



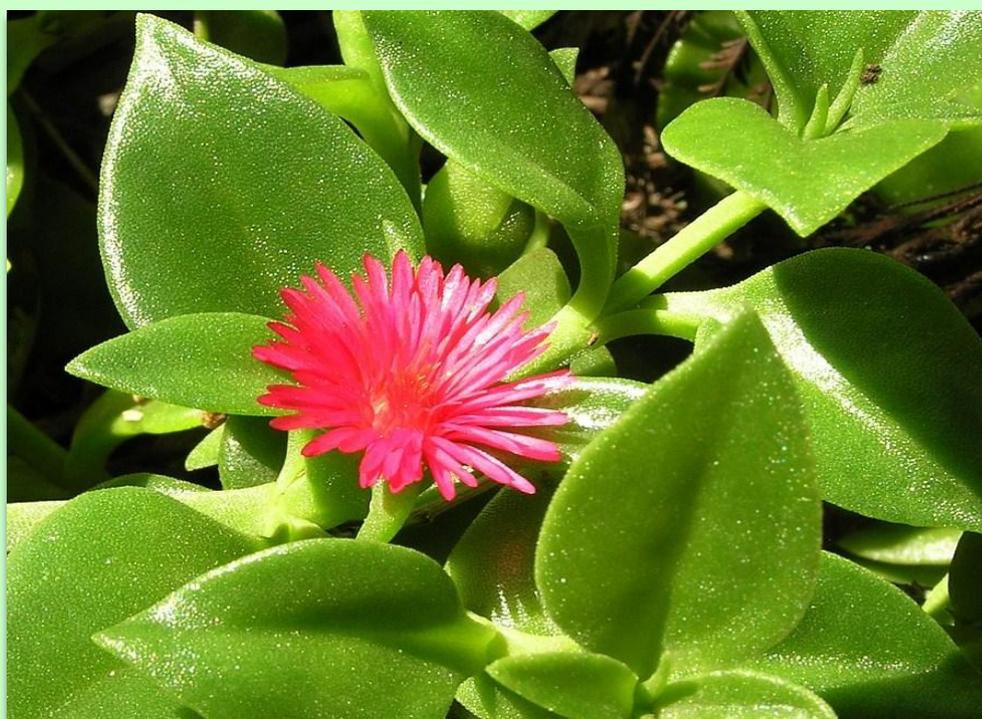
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Characterization and cation exchange capacity of seeds of *Ziziphus spina-christi*

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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 177 μ m 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, contains 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 (*Zizyphus 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 *Zizyphus 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 $C_{16}H_{18}N_3S$ Cl, 319.85 g/mol and the chemical structure of MB is shown in Fig 1.

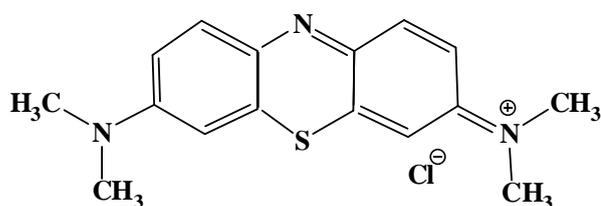


Figure 1: Chemical structure of Methylene Blue

The maximum wave length λ_{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 soluble 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 (01- 0.5 g), particle size (177, 250, 841 and 2000 microns), initial dye concentration ($10\text{--}50 \text{ mg L}^{-1}$) 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 $\text{C}=\text{O}$ 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 $1200\text{--}950 \text{ cm}^{-1}$ 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].

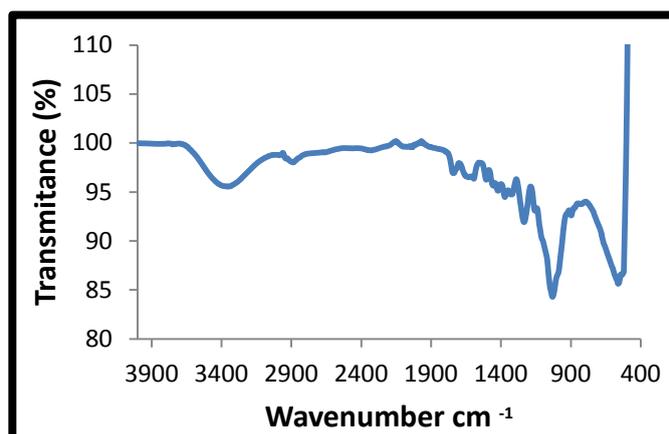


Figure 2: IR analysis of raw Napak Seeds

III.1.2. Thermogravimetric 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 $10 \text{ }^{\circ}\text{C.min}^{-1}$. NS sample exhibited three mass losses. The first one of 5% was related to the loss of water molecules in the range of RT to $100 \text{ }^{\circ}\text{C}$. The second mass loss in the range of 200 to $370 \text{ }^{\circ}\text{C}$, 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 $317 \text{ }^{\circ}\text{C}$ [16-19].

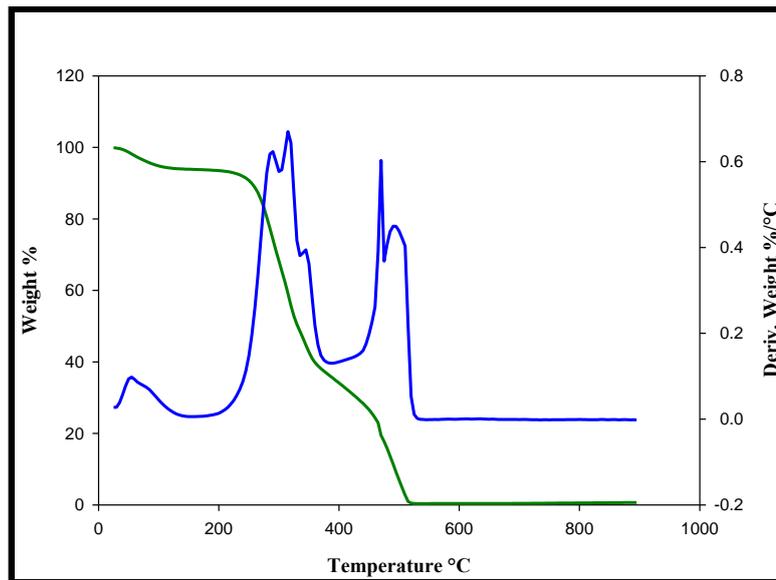


Figure 3: Thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) curves of Napak Seeds.

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 , mg/g) was measured as follows:

$$q_e = \frac{(C_0 - C_e) \times V}{m} \tag{1}$$

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.

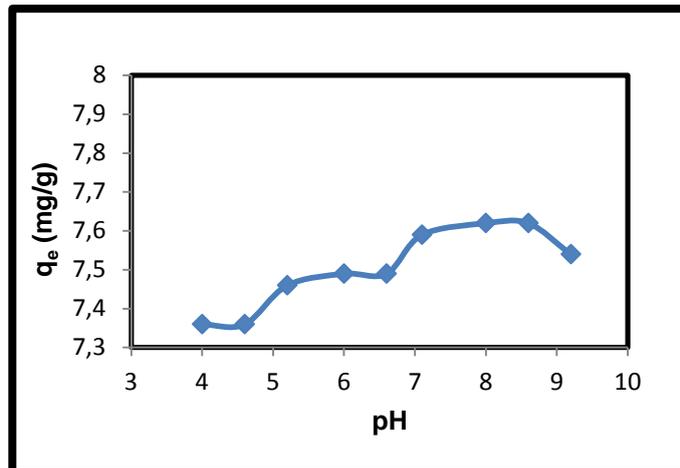


Figure 4: Effect of pH on uptake capacity of MB by Napak Seeds

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.

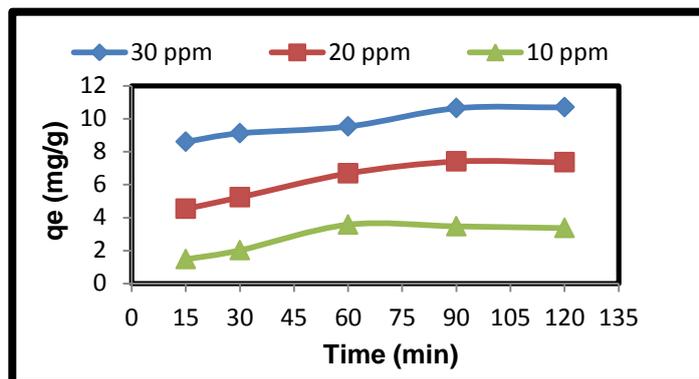


Figure 5: Effect of contact time uptake capacity of MB by Napak Seeds

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:

Influence of particle size on dye sorption was investigated 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.

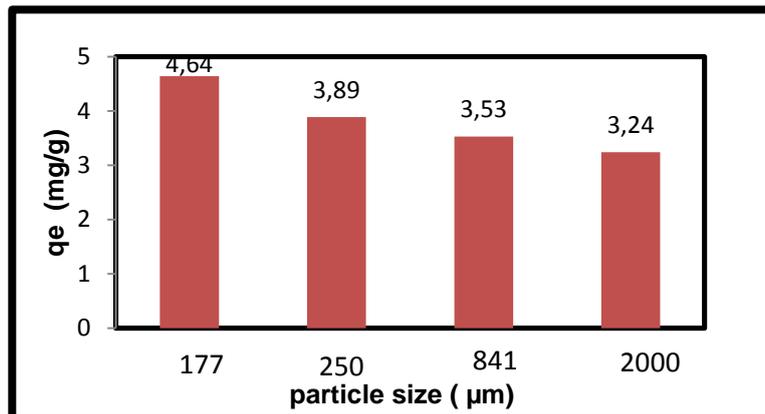


Figure 6: Effect of particle size of Napak Seeds on uptake capacity of MB dye

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].

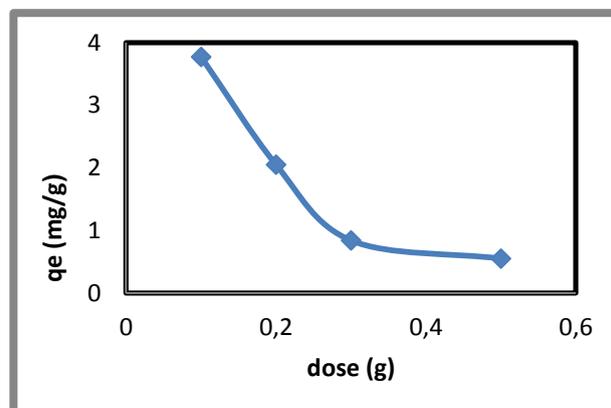


Figure 7: Effect of particle dose on uptake capacity of MB dye by NS

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:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{2}$$

The linear form of Langmuir equation is

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m K_L} \tag{3}$$

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:

$$q_e = K F C_e^{1/n} \tag{4}$$

$$\ln q_e = \ln KF + \frac{1}{n} \ln C_e \tag{5}$$

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.

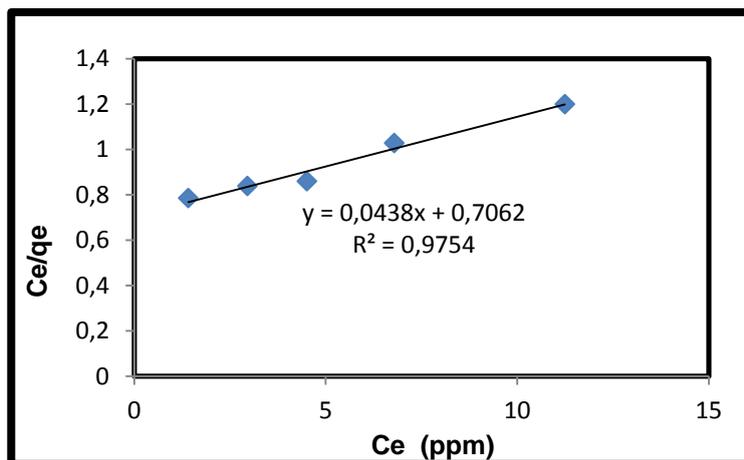


Figure 8: Langmuir isotherm for sorption of MB onto NS.

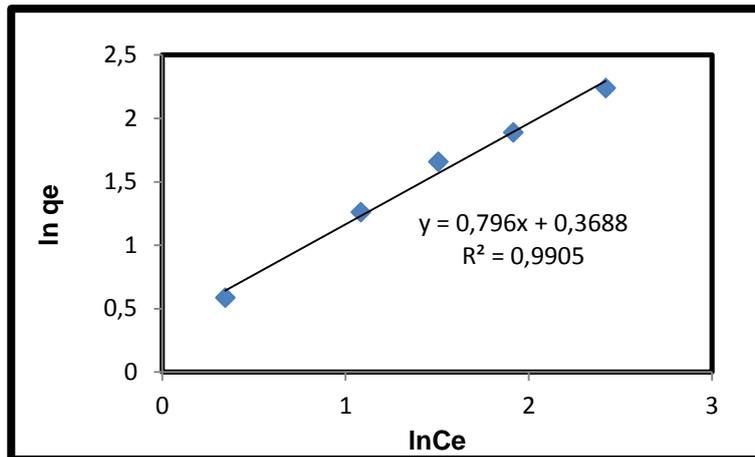


Figure 9: Freundlich isotherm for sorption of MB onto NS

Table 1: Isotherm constants for the sorption MB onto NS

Langmuir parameters				Freundlich parameters		
q _m (mg/g)	K _L	R ²	K _f	n	1/n	R ²
23.25	0.06	0.975	1.4	1.2	0.8	0.99

From the previous isotherms all R² values for Langmuir and Freundlich were great 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 physisorption 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

- [1] Prez-Marin, A.B.; Ballester, A ; Gonzlez, F.; Blzquez, M.L.; Muoz, J.A.; Sez, J.et al. Study of cadmium, zinc and lead biosorption by orange wastes using the subsequent addition method. *Bioresource Technology*, 99 (2008) 8101-8106.
- [2] Benaissa, H., Screening of new sorbent materials for cadmium removal from aqueous solutions. *Hazardous Materials*, 132 (2006)189-195.
- [3] Pino, G.H.; De Mesquita, L.M.S.; Torem, M.L. and Pinto, G.A.S. Biosorption of cadmium by green coconut shell powder. *Minerals Engineering*, 19 (2006) 380-387.
- [4] Ofomaja, A. E. and HO, Y.S. Effect of pH on cadmium biosorption by coconut copra meal, *Journal of Hazardous Materials*,139 (2007) 356–362.
- [5] Blazquez, G.; Hernainz, F.; Calero, M. and Ruiz-Nunez, L.F. Removal of cadmium ions with olive stones: the effect of some parameters. *Process Biochemistry*,40 (2005) 2649-2654.
- [6] Khattri, S.D. and Singh, M.K. Removal of malachite green from dye wastewater using neem sawdust by adsorption. *J Hazard Mater*, 167 (2009) 1089–1094.

- [7] Kyzas, G. Z.; Fu, J. and Matis. K.A. The Change from Past to Future for Adsorbent Materials in Treatment of Dyeing Wastewaters- A review. *Materials*, 6 (2013) 5131-5158.
- [8] Bhatnagar, A. and Sillanpää, M..Utilization of agro-industrial and municipal waste materials as potential adsorbents for water treatment- A review. *Chemical Engineering Journal*, 157 (2010) 277–296.
- [9] Osman, M.A. and Ahmed, M.A.Chemical and proximate composition of (*Zizyphus spina-christi*) nabag fruit, *Nutrition & Food Science*, 39 (2009)70-75.
- [10] Dohrmann, R. Cation exchange capacity methodology I: An efficient model for the detection of incorrect cation exchange capacity and exchangeable cation results. *Applied Clay Science*, 34 (2006) 31-37.
- [11] Meier, L.P. And Kahr, G.Determination Of The Cation Exchange Capacity (CEC) Of Clay Minerals Using The Complexes Of Copper(II) Ion With Triethylenetetramine And Tetraethylenepentamine. *Clays and Clay Minerals*, 47 (1999) 386-388.
- [12] Yukselen, Y. and Kaya, A.Suitability of the methylene blue test for surface area, cation exchange capacity and swell potential determination of clayey soils. *Engineering Geology*, 102 (2008) 38- 45.
- [13] Araújo, C.S.; Alves, V.N.; Rezende, H.C.; Almeida, I.L.; de Assunção, R.M.; Tarley, C.R.; et.al Characterization and use of Moringa oleifera Seeds as biosorbent for removing metal ions from Aqueous effluents. *Water Science & Technology—WST*, 62 (2010) 2198-2203.
- [14] Belala, Z.; Jeguirim, M.; Belhachemi, M.; Addoun, F. and Trouvé, G. Biosorption of basic dye from aqueous solutions by Date Stones and Palm-Trees Waste: Kinetic, equilibrium and thermodynamic studies. *Desalination*, 271 (2011) 80–87.
- [15] Akar, T.; Anilan, B.; Gorgulu, A. and Akar, S.T., Assessment of cationic dye biosorption characteristics of untreated and non-conventional biomass: *Pyraecanthus coccinea* berries, *J. Hazardous Materials*, 168 (2009)1302–1309.
- [16] Pavan, F.A.; Lima, E.C.; Dias, S.L. and Mazzocato, A. C. Methylene blue biosorption from aqueous solutions by yellow passion fruit waste, *J. Hazardous Materials*, 150 (2008), P.P.703–712
- [17] Liu, C.; Yuan, H.; Yang, J. and Li, B., Effective biosorption of reactive blue 5 by pH-independent lyophilized biomass of *Bacillus megaterium*, *African Journal of Biotechnology*, 10 (2011)16626-16636.
- [18] Babiker, M.E.; Aziz, A.R.A.; Heikal, M.; Yusup, S. and Abakar, M. Pyrolysis Characteristics of Phoenix Dactylifera Date Palm Seeds Using Thermo-Gravimetric Analysis (TGA). *International Journal of Environmental Science and Development*, 4 (2013) 521-524.
- [19] Al-Swaidan, H.M. and Ahmad A., Synthesis and Characterization of Activated Carbon from Saudi Arabian Dates Tree's Fronds Wastes, 3rd International Conference on Chemical, Biological and Environmental Engineering, 20 (2011) 25-31.
- [20] Ribeiro, G.C.; Coelho, L.M.; Oliveira, E. and Coelho, N.M. Removal of Cu(II) from ethanol fuel using mandarin peel as biosorbent. *Bio Resources*, 8 (2013) 3309-3321.
- [21] Mayer, Z. A. Apfelbacher, A. and Hornung, A. Effect of Sample Preparation on the Thermal Degradation of Metal-Added Biomass, *Journal of Analytical and Applied Pyrolysis*, 94 (2012) 170–176
- [22] Maurya, N.S.; Mittal, A.K. and Cornel, P. Evaluation of adsorption potential of adsorbents: A case of uptake of cationic dyes, *Journal of Environmental Biology*, 29 (2008) 31-36.
- [23] Doğan, M.; Abak, H. and Alkan, M, Biosorption of Methylene Blue from Aqueous Solutions by Hazelnut Shells: Equilibrium, Parameters and Isotherms. *Water Air Soil Pollut*, 192 (2008)141–153.
- [24] Ben Hamissa, A. M.; Ncibi, M. C.; Mahjoub, B. and Seffen, M. Biosorption of metal dye from aqueous solution onto *Agave americana* (L.) fibres. *Int. J. Environ. Sci. Tech*, 5 (2008) 501-508.
- [25] Znu, Y.; Wang, D.; Zhang, X. and Qin, H. Adsorption removal of methylen blue from aqueous solution by using bamboo share coal, *Fresenius Environmental Bulletin*, 18 (2009) 369-376.
- [26] Lata, H.; Mor, S.; Garg, V.K. and Gupta, R.K. Removal of a dye from simulated wastewater by adsorption using treated parthenium biomass, *Journal of Hazardous Materials*, 153 (2008) 213–220.
- [27] Tumin, N.D.; Chuah, A.L.; Zawani, Z. and Abdul Rashid, S. Adsorption Of Copper from Aqueous Solution by Elais Guineensis Kernel Activated Carbon. *Journal of Engineering Science and Technology*, 3 (2008) 180-189.
- [28] Das, S.K.; Bhowal, J.; Das, A.R. and Guha, A.K. Adsorption Behavior of Rhodamine B on *Rhizopus oryzae* Biomass, *Langmuir*, 22 (2006) 7265-7272.
- [29] Sivasamy, A.; Nethaji, S. and Nisha, L.L, Equilibrium, kinetic and thermodynamic studies on the biosorption of reactive acid dye on *Enteromorpha flexuosa* and *Gracilaria corticata*. *Environ. Sci. Pollut. Res.*, 19 (2012)1687–1695
- [30] Premkumar, M.P.; Kumar, V.V; Kumar. P.S.; Baskaralingam, P.; Sathyaselvabala, V.; Vidhyadevi, T.; et. al. Kinetic and equilibrium studies on the biosorption of textile dyes onto *ovata* seeds, *Korean Journal of Chemical Engineering*, 30 (2013)1248-1256.

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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 Maurbhanj 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 aegyptica*, *Cucumis melo*, *Cucumis mexima*, *Cocinia grandis*, *Tricosanthes cucumerina*, *Tricosanthes tricuspida* and *Momordica charantia* belonging to the family Cucurbitaceae, are mostly wild in nature and commonly used to cure different diseases by these people. *Tricosanthes tricuspida*, (Plate1) locally called as “Mahakaal” is popular of this area due to its attractive red coloured fruits [12]. *T. tricuspida* 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. tricuspida* 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 ethnobotanical 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. tricuspida* were confirmed by cross check with informants. Plant species was confirmed with the Flora's Book and published article [16, 12].



Plate 1: Collection of Plant materials, 1: Fruits of *T. tricuspidata*, 2: Collection of fruits at Similipal Biosphere Reserve, 3: Collection of ripen fruits at Hatibadi village

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 (Plate1).

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

Phyto-chemical 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].

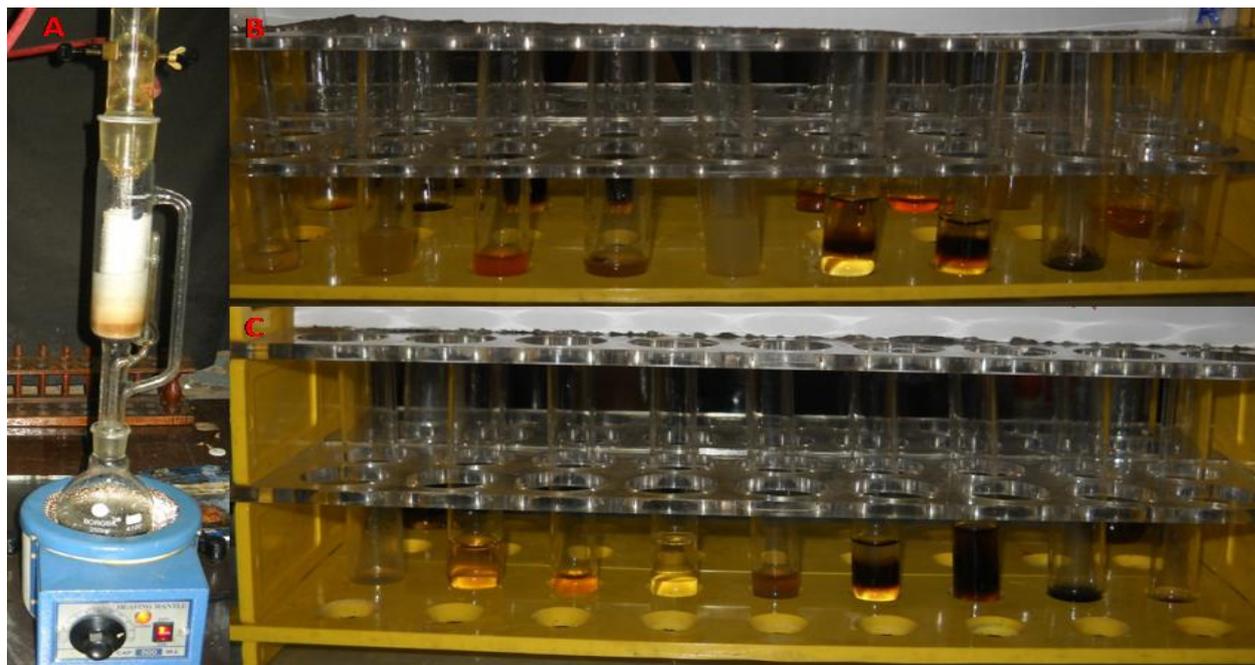


Plate 2: Extract preparation and qualitative estimation of bioactive compounds present in fruit extracts

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 glycolic 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 shaken. 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 μ l per well ranged from 0.25 and 0.5 mg / ml. 100 μ l 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 $35 \pm 2^\circ\text{C}$ 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 \pm 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.

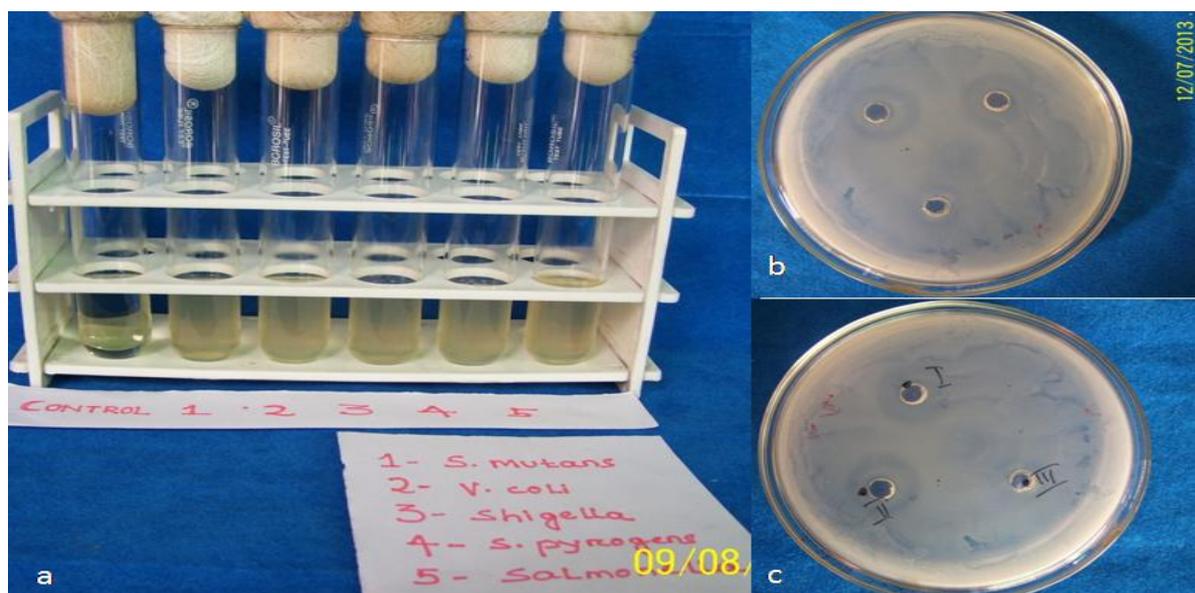


Plate 3: Antibacterial activity, a: broth with control; b & c: zone of inhibition of methanolic extract against MTCC 1926.

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 1: Ethnobotanical values of Mahakaal (*T. tricuspidata* L.) fruit among the rural and tribal communities of Similipal Biosphere reserve and its adjoining areas

Informants	Sex	Age	Collection site	Forms of use(s)	Mode of use(s)
Abhiram Bisad	Male	45	Hatibadi	Paste, external	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.
Laxaman soi	Male	52	Hatibadi	Paste	Dry Fruits are macerated with fruit oil of <i>Azadirachta indica</i> (Neem) and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.
Bajraj Chandra Nayak	Male	38	Hatibadi	Paste	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.
Morgad Nayak	Male	51	Kadlibadi	Paste	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.
Jairam Futi	Male	44	Kadlibadi	Paste	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 <i>Ricinus cumunis</i> (Joda) to cure itching on skin.
Rama Ho	Male	40	Padampur	Paste	Black pulp of ripen fruits are dried and powdered. Powder are mixed with seed oil of <i>Pongamia pinnata</i> (Karanja) and made paste. Paste is applied on lesions of fungal infections on feet, particularly during rainy seasons.
Bnamali Ho	Male	41	Padampur	Paste	Dry Fruits are macerated with fruit oil of <i>Azadirachta indica</i> and camphor. Macerated paste is applied on head and kept it about 1h to kill lice thrice in a week before bath.
Balram Munda	Male	55	Padampur	Paste	Dry Fruits are macerated with fruit oil of <i>Azadirachta indica</i> and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.

Table 2 : Qualitative analysis of bioactive compounds present in fruits of Mahakaal (*T. tricuspidata* L.)

Solvents	bioactive compounds
n-butanol	Terpenoids
Acetone	Flavonoids, Phenolic compounds
Methanol	Flavonoids, Alkaloids, Steroids, Phenolic compounds
Aqueous	Tannin, Saponin, Flavonoids, Phenolic compounds

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.

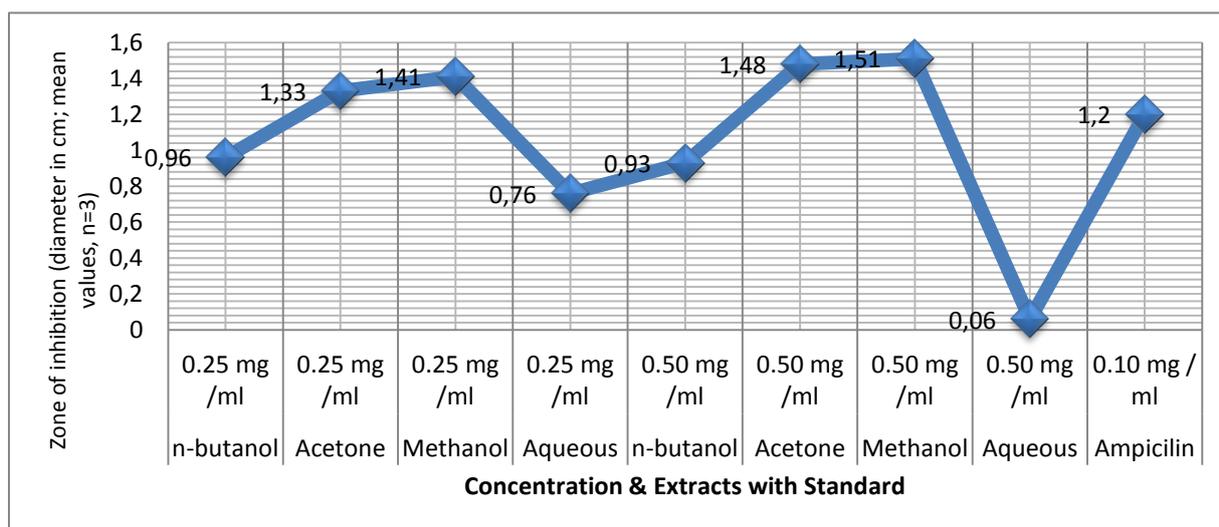


Figure 1: Antibacterial activity against MTCC 1926

Table 3: Antibacterial activity of fruit extracts of Mahakaal (*T. tricuspidata* L.)

Antibacterial activity (Agar well diffusion assay, Zone of inhibition, cm; mean \pm SD; n=3)					
Strain	n-butanol	Acetone	Methanol	Aqueous	Concentration
MTCC 1252	0.73 \pm 0.02	1.20 \pm 0.02	1.18 \pm 0.07	0.76 \pm 0.02	0.25 mg/ml
MTCC 1457	0.91 \pm 0.05	1.21 \pm 0.02	1.25 \pm 0.05	0.76 \pm 0.02	
MTCC 3906	0.88 \pm 0.02	1.16 \pm 0.02	1.28 \pm 0.02	0.78 \pm 0.02	
MTCC 1926	0.96 \pm 0.02	1.33 \pm 0.02	1.41 \pm 0.02	0.76 \pm 0.02	
MTCC *497	0.95 \pm 0.05	1.16 \pm 0.02	1.23 \pm 0.02	0.86 \pm 0.02	
MTCC 1252	0.96 \pm 0.02	1.40 \pm 0.05	1.51 \pm 0.02	0.98 \pm 0.02	0.5 mg/ ml
MTCC 1457	0.98 \pm 0.02	1.41 \pm 0.02	1.43 \pm 0.02	0.95 \pm 0.05	
MTCC 3906	0.96 \pm 0.02	1.46 \pm 0.05	1.48 \pm 0.02	0.98 \pm 0.02	
MTCC 1926	0.93 \pm 0.05	1.48 \pm 0.02	1.51 \pm 0.02	1.06 \pm 0.05	
MTCC *497	0.95 \pm 0.05	1.18 \pm 0.07	1.16 \pm 0.11	0.96 \pm 0.02	
Ampicilin (zone of inhibition, cm, mean \pm SD; n=3)					
MTCC 1252	1.10 \pm 0.00		0.1 mg / ml		
MTCC 1457	1.40 \pm 0.00				
MTCC 3906	1.20 \pm 0.00				
MTCC 1926	1.20 \pm 0.00				
MTCC *497	1.40 \pm 0.00				

(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

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

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.

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VI. REFERENCES

- [1] Saxena, H.O., Brahmam, M.M. The flora of Similiphar (Similipal). Orissa, with particular reference to the potential economic plants, Regional Research Laboratory: Bhubaneswar, India, 1989; pp. 1-231.
- [2] Misra, R. C., Sahoo, H.K., Pani, D. R., Bhandari, D.C. Genetic resources of wild tuberous food plants traditionally used in Similipal Biosphere Reserve, Odisha, India. *Genetic Resource Crop Evolution.*, Doi 10.1007/s10722-013-9971-6, (2013).
- [3] Misra, R.C., Sahoo, H.K., Mahapatra, A. K., Reddy, R.N. Addition to the Flora of Similipal Biosphere Reserve, Orissa, India. *Journal of Bombay Natural History Society.*, 108: 1 (2011) 69-76.
- [4] Kumar, S., Jena, P. K., Tripathy, P.K. Study of wild edible plants among tribal groups of Similipal Biosphere Reserve forest, Odisha, India; with special reference to *Dioscorea* species. *International Journal of Biological Technology.*, 3:1 (2012) 11-19.
- [5] Thatoi, H.N., Panda, S.K., Rath, S.K., Dutta, S.K. Antimicrobial activity and ethnomedicinal uses of some medicinal plants from Similipal Biosphere Reserve, Orissa. *Asian journal of Plant Sciences.*, 7:3 (2008) 260-267.
- [6] Reddy, C.S., Pattnaik, C., Mohapatra, A., Biswal, A. K. Phytosociological observation on tree diversity of Tropical forest of Similipal Biosphere Reserve, Orissa, India. *Taiwania.*, 52:4 (2007) 352-359.
- [7] Mohanta, R.K., Rout, S.D., Sahu, H.K. Ethnomedicinal plant resources of Similipal Biosphere Reserve, Orissa, India. *Zoo's Print Journal.*, 21:8 (2006) 2372-3274.
- [8] Panda, S.K, Rout, S.D., Mishra, N., Panda, T. Folk uses of some medicinal plants by Kol tribes of Similipal Biosphere Reserve, Orissa, India. *International Journal of Biological Technology.*, 2:1 (2011) 16-20.
- [9] Mohanty, B.B. Tribal Culture in Orissa. In: Tribes of Orissa. Scheduled Castes & Scheduled Tribes Research and Training Institute, Bhubaneswar, India, 2004; pp. 1-293.
- [10] Behera, K.K., Mandal, P., Mahapatra, D. Green Leaves for diarrheal diseases used by the tribals of Kenojhar and Mayurbhanj district of Orissa, India. *Ethnobotanical Leaflets.*, 10 (2006) 305-328.
- [11] Rout, S.D., Panda, S.K., Mishra, N., Panda, T. Role of tribals in collection of commercial Non-timber forest products in Mayurbhanj district, Orissa. *Stud Tribes Tribals.*, 8:1 (2010) 21-25.
- [12] Saxena, H.O., Brahmam, M. The flora of Orissa, Vol-2; Orissa Forest Development Corporation Ltd. & Regional Research Laboratory, Bhubaneswar, India, 1995. Pp-752-753.
- [13] Christian, R.V., Brigitte, V.L., Tools and methods for data collection in ethnobotanical studies of homegardens. *Field Method*, 16:3 (2004) 285-306.
- [14] Hawkes, J.G. Crop Genetic Resources, In: field Collection Manual. International Board of Plant Genetic Resources, Rome Italy, 1980.

- [15] Cotton, C.M. *Ethnobotany : Principles and Applications*. Wiley, Chichester, 1996.
- [16] Tripathy, P.K., Kumar, S., Jena, P.K. Diversity and ethno-botanical assessment of some wild cucurbits of Similipal Biosphere Reserve forest, Odisha, India. In *Recent Advances in Plant Biotechnology, Proceeding of the P. Parija Memorial National Conference, Cuttack, India, December 22-23, 2012*; Jena, P.K., Naik, S. K., Barik, D.P. , Department of Botany, Ravenshaw University, Cuttack, India. pp.77-83.
- [17] Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., Phytochemical screening and extraction: A review. *International Pharmaceutical Sciencia*, 1:1 (2011) 98-106.
- [18] Kumar, S. Qualitative studies of bioactive compounds in leaf of *Tylophora indica* (Burm.f.) Merr. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2:3 (2011) 1188-1192.
- [19] Misra, R.C., Kumar, S., Pani, D.R., Bhandari, D.C., Empirical tribal claims and correlation with bioactive compounds: a study on *Celastrus paniculata* Willd., a vulnerable medicinal plant of Odisha. *Indian Journal of Traditional Knowledge*, 11:4 (2012) 615-622.
- [20] Sofowora, A. *Medicinal plants and traditional medicine in Africa*. Spectrum Books Limited. Ibadan, (1993).
- [21] Trease, G.E., Evans, W.C. *Pharmacognosy*, WB Scanders Company, Ltd. London, (1989) 89-300.
- [22] Allen, K.L., Molan, P.C., Reid, G.M., A Survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmaceutical*, 18:1 (1991) 39-44.
- [23] Duvay, B.K, Goyel, R., Parasara, B., Verma, D., Hameja, H., Sharma, D. *Trichosanthes tricuspidata* : Exploration of its medicinal value. *Asian Journal of Pharma Tech*, 2:1 (2012) 26-28.
- [24] Bhandari, S., Dobhal, u., Sajwan, m., bisht, N. S. *Trichosanthes tricuspidata*: A medicinally important plant. *Trees for Life Journal*, 3:5 (2008) 1-4.
- [25] Shweta, S.S., Priyanka, T., Ganesh, G. T., Khadabadi, S.S. Distribution and ancient-recent medicinal uses of *Trichosanthes* species. *International Journal of Phytopharmacy*, 2:4 (2012) 91-97.
- [26] Pasquale, R.D., Circosta, C., Occhiuto, F., Rosa, S., Stefano, S. Pharmacological studies on terpenoids from marine sponges: Analgesic and muscle relaxant effects. *Phytotherapy Research*, 5:2 (2006) 49-53.
- [27] Kawo, A.H., Mustapha, A., Abdullahi, B.A., Rogo, L.D., Gaiya, Z.A., Kumurya, A.S. Phytochemical properties and antibacterial activities of the leaf and latex extract of *Caliotropis procera*. *Journal of Pure and Applied Sciences*, 2:1 (2009) 34-40.
- [28] Mbagwu, F.N., Okator, V.U., Ekeanyanwu, J., Phytochemical screening on four edible legumes (*Vigna subterranean*, *Glycine max*, *Arachis hypogea* and *Vigna unguiculata*) found in Eastern Nigeria. *African Journal of Plant Sciences*, 5:6 (2011) 370-372.
- [29] Middleton, E., Kandaswami, C., Theoharises, T.C. The effect of plant flavonoids on mammalian cells: implication for inflammation, heart disease and cancer. *The American Society for Pharmacology and Experimental Therapeutics*, 52:4 (2000) 673-751.
- [30] Murugan, S., Devi, U.P., Parameswari, N.K., Mani, K.R. Antimicrobial activity of *Syzygium jambos* against selected human pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3 (2011) 44-47.
- [31] Murugesan, S., Pannerselvam, A., Tangavelou, A. C. Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burn. F. *Journal of Applied Pharmaceutical Science*, 1:1 (2010) 42-45.
- [32] Silveira, F., Cibulski, S.P., Varela, A.P., Marques, J.M., Chabalqoity, A., De Costa, F., Yendo, A.C., Gosmann, G., Roche, P.M., Fernandez, C., Ferreira, F. *Quillaja brasiliensis* saponins are less toxic than quill A and have similar properties when used as an adjuvant for a viral antigen preparation. *Vaccine*, 15 :29 & 49 (2011) 9177-9182.
- [33] Mohan, V.R., Kalidas, C. Nutritional and anti-nutritional evaluation of some unconventional wild edible plants. *Tropical and Subtropical Agro ecosystem*, 12 (2010) 494-506.
- [34] Okwe, D.E., Okwe, M.E. Chemical composition of *Spondias mombin* L., plant parts. *Journal of Sustainable Agriculture and Environment*, 6 (2004)140-147.
- [35] Kumar, S., Behera, S.P., Jena, P.K. Validation of tribal claims on *Dioscorea pentaphylla* L. through phytochemical screening and evaluation of antibacterial activity. *Plant Science Research*, 25:1&2 (2013) 55-61.

- [36] Musa, A.M., Aliya, A.B., Yaro, A.H., Magaji, M.G., Hassan, H.S., Abdullahi, M. Preliminary phytochemical analgesic and anti-inflammatory studies of the methanol extract of *Anisopus mannii* in rodents. *African Journal of Pharmacy and Pharmaceutical*, 3:8 (2009) 374-378.
- [37] Majumdar, S.H., Chakaborty, G.S., Kulkarni, K.S. Medicinal potential of *Semecarpus anacardium* nut: A review. *Journal of Herbal Medicine and Toxicology*, 2:2 (2008) 9-13.
- [38] Gloria, A.A., Solomon, S.I. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* Oliv (Guttiferae). *International Journal of Health and Research*, 1:2 (2008) 87-93.
- [39] Mustafa, F., Indurkar, J., Ismail, S., Mordi, m.n., Ramanathan, S., Mansor, S.M. Analgesic activity, toxicity study and phytochemical screening of standardized *Cinnomomum iners* leaves methanolic extract. *Pharmacognosy Research*, 2:2 (2010) 76-81.
- [40] Govindasamy, C., Arulpriya M. Antimicrobial activity of *Acanthus ilicifolius*: skin infection pathogens. *Asian Pacific Journal of Tropical Disease*, 3:3 (2013) 180-183.
- [41] Johansson, I., Thulin, P., Donals, E.L, Teglund, A.N. Getting under the skin: The immunopathogenesis of *Streptococcus pyogenes* deep tissue infections. *Clinical Infections Diseases*, 51:1 (2010) 58-65.
- [42] Misra, B.K. Conservation and management effectiveness of Similipal Biosphere reserve, Orissa, India. *Indian Forester* (2010) 1310-1326.

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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 livers 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 m³, insufficient for a mean daily volume (3000 m³) 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].

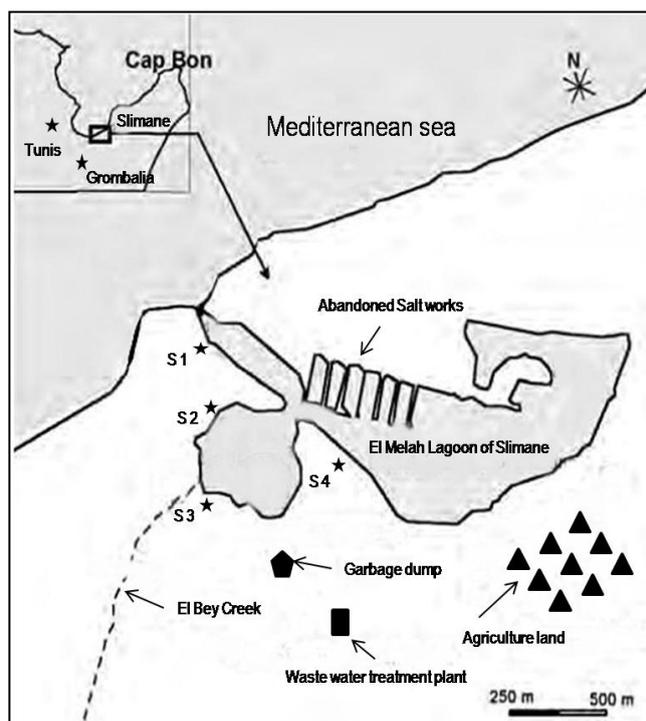


Figure 1: Map of the El Melah Lagoon showing the four sampling sites from where mallards were hunted (S1, S2, S3, S4) and the pollution places around the lagoon.

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.

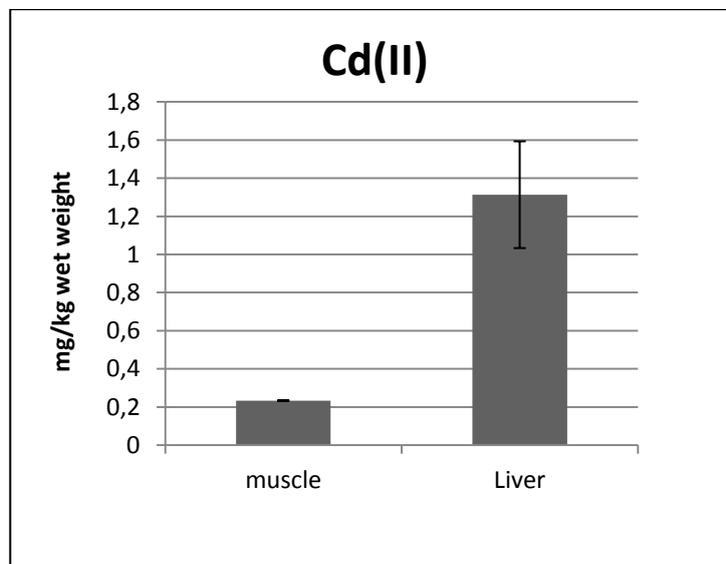


Figure 1: Cd(II) concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)

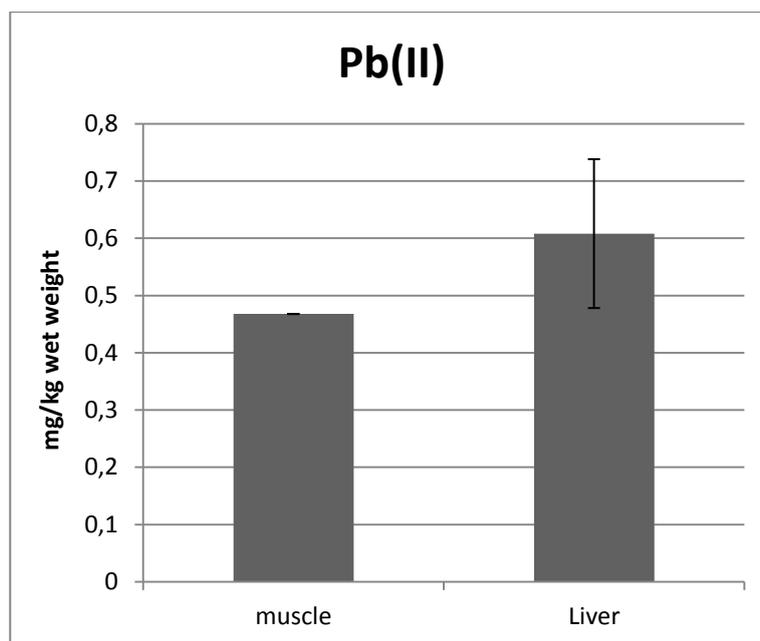


Figure 2: Pb(II) concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)

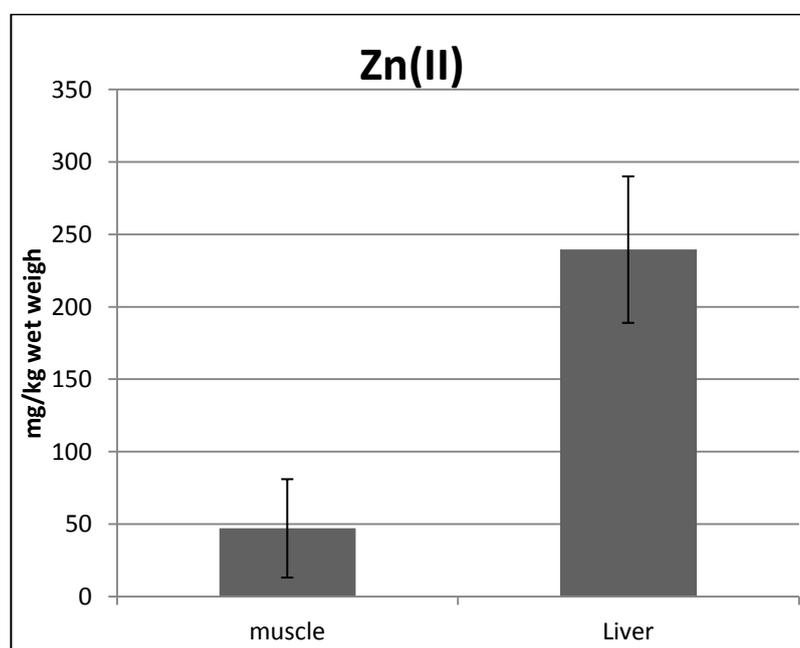


Figure 3: Zn(II) concentrations (mg/kg w.w.) in Livers and muscles of Mallard (n=14)

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

- [1] Gasparik J., Vladarova D., Capcarova M., Smehyl P., Slamecka J., Garaj P., Stawarz R., Massanyi P. (2010). Concentration of Pb(II), cadmium, mercury and arsenic in leg skeletal muscles of three species of wild birds. *Journal of Environmental Science and Health, Part A*, vol. 45, 2010, p. 818-823.
- [2] Eeva Tapio, Eugen Belskii, Alex S. Gilyazov, Mikhail V. Kozlov. (2012). Pollution impacts on bird population density and species diversity at four non-ferrous smelter sites. *Biological Conservation* 150 : 33–41
- [3] Lee D.P., K.-G. Lee, D.-H. Nam (2012). Population declines and heavy metals exposure of Swinhoe's Storm Petrels (*Oceanodroma monorhis*) breeding on the southwest coast of Korea. *Marine Pollution Bulletin*, 64: 2645-2649.
- [4] Knott J, Gilbert J, Hoccom D, Green R. (2010). Implications for wildlife and humans of dietary exposure to Pb(II) from fragments of Pb(II) rifle bullets in deer shot in the UK. *Science of Total Environment*, 409:95–99.
- [5] Baoshan Cui, Qijun Zhang, Kejiang Zhang, Xinhui Liu, Honggang Zhang. (2011). Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta, China. *Environmental Pollution* 159 : 1297-1306.
- [6] Kim J, Koo TH. 2007. Heavy metal concentrations in diet and livers of black-crowned night heron *Nycticorax nycticorax* and grey heron *Ardea cinerea* chicks from Pyeongtaek, Korea. *Ecotoxicology*, 16:411–416.
- [7] Zhang, Jian zhang Ma (2011). Waterbirds as bioindicators of wetland heavy metals pollution *Procedia Environmental Sciences*, 10: 2769-2774.
- [8] Cramp, S. (1998). *Birds of the Western Palearctic, Concise Edition and CD-ROM Set*. EC. 2001. 466/2001. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities.
- [9] Binkowski Łukasz Jakub, Robert Stawarz, M Zakrzewski (2013). Concentrations of cadmium, copper and zinc in tissues of Mallard and Coot from southern Poland, *Journal of Environmental Science and Health Part B*. 410-415.
- [10] Prudêncio M.I., M.I. Gonzalez, M.I. Dias, E. Galan, F. Ruiz (2007). Geochemistry of sediments from El Melah lagoon (NE Tunisia): A contribution for the evaluation of anthropogenic inputs *Journal of Arid Environments*, Volume 69, Issue 2, April 2007, Pages 285-298
- [11] Garcia-Fernandez A.J., Sanchez-Garcia J.A., Gomez-Zapata M., Luna A., (1996). Distribution of cadmium in blood and tissues of wild birds. *Archives of Environmental Contamination and Toxicology* 30 : 252-258.
- [12] KIM J., SHIN J.R., KOO T.H. (2009). Heavy metal distribution in some wild birds from Korea. *Archives of Environmental Contamination and Toxicology*, 56 :317- 324.
- [13] Yamamoto F.Y., F. Filipak Neto, P.F. Freitas, C.A. Oliveira Ribeiro, C.F. Ortolani Machado. (2012). Cadmium effects on early development of chick embryos. *Environmental Toxicology and Pharmacology* 34 : 548-555.
- [14] Kalisinska E., Salicki W., Mys1ek P., Kavetska K.M., Jackowski A. (2004). Using the Mallard to biomonitor heavy metal contamination of wetlands in northwestern Poland. *The Science of the Total Environment* 320: 145e161.
- [15] Măcinic I., Trif Alexandra, Muselin F (2002). Lead level in mallard (*Anas platyrhynchos*). *Veterinary Drug*, Vol. 6 (2) : 80-84.
- [16] Struzynska L, Sulkowski G, Lenkiewicz A, Rafalowska U. 2002 Lead stimulates the glutathione system in selective regions of rat brain. *Folia Neuropathol* 40(4), 203–209.
- [17] Needleman HL, Gunnoe C, Levinton A, et al. 1979 Deficits in psychological and classroom performance of children with elevated dentine lead levels. *New England Journal of Medicine* 300: 689–695.

- [18] Wilson MA, Johnston MV, Goldstein GW, Blue ME. 2000 Neonatal lead exposure impairs development of rodent barrel field cortex. *Proc Natl Acad Sci USA* 97, 5540–5545.
- [19] Florijančić Tihomir, Anđelko Opačak, Ivica Bošković, Dinko Jelkić, Siniša Ozimec, Tanja Bogdanović, Irena Listeš, Mario Škrivanko, Zlatko Puškadija. (2009). Heavy metal concentrations in the liver of two wild duck species: influence of species and gender. *Italian Journal of Animal Sciences*. 8: 222-224.
- [20] Kim J. and Oh J.M. (2012). Metal levels in livers of waterfowl from Korea. *Ecotoxicology and Environmental Safety*. 78, 1 : 162-169
- [21] Nordberg, G.F.; Fowler, B.A.; Nordberg, M.; Friberg, L.T. *Handbook on the Toxicology of Metals*. Elsevier: London, 2007; 445–478, 529–543.

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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} , ΔG_{ads}° , ΔH_{ads}° and ΔS_{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.

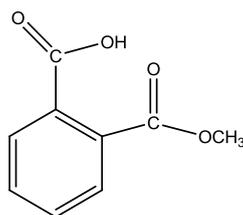


Fig .1: Scheme of acetylsalicylic acid

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.

Table1: The physico-chemical characterization of the decanted water

Parameters	Values
pH	5.7
conductivity (mS/cm)	134.4
TDS (g/l)	67.1
Resistivity(Ω.cm)	7.44
SO ₄ ²⁻ (mg/l)	300
S ²⁻ (mg/l)	0.051
Cl ⁻ (mg/l)	40750
O ₂ dissolved	7.4
Bart test (bacterial corrosion)	Negative

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.

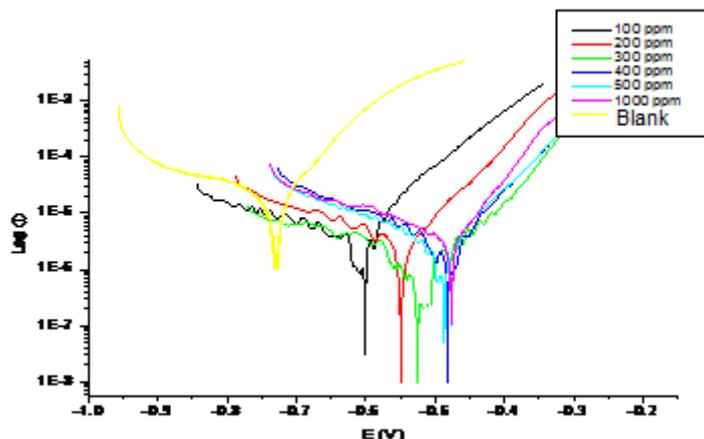


Fig 2: Polarization curves of carbon steel in decanted water in the absence and various concentrations of inhibitor

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:

$$IE\% = (I_0 - I_1 / I_0) \times 100 \tag{1}$$

Where I₀ and I₁ 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.

Inhibitor concentration (ppm)	E _{corr} (mv)	I _{corr} (µA/cm ²)	Θ	%IE
0	-729.4	7.5371	-	-
100	-601.1	1.425	0.810	81.09
200	-549.3	0.914	0.878	87.87
300	-527	0.324	0.9705	97.05
400	-483.2	0.5025	0.933	93.33
500	-487.9	0.6584	0.912	91.26
1000	-476.7	2.335	0.690	69.01

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 Θ on the molar concentration of the inhibitor was calculated using the following equation:

$$\Theta = I_0 - I_1 / I_0 \tag{2}$$

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].

$$C/\Theta = C+1/K \quad (3)$$

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_{inh}/Θ (ppm) were plotted against C_{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.

$$K=(1/55.55)\exp(-\Delta G^{\circ}_{ads}/RT) \quad (4)$$

The negative value of ΔG°_{ads} (-34.832 kJ mol⁻¹) indicate that adsorption of the inhibitor on the metal surface is spontaneous.

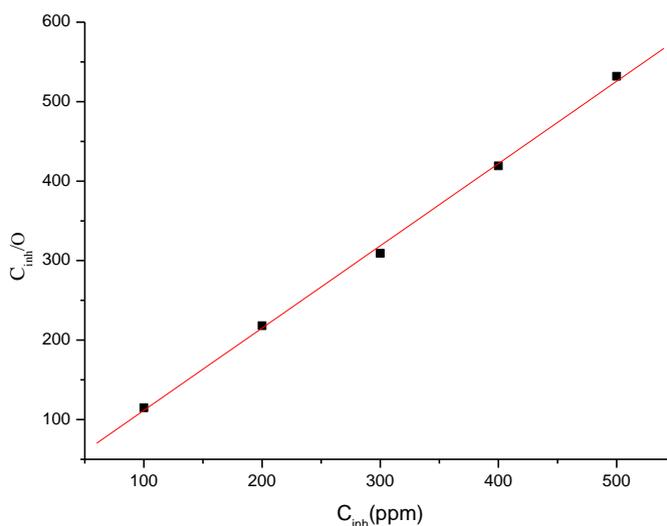


Fig.3. Plot of C_{inh}/Θ versus C_{inh} for carbon steel in decanted water solution in presence of different concentrations of inhibitor

Generally, values of ΔG°_{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 ΔG°_{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_{corr} , I_{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_{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.

Temperature °C	Concentration (ppm)	I_{corr} (mA.cm ⁻²)	E_{corr} (mv)	k	%IE
25	0	7.5371	-729.4	85.92	-
	300	0.324	-527	2.532	97.05
30	0	39.242	-705.3	281.896	-
	300	5.1089	-403.4	36.7	80.95
35	0	55.905	-689.2	401.6	-
	300	14.913	-402.8	107.2	77.55
40	0	129.115	-663.5	927.5	-
	300	34.565	-400.7	248.3	73.22

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

$$k = A \exp(-E_a^*/RT) \tag{5}$$

Where E_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_{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.

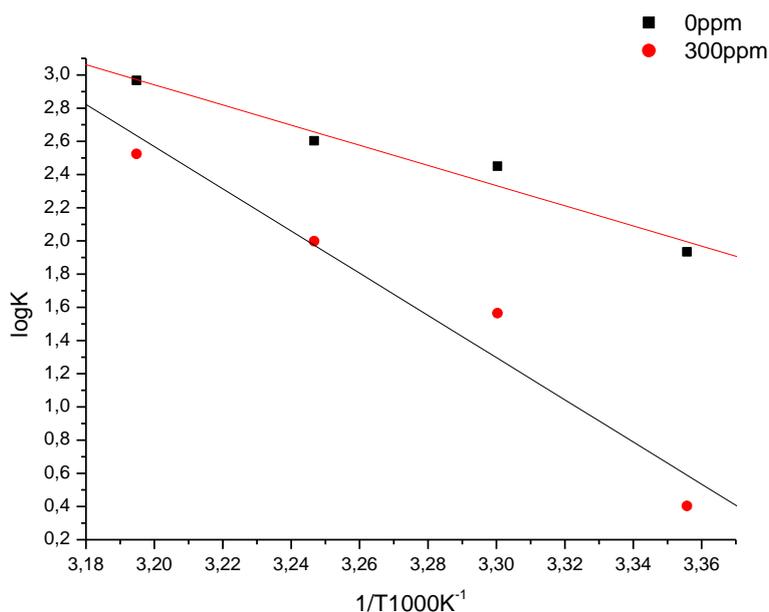


Fig.4. Plot of $\log(k)$ versus $1/T \times 10^3, K^{-1}$ for carbon steel in decanted water Solution in absence and in presence of 300ppm of inhibitor.

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

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.

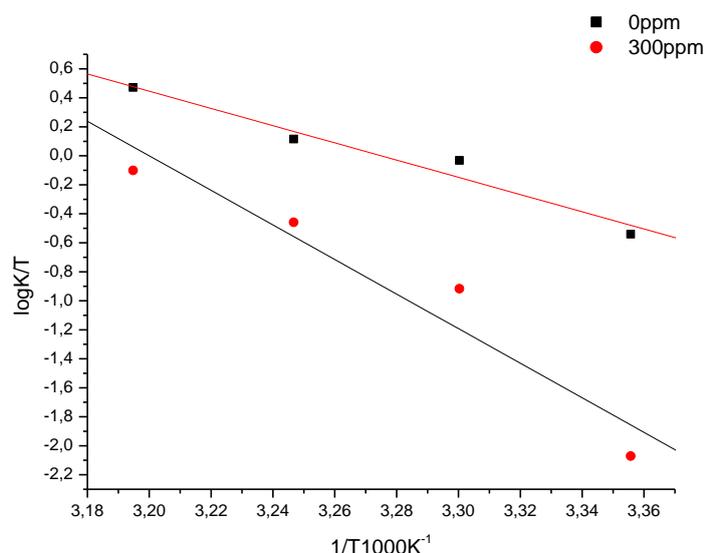


Fig.5. Plot of $\log(\text{corrosion rate}/T)$ versus $1/T \times 10^3, \text{ K}^{-1}$ for carbon steel in decanted water Solution in absence and in presence of 300 ppm of inhibitor.

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

$$\text{Rate} = RT/Nh \exp(\Delta S^*/R) \exp(-\Delta H^*/RT) \quad (6)$$

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.

Concentration (ppm)	$E_a^* (\text{J} \cdot \text{mol}^{-1})$	$\Delta H^* (\text{J} \cdot \text{mol}^{-1})$	$\Delta S^* (\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1})$
0	116.2679	113.7594	286.6372
300	230.735	228.9466	644.7087

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.

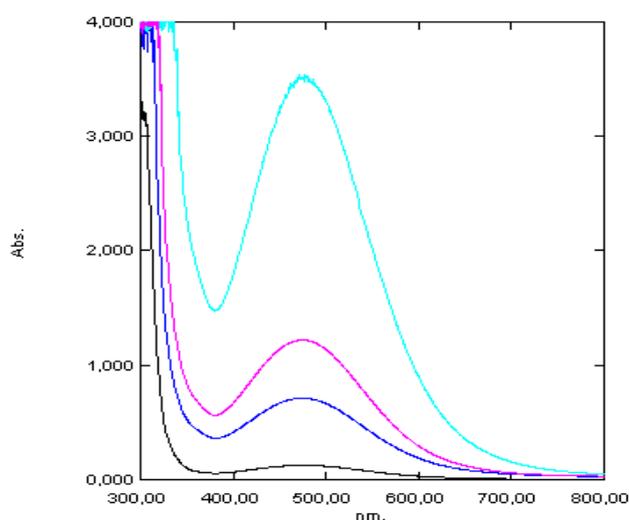


Fig.6. UV-spectra of the solutions containing different concentration of acetylsalicylic acid after 3h immersion of carbon steel.

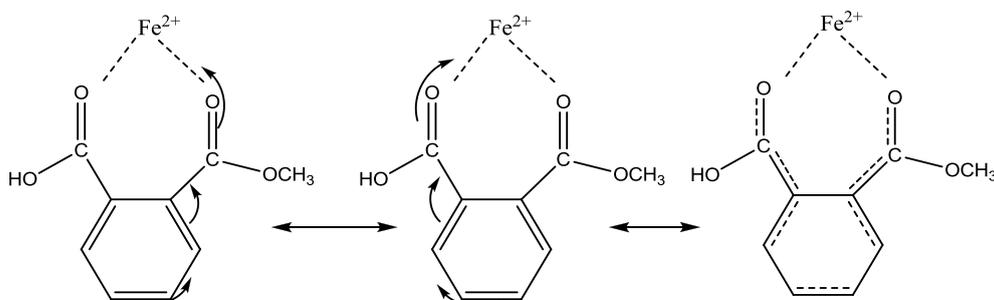


Fig. 7. Schematic representation of adsorption behavior of studied organic compound on carbon steel

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 charge transfer resistance, which could correlate to resistance between the steel and outer helmoholtz 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, $R_s(R_1)$ is the solution resistance, R_p ($R_p=R_f+R_{ct}$) is the polarization resistance, Q_f and $R_f(R_2)$ are constant phase element and the resistance related to the surface film.

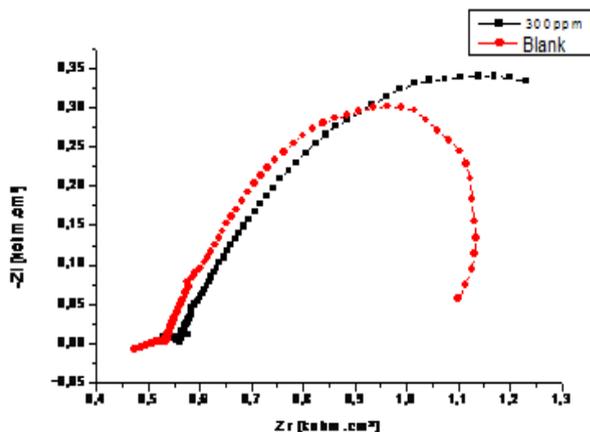


Fig.8. EIS of carbon steel in decanted water in absence and presence of 300ppm

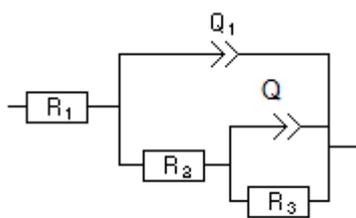


Fig.9. Equivalent circuit used to fit the EIS.

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.

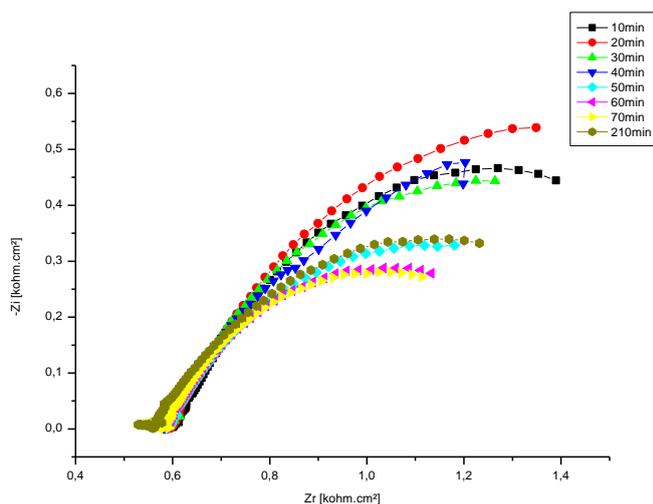


Fig.10. EISs of carbon steel in decanted water in presence of 300ppm of inhibitor at 25°C after different immersion times

A comparison of the results of R_f and R_p with 300ppm 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

Immersion time(min)	$Q_1(\text{Fs}^{-a})$	a_1	R_f (Ohm)	Q (Fs^{-a})	a	R_p
0	0.427e-3	0,66	979	0.241e-6	0,794	785
10	0.356 e ⁻³	0.69	557	0.202 e ⁻⁶	0.63	1 360
20	50.19e-6	0.33	324	1.064e-3	0,71	1 707
30	4,415e-6	0.92	225	1.392e-3	0,672	2072
40	0.1341e-6	0,66	205	1.392e-3	0,67	1 690
50	0.1341e-6	0,67	223	1.114e-3	0.71	926
60	56.74e-6	0,66	285	1.136e-3	0.76	695
70	4,415e-3	0,42	354	1,188e-3	0,67	635
210	0.939 e-3	0,64	1118	7.697e-3	0.52	460

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_a^* , $\Delta G_{\text{ads}}^\circ$ 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 $\Delta G_{\text{ads}}^\circ$ 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

- [1] Trabonelli. G; 'Corrosion Mechanisms', Mansfeld Editor, Marcel Dekker, New York, P.119, 1987.

- [2] Landolt. D., Corrosion et Chimie de Surfaces des Métaux', Presses Polytechniques Universitaires Romandes, pp. 62, 492, 495, 1997.
- [3] Moretti. G ; Guidi. F ; Grion. G; Corros.Sci. 46 (2004) 387-403.
- [4] Sorkhabi. H.A, Seifzadeh. D; Hosseini. M.G; EN, EIS and polarization studies to evaluate the inhibition effect of 3H-phenothiazin-3-one, 7-dimethylamine on mild steel corrosion in 1 M HCl solution, Corros. Sci. 50 (2008) 3363.
- [5] Quraishi. M.A; Jamal. D, Inhibition of mild steel corrosion in the presence of fatty acid triazoles, J. Appl. Electrochem. 32 (2002) 425.
- [6] Elayyachy. M; El Idrissi. A; Hammouti..B, New thio-compounds as corrosion inhibitor for steel in 1 M HCl, Corros. Sci. 48 (2006) 2470.
- [7] Emregul.K.C; Hayvali. M, Studies on the effect of a newly synthesized Schiff base compound from phenazone and vanillin on the corrosion of steel in 2 M HCl, Corros. Sci. 48 (2006) 797.
- [8] Mernari. B; Elattari. H; Traisnel. M; Bentiss. F; Lagrenee. M, Inhibiting effects of 3,5-bis(n-pyridyl)-4-amino-1,2,4-triazoles on the corrosion of mild steel in 1 M HCl medium, Corros. Sci. 40 (1998) 391.
- [9] Wang. L; Evaluation of 2-mercaptobenzimidazole as corrosion inhibitor for mild steel in phosphoric acid, Corros. Sci. 43 (2001) 2281.
- [10] Azhar. M.E; Mernari. M; Traisnel. M; Bentiss. F; Lagrenee. M; Corrosion inhibition of mild steel by the new class of inhibitors [2, 5-bis(n-pyridyl)-1,3,4-thiadiazoles] in acidic media, Corros. Sci. 43 (2001) 2229.
- [11] Roberge. P.R , Corrosion inhibitors, Handbook of Corrosion Engineering, McGraw-Hill, New York, 1999.
- [12] Zhang. Q.B; Hua. Y.X; Corrosion inhibition of mild steel by alkylimidazolium ionic liquids in hydrochloric acid, Electrochim. Acta 54 (2009) 1881–1887.
- [13] Bentiss. F;Traisnel. M; Chaibi. N, Mernari. B; Vezin. H; Lagrenee. M; 2,5-Bis(nmethoxyphenyl)-1,3,4-oxadiazoles used as corrosion inhibitors in acidic media: correlation between inhibition efficiency and chemical structure, Corros. Sci. 44 (2002) 2271–2289.
- [14] Bentiss. F;Traisnel. M; Chaibi. N, Mernari. B; Vezin. H; Lagrenee. M ; 2,5-Bis(4-dimethylaminophenyl)-1,3,4-oxadiazole and 2,5-bis(4-dimethylaminophenyl)-1,3,4-thiadiazole as corrosion inhibitors for mild steel in acidic media, Corros. Sci. 46 (2004) 2781–2792.
- [15] Ali. S.A; Saeed. M.T; Rahman. S.U, The isoxazolidines: a new class of corrosion inhibitors of mild steel in acidic medium, Corros. Sci. 45 (2003) 253–266.
- [16] Yildirim. A; Cetin. M, Synthesis and evaluation of new long alkyl side chain acetamide, isoxazolidine and isoxazoline derivatives as corrosion inhibitors, Corros. sci. 50 (2008) 155–165.
- [17] Savithri.B.V; Mayanna. S, Indian J. Chem. Technol. 3 (1996) 256.
- [18] Martinez.S; Stern. I, J. Appl. Electrochem. 31 (2001) 973.
- [19] Abd El-Rehim. S.S; Ibrahim. M.A.M; K.F. Khaled, Mater. Chem. Phys. 70 (2001) 268.
- [20] Bahrami. M.J ; Hosseini.S.M.A; Pilvar.P, Corros. Sci. 52 (2010) 2793–2803.
- [21] Putilova. I.K; Balezin. S.A; Barasanik. Y.P, Metallic Corrosion Inhibitors, Pergamon Press, Oxford, 1960, p. 30.
- [22] Noor. E.A; Al-Moubaraki. A.H, Thermodynamic study of metal corrosion and inhibitor adsorption processes in mild steel/1-methyl-4[4(-X)-styrylpyridinium iodides/hydrochloric acid systems, Mater. Chem. Phys. 110 (2008) 145.
- [23] Ebenso. E.E; Ekpe. U.J; Ita. B.I; Offiong. O.E; Ibok. U.J; Mater. Chem. Phys. 60 (1999) 79.
- [24] Dinnapa. R.K; Mayanna. S.M; J. Appl. Electrochem. 11 (1982) 111.
- [25] Dinnapa. R.K; Mayanna. S.M; Corrosion 38 (1982) 525.
- [26] Achary G; Arthoba Naik.Y; Vijay Kumar. S; Venkatesha. T.V; Sherigara. B.S; Appl.Surf. Sci. 254 (2008) 5569.
- [27] Solmaz. R et al, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 312 (2008) 7.
- [28] Aljourani. J ; Raeissi. K ; Golozar. M.A; *Corros. Sci.*, 51 (2009) 1836.
- [29] Bhrara. K; Singh. G; Appl. Surf. Sci. 253 (2006) 846.
- [30] Noor. E.A; Al-Moubaraki. A.H; Mater. Chem. Phys.110 (2008)145.
- [31] Gomma. G.K; Wahdan. M.H; Chemistry, third ed., Wiley, Eastern New Delhi, 1995.
- [32] Soliman. M.S; Ph.D Thesis, Alex. Univ., Egypt, 1995.

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V. References

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- [2] Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, Editor 2, Eds.; Publisher: Publisher Location, Country, 2013; Volume 3, pp. 154–196.
- [3] Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2013; pp. 204–215.
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