Novel Removal of Heavy Metals and Antibiotic Tetracycline Using Cost-Effective, Eco-Friendly and Readily Available Natural Sources

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Abstract— **Pollution of Pb^{2+}, Cd^{2+} and antibiotic tetracycline in water bodies is threatening due to their toxicology and costly removal. Our study showed that red beans and green beans, bamboo biochar and SG Al-WTR are extremely efficient in removing metal ions and tetracycline, respectively. Adsorption reached equilibrium in 120 mins under the Freundlich isotherm model. The optimal pH and temperature for beans was pH 6-7 and 25 °C, while the adsorption by bamboo biochar is unaffected by them. Potential adsorption mechanisms were investigated using FT-IR and FE-SEM. Our findings highlight the potential use of these cost-effective, eco-friendly and readily available natural sources in water treatment.**

1.0 INTRODUCTION

Following rapid industrialization and advancements in medicine, the increased discharge of heavy metals and pharmaceuticals in wastewater has become a devastating global concern. Processes such as lead-acid battery recycling and coal mining has caused heavy pollution of lead (Pb^{2+}) and cadmium (Cd^{2+}) in waters [1]. Ranked second in worldwide production and usage, the proliferated use of tetracycline (TC) in the treatment of animal diseases has also resulted in its accumulation in domestic sewage and wastewater treatment plants worldwide [2]. The toxicology of these chemicals leads to severe body complications: neurotoxin Pb^{2+} may lead to genotoxic effects in the brain, bone marrow, liver and lungs. Carcinogen Cd^{2+} induce damages to multiple organs even from minimal exposure. The high biological activity in TC can significantly increase pathogenic bacterial resistance against its intended treatment for severe acne, and potentially fatal sexually transmitted diseases such as syphilis, gonorrhoea and chlamydia. [3] [4]. In this study, the efficacies of several cost-effective and readily available natural sources in wastewater purification have been explored: Red beans (*Vigna angularis*) and green beans (*Vigna radiata*) in Cd^{2+} and Pb^{2+} removal; bamboo (*Bambusoideae*) biochar and aluminium-water treatment residual (Al-WTR) from Singapore, a typical by-product in drinking water treatment process, in TC removal.

1.1 WATER PURIFICATION METHODS

A myriad of commercialized wastewater treatment technologies has been employed to remove heavy metal pollutants in the aqueous phase, such as chemical precipitation, ion exchange and membrane filtration [5]. However, they have high selectivity for their working ranges, energy consumption and convenience— chemical precipitation only works for high

concentrations, and produces sludge which require further treatment; ion exchange only works for low concentrations and is highly sensitive to the pH of the solution; chemical membrane filtration is complex and incurs high cost.

Meanwhile, wastewater treatment processes do not address the removal of pharmaceuticals, despite traces of TC found in the municipal effluents of wastewater treatment plants in both developed and developing nations, such as Iran, Canada and

America [7]. Hence, adsorption studies have been employed as an alternative cost-effective purification technology. Activated carbon (AC) is the most common adsorbent used, where 1g/L attains 66.1%-86% Cd^{2+} removal and 69.6%-83% Pb^{2+} removal, and 2g/L attains 93% TC removal [8]. However, its widespread application is impeded by its high cost [9]. Another recently discovered way is through the use of Al-WTR collected from the drinking water treatment plants in America, where 20g/L attains approximately 80%-90% TC removal at 1 ppm [10].

2.0 MATERIALS AND METHOD

2.1 PREPARATION OF MATERIALS

Red beans and green beans were used in two forms: whole beans, and finely powdered beans that were sieved through a 2.3mm stainless steel sieve. Bamboo biochar provided by Kenaboi Nature Resource Ptd Ltd was grinded into powder form, and sieved through a 2.3mm stainless steel sieve. The Al-WTR used in this study was picked from a drinking water treatment plant in Singapore. Before conducting the experiments, glass bottles and bottle caps were thoroughly washed with deionized water and dried in 95°C -105°C ovens overnight.

2.2 BATCH ADSORPTION STUDIES

The adsorption of heavy metals and TC on the adsorbents was studied through batch tests. 20 ppm stock solutions of Cd^{2+} and Pb^{2+} were prepared by dissolving cadmium(II) chloride and lead(II) chloride in deionized (DI) water, and 1 ppm TC stock was prepared by dissolving pure TC in DI water. With the aid of a VWR MU 6100H pH meter, 37% hydrochloric acid and a pure NaOH tablet dissolved in DI water were used for pH adjustments. In all of the following tests, adsorbents were added to 50 mL of stock in glass containers, and shaken at 150 rpm in a LM-400D orbital shaking incubator for 22 hours. TC solutions were shaken in the dark due to its degradation by solar photolysis [11]. Thereafter, heavy metal solutions were filtered via suction filtration with filter papers of 0.45μm pore size from Pall Corporation, and TC solutions were filtered with filtration syringes from Terumo, with syringe filters of 0.45μm pore size from Cronus. The heavy metal filtrate was then transferred to a 50mL centrifuge tube while the TC filtrate was transferred to two 2mL glass vials. All samples were preserved in a 4°C cold room, until retrieved for testing.

2.3 TESTS

To determine the concentration of metal ions, the Optima 7300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was used. Readings recorded were the average of 3 technical replicates read by the machine, and r^2 values of calibration curves were >0.999. The Liquid Chromatography Tandem-Mass Spectrometer-8030 (LC-MS/MS) was used to determine the concentration of TC, with two glass vials being prepared from each filtrate The average of 2 readings was recorded as the reading for that trial, and r^2 values of calibration curves were >0.99, against blank samples of DI water as internal standards.

2.4 DETERMINING ADSORPTION CAPACITY

The adsorption capacity, also known as q value, is calculated by the following equation: $q_e = \frac{(C_0 - C_e)V}{M}$ where C_o and C_e (ppm) are the concentrations at initial and at equilibrium, respectively. V (L) is the volume of the solution and M (g) is the mass of adsorbent.

2.5 CHARACTERIZATION

The Fourier Transform Infrared Spectroscopy (FT-IR) spectra for adsorbents were recorded in the range of 4000-400 cm^{-1} using a Shimadzu IR Prestige-21 Spectrophotometer. Specimens were first mixed with KBr powder, then grounded in an agate mortar. The powdered mixture was then pressed to form a disc using a Shimadzu MHP-1 press model. Surface images of the powdered red bean, powdered green bean and bamboo biochar before and after adsorption were captured using Field Emission Scanning Electron Microscopy (FE-SEM).

3.0 RESULTS AND DISCUSSION

30 red beans and 30 green beans were used for batch tests under neutral pH and at room temperature to investigate on the removal of heavy metals Cd^{2+} and Pb^{2+}. 150 mg of powdered bamboo biochar and 150 mg of SG Al-WTR were used to investigate on the removal of TC. Comparing figures 1 and 2, red beans had higher adsorption capacities for the removal of Cd^{2+}, while green beans had higher adsorption capacity for the removal of Pb^{2+}, hence their powdered forms were further investigated upon. 50 mg of powdered red bean and 50 mg of powdered green bean were used for batch tests with Cd^{2+} and Pb^{2+} respectively.

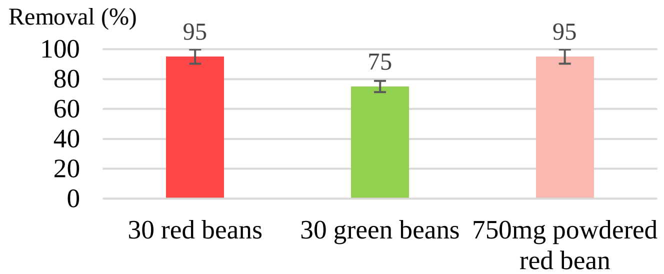

Figure 1. Average removal of Cd^{2+} ions (%)

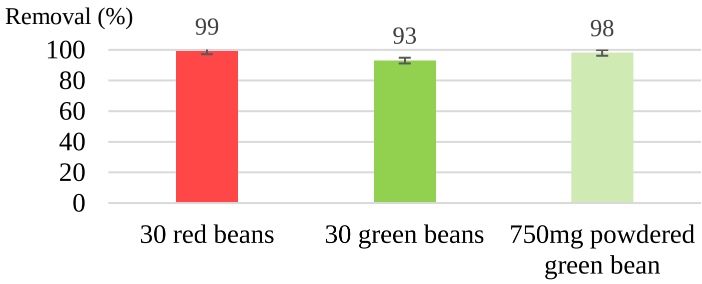

Figure 2. Average removal of Pb^{2+} ions (%)

As observed in figures 1 and 2, the powdered beans achieved comparable or much higher removal rates of heavy metals at a lower mass, therefore they have greater adsorption capacities than whole beans. This is likely due to the increase in surface area available for adsorption.

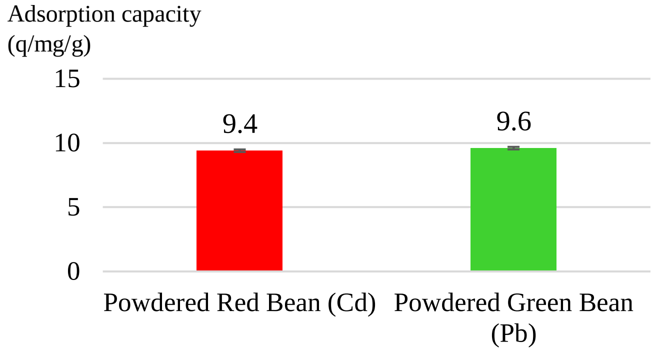

Figure 3: Average adsorption capacity (q value)/mg/g of powdered beans for heavy metals

The adsorption capacity of powdered beans for heavy metals is shown above in figure 3.

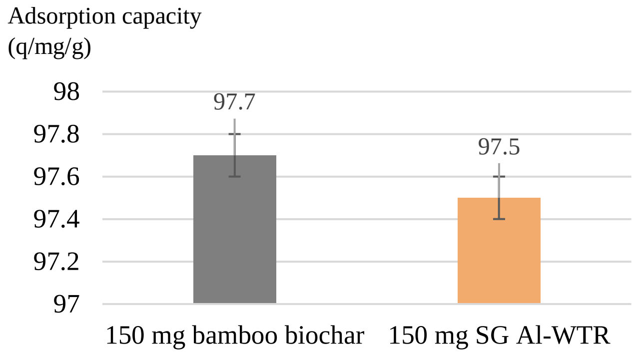

Figure 4: Average removal of TC (%)

As shown in figure 4, bamboo biochar also proved to be more effective in its removal of TC than Al-WTR, as it could achieve higher removal at a lower mass, resulting in it having a greater adsorption capacity.

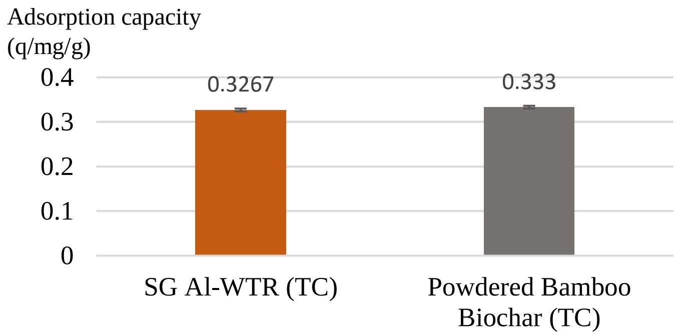

Figure 5: Average adsorption capacity (q value)/mg/g of adsorbents for TC

The adsorption capacity of powdered beans for heavy metals is shown above in figure 5. The adsorption capacities appear low as the concentration of TC tested with adsorbents was only at a low value of 1 ppm, to mimic pollutant levels reported in municipal effluents, hence a low amount of pollutant was available for adsorption. [7]

3.1 STUDY OF REMOVAL EFFICIENCIES

The removal efficiencies of Cd^{2+} by powdered red bean, Pb^{2+} by powdered green bean and TC by bamboo biochar were further studied using 100 mg of powdered beans and 150 mg of bamboo biochar. To obtain each data point, a bottle of adsorbent and pollutant was incubated at 25°C and 150 rpm.

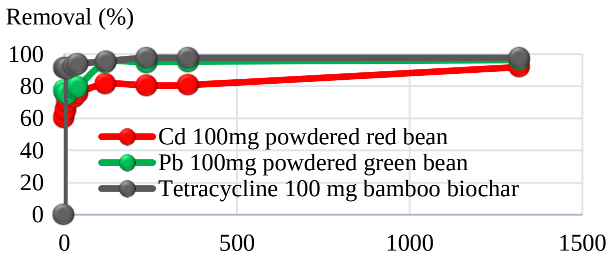

Figure 6: Kinetics of adsorbents

As observed in figure 6, powdered red bean, powdered green bean and bamboo biochar can significantly remove pollutants in the first minute. Subsequently, all adsorbents showed a logarithmic curve. This can be attributed to the initial large number of vacant adsorption sites on the adsorbents' surfaces. Overtime, the remaining vacant surface sites would be increasingly challenging to occupy due to the repulsion between adsorbates on surface and bulk phases [12].

3.2 FE-SEM ANALYSIS

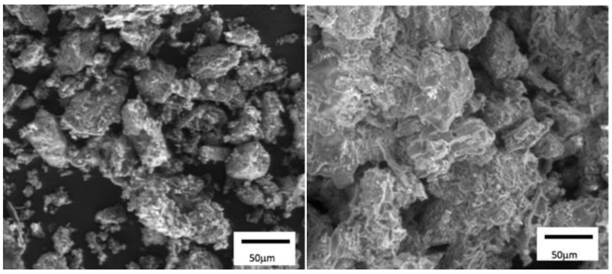

Figure 7a: SEM of powdered red bean

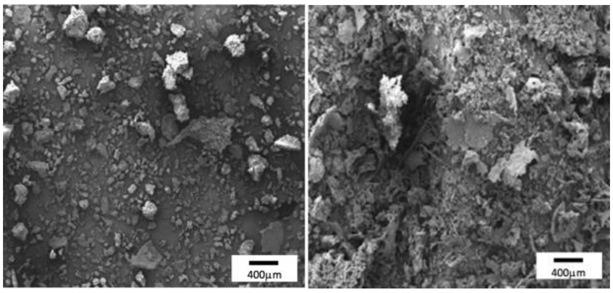

Figure 8a: SEM of powdered green bean

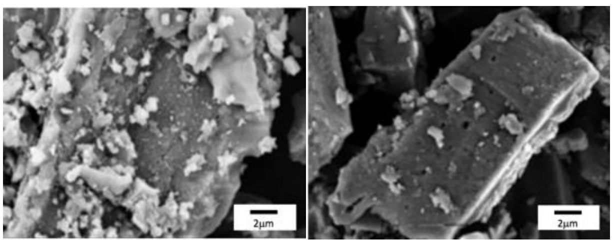

Figure 9a: SEM of powdered bamboo biochar

As shown in figure 7a, the powdered red bean has a fibrous surface, which helps to increase the surface area in contact with Cd^{2+} ions, allowing for adsorption with the maintenance of a high structural integrity. Figure 7b depicts the presence of Cd^{2+} ions forming a layer around the red bean surfaces, indicating that binding sites have been occupied. The transition from figure 8a to figure 8b shows the Pb^{2+} ions coated on the fibrous surface of the powdered green bean. As illustrated in figure 9a, bamboo biochar also has a porous and large surface area for TC molecules to bind on. Figure 9b shows the tendency TC molecules to form clusters when binding to bamboo biochar, as binding sites are strong, explaining its highly efficient removal of TC.

3.3 ADSORPTION ISOTHERMS

The adsorption isotherms were determined by varying the concentrations of adsorbents. All solutions were shaken at 25°C, and experiments were conducted twice for each concentration. Linearized graphs describing the Freundlich isotherms and Langmuir isotherms were plotted to determine the model which best fits each adsorbent [13]. Based on the intercept and r^2 values for both models, it was found that all adsorbents better followed Freundlich isotherm model, as described by the following equation: $q_e = K_F C_e{}^{1/n}$, where q_e is the adsorption capacity of the adsorbent at equilibrium, K_F is an indicator of q_e , C_e is the equilibrium concentration of solute remaining in the solution when amount adsorbed equals q_e , and $1/n$ is the intensity of adsorption.

The Freundlich isotherm tells us the trend of adsorption base on the graph plotted, thereby predicting the performance under different concentration of pollutants. The Freundlich isotherm is also an empirical model that allows for the formation of multilayer of molecules on the surface, agreeable with our findings from FE-SEM analysis. Furthermore, the binding sites of the adsorbents may not be equivalent and adsorbate molecules on adjacent sites can interact with one another.

Pollutant	Material	Varied Quantity	R^2	K_F	1/n
Cd^{2+}	Red Beans	10, 20, 30 beans	0.97	0.209	1.612
	Green Beans	10, 20, 30 beans	0.99	0.024	2.555
	Powdered Red Bean	150, 200, 750 mg	0.99	1.392	1.368
Pb^{2+}	Red Beans	10, 20, 30 beans	0.99	0.557	0.421
	Green Beans	10, 20, 30 beans	0.99	1.087	3.512
	Powdered Green Bean	150, 200, 750 mg	0.99	3.048	0.675
TC	Bamboo Biochar	50, 100, 150 mg	0.99	7.674×10^{14}	9.741
	SG Al-WTR	100, 150, 200 mg	0.99	82867.647	3.504

Table 1: Adsorbents following Freundlich isotherm

As shown in table 1, generally $1/n > 1$, indicating that adsorption processes are more favourable, as even at low concentrations, surfaces can be loaded with high amounts of pollutant and achieve high removal rates. However, the $1/n$ values of green beans in their removal of Pb^{2+} are lower than 1. Since Pb^{2+} ions are bigger than Cd^{2+} ions, we hypothesise that the adsorption of Pb^{2+} takes up more surface area, resulting in $1/n$ values being lower than 1 despite its effective adsorption.[14]

3.4 INFRARED SPECTROSCOPY ANALYSIS

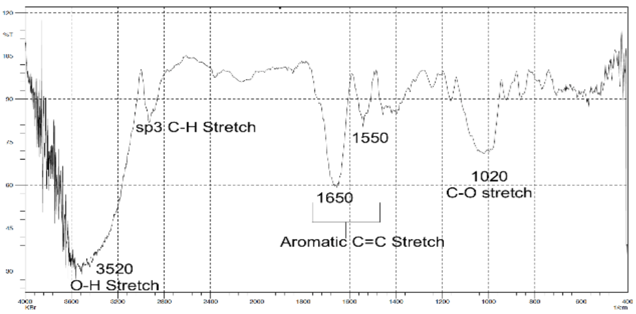

Figure 10: FT-IR Spectra of Red Bean at pH 6-7

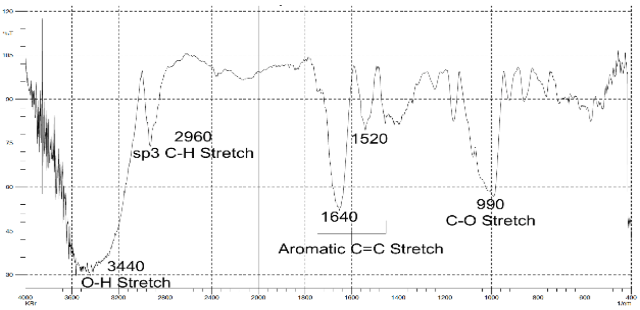

Figure 11: FT-IR Spectra of Green Bean at pH 6-7

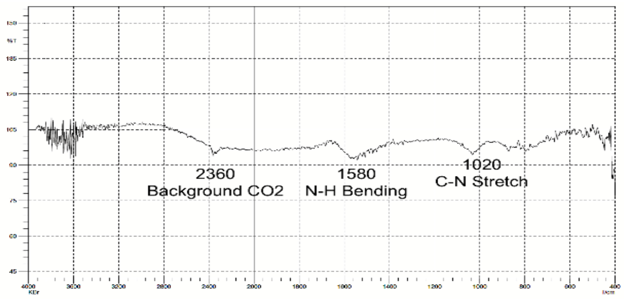

Figure 12: FT-IR Spectra of Bamboo Biochar at pH 6-7

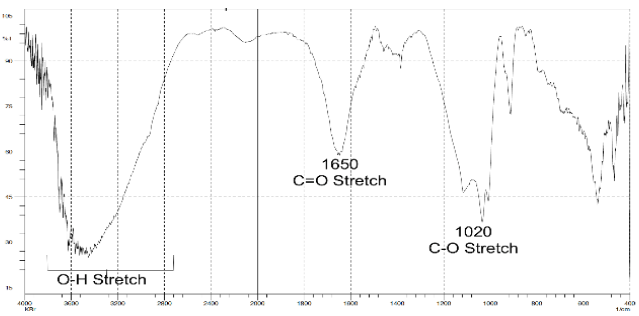

Figure 13: FT-IR Spectra of Al-WTR at pH 6-7

As shown in figures 10-11, the broad bands in the range of 4000-3000 cm^{-1} in both red bean and green bean correspond to the O-H bond stretch of alcohol. Sharp peaks around 3000 cm^{-1} in both beans correspond the sp^3 C-H stretch. Double peaks in the range of 1700-1500 cm^{-1} in both beans correspond to the aromatic C=C stretch. The peak around 1000 cm^{-1} in both beans correspond to the C-O stretch which shows that alcohol groups can be present in the molecule. The presence of aromatic C=C, C-O and O-H indicates the possible presence of phenolic groups, ascertained by research carried out on composition of red beans and green beans. [15-16]Efficient adsorption can thus be explained by the electrostatic attraction between the partially negatively charged oxygen in phenol and the positively charged metal ions. The electron rich aromatic ring also allows for the formation of electrostatic interactions with the positively charged metals ions [17].

In figure 12, the peak at 1580 and 1020 cm^{-1} in bamboo biochar correspond to N-H bending and C-N stretch. Hence, it can be concluded that amine is present, as supported by the findings of our isotherm studies. Amine allows the bamboo biochar to form hydrogen bonds with TC which has amide and alcohol groups [16]. Cation-π interaction, an electrostatic interaction between a cation and the polarizable pi electron cloud of an aromatic ring, was also likely to have occurred between the easily protonated amino group of bamboo biochar and the aromatic ring of TC, facilitating adsorption [19].

In figure 13, the strong and broad peak in the range of 3400-2400 cm^{-1} corresponds to the O-H stretch of carboxylic acid in SG Al-WTR. The peak at 1650 cm^{-1} corresponds to C=O stretch which is part of the carboxylic group, backed by the results of our isotherm studies. The peak at 1020 cm^{-1} corresponds to C-O stretch which is part of the carboxylic groups. The carboxylic acids detected come from the humic acid present in the WTR (appendix table 1) [18]. The carboxylic acids can form dipole-dipole interactions, such as hydrogen bonding, with the amino groups as well as the alcohol groups in TC, allowing for adsorption [2].

3.5 VARIATION OF pH

The effect of pH on the efficacies of selected adsorbents was studied by varying pH of solutions from 3-8, with experiments conducted twice for each pH. A total of 100 mg of powdered red bean and powdered green bean were shaken with Cd^{2+} and Pb^{2+} solutions respectively, and 150 mg of bamboo biochar was shaken with TC. In this section, it can be assumed that the small amounts of concentrated acid or base added do not affect the concentration of pollutants. As the measured pH of Cd^{2+}, Pb^{2+} and TC solutions fluctuates between 6 and 7, pH 6-7 will be used to denote the pH when pure standard stocks are shaken with the adsorbent.

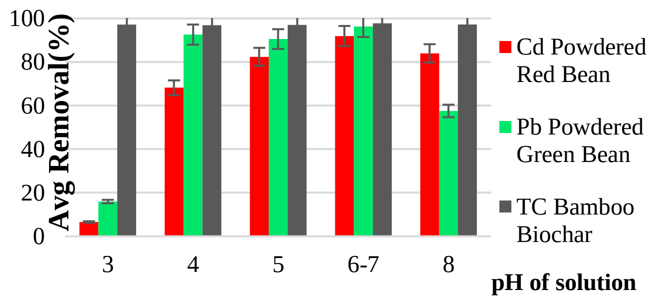

Figure 14: Average percentage removal of heavy metals and TC by adsorbents at different pH

As shown in figure 14, the observed optimal pH for adsorption of both Cd^{2+} and Pb^{2+} is 6-7, and adsorption is significantly lower at other pH levels. This is optimal as no pre-treatment of adsorbents is required to alter the acidity of adsorbents. This can be attributed to the fact that under acidic conditions from pH 3-5, H_3O^+ act as strong competitors for negatively charged binding sites on the powdered beans [21]. The H_3O^+ adsorbed onto the surface of the powdered beans also repels the positively charged metal cations, hence decreasing adsorption. Under alkaline conditions at pH 8, when NaOH is added to the solution, the phenol present in both beans react with NaOH to produce sodium phenoxide and water. The sodium ions generated then compete with the metal ions in the solution for binding sites, resulting in a lower percentage removal [22].

The observed maximum adsorption capacity for TC is at pH 6-7. Contrary to the discoveries of some studies on different adsorbents, TC adsorption by bamboo biochar is not significantly affected by varying pH levels [23-25].

As illustrated in figure 15, at pH <3.3, TC exists as a cation. Under acidic conditions, at pH 3, the amino group in bamboo biochar is protonated and engages in cation-π interaction with the aromatic ring in TC. As the positively charged N^+ ion in TC is distanced from the aromatic ring, the cation-π interaction is able to overcome the weaker repulsion between positively charged TC and bamboo biochar, achieving similarly proficient adsorption. As pH increases from 3.3 to 7, where TC exists as zwitterions, the strength of the cation-π interaction stays unaffected as the aromatic ring and protonated amino group of the bamboo biochar remain intact [26]. Under alkaline conditions, as pH increases to 8, TC exists as an anion, whilst the amino group in bamboo biochar remains as an unprotonated base. Hydrogen bonding between the amino group of the bamboo biochar and the alcohol groups and amine in TC is then formed, resulting in equally high percentage removals.

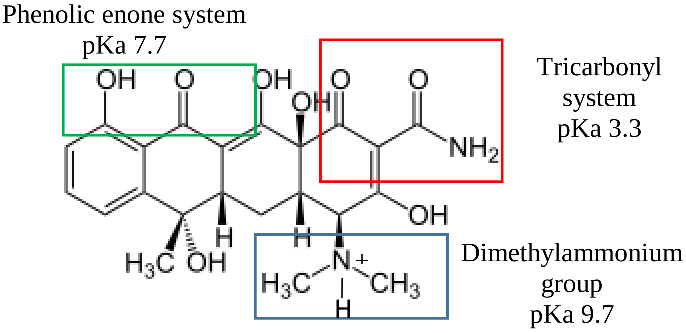

Figure 15: Molecular structure of TC on planar

3.6 VARIATION OF TEMPERATURE

The effect of temperature on the efficacies of selected adsorbents was also studied by varying the surrounding temperature from 25°C-65°C, with experiments conducted twice for each temperature. A total of 100 mg of powdered red bean and powdered green bean were shaken with Cd^{2+} and Pb^{2+} solutions respectively, and 150 mg of bamboo biochar was shaken with TC.

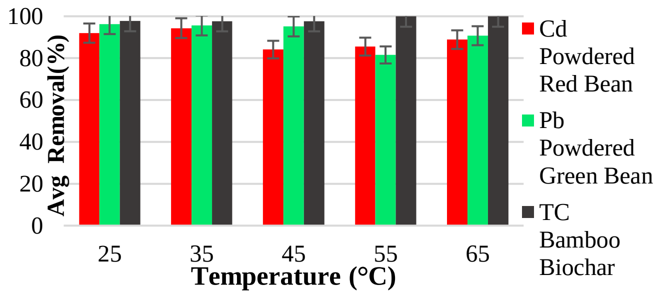

Figure 16: Average percentage removal of heavy metals and TC by adsorbents at different temperatures

As seen in figure 16, the optimal temperature for adsorption of Cd^{2+} by powdered red bean is observed to be at 35°C, and 25°C for the adsorption of Pb^{2+} by powdered green bean. Beyond 35°C, the adsorption of both Cd^{2+} and Pb^{2+} generally decreases. This can be explained by the increased dissociation of phenol in water at higher temperatures, as its dissociation is an endothermic process favoured by high temperatures [27]. This results in the formation of H_3O^+, a potential competitor for binding sites on the powdered beans, causing significant reduction in percentage removal. However, percentage removals are not significantly affected by pH as phenol dissociation in water is low [28].

As temperature increases, the adsorption of TC by bamboo biochar also increases. As the maximum percentage removal has been obtained at 55°C, the increment in percentage removal >55°C could no longer be observed. The increased removal is likely due to the increased strength of cation-π interaction between the bamboo biochar and TC as temperature increases, allowing more TC molecules to be adsorbed [27].

4.0 CONCLUSION & RECOMMENDATIONS

4.1 COMPARISON WITH COMMERCIAL ADSORBENTS

Powered red bean, powdered green bean, powdered bamboo biochar and SG Al-WTR showed to be extremely effective in their removal of Cd^{2+}, Pb^{2+} and tetracycline respectively. It only requires 15 grinded red beans (2g of powdered red beans) in 1L 20ppm Cd^{2+} solution to achieve 94% removal, 30 grinded green beans (2g of powdered green beans) in 1L 20ppm Pb^{2+} solution to achieve 96% removal, rendering them as comparable alternatives to AC, where 1g in 1L 30ppm Cd^{2+} solution attains 86% removal and 1g in 1L 30 ppm Pb^{2+} solution attains 83% removal. 3g of powdered bamboo biochar in 1L 1 ppm tetracycline solution can attain >99% removal and 3g of WTR in 1L of 1ppm tetracycline solution can achieve 98% removal, enabling them to be plausible alternatives to Al-WTR from America, where 20g in 1L 1ppm tetracycline solution attains 80% removal, and AC, where 2g in 1L 1ppm tetracycline solution attains 93% removal. An illustrative comparison is as shown in figure 17:

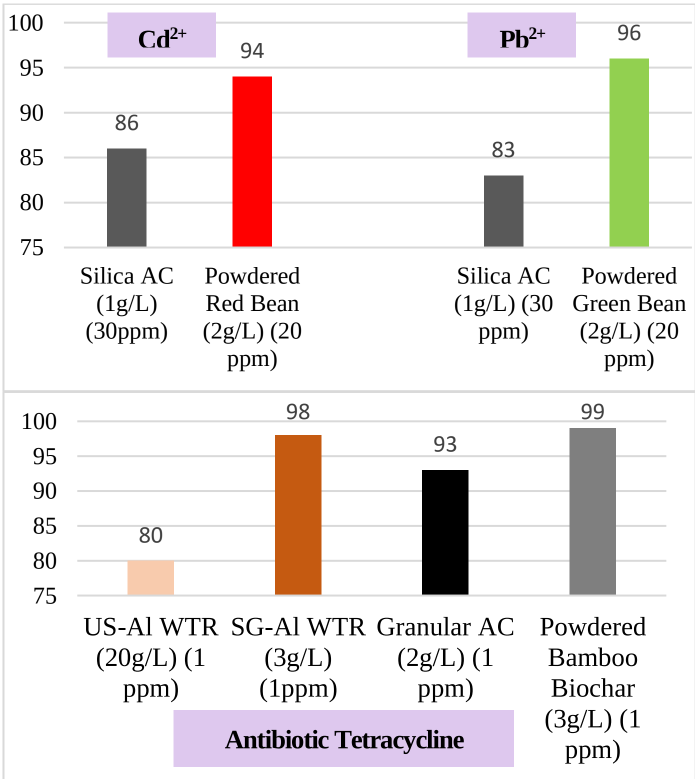

Figure 17: Average removal (%) of different adsorbents

4.2 ECONOMIC ANALYSIS

As apparent in table 2, up to 10 times of the amount used to purchase AC can be saved by using red beans and green beans in their removal of heavy metals, and powdered bamboo biochar to remove antibiotic tetracycline. Furthermore, the discovery made in our study will potentially save millions of dollars incurred from current industrial machinery production of AC.

Pollutants	Adsorbent	Cost to purify 1L of water
Heavy metals (Pb^{2+} and Cd^{2+})	Red Beans (powdered)	USD 0.0002
	Green Beans (powdered)	USD 0.0004
	Activated Carbon	USD 0.0020
Antibiotic tetracycline	Activated Carbon	USD 0.0040
	Bamboo biochar	USD 0.0015

Table 2: Economic Analysis

4.3 COMMERCIALIZATION

Beyond the high removal efficiencies and cost-effectiveness of red beans, green beans, bamboo biochar and SG Al-WTR, they also have vast potentials to be commercialized.

Red beans and green beans are already produced commercially worldwide and are readily available due to their high yield, producing several tonnes per hectare of crops in just 3-4 months. Due to their commercial production, they are not prone to contaminated wastes on their surfaces, explaining their consistencies in attaining high removals of heavy metals. As they are dry products, they can be preserved for a prolonged period of time without rotting, hence enabling them to be extremely practical in industrial wastewater purification.

The bamboo biochar is also an eco-friendly material which is produced globally. Its environmental friendliness lies in how biomass production to obtain biochar is a carbon-negative process, where carbon dioxide is removed from the atmosphere and then released, enabling for long-term sequestration.

The SG Al-WTR can potentially be packaged and sold globally, as they are free and are abundant in local water treatment plants as waste by-products of the water treatment process.

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