Extractability of nitrites from polluted waters using bio-sorbents derived from leaves, barks or stems of some herbal plants

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ABSTRACT

Thermally activated powders of leaves, stems or barks and their ashes of Calotropis Zygantia, Azadiracta Indica and Eichhornia, are found to have affinity towards nitrites at low pH values. The sorption properties of these sorbents have been explored for extracting nitrites from polluted waters by optimizing the physicochemical parameters such as pH, time of equilibration and sorption concentration. The sorption concentration needed for the maximum extraction of nitrite at optimum pH: 2 is less for bark powders and ashes than for leaves powders in the case of Calotropis Zygantia and Azadiracta indica but in the case of bio-materials derived from Eichhornia, the optimum sorption concentration remains the same. The optimum time of equilibration is found to be less with powders of stems or ashes of leaves than with raw powders of leaves for Azadiracta Indica and Eichhornia but in the case of materials related to Calotropis Zygantia, the time remains the same. More than 80% removal of Nitrite is noted with bio-sorbents at optimum experimental conditions. Five fold excess of common ions present in natural waters has less effect on the % of extraction of Nitrite at optimum conditions of extraction. The methodologies developed are tested with the real polluted water samples and are found to be remarkably successful.

Key words: Nitrites; pollution control; bio-adsorbents

INTRODUCTION

Nitrite ion is a potential pollutant found commonly in septic environment in water bodies and is the resultant of incomplete oxidation of nitrogenous matter in waste water [1, 2]. The content of the nitrite in waters is the testimony of the extent and stage of contamination of waters.

The use of agricultural fertilizers and various industrial effluents are the main source of nitrite contamination [1-4]. Nitrite and its salts find many industrial, commercial applications and one of its significant uses is that it is being used as food additive in preserving meat, poultry and fish. The effluents from these have toxic concentrations of nitrites.

Nitrite is highly toxic and is detrimental to the fish. The disease, Methemoglobinemia is a resultant of Nitrites and there is convincing evidence that nitrite causes cancer mortality [2-8]. The maximum limit of allowed concentration of nitrite in drinking water is 0.05 mg L-1 [4, 9]. China in 2005 passed most stringent laws on nitrite pollution to counter the nitrite prone cancer mortality and reduced the permissible limit to 0.005 to 0.002 ppm in bottled water and set a limit of 0.02 ppm for ground water recharge. Russia, Japan and Taiwan also have imposed strict nitrite pollution levels in view of cancer prone epidemiological findings.

The use of agricultural waste products as bio-sorbents for the removal of polluting ions offer a potential alternative to the existing methods of detoxification and recovery of toxic and valuable ions in polluted waters [24-32]. These biological approaches in the recent past have stimulated continuous and expanding research in this field [33-42]. V. Parimal et. al (2007) [43] explored the use of low cost agricultural wastes as sorbents for the control of nitrites.

In the present work an attempt is made to explore the use of leaf, stems or bark powders of some herbal plants in controlling the nitrite concentrations in polluted waters.

**EXPERIMENTAL SECTION**

*Chemicals and solutions:* All Chemicals used were of analytical grade.

- **Stock Solutions of Nitrite:** 500 ppm stock was prepared by dissolving suitable amount of A.R. grade Sodium Nitrite in a suitable volume of double distilled water and it is diluted as per the need.
- **Sulphanilic acid reagent:** 0.6 g of A.R. Sulphanilic acid was dissolved in 100 ml of 20% v/v hydrochloric acid.
- **α-Napthylamine reagent:** 0.48 g of A.R. α-naphthylamine was dissolved in 100 ml of 1.3 % v/v hydrochloric acid.
- **Sodium acetate, 2 M:** 16.4 g of anhydrous sodium acetate was dissolved in 100 ml of distilled water.

*Adsorbents:* Thermally activated powders of leaves, stems or barks of many plants have been studied in this work. It is found that leaves, stems or barks and their ashes of Calotropis Zyzantia, Azadiracta Indica and Eichhornia, have been found to have affinity towards nitrites and hence the methodology presented here, pertains to these sorbents only.

**Fig. No. 1: Plants showing affinity towards Nitrate**

*Calotropis gigantean* is a species of Calotropis, native to South Asian countries and it belongs to Apocynaceae family. It is a large shrub growing up to 3-4 meters tall and its wound healing ability is reported in literature [44]. *Azadiracta indica,* or Neem Tree, is an evergreen tree native to Southeast Asia and it belongs to Meliaceae family. *Eichhornia* is a free-floating perennial aquatic plant native to tropical and sub-tropical countries and it belongs to Pontederiaceae family.
**Sorbent Preparation:** The leaves or stems or barks of *Calotropis Zygantia, Azadiracta Indica* and *Eichhornia* were cut or scrapped freshly, washed with tap water, then with distilled water and then sun dried. The dried materials were powdered to a fine mesh of size < 75 µ and activated at 105°C for 4 hrs in an oven and then these materials were employed in this work.

c) **Adsorption Experiment:**
The Batch system of extraction procedure was adopted [26, 27, 45]. Carefully weighed quantities of adsorbents were taken into previously washed 1 lit/500 ml stopper bottles containing 500 ml /250 ml of nitrite solutions of predetermined concentrations. The various initial pH values of the suspensions were adjusted with dil. HCl or dil. NaOH solution using pH meter. The samples were shaken in Mechanical shakers for a desired period and after the equilibration period, an aliquot of the sample was taken, filtered and the nitrite content in it is determined spectrophotometrically [46].

**Estimation of Nitrites:**
A measured quantity of the nitrite sample was taken into a 50 ml volumetric flask. To it 1 ml of Sulphanilic acid reagent solution was added, mixed well and was allowed to stand at least 3 min and not more than 10 min at room temp in diffused light. Then 1 ml of the α-Naphthylamine solution and 1 ml of 2M-Sodium acetate solution to act as buffer (pH: 2.0-2.5) were added. The solution then diluted to 50 ml and mixed well. After 10 min, but not later than 30 min, the Optical Density of the reddish-purple color developed was measured at 520 nm against blank using U.V and visible spectrophotometer (Systronics make). Previously, a standard graph was constructed between O.D. and known concentrations of nitrite, in conformation of Beer’s law adopting Least Square Method for linear graphs. The measured O.D. values for the unknown solutions were referred to standard graph to determine the concentration of nitrite in un-known samples.

d) **Effect of Interfering ions:**
The interfering ions chosen for study are the common ions present in natural waters, viz., phosphate, sulphate, fluoride, chloride, carbonate, calcium, magnesium, copper and zinc. The synthetic mixtures of nitrite and one of the interfering ions were so made that the concentration of the interfering ions maintained at five fold excess than the nitrite ion concentration. 500 ml of these solutions were taken in stopped bottles and then correctly weighed optimum quantities of the promising sorbents were added. Optimum pH was adjusted with dil. HCl or dil. NaOH using pH meter. The samples were shaken in shaking machines for desired periods and then the samples were filtered and analyzed for nitrites. % of extraction was calculated from the data obtained. The results are presented in the Table No. 1.

e) **Applications of the developed bio-sorbents:**
The effectiveness of the procedures developed in this work with bio-sorbents of interest, have been tested by applying to samples collected from five lakes polluted with nitrite at different places in Bapatla mandalam of Guntur District of Andhra Pradesh. Initially the actual concentration of nitrite present in these samples were analyzed and then these samples were subjected to the extraction for nitrites using the bio-sorbents developed in this work at optimum conditions of pH, equilibration time and sorbent concentration as has been cited in the Table No. 2. The results obtained were presented in the Table No. 2.

**RESULTS AND DISCUSSION**
Of the various bio-adsorbents tried, the adsorbents showing affinity towards Nitrites are presented in the Graph: Nos: A: 1-3; B: 1&2; C: 1&2 and Table No.:1&2. The salient points of our observation are as follows:

1. Nitrite ions show varied affinity towards the powders of leaves, stems and ashes of leaves of *Calotropis Zygantia, Azadiracta Indica* and *Eichhornia*.

2. The general observation is that with these sorbents, the % of extractability of Nitrite increases with time and decreases with the increase of pH (vide Graph Nos. : A: 1-3; B: 1&2 ) for a fixed adsorbent concentration.

3. With the increasing pH, extractability of Nitrite decreases for a fixed adsorbent concentration (vide Graph Nos: B: 1 &2). As for example, in the case of leaves powder of *Calotropis Zygantia*, the maximum extractability is found to be: 42.3% at pH: 10; 54.5% at pH: 8; 59.2% at pH: 6; 68.2% at pH: 4; and 80% at pH: 2. *Azadiracta Indica* leaves
powder is found to extract nitrite to an extent of: 22.4% at pH:10, 34.5% at pH:8; 38.9% at pH:6; 50.6% at pH:4 and 78% at pH:2. Similar trend is found in the case of leaves powders of Eichhornia.

Same trend is found in the case of stem powders as well as with ashes of leaves of the said plants. With the stem powders of Calotropis Zygantia, the maximum extractability is found to be : 48.5% at pH:10, 60.8% at pH:8; 67.3% at pH:6; 78.2% at pH:4 and 90% at pH:2. In the case of Azadiracta Indica stem powders, the maximum extractability is found to be 19.5% at pH:10; 44.2% at pH: 8; 53.5% at pH: 6; 90.8% at pH: 4 and 100% at pH: 2. Eichhornia stem powder is found to extract 40.6% at pH: 10; 51.5% at pH: 8; 62.4% at pH: 6; 78.5% at pH:4 and 86.0% at pH:2.

With ashes of Calotropis Zygantia leaves, the maximum extractability is found to be: 44.6% at pH: 10, 59.2% at pH: 8; 64.3% at pH: 6; 68.7% at pH: 4 and 88.0% at pH:2. And with the ashes of leaves of Azadiracta Indica the
maximum extractability is found to be 18.5% at pH: 10; 39.9% at pH: 8; 47.8% at pH: 6; 79.8% at pH: 4 and 100% at pH: 2. Eichhornia leaves ash is found to extract 39.2% at pH: 10; 52.3% at pH: 8; 60.4% at pH: 6; 85.0% at pH: 2.

4. When percentage removal is studied with respect to the adsorbent dosage at fixed optimum pH: 2 and at optimum equilibration times, the graphs increase up to certain dosage and from then onwards plateaus are obtained (vide Graph Nos: C:1 &2). Further, the optimum dosage needed is found to be in the order of leaves powders > stem powders = ashes of leaves for Calotropis Zygantia and Azadiracta indica. As for example, in the case of Calotropis Zygantia, the sorbent concentration needed for maximum extraction is found to be 4gm/lit for leaves powder, 2 grms/lit for its stem powder and leaves ashes. With Azadiracta indica, 4.0 gms/lit is needed for leaves powder, 1 gm/lit is needed for stem and leaves ashes. All the materials derived from Eichhornia have been found that a
minimum sorbent concentration of 4.5 gm/lit is needed for maximum possible extraction of nitrites from polluted waters (Vide Graph No. C 1&2).

5. Time of equilibration for maximum possible removal of nitrite is found to be less in the case of powders of stems or ashes of leaves than with raw powders of leaves for Azadiracta Indica and Eichhornia (vide Graph No. A: 2 & 3). In the case of all the plant materials derived from Calotropis Zygantia, 6 hrs of equilibration time is essential (Vide Graph No. A: 1).

6. The maximum extractability is found to be 80.0%, 90.0% and 88.0% respectively with the raw powders of leaves, powders of stems and ashes of leaves of Calotropis Zygantia at pH:2 and after an equilibration period of 6 hrs and at optimum sorbent concentrations. 100% of extraction is observed with all plant materials derived from Azadiracta indica at pH: 2 but with varying equilibration times; 6.0 hrs for powders of leaves while 5 hrs for stem or ashes of leaves. 80.0% and 86.0% of extraction of nitrite have been found with the leaves and stem powders of Eichhornia at
pH: 2 and after an equilibration time of 7 hrs while 85.0% has been observed with ashes of leaves of Eichhornia at the same pH but at a reduced equilibration time of 6 hrs.

7. **Interfering Ions**: The extractability of Nitrite ions in presence of five fold excess of common ions found in natural waters, namely, Chloride, Fluoride, Carbonate, Sulphate, Phosphate, Calcium, Magnesium, Copper and Zinc ions, has been studied. These ions show less effect on the extractability of nitrite ions at the optimum conditions of pH, equilibration time and sorbent dosage (Vide Table No. 1).

The remarkable affinity of the sorbents developed in this research work towards nitrite may be due to the naturally existing compounds in the leaves, stems or barks and further probe into this aspect of research is beyond the scope.
of this work. Moreover, these lingo celluloses’ materials have -OH/COOH groups and their dissociation is pH dependent. At high pH values, these groups dissociates as:

\[
\text{Adsorbent-OH} \quad \rightleftharpoons \quad \text{Adsorbent-O}^+ + \text{H}^+
\]

\[
\text{Adsorbent-COOH} \quad \rightleftharpoons \quad \text{Adsorbent-COO}^- + \text{H}^+
\]

and imparts weak cation exchange ability and at low pH values, the dissociation is less favored and protination may also occur as

\[
\text{Adsorbent-OH} + \text{H}^+ \quad \rightleftharpoons \quad \text{Adsorbent-O}^+ \cdot \text{H}_2
\]

and thus resulting weak anion-exchange ability. This is reflected in sorption of nitrite. Nitrite being an anion is adsorbed by these materials at low pH values and hence the high % of removal. At high pH values cation affinity persists and not anion and hence, nitrite anion shows low % of removal.

The decrease in the rate of sorption with the progress in the equilibration time may be due to the more availability of sorption sites initially and are progressively used up with time due to the formation of adsorbate, nitrite, film on the sites of the sorbent and thus resulting in decrease in sorption ability of the adsorbents.

APPLICATIONS
Sample waters at five lakes polluted with nitrite ions, were collected and the successful sorbent developed in this work were tried for the removal of nitrite ions at optimum conditions of extraction. It was found that the sorbents developed in this work were successful in removing nitrites considerably in these samples of water at optimum conditions of pH, equilibration time and sorbent dosage. % removal of nitrite in these samples of water was found to be: 74.9 to 78.5% with leaves powder of Calotropis Zygantia; 84.9% to 88.1% with stem powder of Calotropis Zygantia; 83.1% to 86.4% with ashes of leaves of Calotropis Zygantia. In the case of sorbents derived from Azadiracta Indica, extractability of nitrite was found to be: 93.2% to 96.9% for leaves powder, 90.8% to 93.2% for stem powder and 91.0% to 96.5% for ashes of leaves. With the activated biomaterials of Eichhornia, the % of removal of Nitrite was found to be: 74.2% to 78.0% for leaves powders, 80.2% to 83.3% for stem powders and 79.5% to 83.1% with ashes of leaves (vide Table No.2)
Nitrite conc.: 50 ppm
Equilibration time: 6.0 hrs

% removal of Nitrite

pH

pH Vs % removal of Nitrite
Graph No.B:1

Leaves powder of Calotropis gigantea: 1 gm/lit; 6.0 hrs
Leaves powder of Acaulis indica: 1 gm/lit; 6.0 hrs
Ash of Calotropis gigantea leaves: 2 gm/lit; 6.0 hrs
Ash of Acaulis indica indica: 2 gm/lit; 6.0 hrs

Nitrite conc.: 50 ppm

% removal of Nitrite

pH

pH Vs % removal of Nitrite
Graph No.B:2

Leaves powder of Tylocreia: 4.0 gm/lit; 7.0 hrs
Stems powder of Tylocreia: 4.0 gm/lit; 6.0 hrs
Ash of leaves of Tylocreia: 4.0 gm/lit; 6.0 hrs

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Table No. 1: Effect of interfering Ions on the Extractability of NITRITE with different Bio-sorbents

<table>
<thead>
<tr>
<th>S.No</th>
<th>Adsorbent and its concentration</th>
<th>Maximum Extractability at optimum conditions</th>
<th>Extractability of Nitrite in presence of fivefold excess of (250 ppm) interfering ions at optimum conditions: Conc of Nitrite: 50 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder of Calotropis Zygantia leaves: 4.0 gms/lit</td>
<td>80.0%; pH:2, 6.0 hrs</td>
<td>SO$_4^{2-}$  Phosphates  Cl$^-$  CO$_3^{2-}$  F$^-$  Ca$^{2+}$  Cu$^{2+}$  Zn$^{2+}$  Mg$^{2+}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77.3%  75.2%  76.5%  73.5%  74.6%  78.2%  77.3%  76.9%  75.8%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Powder of Calotropis Zygantia stems: 1.5 gms/lit</td>
<td>90.0%; pH:2, 6.0 hrs</td>
<td>86.4%  83.5%  84.3%  85.4%  88.6%  87.9%  86.3%  85.8%</td>
</tr>
<tr>
<td>3</td>
<td>Ash of leaves of Calotropis Zygantia: 2.0 gms/lit</td>
<td>88.0%; pH:2, 6.0 hrs</td>
<td>86.1%  85.1%  84.1%  82.6%  83.6%  86.0%  83.1%  82.2%  84.0%</td>
</tr>
<tr>
<td>4</td>
<td>Powder of Azadiracta Indica leaves: 4.0 gms/lit</td>
<td>100.0%; pH:2, 6.0 hrs</td>
<td>96.4%  95.4%  94.6%  96.0%  94.5%  97.1%  98.3%  95.7%  97.8%</td>
</tr>
<tr>
<td>5</td>
<td>Powder of stems of Azadiracta Indica: 1.0 gms/lit</td>
<td>100.0%; pH:2, 5.0 hrs</td>
<td>97.8%  95.2%  94.4%  92.5%  93.0%  92.3%  94.8%  96.4%</td>
</tr>
<tr>
<td>6</td>
<td>Ash of leaves of Azadiracta Indica: 1.0 gms/lit</td>
<td>100.0%; pH:2, 5.0 hrs</td>
<td>94.8%  96.6%  93.8%  91.6%  92.0%  95.4%  92.4%  94.6%  96.8%</td>
</tr>
<tr>
<td>7</td>
<td>Powder of leaves of Eichhornia: 4.0 gms/lit</td>
<td>80.0%; pH:2, 7.0 hrs</td>
<td>75.3%  76.4%  77.9%  74.6%  73.1%  75.4%  77.3%  76.5%  78.2%</td>
</tr>
<tr>
<td>8</td>
<td>Powder of stems of Eichhornia: 4.0 gms/lit</td>
<td>86.0%; pH:2, 6.0 hrs</td>
<td>82.5%  80.9%  79.3%  82.3%  83.4%  80.2%  81.3%  82.8%  80.4%</td>
</tr>
<tr>
<td>9</td>
<td>Ash of leaves of Eichhornia: 4 gms/lit</td>
<td>85.0%; pH:2, 6 hrs</td>
<td>81.5%  79.9%  78.3%  76.3%  80.4%  81.2%  82.3%  80.8%  82.4%</td>
</tr>
</tbody>
</table>
Table No.2: % of Extractability Of Nitrites In Polluted Water Samples

<table>
<thead>
<tr>
<th>Bio-Sorbent</th>
<th>Sample 1: Found to have 5 ppm of nitrite</th>
<th>Sample 2: Found to have 6.9 ppm of nitrite</th>
<th>Sample 3: Found to have 7.2 ppm of nitrite</th>
<th>Sample 4: Found to have 8.1 ppm of nitrite</th>
<th>Sample 5: Found to have 6.5 ppm of nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves powder of Calotropis Zygantia</td>
<td>76.0%</td>
<td>77.2%</td>
<td>75.1%</td>
<td>74.9%</td>
<td>78.5%</td>
</tr>
<tr>
<td>at pH:2, Equilibration time: 6 hrs and sorbent concentration: 4.0 gms/lit</td>
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<td></td>
</tr>
<tr>
<td>Stems powder of Calotropis Zygantia</td>
<td>85.3%</td>
<td>86.1%</td>
<td>87.6%</td>
<td>84.9%</td>
<td>88.1%</td>
</tr>
<tr>
<td>at pH:2, Equilibration time: 6 hrs and sorbent concentration: 1.5 gms/lit</td>
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<td></td>
</tr>
<tr>
<td>Ashes of Leaves of Calotropis Zygantia</td>
<td>86.2%</td>
<td>83.1%</td>
<td>85.8%</td>
<td>84.5%</td>
<td>86.4%</td>
</tr>
<tr>
<td>at pH:2, Equilibration time: 6 hrs and sorbent concentration: 2.0 gms/lit</td>
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<td></td>
</tr>
<tr>
<td>Leaves powder of Azadiracta Indica</td>
<td>95.5%</td>
<td>96.9%</td>
<td>94.2%</td>
<td>93.2%</td>
<td>96.0%</td>
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<td>93.2%</td>
<td>92.8%</td>
<td>91.0%</td>
<td>90.8%</td>
<td>92.8%</td>
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<tr>
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</tr>
<tr>
<td>Ashes of leaves of Azadiracta Indica</td>
<td>96.2%</td>
<td>96.5%</td>
<td>94.2%</td>
<td>93.8%</td>
<td>91.0%</td>
</tr>
<tr>
<td>at pH:2, Equilibration time: 5 hrs and sorbent concentration: 1.0 gms/lit</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leaves powder of Eichhornia</td>
<td>76.1%</td>
<td>78.0%</td>
<td>76.0%</td>
<td>75.8%</td>
<td>74.2%</td>
</tr>
<tr>
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<tr>
<td>Stems powder of Eichhornia</td>
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<td>82.2%</td>
<td>81.1%</td>
<td>82.1%</td>
<td>80.2%</td>
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<tr>
<td>Ashes of leaves of Eichhornia</td>
<td>80.0%</td>
<td>82.2%</td>
<td>83.1%</td>
<td>81.7%</td>
<td>79.5%</td>
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CONCLUSION

1. Thermally activated biomaterials derived from *Calotropis Zygantia, Azadiracta Indica* and *Eichhornia*, have been found to have strong affinity towards nitrites at low pH values.
2. % of removal of nitrite is pH sensitive and also depends on sorption concentration and time of equilibration
3. The minimum dosage needed for the maximum removal of nitrite is found to be in the order of leaves powders > stem powders = ashes of leaves for Calotropis Zygantia and Azadiracta indica.

4. Time of equilibration is found to be less with powders of stems or ashes of leaves than with raw powders of leaves for Azadiracta Indica and Eicchorrnia but with all biomaterials derived from Calotropis Zygantia, the same optimum time of equilibration i.e. 6 hrs, is found to be essential.

5. Five fold excess of common ions present in natural waters, viz., Cl\(^{-}\), F\(^{-}\), Sulphate, Carbonate, Ca\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) has less affected the % of extraction of Nitrite at optimum conditions of pH, equilibration time and sorbent concentration.

6. We claim that more than 80.0% and in some cases even 100%, removal of nitrite is possible with the sorbents developed in this work at optimum conditions as has been presented in the Table 1 with the synthetic water mixtures.

7. The suitability of the developed sorbents are tested with respect to five waste water samples collected from polluted waters of five lakes in different places in Bapatla Mandalam of Guntur District of Andhra Pradesh. The procedures are found to be remarkably successful in the removal of considerable amounts of nitrites.

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