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Lead metal ions remediation in vitro, using centrifuge-assisted pectin extract a polysaccharide-based biomolecule.

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Abstract

Sorption of heavy metal ions by different pectin-rich materials such as waste citrus peels and pectin extracts is emerging as a low-cost and double-headed solution technique for metallic contaminant removal and biomass solid waste management. Heavy metal ions present in drinking water and foods have remained a challenge, as the technology to thoroughly remove such heavy metal ions has remained on a higher side of the cost. Nonetheless, the effect of heavy metals on the health of humans taking contaminated water and foods has remained a constant silent threat. The results are almost certainly fatal with many instances a victim hardly knows the cause, as there is no immediate effect after their consumption. This research focused on evaluating the binding efficiency, spectroscopic characterization and extent of sorption. The study investigated the removal of lead, by pectin extract from orange peel wastes. The centrifuge-assisted pectin extract was chemically and spectrometrically characterized, for molecular conformity. The sorption of heavy metal depended on the mass of pectin, pH, and contact time. Uptake was rapid with equilibrium reached after **60 minutes** with high removal of lead solution at **pH 5** with efficiency of **71.0 %**.

Keywords: Pectin, Heavy metals, Health**,** fatal

Introduction

Heavy metals in the environment

Heavy metal contamination in the environment has been a global concern. These minerals can be deposited into the environment through air, water, and soil. Industrial processing such as the mining of minerals has been in existence for thousands of years. One of the byproducts of minerals mined are called heavy metals, among them are lead (Pb), Cadmium (Cd), Arsenic (As), Chromium (Cr) and many others [1]. These minerals can be deposited into the environment through air, water, and soil contamination. Once released into air or water, it may travel long distances before settling on the ground and onto water bodies and transforming by sunlight, air, and water. When it enters the natural aquatic systems, it becomes mobile and bio-available and subsequently a source for human exposure.

Other sources of heavy metal contamination in the environment include battery recycling production plants, cosmetics, and petroleum products [2].

Lead exposure

Lead can affect almost every organ and system of the human body. At high levels, lead may increase reaction time, cause weakness in fingers, wrists, and ankles, and possibly affect memory. Lead may also cause anaemia and damage the male reproductive system. It has proved deadly to exposed children. Based on toxicity and the high possibility of exposure, lead has been listed as one of the toxic heavy metals [3-5]

Lead treatment in water

The recent treatment of lead in aqueous waste streams includes coagulation/precipitation, reverse osmosis, electrochemical techniques, and ion exchange. But these techniques for the removal of heavy metal contamination in water are expensive hence suggesting a new method of using pectin as an effective method [6-8]

Biosorption

Biosorption, sorption onto biological materials, has emerged as a potential alternative method for aqueous removal of transition and heavy metals such as lead. It is a process that involves binding of the metal ions by biological materials. It is a promising technique because very

cheap materials such as industrial waste products or naturally abundant biomass can be employed for the purpose. These biosorbents can be obtained almost free of charge as producers often pay for their disposal. The only considerable cost for these raw materials is the cost of drying, transportation, and solvent for pectin extraction. Several researchers have studied different biological materials for the removal of heavy metal ions such as polymerized corncob, hulls and bran, and brown algae. [1-3, 5, 9-10]

This research focused on pectin extracted from citrus fruit (orange). Pectin is a natural polysaccharide substance present in all fruits and most vegetables. The substance is the major component of the middle lamella and of the primary cell walls of the fruit tissues, where they bring about rigidity and cohesion between cells. They are found associated with physical or chemical bonds with other polysaccharides such as cellulose and hemicellulose [11].

Depending on the age of the plants, pectin is found in two forms; protopectin and pectin acid. Protopectin is insoluble in water as it is linked to other cell components, and pectin acid is soluble in water [11-13].

Protopectin is gradually transformed into pectin by the action of Enzymes such as pectinese and pectin esterase. This is the ripening process that diminishes the cohesion of the cell walls as the lamellae break and induces a softening of the fruit.

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The most abundant pectin polysaccharide is homogalacturonan (HG), a linear homopolymer of α-1, 4-linked glycosidic bond chain galacturonic acid that comprises approximately 65% of pectin.HG is potentially methyl esterified at C-6 carboxyl, maybe O-acetylated at O-2 or O-3, and may contain other potentially crosslinking esters of uncertain structure. HG is present in stretches of approximately 100 GalA residues in length, although the shorter region of HG has been detected interspersed between other pectic polysaccharides [14, 31-34].

Acetylated pectin is not effective for heavy metal binding because they have a lower affinity towards metal ions as compared to carboxylic groups. The presence of a free carboxyl group on the molecule is responsible for the binding of heavy metals. This is because the free

carboxyl group in acid media is protonated (negatively charged) making it easy for the binding to take place effectively.

Along the galacturonic acid chain, several substituents are present: carboxylic acid functions are partially esterified by methanol. The percentage of methyl-esterified groups in the pectin chain is called the degree of methylation (DM) or degree of esterification (DE). Pectin with DE > 50% is called high methylated (HM) pectin, while pectin with DE < 50% is called low methylated (LM) pectin. This factor has a great influence on pectin properties one as the ability to form gels [11-13].

Figure 1.0 below shows: alpha 1, 4-D-galacturonic acid (Molecular Structure of Pectin)

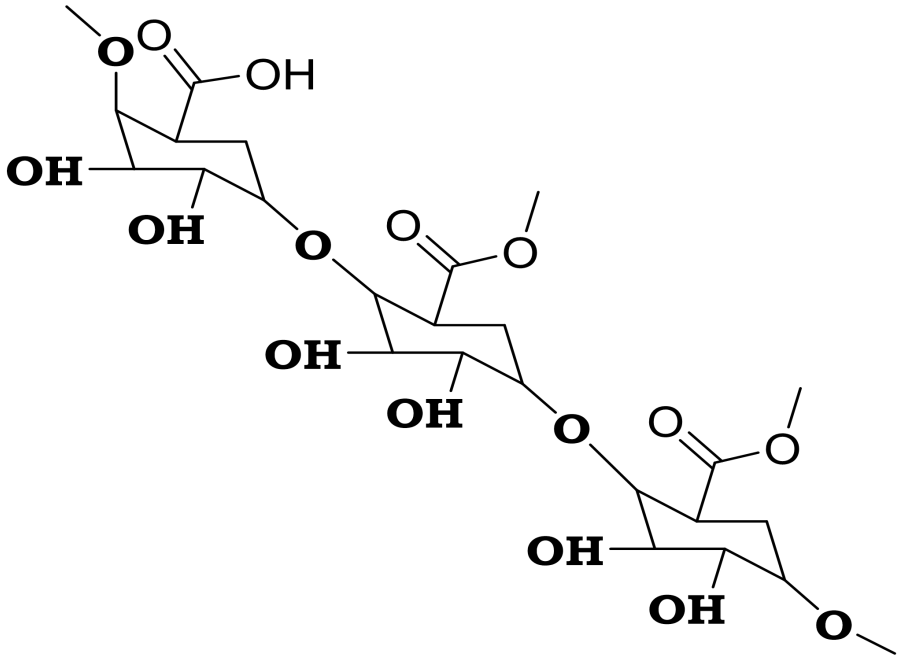

Figure 1.0 shows the molecular structure of pectin.

Several pectin-rich biological materials have been studied for their metal-binding abilities. These materials range from cheap, naturally occurring materials such as lemon fruit peels, orange fruit peels, grapefruit peels, apple fruit peels, and also pectin extract from a variety of plants and its efficiency in terms of binding capacity and disintegration capacity of the material.

Silke and Santosh (2008) studied binding for biosorption of cadmium by pectin-rich fruit (lemon, orange, grapefruit, apple fruit peels) materials and found that citrus peels were most suitable. This was due to higher stability than apple residues and grape skins in combination with good uptake. Kinetic studies showed that equilibrium was reached within 30–90 min, depending on particle size. The metal uptake rate increased as particle size decreased,

indicating mass transfer limitations. There was little impact of particle size on capacity, indicating that biosorption by fruit wastes is not only a surface phenomenon. And also observed metal uptake decreased with decreasing pH, indicating competition of protons for binding to acidic sites [15]. Balaria (2008) Studied biosorption binding for the absorption of lead by citrus pectin and peels. And concluded that the Biosorption of Pb by citrus pectin and orange peels is fast, with equilibrium reached in less than 60 minutes, and it follows pseudo-second-order kinetics [16]. Pb uptake by orange peels depends on pH, ionic strength, and the presence of co-ions but the impact of these parameters depends on sorbent dosage (mass).

Effects of Environmental Conditions

Both binding rate and capacity for biosorption of metal ions depend on several environmental conditions including pH, temperature, ionic strength, initial metal ions concentration, co-ion presence, and sorbent mass.

METHODOLOGY

The methodology involved experimental work which involved the extraction of the pectin. Then it was followed by chemical characterization specifically the presence of binding site testing and general chemical nature. This was followed by a spectroscopic evaluation of the pectin extract to confirm the chemical characterization was showing the said binding sites' functional groups.

Pectin extraction Method used.

The peels of ripe oranges dried and shred to small pieces using a blender to increase the efficiency of extraction. Then the peel powder was measured (30.0 g) and transferred into a beaker (800 mL) containing 450.0 mL of water, and hydrochloric acid 0.06M was added to give a pH of 2.26. The fruit peel samples were then boiled for one hour at a temperature of 85.0 ± 0.5℃ while care was given not to beyond the range. Then the residues were filtered. The filtrate was allowed to cool at 25.0 degrees Celsius to reduce heat degradation of the pectin. Then 250.0 ml 95% isopropanol was added to the extracted pectin while stirring. It was left for 30.0 minutes to allow the pectin to float on the surface and then centrifugation was performed to allow the pectin to sink as opposed to direct filtering which traditionally is done, then removed from the alcohol solution. Then it was dried in the oven at room temperature and broken into smaller pieces.

Chemical characterization

The percentage yield of pectin

The pectin yield was calculated using the equation below:

$$\boldsymbol{Ypectin = \frac{p}{Bi} \times 100} \qquad \text{Equation 1 [17-19]}$$

Where y pectin (%) is the extracted pectin yield in percentage (%), P is the amount of extracted pectin in grams and Bi is the initial amount of orange.

Chemical Characterization using titration

Equivalent weight

A pectin sample of 0.5 g was weighed into a 250.0 ml conical flask and moistened using 5.0 mL ethanol; 1.0 g NaCl was added to the mixture, followed by 100.0 ml distilled water and a few drops of phenol red indicator. Care was taken at this point to ensure that all the pectin had dissolved and that no clumping occurred on the sides of the flask before the solution was then slowly titrated (to avoid possible de-esterification) with 0.1 M NaOH to a pink colour at the endpoint.

Equivalent weight was calculated using the equation below:

$$\textbf{Equivalent weight (EW)} = \frac{\boldsymbol{weight\ of\ the\ sample\ (g) \times 100}}{\boldsymbol{ml\ of\ alkali\ x\ N\ of\ alkali}} \qquad \text{Equation 2 [17-19]}$$

Methoxyl content

Methoxyl (Mec) content is a significant factor in controlling the sensitivity to polyvalent cations, the pectin's setting time, and their usefulness in the preparation of low solid gels, fibers, and films. It was determined by saponification of pectin and titration of the liberated carboxyl group. To a neutral solution titrated for the equivalent weight containing 0.5g of pectic substances, 25mL of 0.25N sodium hydroxide (NaOH) was added, thoroughly shaken, and allowed to stand for 30 mins at room temperature in a flask with a stopper. A 25mL portion of 0.25N HCl (or an amount equivalent to the added base) was added and titrated with 0.1N sodium hydroxide (NaOH) to the same endpoint as the previous one. Methoxyl content was calculated using the following equation:

$$\%\ \boldsymbol{Methoxyl\ content} = \frac{\boldsymbol{mL\ alkali \times N\ alkali\ x\ 3.1}}{\boldsymbol{weight\ of\ sample(g)}} \qquad \text{Equation 3 [17-19]}$$

Anhydrouronic acid

Anhydrouronic acid (AUA) estimation content is essential to determine purity and degree of esterification (DE) and in the evaluation of pectin's physical properties. Pectin, a partly esterified polygalacturonase, contains at least 10% organic material composed of galactose, arabinose, and other sugars. Making use of equivalent weight, methoxyl content, and the alkalinity of the ash data, the anhydrouronic acid was calculated using the following equation:

$$AUA\ (\%) = \frac{176 \times 0.1z \times 100\%}{w \times 1000} + \frac{176 \times 0.1y \times 100\%}{w \times 1000}$$ Equation 4 [17]

Where 176 is the molecular weight of anhydrouronic acid

y = ml (titer) of NaOH from methoxyl content determination.

z = ml (titer) of NaOH from equivalent weight determination.

w = weight of the sample

Degree of esterification (DE)

The DE of pectin was determined according to the formula below

$$\boldsymbol{DE}\ (\%) = \frac{\mathbf{176} \times \boldsymbol{MeC}(\%) \times \mathbf{100}}{\mathbf{31} \times \boldsymbol{AUA}\ (\%)}$$ Equation 5 [17]

FTIR Experiment

Orange pectin was characterized for its surface functional groups using Fourier Transform Infrared Spectroscopy (FTIR).

IR for the liquid gel pectin absorbance data was obtained for wave numbers 400-4000 cm'1. IR data were collected, processed, and analyzed.

Adsorption studies

A solution of lead nitrate ($Pb(NO3)_2$) was prepared in the laboratory whose initial concentration was 141ppm to study the adsorption of pectin with solution lead. This was achieved by experimenting with the effect of the following parameters: pH level, sorbent mass, particle size, and contact time. A volume of 50 ml of 141 mg/L concentration of the lead solution was selected was used to investigate all four parameters. Experiments were conducted in a mechanical shaker at a speed of 150 rpm and at room temperature. The solution was filtered using filter papers and the filtrate solution was analyzed using AAS to measure the concentrations of remaining lead ions. The adsorption capacity or metal uptake

was determined by calculating the concentration of metal retained in the biosorbents (qe, mg/g) using the following formula:

$$qe = \frac{v(Co-Ce)}{m}$$ Equation 6 [17]

Where:

Co = initial metal ion concentration in solution (mg/L)

Ce=final (equilibrium) metal ion concentration in solution (mg/L)

V= solution volume (L) m=sorbent mass (g)

Meanwhile, the percentage removal (%R) known as biosorption efficiency for the metal was evaluated from the following equation:

$$\%\boldsymbol{R} = \frac{\boldsymbol{Co-Ce}}{\boldsymbol{Co}} \times \mathbf{100}$$ Equation 7. [17]

Effect of pH level of solution, sorbent mass, contact time, and particle size

For the varying pH level of the solutions, setups were set to different values at 4 and 5. 0.1M NaOH and HCl were used to adjust the pH to the appropriate levels. The mass sorbent was kept constant at 0.2g and contact time was done at 80 minutes. Meanwhile, for the varying sorbent mass, setups were adjusted to 0.2g, 0.5g, and 0.7g. The pH level was kept at 5 and the contact time of the biosorbents in the solution was kept at 80 minutes. Varying contact times, and setups were done in periods 10, 30, 60, and 80 minutes. The pH level was kept at 5, the sorbent mass was controlled to 0.4g. Particle size setup was 0.355 mm and 1.40 mm. The pH level was kept at 5, the sorbent material was controlled to be 0.3g. In all setups, the solutions were filtered in preparation for analysis through AAS.

RESULTS

Extraction and percentage yield.

Based on equation 1 by Gainer *et al* 1993, above and the various P obtained for pectin, table 1.0 are the results obtained.

Table 1.0 the results of extract trails and masses of pectin obtained

extract	mass of peels (g)	Pectin (g)	Percentage (%)
1	30.0	6.09	20.3%
2	30.0	6.10	20.3%
3	30.0	6.08	20.3%
Average mass extracted		**6.09**	**20.3%**

The yield obtained was still within what other research groups obtained Grainer et al 2019 [20]. The result is still consistent with the result of Maria et al 2021 in their review they stated the range is 6.0 percent to 30.0 percent [19, 35]. This could be attributed to the natural abundance of a molecule in the peels. As it has also been established that different citrus fruits yield different amounts of pectin. Hence it could be noted that the repeated trial at 2.26 pH yielded almost a consistent yield as could be seen from the table. Therefore it could be stated that centrifuging the suspended precipitate of pectin may help if avoiding loss of extracted pectin due to too fine pectin particles sipping through.

Chemical Characterization using titration

Equivalent weight

In this section the titration was performed and four trials were done. Then equation 2 was used to compute the equivalent weight of the extracted pectin. The equivalent weight is the number of acid-base reacting sites in the molecule. These sites are vital because their presence is the possible sorption capabilities of the biomolecule.

Table 2.0 shows the titration values for equivalent mass determination

Trial number	Initial volume titer (ml)	Final volume titer (ml)	Equivalent Weight (g/mol)
1	0	2.5	208.3
2	0	2.6	192.3
3	0	2.4	208.3

4	0	2.5	200.0
Average		**2.5**	**200.0**

The values of equivalent mass show that the sites where sorption could take place with metals are present. The equivalent mass for the orange peel extracts shows that they are within the limits of other work that other people have done so far. [18].

Methoxyl content

The methoxyl content indicates possible sites are reacted with methanol or the degree of esterification D.E less than 50.0% indicates a high carboxylic group which is ideal for sorption of heavy metals. The results from the titration of the D.E. determination could be understood as shown below. The result was in agreement with the determination of others [12-13, 21-26, 30].

Table 3.0 titration results for methoxyl determination and degree of esterification (D.E).

Trial number	**Initial volume titer (ml)**	**Final volume titer (ml)**	**Degree of esterification (D.E) %**
1	0	20.5	12.7
2	0	20.2	12.5
3	0	20.1	12.5
4	0	20.4	12.6
Average		**20.3**	**12.6**

From the values of methoxyl determination or degree of esterification, it is evident that the pectin is low in esters which may make the molecule not a good biosorption molecule. This suggests that the extract may have relatively high absorption abilities.

Purity of extract characterization - Anhydrouronic acid

Based on equations 4 and 5 according to Garnier 1993, also using the average values in tables 1, 2 and 3. The computation of the results is as in Table 4 below.

Colour	EW (g/mol)	MeC (%)	AUA (%)	DE (%)	Yield (%)
Pale yellow	200.0	12.5	80.3	12.5	20.3

The centrifuge-assisted pectin extract shows a high purity and high aptitude for absorption of metal ions, this is evident in the relatively high value of equivalent weight (EW) and lower value of methoxyl content (MeC) or D.E value.

The data outlined above is well correlated with spectroscopic characterization. Which clearly shows the peak absorption for the carboxylic and hydroxyl attached to the carboxylic group. The spectra have a carboxylic acid function group (-RCOOH) at wave numbers at 1690 cm^{-1}. The peak at 3496 cm^{-1} is for hydroxyl group OH. An alky (methyl) group peak is at wave number 2976 cm^{-1}, as shown in Figure 2.0 below.

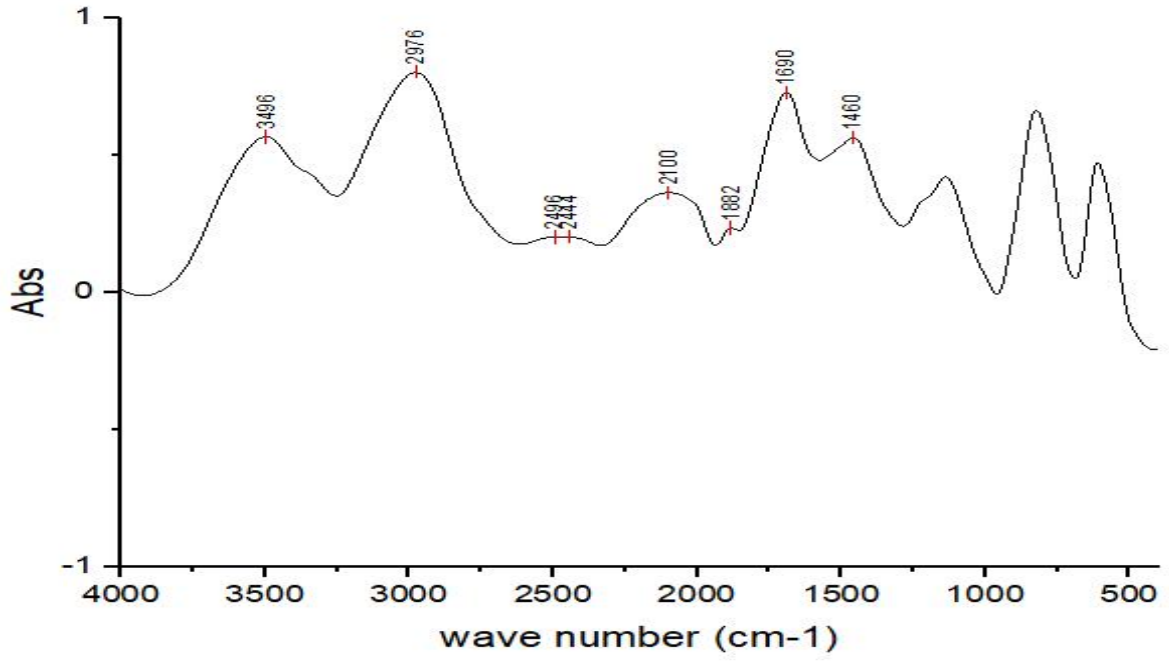

Figure 2.0 The FT-IR spectrum of the centrifuge-assisted pectin.

The spectra indeed confirm the hydroxyl stretch at 3496 cm^{-1}, 2976 cm^{-1}, overtone band combination bonds and broad, then 1690 cm^{-1}, for carbonyl group and 1230 cm^{-1} stretch for C-O bond and finally the 942 cm^{-1} for out of plane O-H bond [21, 28-29].

Sorption evaluation.

In this section, the evaluation followed the general sorption characteristic of the pectin extract, the effect of contact time and sorption ability of the extract and finally the size of the particles and sorption characteristics. The influence of pH on sorption too was investigated.

In vitro tests

General sorption characteristics

The sorption ability of the centrifuge-assisted extracts showed that the sorption depended heavily on the contact time. The trend was found that the longer the time of contact the higher the amount of metal ion removed. The graphic representation of the sorption was as in Figure 3.0 below.

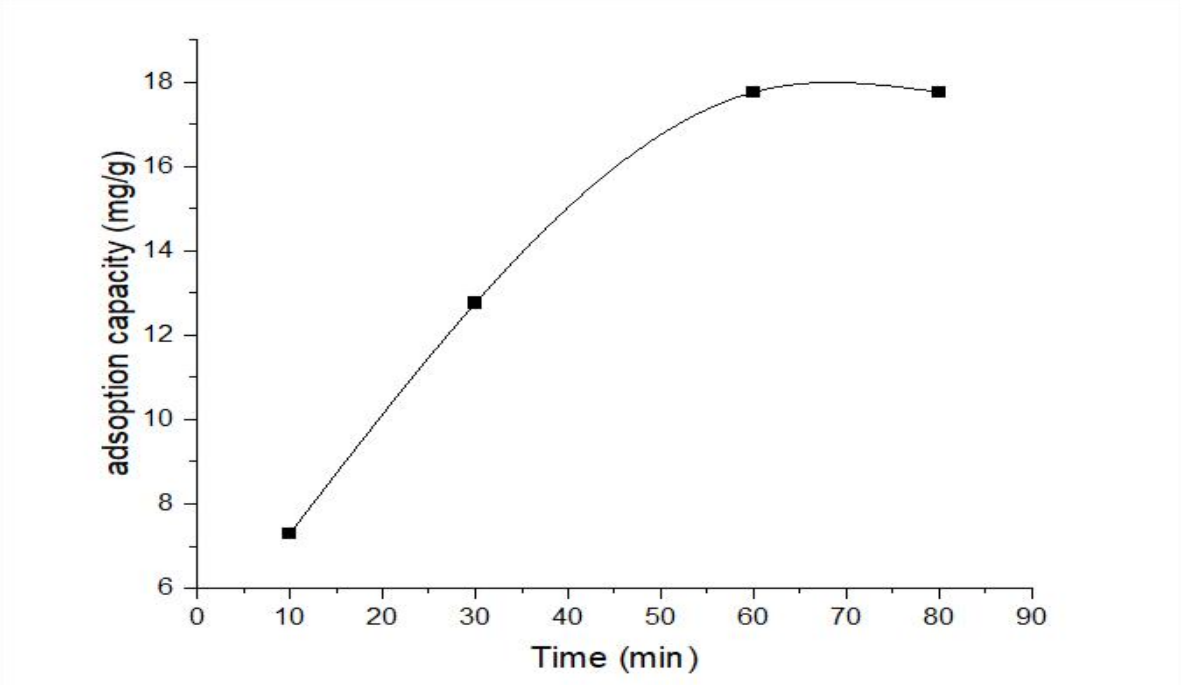

Figure 3.0. Shows the time dependence of the sorption capacity of the pectin extract.

It was found that though the contact time was a factor for the sorption of the lead metal ion in vitro, however beyond 60 minutes the capacity did not improve any further. This could be attributed to the fact that the osmotic pressure of the solution may have changed as a result beyond 60 minutes the osmotic pressure may have favoured bulk solution as favourable direction for diffusing of ions. As it could be seen that as the lead metal ions are binding to the carboxyl sites in the pectin molecule, they may create slightly higher concentrations of metal ions around those areas resulting in molecules diffusing back into the bulky solution hence the decrease seen in the capacity beyond 60 minutes. Equilibrium efficiency was

between 30 to 60 minutes of contact time with pectin material. Comparing the results found by Balaria and Schiewer (2008) also observed a sharp increase in binding between 30 to 60 minutes of contact time between pectin and the lead solution. [23, 27,] Binding efficiency of pectin depended on pH, particle size, contact time, and mass effect

Adsorption evaluation

Effect of sorbent mass

The effect of the sorbent's mass on the sorption capacity of the centrifuge-assisted pectin extract was also investigated. The graph in Figure 4.0 showed the general sorption characteristic of the extract. The sorption capacity decreases with increasing sorbent mass. This can be attributed to sorption site aggregation (forming clusters) at higher sorbent mass resulting in a reduced surface area available for sorption. The clusters reduced the surface area of pectin extract to adsorb the metal ions.

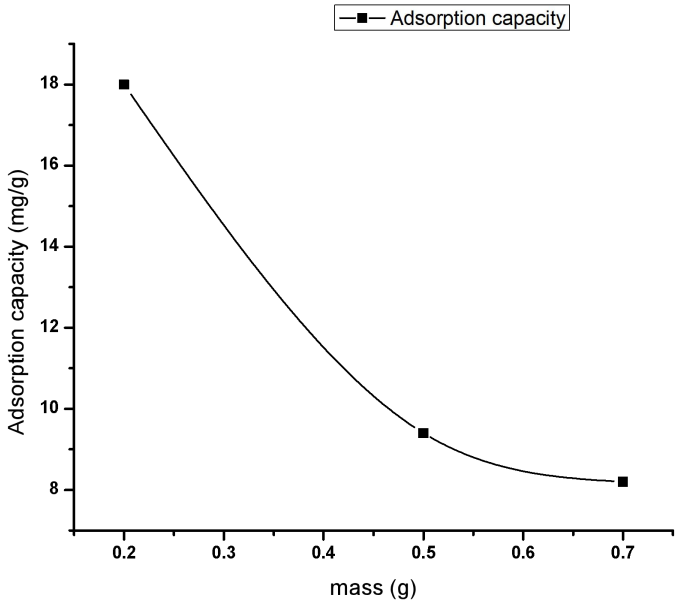

Figure 4.0 Pectin sorption capacity as the mass was changed.

It was generally found that sorption of lead metal ions in vitro was more effective at lower masses than at larger masses of pectin.

Adsorption evaluation

Effect of pH on the sorption capacity of the pectin extract.

The general trend is that at lower pH the sorption capacity of extract was lower than at higher. Figure 5.0 below outlines the general sorption characteristic.

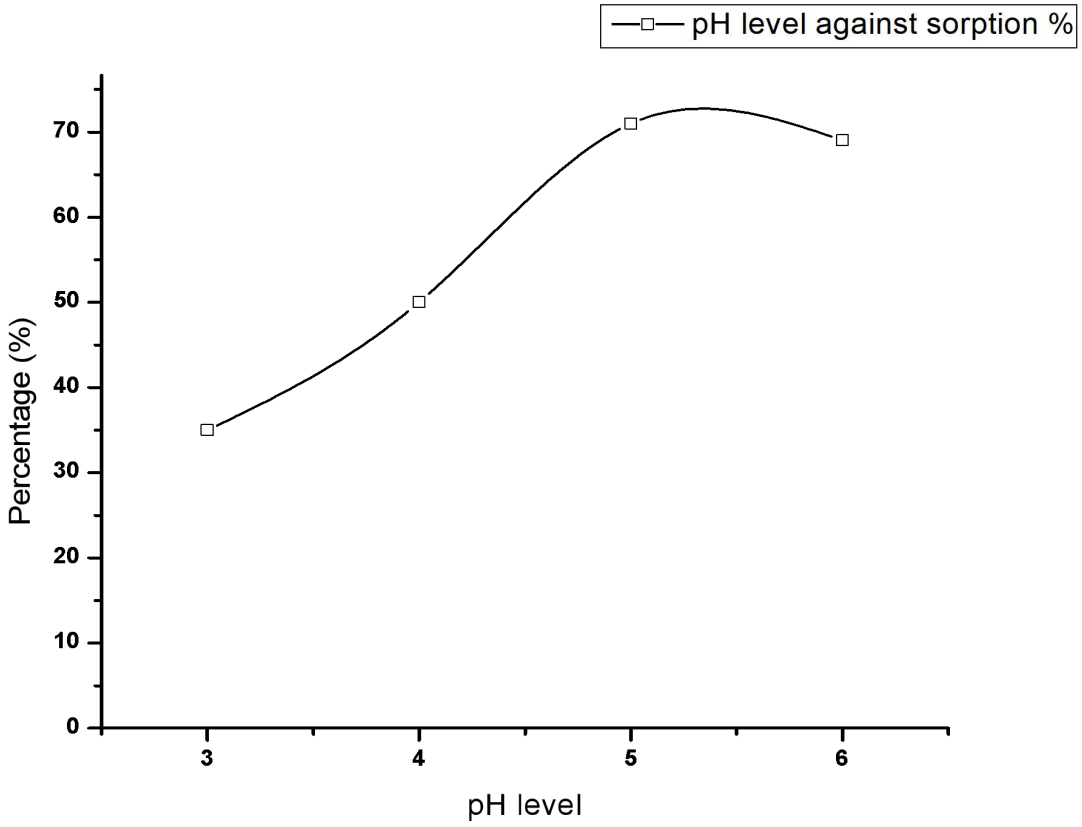

Figure 5.0 shows the sorption percentage against the pH level of the in vitro solution.

The pH level of the solution is a crucial factor in biosorption which affects the solubility of metal ions, surface charge, and availability of binding sites in the biosorbents. At very low pH levels, H+ ions compete with the metal ions for biosorbent binding sites decreasing the chance for metal adsorption. The dissociation of functional groups such as carboxyl on the surface of the biosorbent can also be attributed to the changes in pH. In general, percent removal tends to increase at higher pH for lead ions. The results are consistent with Kumar et al 2015, though they were working on chromium (IV) ions also others on calcium bind behavior [3, 22-23, 35].

Conclusion

Pectin was extracted and it showed an average percentage yield of 20.5% Centrifugation step just after precipitation did not improve the yield. It seemed yield depended on available pectin in citrus and extraction temperature. The functional groups' characteristics revealed that indeed the hydroxyl group attached to the carboxyl were present with peaks at 3496 cm^{-1} responsible for the binding Then the carbonyl bond at 1690 cm^{-1}, for the carbonyl group and 1230 cm^{-1} stretch for C-O bond and finally the 942 cm^{-1} for out of plane O-H bond. This too correlated well with the chemical analysis of the pectin which showed a low degree of esterification DE 12.5%, high percentage purity as evidenced by AUA 80.3% and an equivalent mass of 200.3 g/mol showing that the molecule had a fair amount of carboxylic sites for sorption of metal ions. The extract exhibited potential as an efficient heavy metal sorbent for lead. Metal ion. Uptake by pectin depended on factors such as pH, contact time and mass of pectin used for adsorption. Therefore at optimal conditions, pectin extract showed a 71.0% efficiency in the remediation of lead metal ions in vitro. It can be said that pectin can indeed remove heavy metals from their solutions.

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Conflict of interests

The authors have no conflict of interest that may affect this article.

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Additional Data

THE PICTURES BELOW SHOW THE ORANGE PEELS AND PECTIN EXTRACT

Orange peels

floating pectin in alcohol

Gel pectin

dried pectin