. 2.

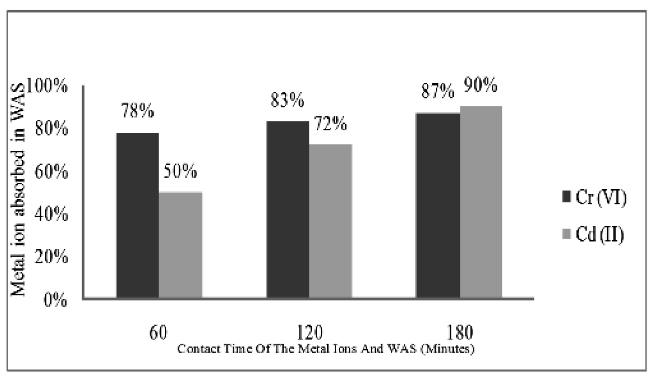

Fig. 2. Showing increase of removal efficiency with increase in agitation time

3. **Effect of biosorbent Dosage:** Higher dose of adsorbent had positive effect on the rate of metal ion removal. Maximum

adsorption was found with 500 mg of WAS, where about 88% Cr(VI) and 86% Cd(II) were adsorbed. However at 450 mg the adsorption was found to be 70% Cr(VI) and 62% Cd(II). Whereas at 475 mg the amount of metal ions adsorbed were 83% and 80% of Cr(VI) and Cd(II) respectively. This has been presented in Fig. 3.

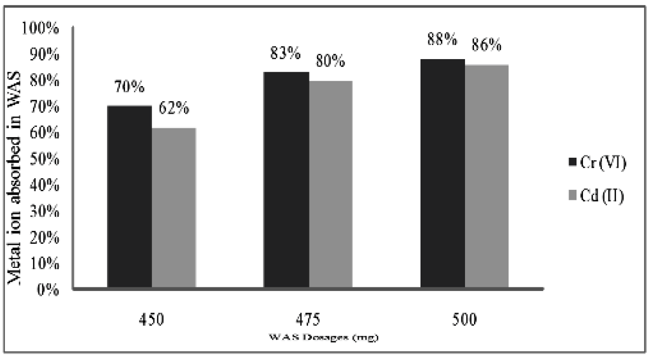

Fig. 3. Showing the positive effect of increase in WAS dosages

4. **Effect of Metal ion Concentration :** With increase in metal ion concentration in the stock solution, the rate of adsorption increased. It was observed that on increasing the metal ion concentration in the stock, the adsorption process also increased. The maximum adsorption was found at 130 (mg/L) which was about 88% for Cr(VI) and about 92% for Cd(II). This has been presented in Fig. 4.

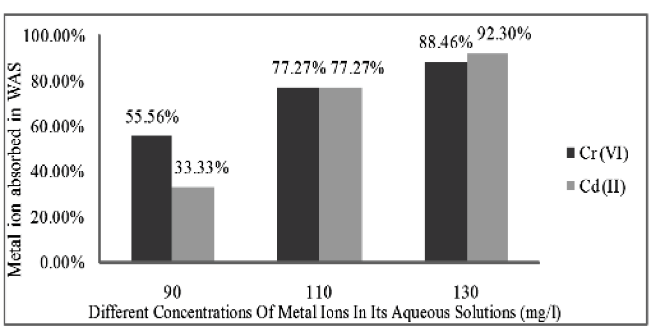

Fig. 4. Showing the effect of different concentrations of metal ion in stock solution with the percent of metal ion absorbed into the WAS

Characterization of Wood Apple Shell (WAS):

Detection of Functional Groups: The characterization of WAS revealed that, it contains various functional groups like- alcohols, esters, amines, proteins and phenols that accounts for the adsorption process of heavy metals on the activated carbon surface. These groups can be involved in complexation reactions with metallic ions and WAS could be viewed as a natural ion-exchange material (Aliya Nur Hasanah et al., 2012).

Results for the estimation of Protein, Phenol and Organic Acid in WAS: Showed the exact quantity of proteins (453.32 µg/mL), phenols (50 µg/mL), and organic acid (0.05 mL) present on WAS that increased the adsorption process of heavy metals on the carbon surface.

Characterization of Seed Protein Content (SPC):

Proximate Composition of SPC: The study of nutritional characteristics of wood apple seed revealed that SPC contains huge amount of protein (75.88g/100g) that would suggest its potentiality for incorporation in food. The carbohydrate content (5.05g/100g) was low and the polyphenol (0.358g/100g) content which accounts for the antioxidant property of the seed and comparable to that of most of the legume seeds (Ravindra, 2013). This has been shown in Table 3.

Table 3. Showing the proximate composition of SPC

Parameters	WSPC sample(g/100g)
Moisture	6.50
Protein	75.88
Carbohydrates	5.05
Polyphenols	0.358

Conclusion:

The present study demonstrated that WAS could be used as an effective adsorbent to treat industrial effluents which are toxic for living world. Hence, instead of using high cost, hazardous chemicals, low cost and non-hazardous, agro-waste material like WAS can be used as an economical as well as effective material to overcome water pollution that can adsorbed about 88% Cr(VI) and about 92% Cd(II) in a stock of 90 mgL^{-1} under acidic conditions.

Similarly, the application of wood apple seed in form of Seed Protein Content (SPC) must be explored in various processed food as its nutritional characters are comparable to that of legumes containing about 75g protein and about 5g carbohydrate per 100g of SPC. Hence, SPC should be used as a supplementary diet to fulfill our daily nutritional requirements as it is a rich source of proteins and has sufficient amount of polyphenol and carbohydrates.

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