EFFECT OF DIFFERENT PREPARATIONS OF *Annona muricata* L. LEAVES ON THE BIOSORPTION OF LEAD, NICKEL AND ZINC FROM AQUEOUS SOLUTION.

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**Abstract**

*Annona muricata* L. gained popularity in the last couple of years and has been acknowledged as a “miracle cure for cancer”. The leaves are commercialized as tea. This study explored the utilization of the leaves as a biosorbent material in the removal of heavy metals Pb, Ni, and Zn in aqueous solution. Different preparations of the leaves were done: air drying (AD), oven drying (OD), drying under low heat using a burner (LHD), and drying in a furnace (FD). Heavy metal analysis after the biosorption experiment was done using microplasma-atomic emission spectroscopy (MP-AES). Results show that percent absorption/adsorption of lead is in the order of AD>FD>LHD>OD. In terms of percent nickel and zinc absorption, same trend was obtained, that is, FD>AD>LHD>OD. Adsorption isotherms, kinetics studies, FTIR and SEM analyses were done to explain the effects of the preparation methods in the biosorption of heavy metals.

**Introduction:**

Heavy metal pollution is a worldwide environmental problem. Lead, arsenic, cadmium, copper, nickel and zinc are among the most common pollutants discharged from various industries. These heavy metals are very harmful to plants, animals and human life because of their high mobility in soil and water. They also have strong tendency for bioaccumulation [1] in the living tissues through processes like breathing workplace air and eating contaminated food grown in soil containing heavy metals [2].

Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death, miscarriage in pregnant women, and damage organs responsible for sperm production in men [3]. Nickel is a nutritionally essential metal needed in trace amounts in several animal species, microorganisms and plants. Too little or too much of this element may cause a number of either deficiency or toxicity symptoms [4]. The mechanisms of nickel toxicity in microorganisms proposed by Macomber and Hausinger [5] are as follows: (1) nickel replaces the essential metals in metalloproteins; (2) nickel binds to catalytic residues of non-metalloenzymes; (3) nickel also binds outside the catalytic site of an enzyme to inhibit allosterically; and (4) nickel indirectly causes oxidative stress. Zinc, when compared to several other metal ions with similar chemical properties, is relatively harmless. Only exposure to high doses has toxic effects such as focal neuronal deficits and lethargy (brain), respiratory disorder after inhalation of zinc smoke and metal fume fever (respiratory tract), nausea/vomiting, epigastric pain and diarrhea (gastrointestinal tract), and elevated risk of prostate cancer [6].
Heavy metals in aqueous solution are usually removed by adsorption, ion exchange, coagulation, floatation, chemical precipitation, reverse osmosis, hyper-filtration, etc. Ion exchange resins are not only costly but creates secondary problems which include regeneration of the adsorbent and recovery of the contaminants. Alternative to the use of costly adsorbents is the use of low cost materials which can be classified as: (1) natural minerals such as coal, clays, sand, mud, etc.; (2) industrial wastes like fly ash, saw dust, biogas slurry, etc.; and (3) biological materials like plant-based adsorbents. Yu et al. [7] reported the removal of Pb(II) and Cu(II) using sawdust. Used tea leaves, cypress, cinchona and pine leaves [8], neem leaf powder [2], papaya wood [9], and cellulosic agricultural materials [10], were used to remove lead (II) ions in aqueous solutions. Treated sawdust from Acacia arabica was also reported to adsorb Cr(IV), Pb(II), Hg(II) and Cu(II) through surface complexation and ion exchange [11]. Biosorption behaviour of compound bioflocculant (CBF) produced by a mixed culture of Rhizobium radiobacter F2 and Bacillus sphaeicus F6 was used for the removal of Pb(II) ions in aqueous solution [12]. Peanut shell biomass was used in the removal of Cu(II) and Cr(III) ions [13]. These bioadsorbents have high versatility, high metal selectivity, high uptake coupled with rapid kinetics of the biosorption systems.

This study was conducted to determine the effect of preparation methods on the biosorption capacities of Annona muricata L. leaves. These methods are drying over low heat using a stove (LHD), oven drying (OD), air drying (AD), and drying in furnace (FD). These biosorbents were used to test their efficiency in the removal of Pb, Ni and Zn ions in aqueous solution.

Methods:-
Reagents and materials:-
All the chemicals used in the experiments were analytical grade without further purification. Pb(NO₃)₂, Ni(NO₃)₂·6H₂O and Zn(NO₃)₂·4H₂O were used in the preparation of 1000 ppm stock solution. AG-WAVECAL-ASL-5 (ICP-OES wavelength calibration solution Plasma Emission standard) was used for calibration.

Preparation of A. muricata (guyabano) leaves:-
Mature guyabano leaves were collected in Central Luzon State University, Science City of Munoz, Nueva Ecija. These were washed repeatedly with water to remove dust and soluble impurities and were allowed to dry at room temperature. Approximately 400 g of guyabano leaves were processed in four different ways. In air drying, the leaves were placed under the shade for five days. In oven drying, the leaves were dried in a laboratory oven for two (2) hr at 105°C. Low heat drying was done by heating the leaves in a pan over a low flame with occasional stirring. In furnace drying, the leaves cut into small pieces were placed in a furnace and heated at 200°C for one (1) hr. The leaves were then converted into powder (guyabano leaf powder, GLP) by grinding in a mechanical grinder. The samples were stored in plastic containers with proper labels.

Adsorption experiments:-
The adsorption experiments were carried out by agitating a pre-weighed amount of the powder with 50 mL of Pb(II), Ni(II) and Zn(II) solution in polyethylene bottles at constant temperature and speed in a mechanical shaker for a predetermined time interval. After adsorption, the mixture was filtered and the filtrate was digested with 5 mL aqua regia for 30 minutes or until the solution volume was reduced to 35 mL. The digested solution was diluted to 50 mL. The remaining heavy metals unadsorbed in the solution were determined using microwave plasma – atomic emission spectroscopy (MP-AES).

Heavy Metal Analysis using Microwave Plasma-Atomic Emission Spectroscopy (MP-AES):-
Agilent 4100 MP-AES was used for the metal determination of Pb, Ni, and Zn in the aqueous solutions. The viewing position and nebulizer pressure were optimized automatically using the Agilent MP Expert software. The instrumental parameters used for sample analysis are listed in Table 1.

| Analyte | Wavelength (nm) | Read time (s) | Nebulizer flow (L/min) | Background correction |
|---------|-----------------|---------------|------------------------|----------------------|
| Pb      | 368.346         | 5             | 0.85                   | Auto                 |
| Ni      | 341.476         | 5             | 0.85                   | Auto                 |
| Zn      | 472.215         | 5             | 0.85                   | Auto                 |
Calibration standards, 1,3,5, 10 and 100 ppm were run prior to the analysis of samples. The calibration curve correlation coefficients are both 0.999 for Zn and Ni and 1.000 for Pb.

**Percent adsorption:**
The amount of Pb, Ni, and Zn ions adsorbed per unit mass of the GLP \((q \text{ in mg/g})\) was computed using the expression:

\[
q = \frac{C_o - C_t}{m}
\]

where \(C_o\) and \(C_t\) are heavy metal concentrations in mg/L before and after adsorption for time \(t\), and \(m\) (g) is the amount of GLP taken for 50 mL heavy metal solution. The extent of adsorption of the GLP in percentage is shown in the equation:

\[
\text{adsorption (\%)} = \frac{C_o - C_t}{C_o} \times 100
\]

**Kinetics of adsorption:**
Adsorption capacity of the GLP may involve chemical reactions between functional groups present on the adsorbent surface and the metal ions forming metal-organic complexes or cation exchange reactions. Other mechanisms such as mass-transport processes, diffusion across the solid particles surrounded by liquid film, and diffusion into macro- and micropores can also account for the adsorption capacity of the adsorbent.

Several kinetic models were tested in this study. These are the pseudo-first-order and second order models (Eqs. 3 and 5) derived by Lagergren [14].

\[
\frac{dq_t}{dt} = k_{ad}(q_e - q_t)
\]

where \(q_t\) and \(q_e\) are the amount adsorbed at time \(t\) and at equilibrium, and \(k_{ad}\) the rate constant of the pseudo-first-order adsorption process. The integrated rate law (Eq 4) allows the computation of the adsorption rate constant, \(k_{ad}\).

\[
\log(q_e - q_t) = \log q_e - \left[\frac{k_{ad}}{2.303}\right]t
\]

The adsorption follows first-order kinetics when a plot of \(\log(q_e - q_t)\) versus \(t\) gives a straight line. If not, the pseudo-second-order kinetics (Eq 5) was used.

\[
\frac{dq_t}{dt} = k(q_e - q_t)^2
\]

where \(k\) is the second-order rate constant. Integrated form (Eq 6)

\[
\frac{1}{q_e - q_t} = \frac{1}{q_e} + kt
\]

can be rearranged into

\[
q_t = \frac{1}{1/kq_e^2} + \frac{t}{q_e}
\]

or, in the linear form,

\[
\frac{t}{q_t} = \frac{1}{h} + \left(\frac{1}{q_e}\right)t
\]

where \(h = kq_e^2\) can be the initial sorption rate as \(t \to 0\). If the adsorption kinetics follows pseudo-second-order, then the plot of \(t/q_t\) versus \(t\) would give a linear relationship where \(q_e, k\) and \(h\) can be calculated without having to know the parameters beforehand.

The absorption data were also analyzed using the Elovich equation

\[
\frac{dq_t}{dt} = a \exp(-\beta q_t)
\]
where $\alpha$ is the initial sorption rate constant and $\beta$ is the desorption rate constant during any one experiment. Elovich equation is simplified by assuming that $\alpha \beta t >> 1$ and by applying the boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_e$ at $t = t$,

$$q_t = \beta \ln(\alpha \beta) + \beta \ln(t)$$

(10)

The intraparticle diffusion model (Eq 11) and its linear form (Eq 12) [14] were also used in this study.

$$q_t = k_p t^{0.5}$$

(11)

$$\ln q_t = \ln k_p + 0.5 \ln t$$

(12)

Adsorption isotherms:
Two models of adsorption isotherms were tested in this study for the metal which showed the highest adsorption capacity toward GLP: the Langmuir isotherm (Eq 13) and the Freundlich (Eq 15) model.

$$\theta = \frac{q_e}{q_m} = \frac{b C_e}{1 + b C_e}$$

(13)

$$\frac{C_e}{q_e} = \frac{1}{b q_m} + \frac{1}{q_m} C_e$$

(14)

where $C_e$ is the concentration of the adsorbate at equilibrium, $q_e$ is the amount adsorbed at equilibrium in unit mass of the adsorbent, $q_m$ is the monolayer capacity, and $b$ is the equilibrium constant.

$$q_e = K_f C_e^n$$

(15)

$$\log q_e = \log K_f + n \log C_e$$

(16)

where $K_f$ and $n$ are known as Freundlich coefficients obtainable from the plots of $\log q_e$ versus $\log C_e$.

Results and Discussion:-
Surface characterization of the GLP biosorbents:-
Figure 1 shows the scanning electron photomicrographs of the four GLP biosorbents. The guyabano leaf powders (LHD, OD, AD and FD) were groups of fine particles which have no regular, fixed shape and with different macro- and micropore sizes. GLP-LHD have micropore diameters ranging from 1.87 to 5.06 μm, 1.49 to 5.08 μm for GLP-OD, 2.23 to 4.04 μm for GLP-AD and 2.02 to 4.41 μm for GLP-FD. Macropores ranging from 9.72 to 16.2 μm were observed in the AD sample (photomicrograph shown as inset in Figure 1 AD).
Figure 1: SEM photomicrographs of guyabano leaf powder at 3500x magnification (inset photograph at 750x)

Fourier Transform Infrared (FTIR) Analysis of the GLP biosorbents:
Figure 2 shows the FTIR spectra of the guyabano leaf powders (LHD, OD, AD and FD). The presence of broad peak of OH near 3400 cm$^{-1}$, C=O stretching at 2300 cm$^{-1}$ and C-O stretching at 1670 cm$^{-1}$, C-H aliphatic stretching at 2900 and 2800 cm$^{-1}$ confirm the presence of functional groups that may have contributed to the biosorption of heavy metals.

Figure 2: FTIR spectra of the guyabano leaf powders
Effect of agitation time and kinetic: -

Table 2: Effect of agitation time on adsorption of Pb²⁺, Ni²⁺, and Zn²⁺ on the different guyabano leaf powder at 30°C (Pb²⁺ = 20 mg/L; Ni²⁺ = 4 mg/L and Zn²⁺ = 6 mg/L).

| TIME (min) | % Adsorption Pb | % Adsorption Ni | % Adsorption Zn |
|------------|-----------------|-----------------|-----------------|
|            | LHD             | OD              | AD              | FD              | LHD             | OD              | AD              | FD              | LHD             | OD              | AD              | FD              |
| 5          | 89.48           | 81.66           | 92.93           | 89.33           | 8.52            | 10.46           | 40.49           | 49.16           | 30.70           | 38.41           | 67.82           | 83.83           |
| 10         | 82.42           | 81.00           | 93.78           | 89.67           | 7.67            | 9.77            | 45.10           | 50.75           | 29.77           | 34.66           | 73.84           | 86.48           |
| 15         | 89.51           | 81.86           | 93.53           | 92.30           | 7.25            | 10.58           | 45.64           | 50.21           | 29.63           | 38.54           | 73.94           | 86.27           |
| 20         | 83.90           | 80.81           | 93.49           | 92.80           | 7.52            | 7.68            | 47.26           | 51.27           | 30.09           | 33.74           | 76.17           | 87.04           |
| 25         | 92.15           | 81.46           | 93.86           | 91.44           | 8.21            | 10.34           | 47.18           | 51.02           | 29.94           | 38.59           | 75.84           | 87.57           |
| 30         | 88.09           | 85.27           | 94.44           | 92.76           | 9.04            | 10.98           | 45.08           | 51.57           | 33.00           | 38.93           | 74.57           | 89.32           |
| 60         | 90.39           | 87.64           | 94.86           | 94.96           | 9.61            | 11.62           | 48.52           | 53.62           | 34.58           | 41.78           | 77.48           | 91.87           |
| Mean       | 87.99           | 82.81           | 93.84           | 91.89           | 8.26            | 10.20           | 45.61           | 51.08           | 31.10           | 37.81           | 74.24           | 87.48           |

Legend: LHD – low heat dried; OD – oven dried; AD – air dried; FD – furnace dried.

Means with the same letter superscript are not significantly different at 5% level using Duncan Multiple Range Test (DMRT).

Results show that percent absorption/adsorption of Pb²⁺ is highest in the GLP-AD after 60 min of agitation but is comparable with GLP-FD with values of 93.84% and 91.89%, respectively, followed by GLP-LHD, 87.99%, and the lowest value was obtained in GLP-OD, 82.81%. In terms of percent Ni and Zn adsorption, same trend was obtained, that is, FD>AD>OD>LHD. Percent adsorption for Ni²⁺ ranged from 7.25% - 53.62% and Zn²⁺ from 29.63% - 91.87%. These results imply that the guyabano leaf powder is more selective toward Pb²⁺ adsorption than Ni²⁺ and Zn²⁺ though the initial concentration of Pb²⁺ was higher compared to the concentrations of Ni²⁺ and Zn²⁺ in the solution. The mechanism of sorption of heavy metals with biosorbents often involves chemical reactions between functional groups on the biosorbents and the metal ions forming organic complexes or cation reaction due to high cation-exchange capacity [14]. It is evident in this study that Pb²⁺ has more capacity to form organic complex with the functional groups present in the guyabano leaf powder compared to Ni²⁺ and Zn²⁺. Added to this effect is the initial concentration of Pb²⁺ in the solution. The amount of Pb²⁺ (20 ppm) may have contributed to high probability of collision between Pb²⁺ with the biosorbent surface, and high rate of Pb²⁺ diffusion on the biosorbent surface [12]. High initial concentration of metal ions accelerates the complexion reaction and reduces mass transfer resistance.

Kinetics of adsorption of Pb²⁺, Ni²⁺ and Zn²⁺ following the Elovich equation and the intraparticle diffusion model and the pseudo-first-order equation of Lagergren with the plots of $q_t$ versus $\ln t$ and $\ln q_t$ versus 0.5 $\ln t$, and $\log(q_e-q_t)$ versus $t$, respectively, did not yield good correlations. Only Pb²⁺ had shown a straight line but the correlation coefficient of 0.911 is not conclusive that the reaction follows pseudo-first order kinetics.

![Figure 3](image-url) - Lagergren plot for adsorption of Pb²⁺, Ni²⁺ and Zn²⁺ on guyabano leaf powder (Powder amount: 0.1 g/L).

Using the pseudo-second-order kinetics, Lagergren plots (Figure 3) of $t/q_t$ versus $t$ yield very good straight lines with correlation coefficients of 0.99 for the three metals in the four GLPs except for Ni²⁺ in GLP-OD with only 0.97. It is...
evident that the adsorptions of the three heavy metals on the guyabano leaf powder follow the pseudo-second-order kinetics. The amount adsorbed at equilibrium, the initial sorption rate and the rate constant of adsorption are presented in Table 3.

Table 3:-The amount adsorbed at equilibrium, initial sorption rate and rate constant of adsorption, $K$ (g mg$^{-1}$ min$^{-1}$) of the Pb$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ following the pseudo-second order kinetics.

| Metal | $q_e$ (mg g$^{-1}$) | $h$ (g mg$^{-1}$ min$^{-1}$) | $K$ (g mg$^{-1}$ min$^{-1}$) |
|-------|---------------------|-----------------------------|----------------------------|
| Pb$^{2+}$ | GLP-LHD  | GLP-OD | GLP-AD | GLP-FD |
| $q_e$ (mg g$^{-1}$) | 3.30 | 3.21 | 3.75 | 3.69 |
| $h$ (g mg$^{-1}$ min$^{-1}$) | 5.46 | 6.85 | 19.61 | 5.95 |
| $K$ (g mg$^{-1}$ min$^{-1}$) | 0.50 | 0.67 | 1.40 | 0.44 |
| Ni$^{2+}$ | | | |
| $q_e$ (mg g$^{-1}$) | 0.07 | 0.09 | 0.39 | 0.43 |
| $h$ (g mg$^{-1}$ min$^{-1}$) | 0.03 | 0.04 | 0.26 | 0.44 |
| $K$ (g mg$^{-1}$ min$^{-1}$) | 5.42 | 4.70 | 1.71 | 2.42 |
| Zn$^{2+}$ | | | |
| $q_e$ (mg g$^{-1}$) | 0.37 | 0.45 | 0.89 | 1.04 |
| $h$ (g mg$^{-1}$ min$^{-1}$) | 0.33 | 0.36 | 0.78 | 1.05 |
| $K$ (g mg$^{-1}$ min$^{-1}$) | 2.41 | 1.78 | 0.98 | 0.97 |

The earlier claim that among the three heavy metals, Pb$^{2+}$ had the highest % adsorption after 60 minutes is supported by the results of the pseudo-second-order kinetics. The amount of Pb$^{2+}$ adsorbed at equilibrium is highest in GLP-AD followed by GLP-FD, GLP-LHD and GLP-OD with values of 3.75, 3.69, 3.30, and 3.21 mg g$^{-1}$, respectively. The highest initial sorption rate, $h$, for Pb$^{2+}$, was observed in GLP-AD with a value of 19.61 g mg$^{-1}$ min$^{-1}$. This implies that guyabano leaf powder is more selective in the adsorption of Pb$^{2+}$ than Ni$^{2+}$ and Zn$^{2+}$.

Adsorption isotherms:-
Lead was used in the study of adsorption isotherm using the GLP-AD biosorbent. This has been chosen in this study since it gave the highest adsorption for Pb$^{2+}$ in the first part of the experiment and it gave the highest initial sorption rate. Langmuir and Freundlich plots are shown in Figures 4 and 5 below.

![Figure 4](image-url)  
**Figure 4:** Langmuir plot for adsorption of Pb$^{2+}$ concentration of 50, 75, 100, 125, and 150 mg/L on guyabano leaf powder (1.2 g/L) and agitation time of 4 h.
Figure 5: Freundlich plot for adsorption of Pb\(^{2+}\) concentration of 50, 75, 100, 125, and 150 mg/L on guyabano leaf powder (1.2 g/L) and agitation time of 4 h.

The adsorption of Pb\(^{2+}\) in GLP-AD follows the Langmuir adsorption isotherm giving correlation coefficient of 0.990. The monolayer capacity of GLP-AD, \(q_m\), has a value of 166.67 mg g\(^{-1}\) and the equilibrium coefficient, \(b\), 0.125 L mg\(^{-1}\). The monolayer capacity of guyabano leaf powder is higher than the one reported by Bhattacharyya and Sharma (2004) on neem leaf powder with a value of 82.0 mg g\(^{-1}\) and is equivalent to \textit{Rhizopus nigricans} (166 mg g\(^{-1}\)) but lower than agricultural waste coirpith (263 mg g\(^{-1}\)) as reported by Kadirvelu and Namasivayam [15].

With regards to the behavior of the biosorbent toward Pb\(^{2+}\), the Freundlich plot have high linearity (R = 0.969). This indicates that the adsorption process of Pb\(^{2+}\) conformed to the empirical Freundlich pattern of adsorption on non-specific, non-uniform, and heterogeneous surface. This is supported by the SEM photomicrographs of the biosorbents (Figure 1). Freundlich coefficient, \(n\), 0.56 is within the range of 0<\(n\)<1 for favorable adsorption. \(K_f\), representing the adsorption capacity, has a value of 24.32 L g\(^{-1}\) which is similar to the result reported by Bhattacharyya and Sharma (2004) using 0.2 g L\(^{-1}\) neem leaf powder.

Conclusion:

The four methods of preparation of guyabano leaf powder, low heat drying, air drying, oven drying and drying in a furnace, have effects on the adsorption capacity of the biosorbents. The kinetics of adsorption is best described by pseudo-second-order kinetics. Langmuir and Freundlich adsorption isotherms gave high correlation coefficients and agreed well with the conditions of favorable adsorption. Among the four preparation methods for the guyabano biosorbent, air drying method is the most feasible. Thus, guyabano leaf powder can be a low-cost biosorbent for the remediation of Pb, Ni, and Zn.
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