**Investigating the adsorption of heavy metal ions and industrial dyes by a lemon peel-zinc oxide nanoparticles-calcium alginate composite and investigating its antibacterial properties**

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## Abstract

Water pollution is one of the huge problems we face in the world we live in today. Over 80% of industrial waste is untreated when discharged, which may contain bacteria, heavy metal ions and industrial dyes that damage the delicate balance in the ecosystem and harm the overall environment. The study focused on the synthesis of lemon peel-calcium alginate-zinc oxide nanoparticle composite and evaluating the adsorption capability and versatility of such a composite in the adsorption of $Cu^{2+}$ and $Zn^{2+}$ ions, as well as the industrial dyes methylene blue, malachite and brilliant green and investigating its antibacterial properties. A comparative analysis was done on the adsorption capability of the lemon peel-zinc oxide nanoparticles-calcium alginate composite against its constituents to analyse the impact of the constituents in the composite on its adsorption capability. In this study, 0.5g of adsorbent was left in 25cm$^3$ of 20ppm pollutant solution in an orbital shaker for 3 hours and the concentration of the remaining solution was evaluated. Compared to its constituents, the composite was shown to have a higher adsorption versatility and capability of some pollutants, displaying an average of 22.3 % better adsorption compared to lemon peels and 31.6% better absorption than sodium alginate in the adsorption of industrial dyes and. Antibacterial tests against *Escherichia Coli* (*E.Coli*) were conducted, and the composite was found to display antibacterial effects of reducing uncountable colonies to an average of 235 colonies for a dilution factor of -5 and 112 colonies for a dilution factor of -6 as well. The composite combines the strengths of its various constituents into a multi-purpose adsorbent. The ease of removal of such a composite due to its solid, round nature, coupled with its adsorption capability and versatility and antibacterial properties, makes it a viable option for wastewater treatment.

## 1. Introduction

Recently, there has been a growing social and ecological concern in environmental pollution, or more specifically, water pollution by heavy metal ions and industrial dyes.

Heavy metals are elements having atomic masses between 63.5 and 200.6, and a specific gravity greater than 5.0 (Srivastava and Majumder, 2008). With the rapid development of industries such as metal plating facilities, the wastewaters contaminated by heavy metal ions are directly or indirectly discharged into the environment increase, especially in developing countries. Unlike organic contaminants, heavy metals are non-biodegradable and tend to accumulate in living organisms with many of such heavy metal ions known to be toxic or carcinogenic. (Fu & Wang, 2011). Due to the heavy metals' industrial applications and with rapid advancements in the world's technology, the amount of human exposure to heavy metal ions has seen an exponential increase which can cause serious health effects, including reduced growth and development, cancer, and in extreme cases, death, even in small amounts. (Barakat, 2011).

As of today, past studies have shown that the most conventional process to remove heavy metal ions in wastewater treatment is chemical precipitation. However, there are also drawbacks of chemical precipitation including its excessive sludge production that requires further treatment, slow metal precipitation, and the long-term environmental impacts of sludge disposal (Aziz et. al, 2008). One major disadvantage would be the need for alkalinity correction. As a result of chemical dosing, alkaline water needs to be supplemented in areas with high acidity. This is to ensure that the pH level of the system is above 7.0 and to minimise corrosion of concrete structures (Haas, Wentzel & Ekama, 2004). As such, more renewable and environmentally-friendly methods of adsorption are required in reducing the environmental effects of heavy metal ions. For example, calcium alginate has previously shown to adsorb heavy metal ions (Papageorgiou et al., 2006).

Similarly, dye removal from textile effluents is a major environmental problem because of the difficulty to treat such streams. (Annadurai, Juang and Lee, 2002). Dyes are harmful to aquatic life in rivers where they are discharged. The occupational exposure of workers in the textile industries is linked to a higher bladder cancer risk. The use of hair colouring products and breast cancer have also been linked. Various agricultural products and by-products have been investigated to remove dyes from aqueous solutions. These include cotton waste, rice husk, bark (Pollard, Fowler, Sollars & Perry, 1992), sugar industry mud (Magdy & Daifullah, 1998), palm-fruit bunch (Nassar, Hamoda & Radwan, 1995), Jackfruit peel (Inbaraj, & Sulochana, 2002), wood (Ho & McKay, 1998), orange peel (Namasivayam, Muniasamy, Gayatri, Rani & Ranganathan, 1996), etc.. The distinct advantage of this method is the lower costs involved. Hence, there is a need to search for more economical and effective sorbents. (Ho, Chiang, & Hsueh, 2005).

Additionally, wastewater often contains harmful bacteria that pose a threat to the safety of humans and other living organisms. Some of these bacteria may be pathogenic or harbour antibiotic resistance or virulence genes harmful to human health. (Varela & Manaia, 2013). E. coli is a common indicator organism to determine the presence of pathogenic microorganisms that may cause illness. (Jamieson et al., 2005) van Elsas, Semenov and Costa reported that the pathogenic forms of E. coli, such as the verotoxigenic (VTEC) (Taylor, 2008), enterohaemorrhagic (EHEC, a subclass of the VTEC class), enteroinvasive (EIEC) and uropathogenic/extraintestinal pathogenic (UPEC/ExPEC) classes, all possess capacities that are harmful to their hosts. The well-known E. coli O157:H7 is an example of a harmful VTEC, which has already caused mortality worldwide. VTEC strains are capable of producing verotoxins (genes denoted as stx) (Taylor, 2008) causing mild to bloody diarrhoea, which eventually culminates in the haemolytic uraemic syndrome. More than 150 serotypes of verotoxin-producing E. coli have been found, but the majority of outbreaks are related to serotype O157. Moreover, the stx genes were found to be transferable to non-pathogenic E. coli strains, allowing these to enhance their virulence (Herold et al., 2004). Therefore, a new, novel and versatile composite would be valuable in wastewater treatment.

## 2. Objectives and hypotheses

### Objectives

1. To investigate the adsorption capability of industrial dyes and heavy metal ions of a lemon peel-zinc oxide nanoparticles-calcium alginate composite.
2. To investigate the antibacterial properties of a lemon peel-calcium alginate-zinc oxide nanoparticles composite.

### Hypotheses

1. The lemon peel-calcium alginate-zinc oxide nanoparticles composite has at least 60% adsorption capability of heavy metal ions and industrial dyes.
2. The lemon peel-calcium alginate-zinc oxide nanoparticles composite demonstrates antibacterial properties.
3. The lemon peel-zinc oxide nanoparticles-calcium alginate composite has a higher adsorption capability of heavy metal ions and industrial dyes than its constituents.

## 3. Methods and Materials

### Materials

| | | | | |
|---|---|---|---|---|
| Lemon peels | Calcium chloride | Deionised water | Sodium hydroxide | Methylene blue |
| Malachite green | Brilliant green | Sodium alginate | Zinc chloride | Copper (II) sulfate |
| Zinc sulfate | *Escherichia Coli* | Potassium bromide | | |

### Apparatus

| | | | | |
|---|---|---|---|---|
| Orbital shaker | Centrifuge | Electronic balance | UV-VIS Spectrophotometer | DR/890 Colorimeter |
| Buchner funnel | Oven | Hotplate | Blender | Pestle and mortar |
| Incubator | Spirit burner | Colony counter | Sieve | FTIR spectrometer |

### Variables

Experiment 1: Adsorption of $Zn^{2+}$ and $Cu^{2+}$ ions

| | |
|---|---|
| Independent variable | Lemon peel-zinc oxide nanoparticles-calcium alginate composite |
| Dependent variable | Concentration of respective heavy metal ion left in the water/ppm |
| Controlled variables | Initial concentration of heavy metal ion solution, time for adsorption, temperature, volume of solution used |

Experiment 2: Adsorption of industrial dyes

| | |
|---|---|
| Independent variable | Lemon peel-zinc oxide nanoparticles-calcium alginate composite |
| Dependent variable | Concentration of respective dye solution left in the water/ppm |
| Controlled variables | Initial concentration of dye solution, time for adsorption, temperature, volume of solution used |

Experiment 3: Antibacterial test

| | |
|---|---|
| Independent variable | Lemon peel-zinc oxide nanoparticles-calcium alginate composite |
| Dependent variable | Number of colonies counted |
| Controlled variables | Volume of cultured E.coli, time for antibacterial effects |

**Preparation of lemon peels**

Lemons were bought, peeled and cut into uniform pieces. Lemon peels were oven-dried, before being blended then ground into a fine powder using a pestle and mortar.

**Preparation of ZnO nanoparticles**

0.1 mol $dm^{-3}$ zinc chloride solution was prepared by dissolving 1.345g of zinc chloride per $100cm^3$ of deionised water. The solution was poured into a beaker and stirred. 1 mol $dm^{-3}$ Sodium hydroxide solution was added dropwise to the zinc chloride solution until pH14 was achieved. The precipitate was separated from the suspension using a Buchner funnel. The precipitate collected was then washed with deionised water until pH7 was achieved, and oven-dried overnight. The ZnO NPs were then ground into a fine powder using a pestle and mortar.

**Synthesis of calcium alginate beads**

A 2% w/v calcium alginate solution was prepared by dissolving 2g calcium alginate per $100cm^3$ of deionised water. The suspension was heated on a hotplate at 50 degrees Celsius and stirred for 15 minutes until all the calcium alginate had dissolved. A $400cm^3$ 4% w/v calcium chloride solution was prepared by dissolving 16g of calcium chloride in $400cm^3$ of deionised water. Using a dropper, the calcium alginate was dropped slowly into the calcium chloride solution to form beads. The resulting beads were soaked in the calcium chloride solution overnight. The following day the lemon peel-zinc oxide nanoparticles-calcium alginate composite beads were filtered and rinsed in deionised water repeatedly until pH neutral. The beads were then oven-dried overnight.

**Synthesis of lemon peel-zinc oxide nanoparticles-calcium alginate composite**

Lemon peel and ZnO NPs were submerged homogeneously in a 1g:1g:100cm$^3$ ratio in sodium alginate solution and stirred on a hotplate for 15 minutes. The suspension was dropped slowly into a 4% w/v calcium chloride solution to obtain the beads. The resulting beads were soaked in the calcium chloride solution overnight. The following day the lemon peel-zinc oxide nanoparticles-calcium alginate composite beads were filtered and rinsed in deionised water repeatedly until pH neutral. The beads were then oven-dried overnight.

**Characterisation of lemon peels**

Fourier-transform infrared (FTIR) spectroscopy was used to analyze the different functional groups of the lemon peels through the KBr pellet method.

**Characterisation of zinc oxide nanoparticles**

Fourier-transform infrared (FTIR) spectroscopy was used to analyze the different functional groups of the ZnO NPs through the KBr pellet method.

**Adsorption of Heavy Metal Ions**

25 cm$^3$ of a 20 ppm solution of $Zn^{2+}$ ions were prepared. 0.5g of the adsorbent (lemon peel, calcium alginate or lemon peel-zinc oxide nanoparticles-calcium alginate composite) was submerged in the solution. The centrifuge tubes were allowed to shake in the orbital shaker for 3 hours. Centrifuge the adsorbent from the solution. The solution was diluted by a dilution factor of 10. To test the amount of heavy metal ions adsorbed, a colorimeter is used to find the amount of copper(II) and zinc ions in the original solution, the processed solution, and a clear solution as control, in units ppm The procedure was repeated for each of the constituents, with 5 replicates carried out per adsorbent.

**Adsorption of dyes**

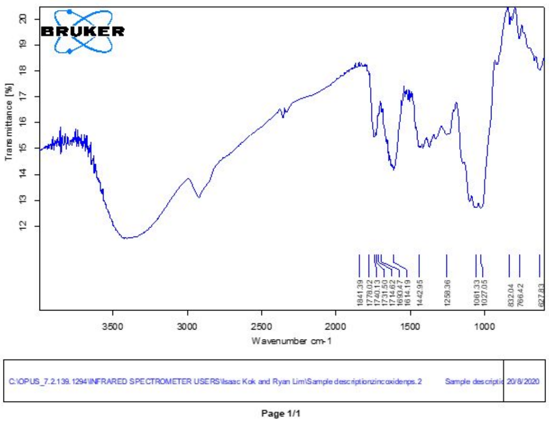
25 cm$^3$ of a 20 ppm methylene blue, brilliant green and malachite green solution was prepared. 0.5g of the adsorbent was submerged in the solution and the mixture was allowed to shake in the orbital shaker for 3h at 200rpm. The adsorbent was separated from the solution by centrifuging the solution at 8000 rpm for 5 min. To test the amount of dyes adsorbed, using a UV spectrophotometer, a calibration curve is plotted with the lambda max of known concentrations of the dye solutions. After, the processed dye is compared with the original dye solution. The procedure was repeated for each of the constituents, with 5 replicates carried out per constituent.

**Antibacterial test**

E.coli was cultured overnight in agar broth. 100 µl of E.coli was added to 0.4g of the lemon peel-zinc oxide nanoparticles-calcium alginate composite and was shaken overnight. Serial dilution up to a factor of -6 was carried out. The -5 and -6 samples were spread onto agar plates then incubated overnight. A colony count test was carried out the next day.

## 4. Results and Discussion

**Characterisation of lemon peels and ZnO nanoparticles by FTIR spectroscopy**



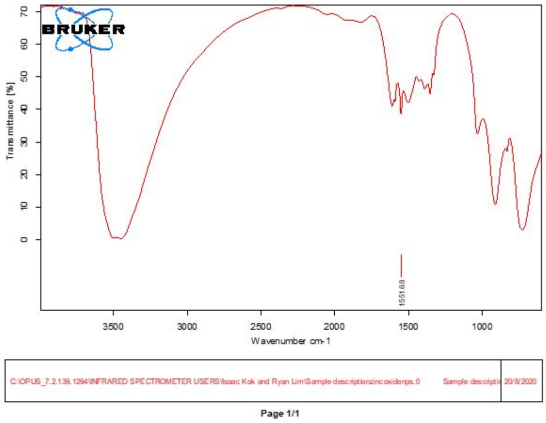




Fig 1a: FTIR spectrum for lemon peels Fig 1b: FTIR spectrum for ZnO nanoparticles

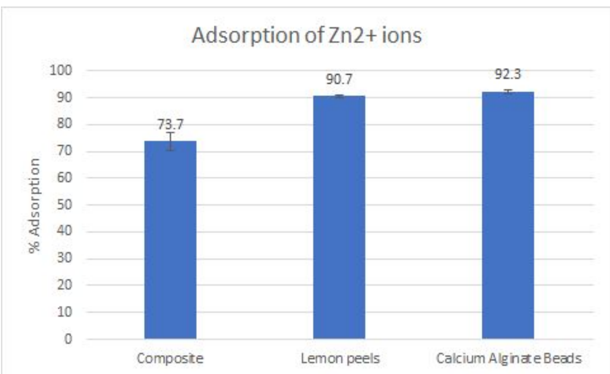
**Analysis of FTIR spectrum for lemon peels**

The band between 3200 and 3550 $cm^{-1}$ indicates O-H stretching in the lemon peels.

**Analysis of FTIR spectrum for zinc oxide nanoparticles**

The tiny peaks at 2300-2400 $cm^{-1}$ in the FTIR spectrum shows some halos surrounding the particles due to the retention of some diol, that remains adsorbed on the ZnO nanoparticles, which corresponds to past findings (Becheri, Dürr, Nostro, & Baglioni, 2007).

**Results from adsorption tests**



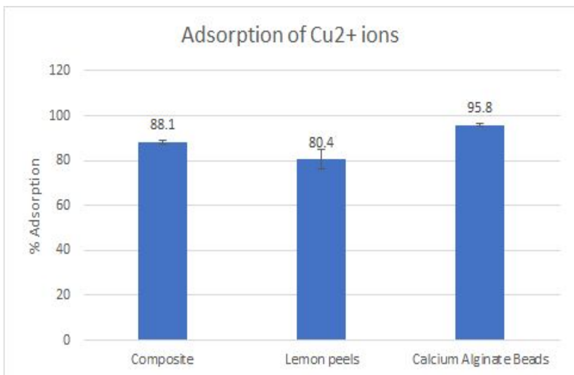
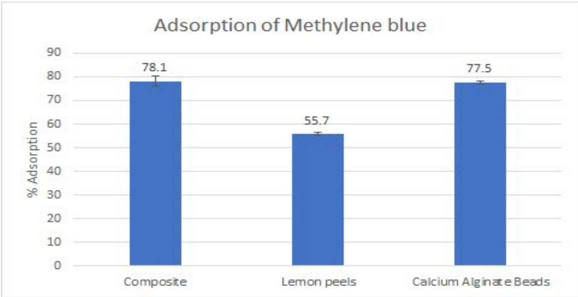



Fig 2a: Absorbents' effectiveness on $Zn^{2+}$ ions Fig 2b: Adsorbents' effectiveness on $Cu^{2+}$ ions



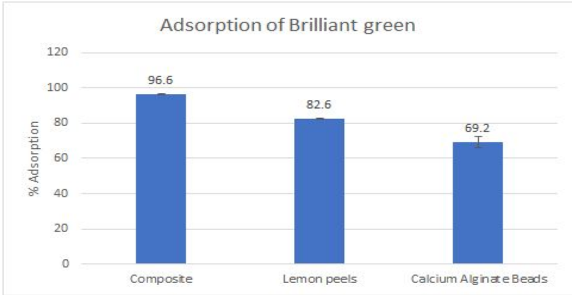




Fig 2c: Adsorbents' effectiveness on methylene blue

Fig 2d: Adsorbents' effectiveness on brilliant green

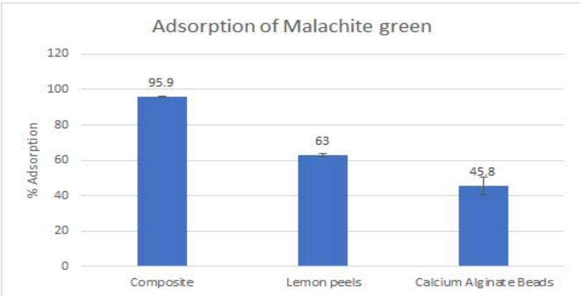



Fig 2e: Adsorbents' effectiveness on Malachite green

## Discussion of results

The respective data sets were compared and analysed using the Mann-Whitney statistical tests to conclude whether a significant difference exists between the adsorption capabilities of the adsorbents. The composite had a higher adsorption capability than lemon peels and calcium alginate beads for Methylene blue, Brilliant green and Malachite green, while the composite had a higher adsorption capability than the lemon peels for $Cu^{2+}$ ions. For $Zn^{2+}$, both constituents performed better than the composite.

### Results of Mann-Whitney U Test

| | Adsorption using composite against adsorption using lemon peels | | | | |
|---|---|---|---|---|---|
| | Zn2+ | Cu2+ | Methylene blue | Brilliant green | Malachite green |
| p-value | 0.012 | 0.138792 | 0.012 | 0.012 | 0.012 |
| Significant difference? | Yes (lemon) | No | Yes (composite) | Yes (composite) | Yes (composite) |

Figure 3a: Results of Mann-Whitney U Test for lemon peel-zinc oxide nanoparticles-calcium alginate composite against lemon peels

| | Adsorption using composite against adsorption using calcium alginate | | | | |
|---|---|---|---|---|---|
| | Zn2+ | Cu2+ | Methylene blue | Brilliant green | Malachite green |
| p-value | 0.012 | 0.011 | 0.676 | 0.012 | 0.012 |
| Significant difference? | Yes (alginate) | Yes (alginate) | No | Yes (composite) | Yes (composite) |

Figure 3b: Results of Mann-Whitney U Test for lemon peel-zinc oxide nanoparticles-calcium alginate composite against calcium alginate

From figure 3a, there was no significant difference between the adsorption of lemon peel-zinc oxide nanoparticles-calcium alginate composite and lemon peels of $Cu^{2+}$ ions, with a p-value of 0.139. There is a significant difference between the adsorption of $Zn^{2+}$ ions, where the lemon peels had significantly higher absorbance than the lemon peel-zinc oxide nanoparticles-calcium alginate composite, with a p-value of 0.012. There is a significant difference between the adsorption methylene blue, malachite green and brilliant green, where the lemon peel-zinc oxide nanoparticles-calcium alginate composites had significantly higher absorbance than the lemon peels, with all having a p-value of 0.012.

From figure 3b, there was no significant difference between the adsorption of lemon peel-zinc oxide nanoparticles-calcium alginate composite and Calcium alginate for Methylene blue, with a p-value of 0.676. There is a significant difference between the adsorption of $Zn^{2+}$ and $Cu^{2+}$ ions, where the Calcium alginate had significantly higher absorbance than the lemon peel-zinc oxide nanoparticles-calcium alginate composite, with a p-value of 0.012 and 0.011 respectively. There is a significant difference between the adsorption of brilliant green and malachite green, where the lemon peel-zinc oxide nanoparticles-calcium alginate composites had significantly higher absorbance than the calcium alginate, with all having a p-value of 0.012.
Overall, the composite has high adsorption capabilities of all five pollutants.

**Colony Count Results**

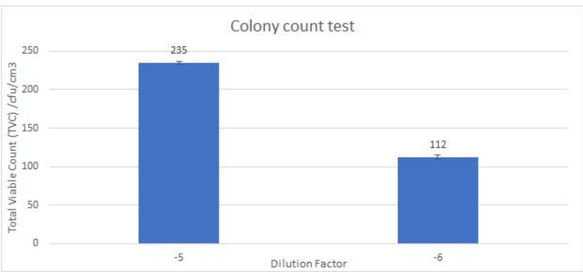



Fig 4a: Graph showing the Total Viable count

Since the control set-up had too many colonies to be counted, compared to the set-up with the lemon peel-zinc oxide nanoparticles-calcium alginate composite beads which had an average of 235 colonies for a dilution factor of -5 and 112 colonies for a dilution factor of -6, lemon peel-zinc oxide nanoparticles-calcium alginate composite showed antibacterial properties.

## 5. Conclusion and recommendations for future work

### Conclusion

This experiment involved the synthesis of a lemon peel-zinc oxide nanoparticles-calcium alginate composite and a comparative analysis of the adsorption of the lemon peel-zinc oxide nanoparticles-calcium alginate composite to its constituents for $Cu^{2+}$ ions, $Zn^{2+}$ ions, methylene blue, malachite green and brilliant green. The composite was hypothesised to have a higher adsorption capability and versatility compared to its constituents. Adsorption tests were carried out with 0.5g of adsorbent and 25cm$^3$ of 20ppm pollutant solution for a period of 3 hours on an orbital shaker at 200 rpm. The lemon peel-zinc oxide nanoparticles-calcium alginate composite was found to have significantly better adsorption of Brilliant green and Malachite green than both lemon peels and calcium alginate while being significantly better than lemon peels for adsorption of Methylene blue. For the heavy metal ions, the lemon peel-zinc oxide nanoparticles-calcium alginate composite performed comparably to its constituents. The lemon peel-zinc oxide nanoparticles-calcium alginate composite has also demonstrated antibacterial properties. As such, with lemon peels, calcium alginate and ZnO NPs as constituents, a cheap composite with a comparable adsorption capability to its constituents and versatility, which is suitable for use in wastewater treatment has been achieved.

### Limitations and future work

Due to time constraints, characterisation using SEM could not be carried out. More replicates could not be done for heavy metal ions, dyes and antibacterial tests. Testing for the adsorption of Zinc oxide nanoparticles against lemon peel-zinc oxide nanoparticles-calcium alginate composite could not be carried out. The ratio of the constituents in the composite was not varied. pH was not varied. The bacterial test should be repeated with a higher dilution factor in order to obtain a countable result for the control.

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