Presentation

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Characterization and cation exchange capacity of seeds of *Ziziphus spina-christi*

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**Abstract:** There are several naturally existing materials have ability to utilize as ion-exchangers. Most of these materials are by-products of waste material from industry or agriculture. Agriculture ion exchange materials include: lemon orange, grapefruit, apple, peas, broad bean, and meddler peels, kernel core, and grape skins. This research deals with the utilization of agriculture waste biomass of napak seed as natural cation exchanger for removal of cationic pollutant from aqueous solution. Methylene blue dye method was used to determine the cation exchange capacity of the stone and it was characterized by IR and TGA methods. The results showed that the highest dye sorption capacity was found at pH 7, the equilibrium time was 60 min, sorbent dose = 0.1g, particle size 177µm and methylene blue concentration range 10-50 ppm. The equilibrium sorption data were analyzed by Langmuir and Freundlich isotherm models.

**Keywords:** cation exchange capacity; *Ziziphus spina-christi*; seeds; methylene blue dye

I. Introduction

Ion exchange materials are classified into synthetic and natural ion exchange materials. Synthetic ion-exchange resins are the main materials used and prepared from organic polymers. The most common examples of these organic polymers include cross-linked polystyrene attached to certain ionizable groups. Natural materials such as clays and zeolites have ion-exchange capability. There is also several other naturally existing materials have been studied by various researchers to determine their ability to utilize as ion-exchangers. Most of these materials are by-products of waste material from industry or agriculture. Agriculture ion exchange materials include: lemon orange, grapefruit, apple, peas, broad bean, and meddler peels, and kernel core [1], coconut shell powder [3] and coconut copra meal [4] and olive stones [5]. Agriculture wastes have been investigated as eco-friendly, low cost and renewable adsorbents for waste water treatment [6]. Agricultural by-products have unique chemical compositions that cause them to be more efficient and feasible option for pollutant removal. These by-products are mainly composed of lignocellulosic materials that consist of three main structural components, which are lignin, cellulose, and hemicelluloses [7]. Also Agriculture wastes, contain lipids, proteins, simple sugars, water, hydrocarbons, and starch [8]. Other polar
functional groups of lignin may be also included, such as alcohols, aldehydes, ketones, carboxylic, phenolic, and other groups [4]. Sidr plant (Ziziphus spina-christi, family Rhamnaceae) is a plant that grows in Saudi Arabia since it prefer coastal, desert, and semi-desert areas. The fruit of this tree is called Nabak. The seeds of Ziziphus spina-christi contains 4.46 % of Moisture, 1.55 % Ash, 7.18 % protein, and 31.8 % fiber, as it contains also a small percentage of fat around 4.25 % and moderate amount of carbohydrates 54.86 % include sucrose, fructose and glucose [9].

The aim of this work is to study the surface characterization of nabak seed (NS) and feasibility of using as natural cation exchange resin. Cation exchange capacity (CEC) is used to described the capacity of a sorbent to exchange cations where negative charge of material is balanced with an index - cation. Then CEC can be determined by measuring the difference between the initial and the remaining content of the index-cation [10]. Cation exchange capacity can be determined by several methods. These method include exchange of the cations by using different types of cations such as methylene blue, K(I), Na(I), Ba(II), ammonium cation, Co(III) hexamine complex, Ag(I) thiourea complex and Cu(II) ethylenediamine complex. Also there is indirect way to determine the cation exchange capacity of the exchanger by exchange with organic cations such as alkyl ammonium [11]. Methylene blue dye is a cationic dye that becomes popular for determining cation exchange capacity due to its easily application, no need special equipment, and it gives accurate results [12].

II. Experimental Section
II.1 Apparatus:

A grinding machine, pH meter (eco Testr pH ), a shaker device (GFL), UV-VIS Double Beam Spectrophotometer (GBC cintra 6), TGA device (SDT 600), IR spectrometer (FTIR-84005 SHIMADZU), sensitive balance (Mettler Toledo), centrifuge device (heraeus sepatech labofuge 200).

II.2. Materials:

Methylene Blue (MB) was purchased from BDH chemicals Ltd pool England. The chemical formula and molecular weights for MB 41 are C16 H18 N3 S Cl, 319.85 g/mol and the chemical structure of MB is shown in Fig 1.

![Figure 1: Chemical structure of Methylene Blue](image)

The maximum wave length $\lambda_{\text{max}}$ of methylene blue dye is 665. Stock solutions MB dye, NaOH and HCl were prepared by dissolving appropriate weight in distilled water and the experimental solutions of the desired concentration were obtained by dilution. The pH of solutions was adjusted with diluted NaOH and HCl using pH meter.

II.3. Preparation of Napak Seeds:

Napak seeds (NS) were collected from Al Madinah Al Monawarah and washed by distilled water to remove dust, water solubl material and other impurities. They were washed and dried for 2 day in the air then grinded by domestic grinder and sieved to obtain particle size 177, 250, 841, 2000 microns then stored in glass bottle in a desiccator for further use.
II.4. Batch sorption experiments

The pH of basic dyes (MB) solutions was adjusted from 4 to 9 by using 0.05 M HCl or NaOH solution. 50 ml of MB dye solution of 20 mg L\(^{-1}\) were shaken with 0.1 g of sorbent (NS) for equilibrium time then the adsorption mixture was centrifuged to separate the seeds from the solutions and the filtrates are analyzed for determine the remain concentration of dyes by UV-Visible In order to characterize the other optimum sorption conditions, experiments were performed varying the following experimental parameters: biosorbent dosage (0.1-0.5 g), particle size (177, 250, 841 and 2000 microns), initial dye concentration (10–50 mg L\(^{-1}\)) and time range (15–180 min).

III. Results and Discussion

III.1. Surface Characterization

III.1.1. IR analysis

IR analysis of NS was done to predict the functional groups that found in it. FTIR spectra for sorbent (Figure 2) show a number of absorption peaks, suggesting complex properties of the NS. The dominant peak at 3360 cm\(^{-1}\) in NS is attributed to O–H or N–H stretching vibrations [13-15]. O–H group may be due to alcohol, phenol and carboxyl of lignin and cellulose and N–H group may be due to amide groups. The peak at 1738 cm\(^{-1}\) is assigned to carbonyl C=O. The carbonyl groups present in protein and the acetyl ester groups of hemicelluloses [14, 16, 17]. The peaks at 1506 cm\(^{-1}\) and 1419 cm\(^{-1}\), may be ascribed to C=C stretching of aromatic skeletal mode [26]. These peaks may also attribute to –C=O of amide group [10]. The peak at 1371 cm\(^{-1}\) are due to C–H bending vibrations. The band at 1234 cm\(^{-1}\) is due to the bending modes of O–C–H, C–C–H and C–O–H [14]. The peaks in the region 1200–950 cm\(^{-1}\) may represent C–O stretching vibrations. The absorption at 897 cm\(^{-1}\) for sidr stones are related to C–H rocking vibrations of cellulose. Consequently, the FTIR results indicate that the NS have different functional groups such as hydroxyl, carboxyl and carbonyl, and amid which may be potential biosorption sites for MB dye [14].

![Figure 2: IR analysis of raw Napak Seeds](image)

III.1.2. Thermogravemetric analysis of Napak Seeds

Figure 3 shows the TGA and derivative thermogravimetric analysis (DTG) curves of NS. The experiments are performed under nitrogen atmosphere and heating rate of 10 °C.min\(^{-1}\). NS sample exhibited three mass looses. The first one of 5% was related to the loss of water molecules in the range of RT to 100 °C. The second mass loss in the range of 200 to 370 °C, was associated to the release of chemical bonded water, lignocellulosic materials (hemicellulose, cellulose) and proteins, and it reached about 60 %. The third mass loss of 35 % occurred at higher temperatures above 400°C, and associated to the decomposition of remaining cellulose and the lignin. Lignin peak is broad and low intensity. The mass loss which occurred in three steps, at temperature range from 200°C to 370°C, shows a maximum at 317 °C [16-19].

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III.2. Cationic Dyes Uptake Capacities:

A batch mode study was carried out to evaluate the uptake capacity of a cationic dye (Methylene Blue MB) from aqueous solution. Factors that affect the biosorption process such as operating parameters such as pH, sorbent dosage, sorbent size, time and initial dyes concentration were evaluated. The uptake capacity at equilibrium \( q_e, \text{ mg/g} \) was measured as follows:

\[
q_e = \frac{(C_0 - C_e) \times V}{m}
\]  

(1)

\( C_0, \) \( C_e \) and \( m \) are the initial concentration, equilibrium concentration and mass of NS, respectively

III.2.1. Effect of pH on uptake capacity of MB by NS.

The pH factor is very important parameter on dye uptake by biosorbent. The pH influences on the surface charge of the sorbent, the dissociation of functional groups on the binding sites, the degree of ionization of the different pollutants and the structure of the dye molecule. The method of sorbate - sorbent interaction depends on the pH of an aqueous medium by two ways:

1. The organic functional groups and unsaturated bonds of dyes alter their ionization potentials at different pH.
2. There are various functional groups on biosorbent surface, so the net charge on biosorbent is also pH dependent [20, 21].

In this study, the initial concentration (20 ppm) of MB was shaken with certain amount of sidr stones (0.1g) size 250 microns at different pH value (4 – 9) for one hour. The results were depicted in Figure 4.

Figure 3: Thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) curves of Napak Seeds.
It was shown that uptake capacity of MB on sidr stones increased slightly with increasing pH and reached a maximum level at the pH of 7.0, and uptake capacity did not change significantly up to pH 9. At low solution pH, the functional groups, on sidr stones are protonated so, the sorbents became more positively charged and the excess of H⁺ ions would compete with cationic dye molecules for the sorption sites. The sorption capacity of the sidr stones decreased slightly at low solution pH and the deprotonated negatively charged surface of sorbents attract more cationic dye molecules. Therefore, the sorption capacity of the sorbents increased at higher pH value [21, 22].

III.2.2 Effect of contact time on sorption capacity of Napak Seeds

Batch tests were done at different contact time and initial concentration with fixed pH (pH = 7) and (0.1g) sorbent dose. The results of sorption capacity are given in Figure 5.

From the results, the sorption capacity of dye increases with increase of contact time. The capacity was found to be rapid at the initial period and then becomes slow with increase in contact time. At low concentration of MB dye (10 ppm) the equilibrium time is 60 min and at high concentration (20 – 30 ppm) the equilibrium time is 90 min (Figure 5). The equilibrium time increases with increasing concentration of dye because at lower dye concentrations, the available sites in biosorbent are high thus, the dye species can easily find biosorbent sites. Reversibly at higher concentrations the available sites of biosorbent become fewer so it takes more time [23].

III.2.3 Effect of particle size of Napak Seeds:

![Figure 4: Effect of pH on uptake capacity of MB by Napak Seeds](image)

![Figure 5: Effect of contact time uptake capacity of MB by Napak Seeds](image)
Influence of particle size on dye sorption was investing in this study by using four different sizes: 2000, 841, 250 and 177 µm. The initial dye concentration (10 ppm) was shaken with 0.1g of each size from Napak Seeds until the equilibrium time. The results are shown in Figure 6.

From the previous figure 6, the MB dye uptake capacity increase with decreasing particle size of Napak Seeds. This result is due to the surface activity and the surface area of the sorbent. The small particles have large surface area and move faster in solution than large particles so in large particles the diffusion resistance to mass transport is higher and most of the internal surface isn’t used for sorption thus lead to decrease capacity [21, 24].

**III.2.4. Effect of Sorbent Dose on the sorption capacity**

The biosorption of Methylene Blue onto NS was studied by varying the sorbent quantity (0.1, 0.2, 0.3, 0.5 g) in the test solution while keep the initial concentration, pH, contact time constant. Figure 7 shows the plot of sorption capacity (mg/g) against sorbent dose (g). As we observed in Figure 7, the dye uptake capacity decreases with increase of the sorption dose. Thus, the smallest quantity of biosorbent (0.1 g) provided optimum sorption capacities, this may be due to overlapping of sorption sites as a result of overcrowding of sorbent particles [25, 26]. Moreover the sorption capacity (the amount of adsorbed per unit mass ) decreases due to the splitting effect of flux (concentration gradient) between adsorbates that lead to a decrease in amount of dye adsorbed onto unit weight of biomass[27].

**III.2.5. Effect of Initial Concentration and Sorption Isotherms**

The sorption was carried out by taking various concentrations of dyes (5-50ppm) and constant amount of NS (0.1 g) and shaken for the equilibrium time. Sorption isotherms provide fundamental
physicochemical data for evaluating the applicability of sorption process. Langmuir and Freundlich isotherms are used to analyze the experimental data. Langmuir's model is based on the monolayer coverage of the adsorbate at specific homogeneous sites of the outer surface of adsorbent [28]. The Langmuir isotherm can be represented by:

\[
q_e = \frac{q_m KL C_e}{1 + KL C_e}
\]  

The linear form of Langmuir equation is

\[
\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m KL}
\]

The Freundlich isotherm gives information about the heterogeneity of the surface, distribution of the sites and their energies as well as multilayer sorption. Freundlich equation and linear form of Freundlich equation are given by the following equations:

\[
q_e = K F C_e^{1/n}
\]

\[
\ln q_e = \ln K + \frac{1}{n} \ln C_e
\]

Where the value of \( n \) can be used also to describe the adsorption. So if the \( n=1 \), the adsorption is linear while if \( n <1 \), it implies that the adsorption process is favoured by chemisorption and if \( n >1 \), the adsorption process is favoured by physisorption [27].

Langmuir isotherm is obtained by plotting the relationship between \( C_e \) against \( C_e/q_e \). While Freundlich isotherm is given from the relation between \( \ln C_e \) against \( \ln q_e \) in Figure 8. The data from these relationships is used to calculate maximum sorption capacity \( q_m \), \( R^2 \) for Langmuir isotherm and \( k_f \), \( n \) and \( R^2 \) for Freundlich these data are shown in table 1.

![Figure 8: Langmuir isotherm for sorption of MB onto NS.](image-url)
Figure 9: Freundlich isotherm for sorption of MB onto NS

Table 1: Isotherm constants for the sorption MB onto NS

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<th>Freundlich parameters</th>
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<td>$q_m$ (mg/g)</td>
<td>$K_L$ R$^2$ $K_f$ n</td>
</tr>
<tr>
<td>23.25</td>
<td>0.06 0.975 1.4</td>
</tr>
<tr>
<td></td>
<td>1.2 0.8 0.99</td>
</tr>
</tbody>
</table>

From the previous isotherms all $R^2$ values for Langmuir and Freundlich were greater than 0.95 indicating almost equal applicability of the two types of sorption isotherm. The maximum MB dye sorption capacity of NS was found 23.25 mg/g. In addition, it can be seen that the numerical value of $n$ is 1.2, it indicates MB is favorably by physisosorption onto NS [30].

IV. Conclusion

This study shows that Nabak stone, an agricultural waste, can be used as natural cation exchanger for removal of cationic pollutant. Sorption capacity MB by NS is affected from several parameters like pH, sorbent dose, sorbent grain size, and initial dye concentration. Both Langmuir and Freundlich isotherm are applicable.

It is therefore recommended in the future to use these stones in the removal of positive ions from aqueous solutions.

V. References


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Validation of Traditional Therapeutic Claims through Phytochemical Screening and Antibacterial Assessment: A Study on Mahakaal (Trichosanthes tricuspidata L.) From Similipal Biosphere Reserve Forest, Odisha, India

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Abstract: Similipal Biosphere Reserve forest is situated in the district Mayurbhanj, Odisha, enriched with the different types of vegetations along with aboriginals. These aboriginals have unique skills in using traditional therapeutic medicines. They use wild plant and their parts in traditional herbal formulations to cure different diseases. Trichosanthes tricuspidata, locally known as Mahakaal is very common to be used as herbal medicine. Fruits of Mahakaal have sound traditional therapeutic values, they have been used against asthma, skin infections, muscular pain and killing the head lice. Phytochemical screening of fruit extracts revealed the presence of major bioactive compounds such as Tannin, Saponin, Flavonoids, Phenolic compounds, Terpenoids etc which indicate its sound pharmacological properties. Antibacterial assessment of fruit extracts also showed excellent activity against two Gram-positive and three Gram-negative bacteria. Methanol extracts showed highest zone of inhibition (1.51 cm) against Streptococcus pyogenes caused skin infections. The experimental works validate the traditional therapeutic claims.

Keywords: Similipal Biosphere Reserve, Mahakaal, Traditional therapeutic values, bioactive compounds, antibacterial activity, validation

I. INTRODUCTION

A Similipal Biosphere Reserve (SBR) forest is the only Biosphere Reserve of the state of Odisha. The name “Similipal” probably derived from abundance of Simili tree (Salmalia malbarica DC. Schott & Endl) in this biosphere [1]. SBR covers an area of 5569 Km², situated (20° 17´ - 22° 34´ N and 85° 30´ – 87° 10´ E) in the centre of Maurubhanj district in
the state of Odisha [2, 3, 4]. Biosphere is enriched with moist deciduous forest (Chahala, Jenabil, Nawana, Bakua etc), dry deciduous forest (Gurguria, Barheipani, Kalyani, Sanuski, Asatakumar, Kusumi, Rajpal, Hatibadi etc) and grass land (Chahala, Ligirdha etc). SBR is rich in wild medicinal plants along with the harbor of orchids [3, 5, 6]. It is also populated with different tribal communities such as Hill-Khadia, Mankidia, Ho, Munda, Bathudi etc. having their own socio-cultural habits and skills [7]. They are known to be primitive medical practitioners in the area as they use various wild phytoresources to cure diseases [8]. Hill-Khadia is a primitive tribe, having unique skills of collection of wild medicines [9, 10]. The plants used as traditional medicine & ailments by these tribes either are found in the peripheral, buffer zones, core or in the locality of tribal villages or hamlets [11]. The plants found in and around the villages are commonly used by them as traditional medicines. The plants mostly belong to the families like Cucurbitaceae, Rutaceae, Convolvulaceae, Moraceae, Myrtaceae, Malvaceae, Solanaceae, Fabaceae etc. Among these families, plants of Cucurbitaceae are quite common in villages of buffer and peripheral zones of SBR. *Luffa actungula, Luffa agyptica, Cucumis melo, Cucumis mexima, Cocinia grandis, Tricosanthes cucumerina, Tricosanthes tricuspidata and Momordica charantia* belonging to the family Cucurbitaceae, are mostly wild in nature and commonly used to cure different diseases by these people. *Tricosanthes tricuspidata*, (Plate1) locally called as “Mahakaal” is popular of this area due to its attractive red coloured fruits [12]. *T. tricuspidata* is a common medicinal plant used by the aboriginals of SBR. Fruits are used against asthma, skin infections, inflammation and joint pains. However, very less documentation / reports are available in literature about the medicinal and pharmacological values of *T. tricuspidata* fruits. Therefore, an attempt was made to document the ethnobotanical values collected from tribal communities of SBR and to validate their claims through phytochemical screening and antibacterial assessment.

II. MATERIAL AND METHODS

The present study is based on the field survey for collecting ethnomedical data and lab work for investigation of bioactive compounds and antibacterial activity. The details of materials used, experimental methods and techniques adopted during the course of the entire investigation are described below.

II.1. Assessment of Ethnobotanical data

The results presented here based on the fieldwork conducted with the rural and tribal communities of adjoining areas of SBR (Jashipur, Karanjia, Bisoi, Kendumundi and Padampur) during 2010-2013. The methodological framework was followed as per standard technique of ethno-biological approaches [13]. The information on plant used as traditional medicine against different diseases and disorders were collected through questioners with different rural and tribal communities [14, 15]. The pharmacological and medicinal properties of *T. tricuspidata* were confirmed by cross check with informants. Plant species was confirmed with the Flora’s Book and published article [16, 12].
II.2. Collection of plant materials

*Fruits of T. tricuspidata* were collected from the village Padampur, Hatibadi and Kalikaparsad peripheral area of SBR. They were washed properly, cut into small pieces and left for air-drying. The dried materials were crushed to powder with mechanical device and kept in airtight container for phytochemical screening and antibacterial activity (Plate 1).

II.3. Preparation of plant extracts

As per polarity index four solvents (n-butanol, acetone, methanol and aqueous) were selected for extraction. Extraction was done using Soxhlet apparatus followed by standard method [17].

II.4. Qualitative detection of bioactive compounds

Phytochemical experiments were carried out on n-butanol, acetone, methanol, and aqueous extracts of *T. tricuspidata* fruits using standard procedure to identify the bioactive compounds as described by different workers [18, 19, 20, 21].
II.4.1. Test of Tannin

0.5 gm of dried powder sample was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. 2 ml of filtrate was taken in a test tube and 3-5 drops of 0.1 % ferric chloride solution were added. The brownish green or blue black colouration indicated the presence of tannins.

II.4.2. Test for Alkaloids

0.5 gm of crude extract was mixed with 5 ml of 1% aqueous HCl on water bath and then filtered. 2-5 drops of Dragendorff’s reagent were added in the filtrate. The occurrence of orange-red precipitate was indicated the presence of alkaloids in the sample extract.

II.4.3. Test for Saponin

0.5 gm of the dried powder was boiled in 15 ml of distilled water and filtered with Whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

II.4.4. Test of Flavonoids

6 ml of dilute ammonium solution was added to portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

II.4.5. Test of Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and then 3 ml of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.
II.4.6. Test of Cardiac Glycosides

5 ml of crude extract was treated with 3 ml of glycial acetic acid. Then 3-5 drops of 1 % ferric chloride solution was added followed by 2 ml of concentrated sulphuric acid. Formation of brown interface indicated the presence of cardiac glycosides.

II.4.7. Test of Phenolic compounds

0.5 gm of extract was treated with 3-5 drops of 1 % ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

II.4.8. Test of Phytosterols

0.5 gm of extract was treated with chloroform and then filtered. The filtrate is treated with 3-5 drops of concentrated sulphuric acid and shakened. The mixture was left for 5 minutes. The appearance of Golden yellow colour indicated the presence of Phyto-sterols.

II.4.9. Test for Steroids

2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulphuric acid was added. Formation of 2 phases (upper red and lower yellow with green fluorescence indicated the presence of steroids.

II.5. Antibacterial activity

The n-butanol, acetone, methanol and aqueous extracts of *T. tricuspidata* fruits were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC *497*) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella enterica typhi* (MTCC 1252). All these used MTCC (Microbial Type Culture Collection) bacterial strain were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antimicrobial activity was done using Agar Well Diffusion assay adopted standard method [22] with slight modification. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (dimethyl sulfoxide) (Sigma) and twofold serial dilutions were made in amount of 100 µl per well ranged from 0.25 and 0.5 mg / ml. 100 µl of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18-24 h. Ampicillin served as standard drug control. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

II.6. Data analysis

Mean and SD (standard deviation) was performed to evaluate triplicate values of zone of inhibition (cm) of samples using Excel, Microsoft Corporation-2010, US.

III. RESULTS AND DISCUSSION

*T. tricuspidata* is very common plant in the peripheral and buffer regions of the SBR. It has a popular name in the locality “Mahakaal” dedicated to lord “Shiva” (Plate1:1). The ethnobotanical survey indicated its sound therapeutic values among the aboriginals of SBR. The aqueous paste of fruits is very effective in headache. The Bathudi tribe of Hatibadi uses it frequently as natural head balm (Table 1). Among the tribe of Kadlibadi, fruits paste are
used to cure mouth inflammation of domestic cattle. Fruits paste is very good for reducing macular swelling and pain. The tribe of Padampur used it against different types of skin infections and also against itching (Table 1). Fruits are very popular among the tribal women as it is used to kill the head lice and to cure dandruff problems. The medicinal values of this plant reported by different researchers throughout the world [16, 23, 24, 25]. The qualitative screening of bioactive compounds revealed that fruits of *T. tricuspidata* are rich in secondary metabolites, which is indicative of its pharmacological values. Terpenoids present in n-butanol extract of *T. tricuspidata* fruit showed the analgesic properties [26]. Flavonoids present in acetone, methanol and aqueous extracts (Table 2) showed its antibacterial [27], anti-cancer [28], and anti-inflammatory activity [29]. Presence of tannin and saponin in aqueous extract (Table 2) showed the anti-microbial [30, 31] activity and toxic properties [32] of *T. tricuspidata* L. fruits.

The anti-bacterial activity showed excellent zone of inhibition against two gram positive and three gram negative bacteria. At concentration 0.25 mg/ml, the methanol extract of *T. tricuspidata* L. fruit showed highest zone of inhibition against MTCC 1252 (1.18 cm), MTCC 1457 (1.25 cm), MTCC 3906 (1.28 cm), MTCC 1926 (1.28 cm) and MTCC 497 (1.41 cm) followed by acetone extract, n-butanol and aqueous extract (Plate 3).

At the concentration 0.5 mg/ml the methanolic extract showed highest activity than other extracts (Table 3). The ethnobotanical claims that fruits preparations are used against various diseases are validated by the present experiment. The presence of bioactive compounds correlated to the therapeutic properties of fruits. Presence of flavonoid might be responsible to swellings and muscular pain [39]. Presence of tannin and saponin might be responsible for skin infections [40] and antimicrobial activity [30] (Table 4).
<table>
<thead>
<tr>
<th>Informants</th>
<th>Sex</th>
<th>Age</th>
<th>Collection site</th>
<th>Forms of use(s)</th>
<th>Mode of use(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abhiram Bisad</td>
<td>Male</td>
<td>45</td>
<td>Hatibadi</td>
<td>Paste, external</td>
<td>Paste of two matured green fruits is macerated with about 10 ml of hot water. Macerated paste is then applied thickly on forehead to reduce headache.</td>
</tr>
<tr>
<td>Laxaman soi</td>
<td>Male</td>
<td>52</td>
<td>Hatibadi</td>
<td>Paste</td>
<td>Dry Fruits are macerated with fruit oil of <em>Azadirachta indica</em> (Neem) and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.</td>
</tr>
<tr>
<td>Bajraj Chandra Nayak</td>
<td>Male</td>
<td>38</td>
<td>Hatibadi</td>
<td>Paste</td>
<td>Green newly 10 days fruits are cut in small pieces. Pieces are kept in small amount of water and kept overnight. Swelled fruits are macerated and make paste with same water. Paste is applied as natural ointment on swelling parts of the body to reduce pain and inflammation.</td>
</tr>
<tr>
<td>Morgad Nayak</td>
<td>Male</td>
<td>51</td>
<td>Kadlibadi</td>
<td>Paste</td>
<td>Seeds of ripen fruits are washed and dried. Dried seeds are powdered using traditional mortar pestle of stone. Approx 200 grams of powder are macerated with water and make paste. Paste is applied on outer parts of mouth of cattle to cure swelling.</td>
</tr>
<tr>
<td>Jaira Futi</td>
<td>Male</td>
<td>44</td>
<td>Kadlibadi</td>
<td>Paste</td>
<td>Ripen fruits are dried with pulp and seeds. Dried fruits are powdered using the traditional grinder. Approx 100 gm powder is macerated with about 10 ml seed oil of <em>Ricinus communis</em> (Joda) to cure itching on skin.</td>
</tr>
<tr>
<td>Rama Ho</td>
<td>Male</td>
<td>40</td>
<td>Padampur</td>
<td>Paste</td>
<td>Black pulp of ripen fruits are dried and powdered. Powder are mixed with seed oil of <em>Pongamia pinnata</em> (Karanja) and made paste. Paste is applied on lesions of fungal infections on feet, particularly during rainy seasons.</td>
</tr>
<tr>
<td>Bnamali Ho</td>
<td>Male</td>
<td>41</td>
<td>Padampur</td>
<td>Paste</td>
<td>Dry Fruits are macerated with fruit oil of <em>Azadirachta indica</em> and camphor. Macerated paste is applied on head and kept it about 1h to kill lice thrice in a week before bath.</td>
</tr>
<tr>
<td>Balram Munda</td>
<td>Male</td>
<td>55</td>
<td>Padampur</td>
<td>Paste</td>
<td>Dry Fruits are macerated with fruit oil of <em>Azadirachta indica</em> and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.</td>
</tr>
</tbody>
</table>
Table 2: Qualitative analysis of bioactive compounds present in fruits of Mahakaal (T. tricuspidata L.)

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Bioactive compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-butanol</td>
<td>Terpenoids</td>
</tr>
<tr>
<td>Acetone</td>
<td>Flavonoids, Phenolic compounds</td>
</tr>
<tr>
<td>Methanol</td>
<td>Flavonoids, Alkaloids, Steroids, Phenolic compounds</td>
</tr>
<tr>
<td>Aqueous</td>
<td>Tannin, Saponin, Flavonoids, Phenolic compounds</td>
</tr>
</tbody>
</table>

The presence of saponin might be responsible to kill the lice. The antibacterial activity of methanolic extract against *Streptococcus pyogenes* (MTCC 1926) showed that fruits are effective against the various skin infections (Figure 1) as *S. pyogenes* are responsible for skin infections [41]. The experimental results validated the traditional therapeutic claims (Table 4) to be used fruit preparations against aforesaid diseases.

Figure 1: Antibacterial activity against MTCC 1926
### Table 3: Antibacterial activity of fruit extracts of Mahakaal (T. tricuspidata L.)

<table>
<thead>
<tr>
<th>Strain</th>
<th>n-butanol</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Aqueous</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTCC 1252</td>
<td>0.73 ± 0.02</td>
<td>1.20 ± 0.02</td>
<td>1.18 ± 0.07</td>
<td>0.76 ± 0.02</td>
<td>0.25 mg/ml</td>
</tr>
<tr>
<td>MTCC 1457</td>
<td>0.91 ± 0.05</td>
<td>1.21 ± 0.02</td>
<td>1.25 ± 0.05</td>
<td>0.76 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MTCC 3906</td>
<td>0.88 ± 0.02</td>
<td>1.16 ± 0.02</td>
<td>1.28 ± 0.02</td>
<td>0.78 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MTCC 1926</td>
<td>0.96 ± 0.02</td>
<td>1.33 ± 0.02</td>
<td>1.41 ± 0.02</td>
<td>0.76 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MTCC *497</td>
<td>0.95 ± 0.05</td>
<td>1.16 ± 0.02</td>
<td>1.23 ± 0.02</td>
<td>0.86 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MTCC 1252</td>
<td>0.96 ± 0.02</td>
<td>1.40 ± 0.05</td>
<td>1.51 ± 0.02</td>
<td>0.98 ± 0.02</td>
<td>0.5 mg/ml</td>
</tr>
<tr>
<td>MTCC 1457</td>
<td>0.98 ± 0.02</td>
<td>1.41 ± 0.02</td>
<td>1.43 ± 0.02</td>
<td>0.95 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>MTCC 3906</td>
<td>0.96 ± 0.02</td>
<td>1.46 ± 0.05</td>
<td>1.48 ± 0.02</td>
<td>0.98 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>MTCC 1926</td>
<td>0.93 ± 0.05</td>
<td>1.48 ± 0.02</td>
<td>1.51 ± 0.02</td>
<td>1.06 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>MTCC *497</td>
<td>0.95 ± 0.05</td>
<td>1.18 ± 0.07</td>
<td>1.16 ± 0.11</td>
<td>0.96 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Ampicilin (zone of inhibition, cm, mean ± SD; n=3)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampicilin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTCC 1252</td>
<td>1.10 ± 0.00</td>
</tr>
<tr>
<td>MTCC 1457</td>
<td>1.40 ± 0.00</td>
</tr>
<tr>
<td>MTCC 3906</td>
<td>1.20 ± 0.00</td>
</tr>
<tr>
<td>MTCC 1926</td>
<td>1.20 ± 0.00</td>
</tr>
<tr>
<td>MTCC *497</td>
<td>1.40 ± 0.00</td>
</tr>
</tbody>
</table>

(MTCC: Microbial Type Culture Collection; SD: standard deviation; mg: milligram; ml: milliliter; MTCC 3906: Vibrio cholera; MTCC 1252: Salmonella enteric typhi; MTCC 1457: Shigella flexneri; MTCC 1926: Streptococcus pyogenes; MTCC 497: Streptococcus mutans)
Table 4: Validation of traditional claims against Mahakaal (T. tricuspidata L.) fruits

<table>
<thead>
<tr>
<th>Traditional claims</th>
<th>Correlation with bioactive compounds</th>
<th>Correlation with antibacterial activity</th>
<th>Supporting literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin infections</td>
<td>Presence of saponin in aqueous; tannin in aqueous; flavonoids in acetone and methanol extracts might be responsible.</td>
<td>Methanolic extract showed highest zone of inhibition (1.51 ± 0.02) against <em>Streptococcus pyogenes</em> (MTCC 1926).</td>
<td>Mohan and Kalidas, (2010) [33]; Okwe &amp; Okwe, (2004) [34]; Kumar <em>et al.</em>, (2013) [35].</td>
</tr>
<tr>
<td>Kill lice</td>
<td>Presence of saponin in aqueous extract might be responsible to kill lice due to foam forming capacity (needs further study)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduce swelling</td>
<td>Presence of flavonoids in acetone and aqueous extracts might be responsible to reduce swelling.</td>
<td></td>
<td>Musa <em>et al.</em>, (2009) [36]</td>
</tr>
<tr>
<td>Reduce pain</td>
<td>Presence of flavonoids in acetone, methanol and aqueous extract; tannin in aqueous extract might be responsible</td>
<td></td>
<td>Majumdar <em>et al.</em>, (2008) [37]; Gloria and Soloman, (2008) [38]; Musa <em>et al.</em>, (2009) [36].</td>
</tr>
</tbody>
</table>
IV. CONCLUSION

The present study highlights the medicinal values of Mahakaal (T. tricuspidata L.) fruits and its uses among the populace of a protected area, Similipal Biosphere Reserve. The studies scientifically validate the tribal claims on the ethnomedicinal uses of fruits. Further present study suggest to isolate the bioactive compounds which actually active against above mentioned claims to formulate drugs. Such study also highlights the medicinal values of the wild plants and gives attention towards the possible awareness and to make policies for conservation of such wild resources.

V. ACKNOWLEDGEMENTS

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VI. REFERENCES


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Zn(II), Pb(II) and Cd(II) levels in livers and muscles of wild duck (Anas platyrhynchos) hunting in El Melah Lagoon (NE Tunisia)

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Abstract: We investigated the levels of Zn(II), Pb(II) and Cd(II) in livers and muscles of the wild duck (Anas platyrhynchos) hunting in El Melah lagoon in northeastern Tunisia from December 2010. Analysis of variance shows that no significant differences in metals levels were found between samples, but it should be pointed out that the number of samples was small. HSD Tukey test show that the amount of Cd(II), Zn(II) and Pb(II) in the levers was higher than in the muscles. The results obtained suggest the importance of wild ducks as bioindicators of heavy metal pollution.

Keywords: Heavy metal, breast muscles, livers, Anas platyrhynchos, Eastern Tunisia.

I. Introduction

Pollutants such as Pb(II) and Cd(II) can have adverse effects on various physiological systems at environmentally relevant concentrations [1]. Previous studies have shown that heavy metals can also have a negative influence on the reproduction and general health of some birds [2, 3].

Because industrial and municipal waste streams, mining runoff, and other anthropogenic sources of metal contamination often end up in wetlands, waterfowl may be exposed to environmental pollutants. Exposure of waterfowl to heavy metals may occur through ingestion of Pb(II) shot gun pellets [4], contaminated sediments [5] or by food chain transfer [6]. As a result, waterfowl may accumulate metals in their tissues and could be used as bioindicators of contamination [7].

One from the most visible and biodiverse group of animals living on water areas are birds. Mallard (Anas platyrhynchos) is the commonest duck in the world [8]. This species shows a great ecological plasticity and is observed in majority types of wetlands including urban habitats. Mallard is proposed as an effective biomonitor of metal pollution, because samples
are easy to collect, the species is spread all over the world, the literature needed for comparison is available from different parts of the world [9]. Our study was undertaken in order to study concentrations of Zn(II), Pb(II), and Cd(II) metallic ions in muscle and liver of Mallards from El Melah lagoon (NE Tunisia).

I. 1. Study area

El Melah lagoon is a small coastal confined area (200 ha) located near Slimene (NE Tunis) and connected to the Mediterranean Sea through an artificial outlet. Diverse anthropogenic activities are present in El Melah Lagoon. The treatment station of Slimene drains directly to the western, confined area of the permanent lagoon waters. This station, constructed in 1992, has a capacity of 2500 m$^3$, insufficient for a mean daily volume (3000 m$^3$) of urban sewage actually produced by this town and the surrounding areas [10].

The industrial wastes of Grombalia, located in southeast Slimene, are also partially discharged to the catchment area of Oued El Bey, and can be transported to the lagoon by rain during the rainy season or through subterranean flows. Other industrial residues, derived from a parapharmaceutical plant, are also delivered to the northeastern sector of the lagoon. In addition, the southern border of the lagoon receives the liquid/solid residues of a broad area occupied by farming activities [10].

Other strong environmental impacts are produced by the solid residual deposits of Slimene, located in the southeastern corner of the lagoon. These residues can be washed in by the periodic rains and some dangerous metals could be transported to the aquifers and finally to the lagoon. In addition, remains of bricks, glazed tiles, concrete, cement or scrap-iron are dumped and stockpiled along the road that joins Slimene to the beach. Similar residues are also observed in the inner areas of the littoral dune strand, near the tourist resort of Solimar. Finally, some old, abandoned saltworks are found in the central lagoon [10].

![Figure 1: Map of the El Melah Lagoon showing the four sampling sites from where mallards were hunted (S1, S2, S3, S4) and the pollution places around the lagoon.](image_url)
II. Experimental Section

Samples (about 50-70 g) of liver and muscles were taken from 14 mallards, collected in the hunting area at the El Melah lagoon (NE Tunis). All birds were shot using Pb(II) shot during the hunting season in 2009 and individually put in labeled plastic bags.

Liver and muscles samples were taken within 2 hours after death, carefully dissected to avoid external contamination, weighed, stored in chemically clean plastic bags, and kept at -20 °C until they were analyzed.

After defrosting, one aliquot (approximately 500mg of fresh hepatic tissue and muscle) was dried at 105 °C for 24 h and weighed. A volume of 2ml of an acid mixture (perchloric, nitric and sulphuric acids, 8:8:1, trace analysis quality, Scharlau) was added to the sample for mineralization. This process was realized in digestion tubes previously washed in a 10% solution of nitric acid, using an automatic digester, programmed to rise from room temperature to 370 °C in 5.5 h, according to the general method proposed by Garcia-Fernandez [11]. Digested samples were subsequently added with 200 ml of HCl Suprapurs (Merck), and diluted in deionized water to a final volume of 20 ml.

Concentrations of different inorganic elements (Zn, Pb and Cd) were determined by means of inductively coupled plasma-mass spectrometry (ICP-MS). Each analysis was carried out in duplicate. Final concentrations in samples were expressed referring to dry weight. All samples were run in batches that included blank and initial calibration standards.

A statistical package (SPSS for Windows, V. 13.0) was used to analyze the results. Comparisons among the different tissue for each heavy metal were realized using the non-parametric Kruskal Wallis’s test. In order to determine which tissues were significantly different from each other, a post hoc comparison with the Dunn’s test was carried out.

III. Results and Discussion

Cd(II) concentrations varied according to tissues (Figure 2). Liver was the main internal organ for Cd(II) accumulation in mallard. The high Cd(II) accumulation in Liver demonstrates the role of this organ in the detoxification process and storage of nonessential elements. In contrast, muscle represented minor sites of Cd accumulation, as previously described [12, 1]. Cd(II) is considered one of the most toxic metals. Cd(II) in the environment has mainly an anthropogenic origin. More than a thousand tonnes of Cd(II) have been emitted to the atmosphere in Europe, mining and smelting being the major sources [11]. In different habitats, birds accumulate varying amounts of Cd(II). The metal concentrates mainly in kidneys, and also in the liver. This pattern was also confirmed by the Mallards from El Melah lagoon considered in this study. Chronic exposure to Cd(II) is known to increase birds susceptibility to disease or other kinds of stress and to reduce reproductive success [13].

Like Cd(II), Pb(II) is an element that plays no role in metabolic processes of animal organisms. It is an extremely toxic element with a wide range of harmful effects. Exposure to Pb(II) may cause kidney and nervous system problems. It can also inhibit heme synthesis. The mean of Pb(II) concentration determined for the mallards was 0.608 mg/kg w.w. in the liver and 0.467 mg/kg w.w. in muscles (Figure 3). Similar results were obtained by Kalisinska et al. [14], who investigated contents of iron, Zn(II), copper, manganese, nickel, Pb(II) and Cd(II) in selected tissues and organs of young and adult Mallards from two regions of northwestern Poland, and by Măcinic et al. [15], who studied Mallard (Anas platyrhynchos) from a hunting ground in Romania.
It should be stressed that Pb is a serious neurotoxin; a small amount penetrating the brain may alter the bird’s behaviour up to the extent of endangering the survival and precluding reproductive success. Higher Pb(II) contents result in visibly disturbed functioning of the central and peripheral nervous systems [16] Developing nervous system has long been recognized as a primary target site for Pb-induced toxicity [17, 18]. For those reasons, ecotoxicological research should pay more attention to the avian brain. In many cases the Mallard, have been used to assess wetland Pb(II) pollution [14, 19, 20].

Zn(II) belongs to the group of trace elements but in higher concentrations can be harmful to organisms [9, 21] Also, its deficiency causes loss of appetite, loss of body weight, impairment of epidermal products, impairment of reproductive functions and changes in bones, especially in birds due to osteogenesis [21]. The concentration of Zn(II) (figure 4) determined in the liver of the mallards was within the range of 93.8 mg/kg w.w to 302.7 mg/kg w.w. (mean 239 mg/kg w.w.). The Zn content in duck muscles ranged from 41.5 to 51.8 mg/kg w.w. Zn concentrations in the liver of the birds appear to have toxic levels. The highest concentrations of this metal occurred in liver what was observed also by Kalisinska et. al. [14] and Binkowski et al. [9]. Concentrations of Zn(II) in bird body may be influenced by the food quality and molting season.

Figure 1: Cd(II) concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)
IV. Conclusion

The El Melah Lagoon’s water has been observed to deteriorate in quality very rapidly because of the anthropogenic activities. Industrial, municipal and agricultural wastes are additionally discharged to El Melah Lagoon. These may be the possible causes of the high metal amounts observed in mallard’s tissues. Levels of heavy metal varied depending on different tissues. The results of this study indicated that accumulation of heavy metals was higher in liver than muscles.
We can conclude there should be effort to protect El Melah Lagoon from pollution to reduce environmental risks and this study may provide valuable data for future research. The main topics that may be needed to be investigated are control of all discharges, regular observation of pollutants, evaluation of effect of pollutants on Lagoon’s ecosystem over the long term, coordinating the pollution source and preventing inflow of pollutants to the Lagoon.

V. References


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Corrosion inhibition of carbon steel A 283 C using the acetylsalicylic acid

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Abstract: The corrosion inhibition characteristics of acetylsalicylic acid on carbon steel has been studied using electrochemical measurements. Results showed that: The inhibition efficiency values give maximum inhibition at the concentration of 300 ppm and decrease back. They decreased with increasing temperature. Polarization studies showed that this compound is an anodic inhibitor for carbon steel in decanted water from a tank bottom hydrocarbon storage (East Region Transport: Skikda Algeria). The inhibition occurs through adsorption of the inhibitor molecule on the metal surface. The adsorption of the inhibitor on the metal surface is found to obey Langmuir’s adsorption isotherm. The values of thermodynamic parameters, such as $K_{ads}$, $\Delta G_{ads}$, $\Delta H_{ads}$ and $\Delta S_{ads}$ are calculated.

Keywords: Corrosion, Carbon Steel, Acetylsalicylic Acid, Inhibition, Decanted Water, Adsorption.

I. Introduction

Carbon steel is used in mass amounts in marine applications, chemical processing, petroleum production and refining. These applications usually induce serious effect on equipments, tubes and pipelines [1-3]. The protections of these materials are generally secured by inhibitors used for their rapid action. Particularly organic compounds containing heteroatoms are usually employed. Generally, they act by adsorption on the metal surface which takes place through heteroatoms such as: nitrogen, oxygen, phosphorus, sulphur, triple bonds or aromatic rings…. The interactions between an organic inhibitor and a metal surface are principally physical adsorption and/or chemisorptions [4-14]. Recently, many studies are interested at the investigation of pharmaceutical compounds. In fact, these compounds offer interesting possibilities for corrosion inhibition and are of particular interest because of their safe use, high solubility in water and containing electronegative atoms in their molecules. These compounds should be good corrosion inhibitors [16-16].

Based on the foregoing points, the aim of this work is to study the inhibition efficiency of the acetylsalicylic acid **fig.1** in decanted water from a tank bottom hydrocarbon storage (East Region Transport: Skikda Algeria) using chemical (polarization and impedance) measurements. The mode of adsorption and the corrosion inhibition, mechanism are also discussed.
II. Experimental section

The electrochemical measurements are carried out with a three-electrode assembly: a platinum electrode against a reference electrode saturated calomel electrode and the working electrode of 1 cm² cross-section, made in the laboratory from steel pipes. The steel selected in this study is a carbon steel A283 grade C according to ASTM whose composition is as follows: C = 0.18%, S = 0.05%, P = 0.06%, Cu = 0.20% and Fe. Before the measurements, the electrode is polished successively with emery paper up to 1200 grade, then rinsed with distilled water, cleaned and degreased with acetone and dried in the open air.

The corrosive medium is decanted water in the bottom of storage tank of crude oil (Transport Region of Skikda Algeria). The physico-chemical characterization of the water is given in table 1. The electrochemical measurements were performed using a VoltaLab 40, with a PGZ301 potentiostat controlled by a computer and software VoltaMaster 4.

Table 1: The physico-chemical characterization of the decanted water

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.7</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>134.4</td>
</tr>
<tr>
<td>TDS (g/l)</td>
<td>67.1</td>
</tr>
<tr>
<td>Resistivity (Ω.cm)</td>
<td>7.44</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/l)</td>
<td>300</td>
</tr>
<tr>
<td>S²⁻ (mg/l)</td>
<td>0.051</td>
</tr>
<tr>
<td>Cl⁻ (mg/l)</td>
<td>40750</td>
</tr>
<tr>
<td>O₂ dissolved</td>
<td>7.4</td>
</tr>
<tr>
<td>Bart test (bacterial corrosion)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

III. Results and discussion

III.1. Polarization measurements

Fig. 2 represents potentiodynamic polarization curves for carbon steel in decanted water in the absence and presence of various concentration of acetylsalicylic acid. As can be seen the addition of the inhibitor molecules inhibit the metal dissolution.
FIG 2: Polarization curves of carbon steel in decanted water in the absence and various concentrations of inhibitor

Table 2 shows the electrochemical corrosion parameters, such as corrosion potential ($E_{corr}$), corrosion current density ($I_{corr}$), obtained by extrapolation of the tafel lines and the inhibition efficiency (IE%) which was evaluated from the relation:

$$\text{IE}\% = \left(\frac{I_o - I_i}{I_o}\right) \times 100$$

Where $I_o$ and $I_i$ are uninhibited and inhibited current densities respectively. The obtained efficiencies given in table 2 indicate that the acetylsalicylic acid act as effective inhibitor. The addition of this compound induces a decrease in current and in the presence of this compound the corrosion potentials shifted to the positive direction compared to the uninhibited alloy. So, it could be concluded that the acetylsalicylic acid is an anodic inhibitor of carbon steel in decanted water. Furthermore, the corrosion inhibition efficiencies show a maximum efficiency (97%) at a concentration of 300 ppm, table 2.

Table 2: The electrochemical parameters of carbon steel in decanted water containing different concentrations of inhibitor at 25°C.

<table>
<thead>
<tr>
<th>Inhibitor concentration (ppm)</th>
<th>$E_{corr}$(mv)</th>
<th>$I_{corr}$( $\mu$A/cm$^2$)</th>
<th>$\Theta$</th>
<th>%IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-729.4</td>
<td>7.5371</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>-601.1</td>
<td>1.425</td>
<td>0.810</td>
<td>81.09</td>
</tr>
<tr>
<td>200</td>
<td>-549.3</td>
<td>0.914</td>
<td>0.878</td>
<td>87.87</td>
</tr>
<tr>
<td>300</td>
<td>-527</td>
<td>0.324</td>
<td>0.9705</td>
<td>97.05</td>
</tr>
<tr>
<td>400</td>
<td>-483.2</td>
<td>0.5025</td>
<td>0.933</td>
<td>93.33</td>
</tr>
<tr>
<td>500</td>
<td>-487.9</td>
<td>0.6584</td>
<td>0.912</td>
<td>91.26</td>
</tr>
<tr>
<td>1000</td>
<td>-476.7</td>
<td>2.335</td>
<td>0.690</td>
<td>69.01</td>
</tr>
</tbody>
</table>

III.2. Adsorption Isotherm

The nature of inhibitor interaction on the corroding surface during corrosion inhibition of metals has been deduced in terms of adsorption characteristics of the inhibitor [17].

The dependence of the degree of surface coverage $\Theta$ on the molar concentration of the inhibitor was calculated using the following equation:

$$\Theta = \frac{I_o - I_i}{I_o}$$

(2)
The values of (Θ) have been inserted in the table 2. The degree of surface coverage was found to increase with increasing the concentration of additive compound. The data were tested graphically by fitting it to Langmuir isotherm which is given by equation 3 [18].

\[
C/\Theta = C + 1/K
\]  

(3)

Where C is the inhibitor concentration (ppm) K is the adsorption equilibrium constant and Θ is the degree of coverage in the metal surface. When \(C_{\text{inh}}/\Theta\) (ppm) were plotted against \(C_{\text{inh}}\) (ppm) a straight line was obtained which suggest that the adsorption of inhibitor obeys the Langmuir adsorption isotherm Fig 3. The value of the equilibrium constant K has been calculated, then the standard free energy can be deduced by using equation 4.

\[
K = (1/55.55)\exp(-\Delta G_{\text{ads}}^°/RT)
\]  

(4)

The negative value of \(\Delta G_{\text{ads}}^°\) (-34.832 kJ mol-1) indicate that adsorption of the inhibitor on the metal surface is spontaneous.

Generally, values of \(\Delta G_{\text{ads}}^°\) around -20 kJ mol-1 or lower are consistent with the electrostatic interaction between charged molecules and the charged metal surface (physisorption), those around -40 kJ mol-1 or higher involve charge sharing or transfer from organic molecules to the metal surface to form a coordinate type of metal bond (chemisorption) [19]. The value of \(\Delta G_{\text{ads}}\) is less than 40 kJ mol-1 is commonly interpreted with the presence of physical adsorption by the formation of an adsorptive film with an electrostatic character [20-21]. However, some researchers have reported that adsorption of inhibitor molecules is obeying a comprehensive adsorption (physical and chemical adsorption) for the same values [22-23].

III.3. Effect of temperature

The effect of temperature on the various corrosion parameters \(E_{\text{corr}}, I_{\text{corr}}, k\) and IE was studied in decanted water at temperature range 25-40°C in the absence and presence of 300ppm of the inhibitor. The results were listed in table 3. An inspection of table 3 shown that as the temperature increases, the values of \(I_{\text{corr}}\) increases and IE decreases. This proves that the inhibition occurs through a physical adsorption on the metal surface.
Table 3: The effect of temperature on the corrosion parameters of carbon steel in decanted water in absence and presence of 300ppm of inhibitor.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Concentration (ppm)</th>
<th>$I_{\text{corr}}$ (mA.cm$^{-2}$)</th>
<th>$E_{\text{corr}}$ (mv)</th>
<th>k</th>
<th>%IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0</td>
<td>7.5371</td>
<td>-729.4</td>
<td>85.92</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.324</td>
<td>-527</td>
<td>2.532</td>
<td>97.05</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>39.242</td>
<td>-705.3</td>
<td>281.896</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>5.1089</td>
<td>-403.4</td>
<td>36.7</td>
<td>80.95</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>55.905</td>
<td>-689.2</td>
<td>401.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>14.913</td>
<td>-402.6</td>
<td>107.2</td>
<td>77.55</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>129.115</td>
<td>-663.5</td>
<td>927.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>34.565</td>
<td>-400.7</td>
<td>248.3</td>
<td>73.22</td>
</tr>
</tbody>
</table>

The activation energy of the corrosion process was calculated using the following equation:

$$k = A \exp(-E^{-\bullet}_{a}/RT)$$

(5)

Where $E^{-\bullet}_{a}$ is the activation energy, A is the frequency factor, T is the absolute temperature, R is the gas constant and k is the rate of metal dissolution reaction which is directly related to corrosion current density $I_{\text{corr}}$ [24]. Plotting log(k) versus 1/T, the value $E_{a}$ can be calculated from the slopes of straight lines obtained from fig 4. The values of $E_{a}$ are listed in table 4.

The activation energy is higher in the presence of additives than in its absence. This observation further supports the proposed physisorption mechanism. Unchanged or lower values of $E_{a}$ in inhibited systems compared to the blank have been reported to be indicative of chemisorptions mechanism, while higher values of $E_{a}$ suggest a physical adsorption mechanism [25]. This type of inhibitors retards the corrosion process at ordinary temperature whereas the inhibition is considerably decreased at

Fig.4. Plot of log (k) versus 1/T$^{1000}$, $K^{-1}$ for carbon steel in decanted water Solution in absence and in presence of 300ppm of inhibitor.
elevated temperature [26, 27]. Plot of log (k/T) versus 1/T for carbon steel in decanted water in absence and presence of acetylsalicylic acid is shown in fig. 5.

![Graph showing log (k/T) versus 1/T for carbon steel](image)

**Fig.5.** Plot of log (corrosion rate/T) versus 1/Tx10^3, K for carbon steel in decanted water Solution in absence and in presence of 300 ppm of inhibitor.

As shown from this figure, straight line was obtained according to transition state equation (6):

\[
\text{Rate}=\frac{R}{\hbar} \exp(\frac{\Delta S^*}{R}) \exp(-\frac{\Delta H^*}{RT})
\]  

(6)

Where h is Plank’s constant, N is Avogadro’s number, ΔH* is the activation enthalpy and ΔS* is the activation entropy. The calculated values are given in table 4.

**Table 4: Activation parameters of the corrosion of carbon steel 283 °K in decanted water in absence and presence of 300ppm of inhibitor.**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Ea (J.mol⁻¹)</th>
<th>ΔH (J mol⁻¹)</th>
<th>ΔS (J.mol⁻¹K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>116.2679</td>
<td>113.7594</td>
<td>286.6372</td>
</tr>
<tr>
<td>300</td>
<td>230.735</td>
<td>228.9466</td>
<td>644.7087</td>
</tr>
</tbody>
</table>

These values indicate that the presence of the additive increases the activation enthalpy ΔH* and the activation entropy ΔS* for the corrosion process. The increase in the activation enthalpy (ΔH*) in presence of the inhibitor implies that the addition of the inhibitor to the decanted water increases the height of the energy barrier of the corrosion reaction to an extent depends on the type and concentration of the present inhibitor. The entropy of activation (ΔS*) in the blank and inhibited solution is large and positive. This increase in the entropy was caused by imperceptible replacement of water molecules due to the adsorption of inhibitor molecules on carbon steel, decreasing the extent of the dissolution reaction [28, 29].

**III.4. UV-vis spectroscopic investigation**

In order to confirm the possibility of the formation of inhibitor-Fe complex, UV-vis adsorption spectra obtained from the corrosive solution in the presence of different concentrations of acetylsalicylic acid after 3 h of the metal immersion are shown in fig 6. It is clearly seen that the charge transfer has been completely appeared and absorption maxima
at 495nm indicating complex formation with Fe$^{2+}$ ions which have a pink color. In Fig.7, we propose the schematic representation of adsorption behavior of studied organic compound on carbon steel.

Fig.6. UV-spectra of the solutions containing different concentration of acetylsalicylic acid after 3h immersion of carbon steel.

Fig. 7. Schematic representation of adsorption behavior of studied organic compound on carbon steel

III.5. Electrochemical impedance spectroscopy.
III.5.1. Effect of the inhibitor concentration.

In order to understand the inhibition behavior, electrochemical impedance spectroscopy was employed to investigate the influences of acetylsalicylic acid on the corrosion of carbon steel. Fig.8. presents Nyquist diagrams of the steel measured in absence and in presence of 300ppm of the inhibitor. The Nyquist plots consisted of two capacitive loops. The one at low frequency (in the right part of the figure) was related to change transfer resistance, which could correlate to resistance between the steel and outer helmholtz plane [30-31]. Conversely the one at high frequency (in the left part of the figure) was attributed to the adsorbed film resistance due to adsorption of the inhibitor and all other accumulated products [32]. The EIS results were simulated using equivalent circuit shown in Fig.9. In this equivalent circuit, $R_s(R_i)$ is the solution resistance, $R_p$ ($R_p=R_t+R_{cl}$) is the polarization resistance, $Q_f$ and $R_f(R_2)$ are constant phase element and the resistance related to the surface film.
III.5.2. The effects of immersion time on the inhibitor performance.

To investigate the inhibitor adsorption kinetics and determine the time needed for the inhibitor to reach its maximum inhibition efficiency, EIS experiment were carried out in the presence of 300ppm of acetylsalicylic acid in decanted water at different immersion times at 25°C Fig 10.
Fig. 1. EISs of carbon steel in decanted water in presence of 300 ppm of inhibitor at 25°C after different immersion times

A comparison of the results of $R_f$ and $R_p$ with 300 ppm of inhibitor at different immersion times is presented in Table 6.

**Table 6.** EIS parameters obtained from the corrosion of carbon steel in decanted water containing 300 ppm of the inhibitor for different immersion times at 25°C

<table>
<thead>
<tr>
<th>Immersion time (min)</th>
<th>$Q_f (F s^{-3})$</th>
<th>$a_1$</th>
<th>$R_f$ (Ohm)</th>
<th>$Q (F s^{-3})$</th>
<th>$a$</th>
<th>$R_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.427e-3</td>
<td>0.66</td>
<td>979</td>
<td>0.241e-6</td>
<td>0.794</td>
<td>785</td>
</tr>
<tr>
<td>10</td>
<td>0.356 e-3</td>
<td>0.69</td>
<td>557</td>
<td>0.202 e-6</td>
<td>0.63</td>
<td>1360</td>
</tr>
<tr>
<td>20</td>
<td>50.19e-6</td>
<td>0.33</td>
<td>324</td>
<td>1.064e-3</td>
<td>0.71</td>
<td>1707</td>
</tr>
<tr>
<td>30</td>
<td>4.415e-6</td>
<td>0.92</td>
<td>225</td>
<td>1.392e-3</td>
<td>0.672</td>
<td>2072</td>
</tr>
<tr>
<td>40</td>
<td>0.1341e-6</td>
<td>0.66</td>
<td>205</td>
<td>1.392e-3</td>
<td>0.67</td>
<td>1690</td>
</tr>
<tr>
<td>50</td>
<td>0.1341e-6</td>
<td>0.67</td>
<td>223</td>
<td>1.114e-3</td>
<td>0.71</td>
<td>926</td>
</tr>
<tr>
<td>60</td>
<td>56.74e-6</td>
<td>0.66</td>
<td>285</td>
<td>1.136e-3</td>
<td>0.76</td>
<td>695</td>
</tr>
<tr>
<td>70</td>
<td>4.15e-3</td>
<td>0.42</td>
<td>354</td>
<td>1.188e-3</td>
<td>0.67</td>
<td>635</td>
</tr>
<tr>
<td>210</td>
<td>0.939 e-3</td>
<td>0.64</td>
<td>1118</td>
<td>7.697e-3</td>
<td>0.52</td>
<td>460</td>
</tr>
</tbody>
</table>

It can be seen that $R_p$ increases in the first 30 minutes and then decreases. This is probably due to the formation of a corrosion product film on the metal surface in the initial stage of immersion. Extended immersion may lead to damage the film, probably because of the development of localized corrosion on the steel surface. $Q_f$ values are low and are consistent with the presence of a protective film on the metal surface formed by the inhibitor.

**IV. Conclusion**

1. The corrosion of carbon steel in decanted water can be inhibited using the acetylsalicylic acid.
2. The inhibition process follows Langmuir adsorption isotherm and corresponds to physisorption phenomenon.
3. The decrease in corrosion current density ($I_{corr}$) and the increase in inhibition efficiency (%IE) with increasing the additive concentration are proved that the tested compound act as inhibitor for carbon steel in decanted water.
4. Polarization studies showed that this compound is an anodic inhibitor for carbon steel in decanted water.
5. The thermodynamic values obtained from this study $E^\circ_{a}$, $\Delta G^\circ_{ads}$ indicate that the presence of the inhibitor increases the activation energy of corrosion and consequently decrease the rate of dissolution of carbon steel in decanted water. The negative value of $\Delta G^\circ_{ads}$ indicates the spontaneous adsorption of the inhibition on the surface of carbon steel.
6. The UV-vis studies reveal the formation of the Fe-inhibitor complex which may be also responsible for the observed inhibition.
7. The equivalent circuit was selected based on properties of the EIS Nyquist diagrams
8. in the presence of the inhibitor, the double layer capacitance decreased which confirmed adsorption of the inhibitor molecules on the steel surface.

**V. References**

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Tel.: + ; Fax: +.

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Main text paragraph.

II. Experimental Section
Main text paragraph.

III. Results and Discussion
Main text paragraph.

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Figure 1. (a) Add a descriptive label of the figure here. (b) Add a descriptive label of the
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IV. Conclusions
Main text paragraph.

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