BIOREMEDIATION OF POLLUTANTS RESPONSIBLE FOR VOLATILE EMISSION OF KALA SANGHIAN DRAIN

Thesis Submitted For the award of Degree of

DOCTOR OF PHILOSOPHY

In

(BOTANY)

By

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2023

DECLARATION

I, hereby declare that the presented work in the thesis entitled "**BIOREMEDIATION OF POLLUTANTS RESPONSIBLE VOLATILE EMISSION OF KALA SANGHIAN DRAIN "**in fulfillment of the degree of **Doctor of Philosophy (Ph. D)** is an outcome of research work carried out by me under the supervision of Dr. Anand Mohan, working as Associate Professor, in the School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgments have been made whenever the work described here has been based on the findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that **Anju Mangotra** has completed the Ph.D. Botany titled, **"BIOREMEDIATION OF POLLUTANTS RESPONSIBLE FOR VOLATILE EMISSION OF KALA SANGHIAN DRAIN "**under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma.

The thesis is fit for submission for the partial fulfillment of the condition for the award of the degree of Ph.D. in botany.

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ABSTRACT

Water contamination in today's scenario stands amid profound apprehension and massive introspection. The world of gigantic challenges is escorting a new dimension towards the treatment of contaminated water.

Water contamination does not concise itself to a single region, rather it spread its arms towards the large population in a specific geographical region. The deleterious effect imposed by nature and human interference has opened severe water challenges. Industrial effluents play a substantial role in manipulating the water quality index. Most industries are very reluctant about the proper treatment of wastewater before discharging it into the water bodies. Improperly treated wastewater adds hazardous pollutants to the environment. Organic pollution is always evident, and the condition becomes worse with the occasional discharge of raw sewage and effluents from industries. The highly polluted drains of developing countries are impairing the normal parameters of the water bodies and Punjab is the most targeted one. The drains near the industries become the bed of carcinogenic and mutagenic compounds. The Kala Sanghian Drain carries the most lethal compounds in high quantity and is located in Jalandhar, Punjab state of India. This drain contains the pollutants from leather industries, motor bearing industries, electroplating, and textile industries.

The primary objective of the present study was to find out the extent of pollution caused by the wastewater of tannery, textile, and electroplating industries into the Kala Sanghian Drain.

The study further gained by the remediation of toxic volatile organic compounds which are accountable for the volatile emission of Kala Sanghian Drain by preparing the innovative hybrid material. To analyze the variations in the physicochemical parameters of wastewater; samples were collected from different sites. Resulting estimates revealed that the values of physicochemical parameters are much higher than the standards set by World Health Organization and are detrimental to human and animal consumption and they can prove hazardous for communities residing in the vicinity.

To identify and analyze the volatile organic compounds released by the industries into the wastewater of the Kala Sanghian Drain, the samples were collected from the selected sites of the drain and were analyzed using gas chromatography with tripled quadrupole mass spectrometry (GC-QqQ-MS/MS) system. This method has given satisfactory results on the spectrum of compounds with different polarity, and volatility as well as having different retention times. On analysis of all volatile organic compounds, dichloromethane, heptane, and cis-1, 2-Dicholoroethene has been observed as the most frequently occurring VOCs in the sample matrix. The volatile organic compounds detected in this drain revealed the type of industries that discharge these effluents. These become a major source of deterioration of the physiology of aquatic and terrestrial entities by cyclical series of pollution events. Some of the detected VOCs behave as carcinogens and mutagens and therefore lethal for the aquatic and terrestrial community. These volatile organic compounds detected from the wastewater are also responsible for the deteriorated taste and odor of the drain.

To evaluate the efficiency of conventional and bioremediation methods in the removal of dichloromethane, four types of adsorbents were prepared from coconut shells and rice husks by applying standard methods. These four adsorbents were coconut shell activated carbon (CSAC), raw rice husk (RRH) (untreated), rice husk chemically treated with 0.5 M NaOH (RHN), and rice husk heated at 300 °C (RHH). These four adsorbents come under the conventional method. The physicochemical properties of these adsorbents were evaluated by applying standard methods. The characterization of CSAC, RRH, RHN, and RHH was investigated by using sophisticated FTIR and FESEM instruments. The UV-Visible spectrophotometer was used to find out the final concentration

Adsorption capacities and removal percentages of samples after treatment with adsorbents were investigated by batch studies. Langmuir and Freundlich adsorption isotherms were used for the evaluation of the adsorption process and the experimental data best fitted the Freundlich model. The most fitting elucidation about the adsorption kinetics for the dichloromethane on adsorbents prepared from coconut shells and rice husks has been given by Pseudo-second-order. Adsorption studies were done by

considering parameters viz., initial pH, and initial concentration of adsorbate, dosage of adsorbent, and contact time on dichloromethane adsorption. In the bioremediation (biodegradation) method, culture, enumeration, and identification tests were done by microscopy and haematocytometer technique. The genus level identification of bacteria was done by biochemical tests and species level using 16s rRNA sequence analysis. GC-FID instrument was used to evaluate the final concentration of dichloromethane.

The result of the final concentration of dichloromethane treated with four adsorbents- coconut shell activated carbon (CSAC) raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN), rice husk heated at 300° C (RHH) (conventional method) and bacteria (bioremediation) was 69.3 mg/L, 74 mg/L, 41.2 mg/L, 37.3 mg/L, 15.33 mg/L respectively. The removal percentage of dichloromethane with four adsorbents- Coconut shell activated carbon (CSAC), raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN), rice husk heated at 300° C (RHH) and bacteria were 65.35 %, 63%,78.4%, 81.35%, and 92.3% respectively.

The preparation of an innovative hybrid material was done by using agricultural byproducts and microorganisms. Four types of hybrid materials were prepared by using coconut shells, rice husk, MOF (UiO66), and bacteria *(Pseudomonas aeruginosa). Pseudomonas aeruginosa* acted as an efficient medium to degrade the volatile organic compound (dichloromethane) with a removal efficiency of 93 % (by taking 50 mg/L of initial conc. of DCM). The efficiency increased from 94 % to 96 %, when the *Pseudomonas aeruginosa* and rice husk were used for biodegradation. The activated carbon, which is prepared from the coconut shell, accelerates adsorption to 99%. It shows activated carbon prepared from the coconut shell along with *Pseudomonas aeruginosa* acted as a good adsorbent to biodegrade or adsorb organic compound (dichloromethane). The removal efficiency *of Pseudomonas aeruginosa* along with MOF (UiO66) completely mirrors the combination of *Pseudomonas aeruginosa* along with rice husk. It showed a removal efficiency of 96%. On the contrary, *Pseudomonas aeruginosa* when combined with rice husk, activated carbon, and MOF (UiO66), speeds up the biodegradation ability of *Pseudomonas aeruginosa* by 98%. The result obtained proves

that the combination of adsorbents from agricultural origin, chemical world, and microorganisms speed up the rate of biodegradation and acted as a potent hybrid material for the removal of volatile organic compounds (DCM).

ACKNOWLEDGMENT

Foremost, I would like to express my thanks to God, the Almighty, for showering His blessings to complete my research successfully. It's my privilege to acknowledge and conveys my gratitude to all those persons who helped and assisted me during my research work.

I would like to express my gratitude to my research supervisor, Dr. Anand Mohan, Associate Professor (Lovely Professional University, Phagwara, Punjab) for providing invaluable guidance during my research work. I am honored to use his immense knowledge and plentiful experience throughout my research work. His patience, zeal, integrity, vision, affectionate encouragement, and positive criticism have profoundly motivated me and made me enthusiastic throughout the academic research work. He has given me the guideline to conduct and present the whole academic research understandably. It was a great esteem and privilege to work and study under his guidance.

I wish to express my thanks to my previous supervisor Dr. Sai Murali and previous Co-supervisor Dr. Shailesh Kumar Singh for their support and guidance in the research work.

I would like to accord my sincere thanks to Dr. Neeta Raj Sharma (HOS), Department of Bioengineering and Biosciences who has provided me with all lab facilities.

I take this chance to extend my thanks to Dr. Joydeep Dutta (HOD), Department of Bioengineering and Biosciences, who kept a consistent observation of the progress of my research work and offered their kind support at every phase of the work. I am honored to have him as the Head of our Department.

My earnest thanks also go to Dr. Harpreet Kaur (HOL), Department of Chemistry, for providing me with kind support and lab facilities in the research work.

My sincere thanks to Mr. Manoj, Mr. Sunny, Mr. Sandeep, and Mr. Surya, for their assistance in the lab, and this list would not be complete without thanking all the non-teaching staff of Bioengineering and Biosciences for their cooperation.

My warm thanks also go to my lab-mates for keeping my morale high in every hurdle and disheartening stage of my research work.

Most importantly, I am immensely obliged to my parents who firmly stand by me throughout the difficulties of my whole research days. I am highly thankful to them for their love, care, and financial and moral support without which this research work could not be possible.

Finally, there are some persons for whom I will always be grateful and I have a shortage of words to show my gratitude and those are my sister Mrs. Chander Mangotra and brother Mr. Rakesh Mangotra. Without their supernumerary support and seamless encouragement, I could not able to complete my research. I am also thankful for their moral and financial support during the intact course of my research work.

I still remind that how the beautiful smiles of my nephew Himanshu Kumar relieved me from stress and charged me up for doing my work even more enthusiastically. Special thanks to him for relieving my stress with his innocent behavior during my entire research work.

Last but not least, I extend my thanks to all the people who have supported me directly or indirectly to complete this research work

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CHAPTER 1 -INTRODUCTION

1.1. Water crisis

Water is biologically mandatory, non-reciprocal, and is the most caring resource. Moreover, its safety, and availability are debated on most of the platforms [180, 375]. Its importance can be judged by its inclusion in the sustainable development goals as SDG6 [318]. It has been envisaged that by 2080, fifty percent of the population of the world will face an acute shortage of water. Asia will be affected more due to its instantaneous inflation in temperature, variation in precipitation patterns, and the seamless escalation in the human population [7]. Water has multi-aspect applications, and it has inevitable importance in all fields of life. Despite the perception that water has inevitable importance, it is intermittently polluted and contaminated [318]. As the resources of freshwater are limited, its management in a sustainable manner is a global challenge. According to WHO, billions of people around the world is facing shortage of clean drinking water and more than 800 million of population are not having the availability of clean drinking water [246]. It has been evaluated that nearly 50 percent population of world will face a drinking water shortage by 2025 [161, 359]. Approximately 33% of the population of the world is facing water stress and it will reach 67% by 2050 if the alarming situation is not controlled in time. The disaster does not end over here, every year contaminated water leads to loss of life of more than 3,61,000 children (below 5 years of age) each year [27, 245]. The natural disruption in the hydrological system, poverty-stricken governance, urbanization, blast in population growth, and hike in economic growth are the factors responsible for this shortage [179, 297]. Despite this, the human activities bring noticeable change in the physicochemical properties of hydrology of landscape around the globe [180]. Industries are accountable for most of the contamination of water bodies and manipulation of quality index. Many countries, including China, have achieved remarkable economic growth by promoting export-oriented industrialization and posing threats to sustainable development [52].

By considering these bitter realities, water needs to be a spectacle through a lens that broadens our perspective on the relationship that exists between social and environmental issues [318]. In light of the above-mentioned facts, it must be emphatically endorsed to make environmental protection norms sincerely and further implement them strictly with regular monitoring of various quality parameters. Quality regulations and proper vigilance must be enforced on industries to set up and regulate properly the effluent treatment plants.

1.2. Properties of water

The high intermolecular hydrogen bonding between the water molecules is responsible for the various properties of water such as viscosity, melting point, boiling point, slow cooling, and heating [357]. Water is a compound with the chemical formula H_2O. It acts as an amphoteric molecule. Normally, it is thermally stable but at high temperatures, it dissociates into hydrogen and oxygen gas. It is neutral [115]. Water can dissolve a variety of substances. It is very essential for various metabolic reactions to take place inside the cytoplasm of cells of living organisms. It transports hormones, minerals, and vitamins throughout the body of living organisms. It has the property to dissolve oxygen which is a lifeline for living beings [115]. The water quality indicator is a significant factor that can be used to assess whether river waters are suitable for various applications, including agricultural and domestic utility. It gives commentary on the water quality to decision-makers and environmental conservationists [111]. The water quality index also depicts the overall water quality stature and stands on several parameters of water quality at a definite time and location [370]. Turbidity, pH, total suspended solids (TSS), total dissolved solids (TDS), electrical conductivity, alkalinity, dissolved oxygen, and biological oxygen requirement are few of the physicochemical factors that are included. Water quality indices act as an important and useful tool for water quality evaluation [111, 320].

1.3. Groundwater contamination

Water is stored in various water compartments on Earth, namely, oceans, glaciers, ice, snow, groundwater, rivers, streams, springs, ponds, lakes, wetlands, and the atmosphere. The oceans cover most of the part of Earth. A glacier holds about 75% of the fresh water on the planet. Groundwater is connected to the surface water. Rivers make up only 0.2 percent of the freshwater of the whole world. Streams discharge the fresh groundwater into springs and streams that later on merge into the oceans. The pond is a reservoir of water. The lakes get water from the underground water, rivers, and streams. A wetland is covered by either fresh or salty water. Water is located in the troposphere of the atmosphere [143]. Water has great significance in the life of living organisms. It has a massive role in all forms of life. It's a prime factor in the physiochemical processes of all organs and organ systems of living beings. Generally, it is used in cleaning, cooking, washing, bathing, irrigation, and all chemical processes in the industry. In short, it is very important for socio-economic progress [254]. The hydrological cycle is the movement of water between the atmosphere, lithosphere, hydrosphere, and biosphere**.** Water is found in various water compartments. The various processes such as condensation, evaporation, precipitation, sublimation, and transpiration take place in the hydrological cycle [211]. The groundwater moves from the high pressure to the lower pressure. It is found in the aquifers. Groundwater is polluted by domestic, agricultural and industrial wastewate**r.** The groundwater quality is acceptable for almost every use but its quality can be deteriorated by highly ambitious human-induced activities including urbanization and industrialization. Water is unacceptable for drinking purposes, if the quantity of dissolved minerals in it will be more than the permissible limit [194, 295].

1.4. Industries as a centric candidate in water pollution

The main challenges for the health and green environment are rapid industrialization and urbanization. The contamination of the environment by hazardous pollutants is caused by both natural and anthropogenic activities. To address these problems, focused research is necessary to counteract their prejudicial effects on living organisms [52]. In economic development, high levels of industry are being set up to accelerate the release of toxic wastewater into the environment and as a key factor for change in environmental quality.

The economic development manipulates the water quality index and deteriorates the normal physiological functions of living organisms. It is occurring at the cost of environmental health and the safety of flora, fauna and human [164]. Many organic compounds including naphthalene impose a bad effect on the physiology of aquatic organisms such as goldfish [104].Water having the properties of poisonous, radioactive, explosive, carcinogenic, mutagenic, teratogenic, or bio-accumulative is considered toxic water. The source of contamination includes chemical processes, improperly disposed wastewater from industrial plants as well as surface runoff containing pesticides used in agricultural areas [71, 220, 281]. The outburst of certain water-borne diseases is the repercussion of the contaminated water and also leads to endangering of aquatic flora and fauna. Tanning industries, electroplating industries, and textile industries are among the important industries contributing to this major pollution event [169, 185]. Processing of raw hides every year releases a huge quantity of untreated effluents causing air and water pollution [140]. Pollutants released from the tanning industry include organic and inorganic compounds, dyes, and heavy metals which affect aquatic life, vegetation as well as human health [307, 374]. These chemicals have a deleterious impact on the aquatic environment and make it essential to remove these malodorous contaminants from wastewater [114, 140]. A strong odor, dark brown color, high pH, heavy metals, chromium, especially high concentrations of total dissolved solids (TDS), biological oxygen demand (BOD), and chemical oxygen demand (COD) are characteristic of the wastewater produced from the tannery industry [293]. Tannery wastewater having plant nutrients like nitrogen and phosphorus causes eutrophication with the repercussion of harmful algal blooms in water bodies [50]. Also, a large turbidity restricts the absorption of sunlight and it acts as a barrier to photosynthesis**.** This results in significant environmental impacts for water, land and the environment [253]**.** Low biodegradability and high concentration of contaminants in tannery wastewater are unavoidable matters and make it important to treat it first and dispose of it safely into the environment [253]. Electroplating industries are responsible for releasing heavy metal-laden effluents into the environment. It becomes an alarming issue for environmental health [8]. Heavy metals being non-biodegradable tend to accumulate in the organs of living organisms. These are very toxic and causes carcinogenicity [242].

Textile effluents give a big contribution to environmental pollution due to the use of about 20,000 dyes and chemicals. Most of them are carcinogens. Due to pertinacious and bio-accumulative in nature, dyes are hazardous for aquatic organisms and mammals. Chemicals after evaporation get mixed in the air and become a massive source of teratogens [171]. Dyes represent the most serious contaminants in water bodies among different chemicals used for textile production. The complex composition of textile wastewater exceeds the permissible limit of the parameters of water. The dye causes turbidity and hinders the photosynthetic process and its toxicity causes hazardous effects on flora and fauna. It has been documented as a carcinogen and mutagen for humans [86, 164, 221].

1.5. Techniques to analyze the physicochemical properties of water

The water quality indicator is determined by using laboratory instruments and following standard protocols. The important quality parameters are temperature (digital thermometer), water pH (digital desktop pH meter), conductivity (conductivity meter), total dissolved solids (TDS) (gravimetric determination and TDS meter), and dissolved oxygen (Winkler's titration) while Biological Oxygen Demand (BOD) is analyzed by using the relationship BOD -DO - DO5, in 5 days (amount of dissolved oxygen present before and after the incubation period) [3, 134]**.**

1.6. VOCs and pollution

Industrial wastewater is a massive threat to the ecosystem. The discharging of industrial effluents into the environment without treating them properly is the main challenge nowadays faced by the research world. The untreated or partially treated effluents have the colossal potential to manipulate the water quality index and bring changes to the normal physicochemical properties of the receiving water bodies. These have become a major source of deterioration of the physiology of aquatic and terrestrial entities because of cyclical series of pollution events. The volatile organic compounds are malodors for the environment and industries are one of the sources to release them. The main sources of emission of volatile organic compounds are petroleum refineries, chemical industries, textile industries, pharmaceutical industries, printing presses, solvent processes, insulating materials, etc. [191, 196, 204, 379]. High pressure and low boiling point are characteristics of the volatile organic compounds. These are volatile and effortlessly evaporate at room temperature [379, 408]. Alkanes, aromatics, ethers, ketones, halogenated hydrocarbons, paraffin, alcohols, alcohols, aldehydes, olefins, and sulfur-containing compounds are examples of VOCs [196, 408]. The characteristics of VOCs that make them liable to manipulate the purity of environment are toxicity, volatility, persistence, and solubility [379]. These volatile compounds for their toxicity have gained tremendous attention worldwide in the field of water science [269, 271]. Volatile organic compounds in water are a burning issue in the context of public health. Some of these compounds, even in small quantities contain a high amount of organic substances and can prove lethal for living beings. Many VOCs have carcinogenic, teratogenic, and mutagenic properties [187, 196, 200, 236, 408]. VOCs at high concentration are responsible for a headache, nausea, dizziness, skin mucosa and damage to respiratory, blood, and nervous system, cancer of the rectum, colon, urinary bladder, low birth rate unpremeditated abortion [146, 196]. The epidemiological studies validated the connection between lymphohematopoietic cancer and carbon tetrachloride (CTC) exposure, cancer of the esophagus, and cervix with tetrachloroethylene (PCE) exposure, cancer of kidney and liver with trichloroethylene (TCE) exposure [187] .

For the safety of public health, various organizations applied a check on the concentration of toxic VOCS by establishing a drinking advisory.BIS has not given any relaxation in the volatility of organic compounds in drinking water. Benzene, carbon tetrachloride, 1,2-dichlorethane, 1,4-dichlorobenzene, 1,2,1,4-dichlorobenzene, chloroform, 1,2-dibromoethane, tetrachloroethylene and trichloroethyne are transitionally categorized as carcinogens. VOCs are accountable for the formation of haze, photochemical smog, ozone depletion, and global warming, and secondary aerosols become a threat to human health and ecology. VOCs act as carcinogens, teratogens, and mutagens for living organisms [48, 133, 202, 380, 395].

1.7. Identification techniques for Volatile organic compounds

The potential of science and technology in cleaning up VOCs would make scientists more interested in the study of these toxins [379]. For the purpose of determining toxic organic compounds, having undesirable taste and smell, many extraction techniques have evolved. The versatile tools such as, solid-phase micro-extraction (SPME), liquid-liquid micro-extraction, headspace (HS), and purge and trap (P &T) have proven to be the best for analyzing different type of organic and inorganic compounds [275, 392]. Purge and Trap-GC/MS is the most widely used technique to determine BTEX and toluene in wastewater [216].The analytes can be detected on sensitive, critically, and selective basis during the multiple reactions monitoring carried out by GC-MS/MS method.To determine the trace pollutants in the sample matrix, multiple reactions monitoring has been extensively used [32, 408].

1.8. Principle of GC/MS

A logical method for analysis of the organic compounds present in the sample is Gas chromatography-mass spectrometry (GC/ MS. It is used to detect the biochemical mixtures and organic components present in the sample [216]. In gas chromatography, there is a mobile phase which is carrier gas. The carrier gas used in this instrument is nitrogen or helium. The carrier gas carries the mixture through the stationary phase. The other phase is the stationary phase. The stationary phase consists of a column made up of glass or metal tubes. The column is coated either with solid or high-boiling polymer. At specific times, the compounds are adequately separated and escaped from the column. The other component of this instrument is the Mass spectrophotometer; it is the detector for GC. The function of the Mass spectrophotometer is the breaking of each segregated compound into its ionized fragments. The fragmentation pattern is specifically related to a particular component of the sample. GC-MS is a logical, sensitive, and efficient tool to get molecular fingerprinting. It is so distinctive that it is frequently pointed out as a molecular fingerprint.

GC and MS complement their work. GC can separate components of the sample and MS has the feature to identify the compounds present in the sample and give comprehensive information about them. GC can segregate even volatile and semi-volatile compounds with high resolution [116, 130]. The mass spectrophotometer, attached to GC, identifies and quantifies them. The data was later analyzed on the computer [216]. GC can segregate even volatile and semi-volatile compounds with high resolution [116, 130].

1.9. Physicochemical techniques for removal of VOCs

For decades, various conventional methods such as reverse osmosis, coagulation, ozonation, distillation filtration, electro dialysis, ion exchange, irradiation and precipitation, adsorption, and solvent extraction are in practice to treat wastewater [9, 36, 57, 343, 409].

But these techniques are not well appreciated due to their certain limitations of low efficiency, high possibility of production of secondary pollutants, and less flexibility [80, 317, 321]. Besides this, various physicochemical techniques are in practice, such as API separators, electro-dialysis methods, catalytic vacuum distillation, sedimentation, advanced oxidation processes(AOPs) like DAF and Electro-oxidation membrane process, and the use of activated carbons [75]. There are drawbacks to some of these methods, such as increased sludge production, chemical consumption, need for physicochemical monitoring, assessment of wastewater, requirement of adjunction of unrecyclable chemicals with low removal of certain chemicals. The limitations list extends to high energy costs in the transportation and management of oxidants [81].

1.10. Adsorption method for removal of VOCs

Adsorption is a kind of technology for water treatment that purely focuses on the contaminants and their conversion to other phases. It is a process entirely based on relation between the molecules of adsorbate and surface chemistry of adsorbents.

The absorption potential of an adsorbent prepared from carbon for chemical compounds hangs on the size and molecular weight, polarity of adsorbate, nature of adsorbate, and functionality. The pore size, functional groups, and structure reveal the efficiency of the adsorbent**.** In addition to this, the solution's conditions and its attributes like pH, ionic strength, and temperature also reveal its potential [333].

The mechanism behind the binding of adsorbate atoms on the adsorbents is governed by various forces such as induced dipole, dipole-dipole, Vander-waals, donor-acceptor forces, and hydrogen bond forces. The electrostatic and covalent interactions and the hydrophobic effect have a substantial role in adsorption [88].

1.11. Adsorbents

Adsorption has achieved a unique place due to its simplicity in design, easily operated, inexpensiveness, easily accessible, yielding no secondary byproducts, and stability in different environmental conditions. This method is efficient to treat the wastewater having organic and inorganic compounds [15, 70, 80, 284, 298]. Industrial effluents, including VOCs, are discharged into the water bodies and bring alteration in the various properties of water **[119].** It has become a key area of research to investigate the tecniques to remove VOCs from the water courses. Various allotropic forms of carbon bear several positive attributes viz., high surface area, ease of avilability, hydrophobic nature, porosity with extensive distribution, low concentration effectiveness, re-usuability, low energy requirement and probability of product repossession which provide greater scope for utilization in removal of VOCs by effective adsorption [248]. Activated carbon is one of the forms of carbon which has extreme porosity, active surface and high surface reactivity which provide a wider scope for its utilization in the purification of organic compounds (194-196). Due to this reason it is getting momentum for removal of a wide array of VOCs including methylisoborneol, geosmin, toluene, xylene, benzene, methyl ter-butyl ether, dichloromethane, trichloroethylene, trichloroacetic acid, mesitylene [22, 127, 198, 263, 379, 405, 408, 410].

Till now a diverse form of agricultural products such as rice bran, sugarcane bagasse, walnut shell, wheat shell, cotton seed shells, rice husks, hazelnut shells, barley husks, soybean hulls, maize (corn) cob, sunflower stalks, banana stalk, citrus peel, rice hulls, sawdust, wheat bran, coconut shells and bark of the trees have been used in the removal of chemical pollutants [36, 343, 409].These adsorbents attract the attention of the research world due to their easy availability, high adsorption capability, effective performance, and non-toxic for the environment [1].

1.12. Mechanism of adsorption

Absorption is a technology that deals with wastewater treatment. It is the mechanism to concentrate the pollutants and transfer them to another phase. Adsorption is based on adsorbate and adsorbent interaction. The adsorption capacity of an adsorbent made up of carbon depends on various factors like functionality, molecular weight, size and polarity of adsorbate and ionic strength, pH, and temperature of the adsorbent [282]. The adsorption energies increased by Van der Waals and dipole-dipole forces. On the surface of the adsorbents, these forces control the movement, binding, and adsorption of various organic compounds [282]. Besides this, different types of interaction, covalent and electrostatic interactions, bonds like p-p, and hydrophobic effect also play a pivot role in the adsorption [88]. Carbon-based materials comprise AC, carbon nanofibers (CNFs), graphene, biochar (BC), carbon nanotubes (CNTs), and carbon aerogels (CAs) [282]**.** The speed of adsorption depends on the physical characteristics of the adsorbent, aqueous solution properties, and composition of the adsorbent [282]. Physisorption and chemisorption are two types of adsorption [148, 381]. Chemisorption is more specific and has high activation energy as compared to physisorption [282]. In ion exchange, interfacial equilibrium, Van der Waals interactions between the charged molecules, and the functional groups play a decisive role [282].

1.12.1. Adsorption equilibrium isotherm

The experimental results are analyzed by isotherm models. It supplies information about the rate at which the process takes place [4, 39, 266]. The adsorption isotherms models under certain conditions provide essential information for scrutinizing the movement of ions between aquatic environments and to the solid surface. Vital information such as the extent of interaction between adsorbate and adsorbents, adsorption capacity, and the bond energy can be extracted after analyzing the isotherm equilibrium models [12, 34, 266]. The experimental data can be analyzed by various models such as Languimer, Temkin, Freundlich, Redlich-Peterson, Henry's law, and Dubinin-Radushkevich model [12, 14, 109, 266, 316, 402].

1.12.2 Adsorption kinetics model

The adsorption kinetic models reveal the exact nature of adsorption, which is purely a physicochemical phenomenon. The rate and mechanism of adsorption is analyzed by the adsorption kinetic models [282].

The adsorption process involves a complex mechanism between adsorbate and adsorbent which follows the concept of rate laws and can be described by various different rate equations [266]. In the pseudo-first-order, equilibrium time and defined time are kept under consideration. The difference in the adsorption of adsorbate molecules on the surface of adsorbents in equilibrium time and defined time is calculated. The chemisorption significantly participate in adsorption, according to pseudo-second order model [261, 287]. The intra-particle diffusion model (IPD) has been given by Weber and Morris and it has been enforced in the analysis of the kinetics of adsorption. The straight-line between qt versus $t^{1/2}$ reveals the control of intraparticles diffusion on the adsorption process. On the contrary, multilinear plots endorsed the influence of multiple parameters on the sorption process [12, 14, 107, 137, 352, 353].

1.13. Bioremediation methods

Bioremediation means remediation by the use of plants or microbes. The former method is called phytoremediation and the latter one is known as microbial remediation [135, 163]. The bioremediation method involves the microbial degradation of extremely toxic compounds into less toxic forms with the release of some byproducts. The living organisms under consideration in this method are bacteria, fungi, or plants that cause degradation or detoxification of substances lethal to human physiology and the surrounding environment. The remediation occurs either by the local microorganisms or exotic one. By using metabolic reactions, living organisms transform the highly toxic compound into a less toxic compound [135, 312].

1.13.1. Types of bio-remediation

Phytoremediation

The two main types of bioremediations are Phytoremediation and microbial remediation. These methods are used in the biodegradation of contaminants in wastewater. Phytoremediation is further divided into five types-Phytoextraction, Phytofiltration Phytoimmobilization, Phytostabilization and Phytodegradation, Phytoextraction (also known as phytoaccumulation, phytoabsorption, or phytosequestration). In this type of bioremediation, the plant's roots absorb the metal ions and carry them to the other parts of a plant and later on concentrate them into the cells [339]. Phytofiltration is a type of remediation in which roots are used to remove toxic substances or excess nutrients from wastewater, surface water, and groundwater. This method is also known as rhizofiltration [339]. The mechanism behind this method is the formation of certain chemicals in the roots that is further responsible for the adsorption of pollutants. Phytostabilization or Phytoimmobilization is the process of reducing the movement of pollutants through accumulation by roots or immobilization within the rhizosphere after forming the insoluble compounds in the root zone [339]. Phytovolatilization is the process of uptaking pollutants from plant roots and their transformation into volatile compounds and eventually releasing these compounds into the atmosphere. It occurs due to its transpiration and metabolic pull [339]. Phytodegradation or Phytotransformation involves the trapping of water and nutrients from the water or soil and degrading the pollutants directly either through the release of enzymes from roots or through metabolic activities. Therefore, it leads to the degradation, inactivation, or immobilization of pollutants in the roots as well as shoots of plants [339]**.**

Microbial bioremediation

This type of bioremediation involves the use of microbes and their derivatives. The mechanism behind microbial bioremediation is the utilization of the ability of microorganisms to secrete a variety of enzymes. These enzymes degrade the pollutants and convert them into less harmful products [29, 135, 312]. *Pseudomonas putida*, *Thiobacillus thioparus, Xanthomonas sp, Thiobacillus denitrifans, Thiobacillus ferroxidans Acidithio bacillus, Metallibacterium* sp, *Thionomas Thiobacillus thioparus* strain E6, and *Bacillus cereus* can degrade hydrogen sulfide (H_2S), methanethiol (MT), dimethyl sulfide (DMS) and p-cresol [213, 319]. *Thiobacillus thioxidans* can degrade VOCs and oxidize sulfur [76, 382].

1.13.2. Biological methods under microbial bioremediation

1. Bio-filtration

The principle of bio-filtration is the decomposition of unwanted volatile organic compounds into dissolved mineral products by microorganisms under controlled conditions. The bed material is considered the heart of this process. The choice of bed material depends upon the compounds to be degraded. The prominent microorganisms used in biofiltration are aerobic ones. The degradation of pollutants releases carbon and energy and hydrogen and oxygen are provided by the air. The nutritional requirements are fulfilled from the bed materials. pH and moisture plays a prominent role in the bio-filtration process. The bio-filtration process is capable to almost all types of chemicals. It has gained more priority over wet-scrubber technology [200, 217].

2. Bio-trickling filters

In this type of filter, nutrient-rich water, showers over the packing material namely, perlite, and lava rock [280]. This technique is economically best due to less cost of operation, less use of chemicals, the precise removal efficacy of contaminants present in the sample matrix, and being environmentally friendly [368]. In the bio-trickling method, activated sludge is considered best due to the existence of various types of bacteria. Organic compounds such as ammonia, methanethiol, and hydrogen sulfide have been removed by this method [225]**.**

Bio-scrubber

Bio-scrubber is a technique that comes under the category of microorganism remediation. The working of bio-scrubber technique is derived from other adsorption and biodegradation methods. In this method, microorganisms that are either autotrophic or heterotrophic convert pollutants into less harmful compounds [290].

1.13.3. Theoretical aspects of the bioremediation method

Bio filters have been in practice since the early 1980s and have inspiring history in the removal of organic compounds.

Bioremediation mechanisms can be understood in depth with the help of kinetic models and rate kinetic models. These models predict the behavior and nature of a particular bio-filter system. These act as tools to forecast the efficiency of the biofilter column at certain operating conditions.

1.14. Mechanism of bio-remediation

The conversion of malodorous compounds into non-malodorous compounds in water and soil using the microbial is known as bio-remediation.

In the modern definition, bioremediation is a biological system that is used to kill or minimize the concentration of toxic contaminants or chemical compounds present in a contaminated site. Therefore, some definitions of bioremediation limit themselves to the use of microbes only, while others consider the incorporation of all the biological entities including plants (phytoremediation).

1.15. Study site

As we discussed earlier, Industrialization and development are the necessary evils of the present scenario. Urbanization and industrialization are the immense sources of pollutants in the environment especially for water bodies. Despite the strict rules formed by the government for the construction of water treatment plants as a prerequisite of every industry, the discharge of untreated effluents is still carried out. The highly polluted drains of developing countries are impairing the normal parameters of the water bodies and Punjab is the most targeted one. The drains near the industries become the bed of carcinogenic and mutagenic compounds. The Kala Sanghian Drain carries the most lethal compounds in high quantities located in Jalandhar, Punjab, India. The leather industries, motor bearing industries, electroplating, and textile industries releases pollutants into this drain. Among these malodorous compounds, volatile organic compounds are the most pestilential for aquatic and terrestrial communities.

Kala Sanghian drain is a 45 km drain that extends from Bulandpur to Bein. It passes through the Jalandhar, Kapurthala, and Nakodar districts of Punjab. Jalandhar is located at 31.3260° N, 75.5762° E. Kapurthala is located at 31.3656° N, and 75.2946° E. Nakodar is located at 31.13° N 75.47° E. The climates around these regions are almost similar with high humidity and monsoon-influence.

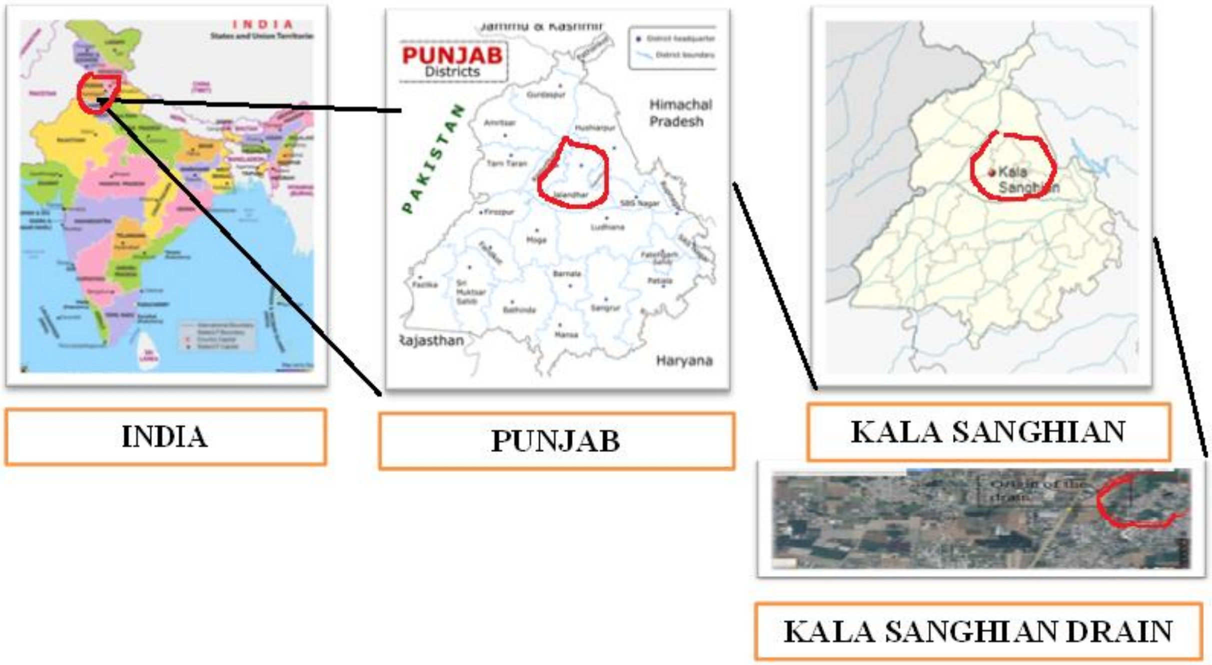


Fig.1 India, Punjab, Kala Sanghian, and Kala Sanghian Drain

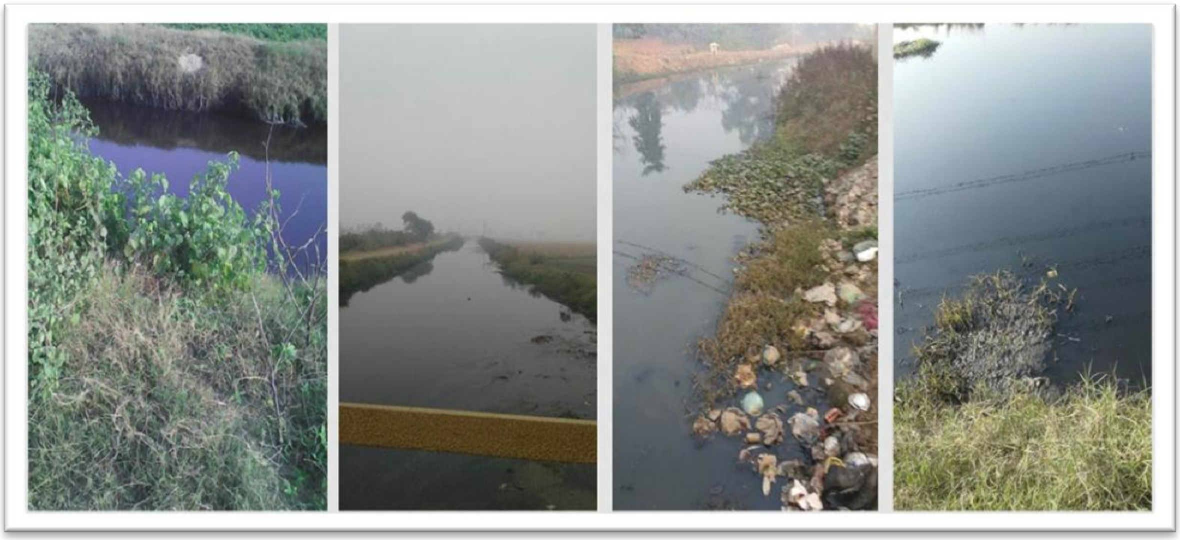


Fig. 2(a) Sites of Kala Sanghian Drain

Fig. 2 (b) Sites of Kala Sanghian Drain

The aim of the present work was the identification and analysis of VOCs, conventional and bioremediation methods used to remediate the volatile organic compounds, and the preparation of innovative hybrid material for the remediation of organic compounds responsible for the volatile emission of Kala Sanghian Drain.

CHAPTER 2-LITERATURE REVIEW

2.1. Physiochemical parameters of wastewater

Industrialization and urbanization are substantial factors responsible for adverse impacts on a healthy and green environment. Natural as well as anthropogenic activities cause the contamination of the environment with hazardous pollutants. Their inauspicious effects on the physiology of living organisms require focused research to solve these problems [71, 75, 77, 142]. Economic development is the main root of releasing toxic wastewater into the environment. This is decisive for alterations in environmental quality due to economic development which ultimately threatens both human beings and the environment on a big scale [164].

Industries deteriorate the water quality index and alter the normal physicochemical properties of water. Therefore contaminated water has properties different from normal water. The industries such as tanneries carry out the processing of hides and discharge loads of wastewater per day. This changes the physicochemical and biological properties of the aquatic environment. Due to having salts, a high load of organic compounds leads to high pollution of water. Dark brown color, unpleasant smell, high total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity, biological oxygen demand (BOD), chemical oxygen demand (COD), salinity, alkalinity nitrogen, sulfate, sulfide, and heavy metals shall be regarded as contaminated water. The high dissolved organic content utilizes oxygen of water bodies and leads to anaerobic fermentation and high BOD level. This anaerobic fermentation subsequently causes the formation of organic acids, which decrease water’s pH after hydrolysis. The contaminated water also elevates the TDS, COD, and BOD and starts to interfere with the physiology of aquatic organisms [51, 174, 251, 340]. The wastewater from pharmaceutical industries also contains high BOD and COD and deteriorates the physiological functions of the water organisms [159, 267].

2.2. Volatile organic compounds in Industrial wastewater

Industrial wastewater is not just degrading the water’s physicochemical and biological properties but also releases many inorganic and organic compounds into the aquatic environment [51]. Besides this, large amounts of toxic chemicals such as surface-active compounds, phenolic compounds, pesticides, synthetic tannins, chrome salts, vegetable and azo dyes, and sulfonated oils are also drained by these types of industries into the environment [51, 174, 251, 340]. In the textile industry wastewater is characterized by the presence of tetradecane, ethylmethylenecyclohexane, decane, undecane, dodecane, naphthalene, decahydro-1,5-dimethyl, tridecane, and hexadecane[112].The textile effluents also contain nitrogen-containing compounds (NOCCs) and oxygen-containing compounds (OCCs), aliphatic hydrocarbons (AIHs), halogenated hydrocarbons (HHs), aromatic hydrocarbons (AHs). The textile industry also discharges organic compounds that are volatile in nature such as BTEX, detergents and esters into the environment [200]. Pharmaceutical industries emitted volatile organic compounds namely chloroform, toluene, and chlorobenzene into the biosphere [159]. Electroplating industry wastewater also releases heavy metal ions, oils, and grease, highly toxic cyanide (CN), organic solvents into the ecosystem [267].

VOCs are designated as the compounds having a low water solubility, high vapor pressure and low boiling point. These particularly easily evaporate at room temperature [379, 408]. Alkanes, aromatics, ethers, ketones, halogenated hydrocarbons paraffin, alcohols, aldehydes, olefins, and sulfur-containing compounds come under the category of volatile organic compounds [196, 408]. The characteristics of volatile organic compounds, which make them liable to alter the purity of the environment are volatility, toxicity, solubility, and persistence [379]. The VOCs has the ability to deteriorate the purity of the environment [269, 271]. Aliphatic and aromatic hydrocarbons are volatile organic compounds. The aromatic hydrocarbons contain their halogenated derivatives, alcohols, aldehydes, formaldehyde, and ketones are halogenated derivatives. These volatile organic compounds have the peculiar characteristic to get converted into gas or vapor without any chemical change. These make the climate hot by trapping the heat, imposing colossal effects on human health, ecology, and climate change [378]. Volatile organic compounds are compounds with a low boiling point. Saturated hydrocarbon is a kind of volatile organic compound that includes ethane, propane, butane, and pentane, and unsaturated hydrocarbon includes ethylene and propylene [391]. Aromatic hydrocarbons include toluene, xylene, benzene, and ethylbenzene[124].Dichloromethane, trichloromethane, and trichloroethane are included in the category of halogenated hydrocarbons [244]. Butanol, ethanol, methanol, and isoprene are categorized as alcohols [175]. Aldehydes include formaldehyde, acetaldehyde, butyraldehyde, and propionic aldehyde [329]. Ketones include acetone, butanone, and methyl ethyl ketone [408]. Based on the degree of polarity, VOCs are divided into polar and nonpolar VOCs [407, 408].

Industrial wastewater is a big menace to the ecosystem. Partially or untreated industrial effluents are a massive threat to the environment nowadays faced by the research world. The effluents have the enormous potency to deteriorate the physicochemical and biological attributes of the water bodies. These malodors of industries become a crucial source of worsening of the physiological functions of aquatic and terrestrial species by series of pollution occurrences. The industries such as petroleum refineries, chemical industries, textile industries, pharmaceutical industries, printing presses, solvent processes, and insulating materials are contributing to the contamination of the ecosystem [191, 196, 204, 379, 408].

Tannery, electroplating, textile, pesticides, and textile industries are the main industries giving a contribution to this water pollution event [91, 178, 288, 311, 361]. Tannery industries based on leather are releasing enormous quantities of effluents into the environment [140]. The leather is non-biodegradable and is more resistant to chemicals, heat, and microbes [73]. Aquatic life, vegetation and human lives are adversely affected by the chemicals released by the tanning industries [87]. The tannery effluents have high physicochemical parametric values with odorous smell and strong coloration[401].The tannery wastewater with nutrients leads to the endorsement of the eutrophication of water bodies [96]. High turbidity causes blockage in the path of sunlight and proves pernicious to aquatic life. The effluents of tannery wastewater are non-biodegradable, so it becomes a prime duty for industrialists to treat first properly and dispose of it safely into the environment [229].

2.3. Sources and applications VOCs

The volatile organic compounds percolate in the environment due to urbanization, industrialization, reckless human activities, and from biogenic sources [71, 133, 142, 151, 232]. Some volatile organic compounds including methane are generated from dairy industries and wastewater treatment plants [16, 99]. The oxygenated VOCs are emitted from vegetation [59, 192, 257, 276]. The other sources responsible for the emission of VOCs include fossil-fueled power plants, solvent usage, industrial plants, fuel storage, vehicle, transport, and landfills [189, 205, 237, 257, 310]. Agriculture activities such as the use of organic fertilizers, chemicals controlling pests and biomass consumption account for around 14% of VOCs emission. Transportation of 28% of VOCs into the environment and the industries have the upper hand with the contribution of 43% [408]. In the preparation of products for commercial use, volatile organic compounds are applied. The use of acetone in making of paints and varnishes is widespread [122]. Benzene is widely applied for the manufacture of chemicals, e.g. cumene, ethylbenzene, styrene, and alkyl benzene [31, 67]. Butanol is utilized in the synthesis of lacquers, paints, gums, vegetable oils, alkaloids, coatings, dyes, camphor, antibiotics, hormones, and vitamins. It is also used in the synthesis of paint, cellulose esters, removers, extraction of drugs, polishes, industrial cleaning products, fruit essences, perfumes, dyestuffs, flavors, and lacquers [344, 404].Chloroform is used as a solvent for resins and in the preparation of chlorodifluoromethane as a refrigerant. It is also used in pharmaceuticals as an extraction solvent [234]. Ethyl benzene is used in insecticides, herbicides, disinfectants, paints, dyes, resins, and pharmaceuticals. Their application extends and is used as an intermediate in the production of dichloromethane, and methyl ethers [125]. Methyl ethyl ketone is used in the preparation of paints, varnishes, and as a solvent [240].Pyridine is used in the pharmaceutical and polycarbonate resin industries. It is also used in the manufacture of insecticides, pharmaceuticals, herbicides, and dyeing [270].

2.4. Concentration of VOCs in samples

2.4.1. VOCs in surface water and industrial wastewater treatment plants

2.4.1.1. Ubeji Creek of Delta State, Nigeria

Petroleum causes pollution in marine water through spills. The hydrocarbons such as xylene, benzene, ethylbenzene, and toluene in petroleum are released into the marine water. These compounds spoil the purity of water and produce difficulties in the existence of aquatic organisms. As per a study, BTEX detected in surface water, sediment, and industrial wastewater treatment was 5.57 ± 0.62 mg/kg [28].

2.4.1.2. Taiwan (volatile organic compounds in wastewater treatment plants

Wu, Feng, Sree, Chiu, & Lo, identified acetone, dimethyl sulfide, and isopropanol (IPA) in the samples collected from the wastewater treatment plants. These wastewater treatment plants were located at Science Park in Hsinchu, Taiwan. The maximum concentration of these compounds was acetone (400.4 ppb), dimethyl sulfide (641.2 ppb), and isopropanol (22.8 ppb) [367].

2.4.1.3. South Korea (industrial effluents)

In this study, eleven volatile organic compounds were investigated at thirty-six sites in five areas near factories releasing wastewater. The sample testing was done for the presence of 35 VOCs but only 29 pollutants were detected. The most frequently detected compounds at low concentrations were organochlorine pesticides (OCPs). On the contrary volatile organic compounds (VOCs) and phthalates were found at higher concentrations. This proves that the factories around this site are the main source of these chemicals present in sample matrices. The samples collected from rivers contain volatile organic compounds in different concentrations. 2, 6-Dinitrotoluene, 2, 6-Dinitrotoluene, 1, 3-Dichloropropylene and 1, 1-Dichloroethylene have concentration less than 1 µg/L. **1**, 1, 2, 2-Tetrabromoethane and 2-cis-Dichloroethylene have concentration less than 3 µg/L.The compounds with highest concentrations were 1,2-Dichloroethane (8.01 µg/L), and 2,4-Dimethylphenol (12.7 µg/L) [74].

2.4.1.4. Petrochemical Industry in China

Shen took samples from the chemical industry’s wastewater treatment plants. Four locations were selected for the collection of samples. The quantification of collected sixteen samples was done. The quantification revealed a total concentration of 36,302 g/m3 of organic compounds in samples. The mass concentrations of volatile organic compounds varied from 132 to 1377 g/m3 in oil refinery units [299].

2.4.1.5. Polypropylene-producing industry

In this research, 192 samples of contaminated water and 72 samples of desorption samples were collected and evaluated. The concentration of five phenols was observed as Irganox 1076 (380 ppm), DTF (366 ppm), Etanox 330 (396 ppm), Irganox 1010 (331), and Cyanox 1790. (330ppm)[142].

2.5 Sampling of VOCs

Activated charcoal: In traditional air sampling, the contaminants are trapped by passing the polluted air through a glass tube having sorbent. Before the instrumental analysis, to desorb the adsorbed pollutants, thermal desorption is carried out [101].

Tenax

Activated charcoal is best when the analytes are present at ppm level. But if the contaminants are present in a very low concentration such as in ppb level, in that case, Tenax becomes the most preferable choice for sampling. It is more stable above 250°C as compared to activated charcoal. It is extensively used in the thermal desorption. The collected analytes are later on analyzed by GC-MS [26, 101].

Multisorbent systems

In this system, charcoal and Tenax (a porous polymer resin) combine to form a multi-sorbent system. The Tenax collected the majority of volatile organic compounds. On the other hand, activated charcoal collected compounds that are more volatile. These types of systems widen the application of a collection of a broader range of chemicals [158].

Direct (whole air) sampling

Whole air sampling is typically done with canisters or Tedlar bags. In this sampling, a" snapshot" of an entire air sample is taken and tested for chemicals present in it. In this method, the air is pumped and filled into the sample bag [390].

2.6 Analysis of VOCs

2.6.1 Analysis of air samples

GC-MS/GC-FID

Gas chromatography (GC) is applied to identify and analyze the quantity of organic compounds in the sample. The GC is based on the principle that when a sample is injected into the GC-MS instrument, the mixture traveled through a column with the help of Helium or Nitrogen gas (inert gas). After coming out of the column, the separated compounds move toward the mass spectrophotometer. The mass spectrophotometer breaks each component of the sample into its ionized fragments [177].

Samples are generally analyzed by first using the gas chromatograph to separate the samples into their components. After that, with the help of detectors, namely, Mass spectrophotometer (MS), Flame ionization detector (FID), and Electron capture detector (ECD), the separated components are identified [168, 172, 354].

Olfactory analysis

In this analysis, substances, processes, and sources of the odorous substances have to be found. Well-trained odor experts participate in this analysis. Judgments are given by this analyst about the possible impact on the health of these compounds present in the air directly. In olfactory analysis, a team of expert panelists in odors evaluate the possible disease that can happen due to the inhalation of a particular air sample [149].

2.6.2. Analysis of water samples

To analyze volatile organic compounds, closed-loop stripping analysis and purge and trap methods are in practice.

Closed loop stripping analysis

In this type of analysis, gas in a closed circuit is pumped via the water phase. As a result of it, the organic compounds get trapped in a sorbent. The sorbent holds the volatile organic compounds and gas returned to repurge the water sample.

This method is considered very simple, speedy, sensitive, and comparatively problem free. There is a limitation in the range of analyzing compounds by this method [197].

Purge-and-trap method

Purge and trap, used to separate volatile organic compounds in samples with complicated grids, is a very sensitive and well controlled headspace technique. These volatile organic compounds transfer on to a sorbent trap. In the sorbent trap, these are concentrated and introduced into an instrument that may be GC-MS/GC-FID/GC-ECD [95].

2.7. Factors influencing the detection of VOCs

The type of capillary column is chosen very carefully as it is directly related to the efficiency of VOCs chosen for analysis. The choice of the capillary column is dependent upon its internal diameter, because it has a major impact on the analysis of VOCs [302].

Recently research world starts using capillary columns with wide-bore with 0.53 mm and 0.75 mm internal diameter due to the attribute of operating this capillary column at a flow rate with a purge-and-trap system [252, 314]. Flow rates of 1 ml-3 ml are often used to operate narrow bore capillary columns. It has been reported that higher flow rates substantially decrease the segregation efficacy [336].

2.8. Toxicity of VOCs in Living Beings and the surrounding environment

In human beings, VOCs namely, trimethyl benzene, cis 1,2-dichloroethene, _2-chlorotoluene, dichloromethane, bromobenzene, cumene, ethyl benzene, 1,1,1-trichloroethane, and tetrachloromethane deteriorate the physiology of living beings and flare up adverse health effects on development, reproduction, nervous and endocrine system [223, 224, 338, 362]. Many VOCs have carcinogenic, teratogenic, and mutagenic properties [187, 196, 200, 236, 408]. VOCs in high concentration are responsible for headache, nausea, dizziness, skin mucosa, and interference in the respiratory, blood, and nervous system. These compounds cause cancer of the rectum, colon, and urinary bladder. These also lead to low birth rates, unpremeditated abortion [146, 196, 392]. Cations and anions are produced as a repercussion of the degradation of organic compounds. These ions suppress the growth of some species and lead to the growth and development of other species [174, 251, 340]. The effects of chemicals released from industries cause impairment in the physiology of goldfish. The toxic compounds cause the malfunctioning of the antioxidant and metabolic enzymes of the fish [104]. In monkeys, dogs, Guinea pigs, and rabbits, these chemicals show repercussions of, increase organ weights, rising metabolic activity of enzymes, histopathological alteration, slowing down of neurological functions, narcosis, alteration in motor function, and physiological variations in heart, and liver, lungs, and kidneys [386]. In birds, these chemicals affect the hens severely after causing degeneration of peripheral nerves, axons, and spinal cord. Exposure to aromatic volatile organic compounds causes platelets decrease and increase in blood osmatic pressure in the embryos of the hens [100]**.** In invertebrates such as Rainbow trout, Daphnia, and Ceriodaphni, these effects on the reproductive system were noted and in some cases, it also leads to mortality [49, 277]. In plants namely, *Lilium longiflorum, Raphanus sativa, Lactuca sativa, Linum strictum Petunia hybrida, Phaseolus petunia*, the symptoms such as epinasty, chlorosis, reduction in dry weight, decreased flowers per plant, speedy leaf drops have been observed [346, 372]. In aquatic plants such as *Cyprinus carpio, Daphnia magna, and Oncorhynchus mykiss,* mortality cases have been seen when it is exposed to bromoform, chloroform, and naphthalene at 1.8ng/L, 1.8 ng/L, and 0.11 mg/L respectively [150]. Moreover, mortality has been analyzed in *Oncorhynchus mykiss* on its exposure to toluene at 0.0029 mg/L [150]. Xylene at 10.4 mg/L proves lethal in *Lepomis macrochirus* [150].

Despite having a bad health impact on living organisms, it also causes unavoidable environmental issues. In the presence of nitrogen oxides, high level of air pollution induces volatile organic compounds to oxidize**.** The smog has deleterious effects on the lungs. It also causes damage to the crops. This ‘urban soup’ outcome of industrialization and urbanization consists of active, biologically, and ecologically volatile organic compounds that cause ozone depletion. The unreactive toxic compounds present in the upper atmosphere cause global climate change by trapping solar radiation [20, 196, 378, 395, 408].

2.9. Removal of VOCs by adsorbents from agricultural products

As per previous literature, adsorbents prepared from agricultural wastes have high carbon content and less inorganic content. Many agricultural products such as fruit stones, kola-nut testa, almond green hull powder, date pits, almond shells, bean husks, cassava peel, jute fiber, olive stones bagasse, coffee husk, plum kernels, rice husks, bamboo steam, palm-tree cobs have been reported as appreciable adsorbents for the biosorption of chemicals [322, 377]. These are available at affordable prices. The carbon obtained from these agricultural products can give better performance in both wastewater and drinking water purification. These adsorbents are more effective at removing phenols, dyes, heavy metals, and a wide range of organic compounds [210]. Numerous agricultural wastes such as tea waste, coconut coir pith, wheat husk, rice husk, and wheat bran attained high appraisal in the remediation of organic and inorganic malodors because of having attributes of accessibility, adsorption efficiency, and low expenditure [190].

2.10. Adsorption-a weapon to counter the volatile organic compounds

Industrial effluents containing VOCs releases into the aquatic ecosystem and deteriorate its physical and chemical environment [119]**.** The researchers’ top priority is to work on removing volatile organic compounds from the watercourses. In the present era of pollution all around, there has been a substantial effort taken by the research world to deal with it. Candidly, the industrial chemical processes not only consume too much water, but rather it releases contaminated effluents too into the water bodies [235]. In the crowd of various purification methods, adsorption comes out with the promises of decolonization, separation, and purification of malodors from sample matrices [82]. This method is applicable in low concentrations of pollutants and diverse forms of pollutants too. The adsorbents used in the adsorption are available in versatile forms with low cost, are easy to operate, remove pollutants in both liquid and gaseous phases, and are environment friendly. Industrial, healthcare and pharmaceutical processes use it for a variety of applications [190].

Activated carbon is an effective adsorbent because of its high carbon, low moisture and low ash [227]. The activated carbon has large surface area, so it can remove chemicals, that are present, even in low concentration [248]. The carbon modified by certain chemicals or heat treatment is appreciating more in the adsorption of contaminants in the wastewater due to having attributes of highly porous, large surface area, and strong reaction on the surface. The organic compounds are organized into five categories (a)Aliphatic hydrocarbons (b) Aromatic hydrocarbons (c) Halogenated VOCs (d) Oxygenated organic compounds (e) S, N-containing compounds [379]. These adsorbents play an efficient role in the remediation of 1,1-dichloroethene, trichloroethylene, trichloroethene, tetra chloroethylene, tetrachloroethene, 1,1,2-trichloroethane, 1,1-dichloroethene and aliphatic hydrocarbons [379]. These are prepared either from plant materials or from decomposed plant matter such as coal, peat, and lignite [379]. Agronomic products have been extensively used in practice for the preparation of activated carbon due to their reasonable cost and easy accessibility in abundant quantity. For purification, these can be used in the form of powder, granules, or sticks. Numerous modern oxidation processes are in practice to treat volatile organic compounds. In terms of cost, economic compatibility, sustainability, efficiency, and eco-friendly nature, activated carbon is the most preferred choice [190].

2.10.1. Factors affecting the textural characteristics of activated carbon

Numerous factors affect the textural features of activated carbon. These are listed as activating agent, carbonizing temperature, carbonizing time, the mass ratio of precursor activating agent, and heating speed. The chemical agents usually used in the activation of carbon are Sodium hydroxide, Zinc chloride, Potassium hydroxide, Phosphoric acid (H_3PO_4), and Potassium carbonate [190]. In physical activation, the temperature is less as compared to chemical activation. In the preparation of activated carbon, The temperature needed for the carbonization process is usually higher than 400 degree Celsius and range from 120 to 1000 degree Celsius [121]. The activation temperature and carbonizing time are the decisive factors in yield percentage. The yield percentage has correlation with activation temperature and holding time. The saturation of agricultural and industrial waste leads to the formation of activated carbon with optimum porosity by using less amount of activating agent. An important factor in preparing the activated carbon is the ratio between the mass of the precursor used to activate and the activating agent. Normally, 400°C is considered optimal temperature and 1:1 is optimal mass ratio for the activation of carbon for one hour. In Physical activation, raw material is carbonized at high temperatures and gasified by appropriate oxidants. However, in chemical activation low temperature at short activation time results in high porosity and greater yield with high carbon content [332]. Heating speed also affects the porosity of carbon. Low speed resulted in activated carbon with good porosity [190].

2.10.2. Factors affecting the adsorption capacity of activated carbon

Two factors control the feature of adsorption capacity. First is the raw material used and second one is the structure of carbon. Generally, vegetables, animals and minerals are uses in the preparation of activated carbon. These can be agricultural byproducts such as wall nut shells, coconut shells, wood, or materials belonging to minerals such as anthracite. However, availability of minerals for the formation of activated carbon is not easy. By contrast, due to the easy availability and low cost combined with a wide range of uses, agricultural byproducts have been more appreciated. Previous literature revealed that carbon having more porosity and a more heterogeneous nature is prepared from vegetables by using physical or chemical activations as compared to activated carbon production from coal [190].

2.11. Removal of VOCs by coconut shell activated carbon

A tried and tested material with high adsorption capability for the removal of VOCs is activated carbon prepared from coconut shells. It has been found effective for removal of VOCs like methylisoborneol, geosmin, toluene, xylene, benzene, methyl ter-butyl ether, trichloroethylene, trichloroacetic acid, mesitylene from various samples [44, 207]. The texture and surface chemistry of activated carbon is responsible for its high adsorption capacity [188].

2.12. Removal of VOCs by Rice husk as adsorbent

Rice husk as adsorbent have the potency to treat organic compounds including volatile organic compounds. This demonstrates extensive applications in various fields due to its appreciable nano structure and functional patterns.

These are preferable due to its property of getting economical cheap and sustainable carbonaceous material from it. The literature survey endorsed its property of fast kinetics and praiseworthy adsorption capacities [21, 264, 296, 300].

2.13. Removal of VOCs by MOF (Metal-organic framework)

The MOFs are the compounds having metal clusters and organic molecules. The research community was drawn by the huge adsorption properties of these materials, viz., high temperature stability, large surface area and significant porosity [274]. There are many organometallic structures that have the potential to adsorb chlorinated and non-chlorinated volatile organic compounds [69, 351, 379]. MOF capability as a porous material has been tested and conducted for the adsorption of toluene [145, 351]. The adsorption capacity of MOF UiO-66 has been analyzed in the previous study. Various

MOFs have been used in the studies by Vikrant for the adsorption of eight volatile compounds by MOF and they have given exceptional adsorption performance [247].

2.14. Bioremediation

An effective method for removing volatile organic compounds is the bioremediation by microorganism. For organic compounds treatment, adsorption and biodegradation are usually used by the scientific community. Due to their effectiveness, wide applicability, easy operation, convenience in use, and simplicity in design, these techniques are being taken more seriously. [82]. In biodegradation, microbes cause the mineralization of compounds by using their metabolic pathways. Due to its coherence, economic, multi-faceted, and harmless to the environment, it is a preferred method used in the purification and detoxification of organic compounds in contaminated wastewater [11, 83]. Bacteria have the potency to biodegrade the organic compounds in wastewater. The research community is successful in treating chlorinated volatile organic compounds using bacteria [11, 83]. The type of microorganisms used in the process, predicts the rate of biodegradation. Apart from these, physical and environmental factors namely, pH, moisture, temperature, and nutrients also control the rate of biodegradation [5, 33]. Using indigenous microbes is considered to be economically cheap, environment-friendly and energy efficient. The most alluring benefits of biological processes are their non-toxic end products. The end products of biodegradation by microbes accumulate back into the environment.

Bringing certain alterations in optimizing factors of a particular area can improve the biodegradation efficiency of indigenous microorganisms[305]. The utilization of microorganisms in the elimination of organic compounds is in exercise for many years. There are many potential microorganisms that have the potency to remove the chemicals from wastewater, e.g. *Micromonospora albus, Xanthobacter autotrophicus Enterobacter oryzendophyticus*, *Mycobacterium vaccae, Arthrobacter sp, Acinetobacter calcoaceticus, Cephalosporium* sp, *Gordonia amicalis*, *Nocardioides sp*, *Pseudoxanthomonas sp,* *Mycobacterium austroafricanum, Actinomyces globisporus, Micrococcus albus,* and *Hyphomicrobium sp,* and *Nocardia sp* [176, 193, 214, 331, 341].

In the crowd of these efficient bacteria, *Pseudomonas aeruginosa, a* gram (-) bacterium, emerged as one of the most efficient bacteria in the world of biodegradation. It can survive almost in every environmental condition with huge metabolic diversity and can grow easily in an extensive variation of conditions and temperatures. It is an obligate aerobic, non-fermenting, saprophytic, gram-negative bacilli [94, 330]. Some members of Pseudomonas are very different in ecology and genetics and cause human infections. On the contrary, some other strains of Pseudomonas bioremediate the pollutants present in the environment. There is a certain genus of Pseudomonas that snubs the growth of insects and pests [335]. This bacterium is able to degrade the oxygenates, used as fuel additive [30, 214, 331] and biosorption of nickel ($Ni^{2+)}$, Cadmium (Cd^{2+}), copper ($Cu^{2+)}$, and mercury (Hg^{2+}) [64, 136, 152, 325]. Pseudomonas is also been found to degrade benzene, styrene, toluene, ethyl benzene, phenol and xylene [35, 72, 103, 113, 170],

2.14.1. Biodegradation by bacteria

Biodegradation is a natural process where microorganisms' biodegradation property is utilized in the breaking down of main compounds into their end products via intermediates. The end products produced after the completion of biodegradation return to the environment.

In biodegradation, the microbes convert the pollutants or organic compounds into simpler substances by decomposition [108]. Identification of several physicochemical and biological factors has been carried out earlier that control the rate of biodegradation. These factors include the availability of inorganic nutrients, microbial adaptation, pollutant concentration, temperature, pH, and water composition. The available organic pollutant for microbes determines the rate of reaction. The reaction rate is governed by availability of organic pollutants to the bacterium and their temperature. The uptake of various organic compounds by the microbes also depends upon the biosurfactants released by various microorganisms [304, 305].

2.15. Mechanism of adsorption by Rice husk

Rice husk is available in large quantities. It is easily accessible from rice milling and agro-based industries. It is a cellulose-based material that contains volatile matter (60–65%), cellulose (40%), fixed carbon (10–15%), lignin (21%), and hemicellulose (21%) with silica and protein. Among all these materials, lignin plays a substantial role in the adsorption process [182, 218]. Mesoporous silica is widely used in the treatment of wastewater and air-purification, due to high stability, unique pore structure, high surface area, high total volume, and pore size distributed uniformly. Silica's adsorption capacity is made possible by the presence of potent functional groups [38].

2.16. Mechanism of adsorption by coconut shell activated carbon

In the crowd of adsorbents from agricultural byproducts, coconut parts such as shell and coir emerged as efficient adsorbents for the treatment of organic compounds [157]. Coconut shell is an agricultural product, easily accessible, and economically cheap, has low ash content and high strength with dense structure. Coconut shell consists of cellulose, hemicellulose, lignin, and moisture which are potential sources of carbon. Coconut shell is an agricultural product, a rich source of fiber, and has an average composition of cellulose (36%), hemicellulose (25%) and lignin (28%) [118]. It has the efficiency to adsorb dyes, heavy metal ions, and organic compounds from wastewater [45, 92, 157]. Due to the presence of lignin, cellulose, and low inorganic impurities, the adsorbent prepared from the coconut shell has high porosity and surface area [376].

The functional groups, carbonyl and hydroxyl that can interact with contaminants, are present in these polysaccharides. The coconut shells are a praiseworthy adsorbent material due to this property [260].

2.17. Mechanism of adsorption by MOF (UiO66)

Besides the adsorbents from the plant origin, certain other adsorbents have good capability to adsorb volatile organic compounds. Metal-organic framework is one of them. The MOF is a composite material with both organic and inorganic properties. This is a porous substance composed of positive ions connected by organic linker molecules. The linker's arms are joined together by the metal ions to form nodes to produce a repetitive cage-like structure. The hallow structure of MOFs is responsible for its large surface area and preferable choice for adsorption. The linkers such as copper, zirconium, and aluminum are used in the formation of MOFs [68, 326, 387, 394, 400]. MOFs are thermally and chemically stable. They' are very well suited for adsorption because of large surface area and strong ion exchange capacities [186]. The porosity and chemical stability makes the metal−organic frameworks (MOFs) a new class of adsorbents. These are specially tailored for particular applications by bringing forth the changes in metallic clusters and organic linkers and moving them one step ahead to other adsorbents. MOFs are imbedded with positive attributes such as synthetic tenability, porosity, uniform pore structure, and stability with diverse applications in adsorption, catalysis, gas storage, and bio-medical imaging, MOFs act as appreciable adsorbents for volatile organic compounds [69].

2.18. Literature gap

In the light of literature survey, it is quite clear that a handful of studies are reported related with removal of VOCs using adsorbents prepared from agricultural products and microorganisms. The treatment of heavy metals in wastewater has been the subject of most of the studies. There are very few research studies on VOC remediation by using the above-mentioned adsorbents and microorganisms. Removal of the VOCs has been seen in previous studies, where the activated carbon from different agricultural products has been used. Various strains of bacteria also have been practiced by various researchers for the removal of VOCs. But no one has yet touched the idea of preparing an innovative hybrid material by combining the properties of agricultural byproducts, metal-organic framework, and microorganisms in the removal of VOCs from wastewater.

It's a new approach completely. Limited studies are reported on the mechanism of the bioremediation of VOCs. Most of the studies are limited to the use of either activated carbon or pure strain of microorganisms or using adsorbents of plant's origin for the removal of VOCs from wastewater.

2.19. Scope of the present study

There is a necessity to observe the contaminated wastewater of the Kala Sanghian Drain. In terms of the disposal of toxic wastewater in this drain, it impairs the organ system of living organisms and deteriorates the purity of environment as a whole. There is an unavoidable necessity to remove the malodorous volatile organic compounds that act as carcinogens, teratogens, and mutagens. The research studies on the identification of VOCs and their remediation by biological means as well as by attempting different types of hybrid materials must be carried out.

In the present study, conventional and bioremediation experiments were applied to remove the volatile organic compounds emitted in the drain. Different isotherms models were fitted using experimental data. The models helped in the apprehension of the mechanism of removal of the compounds. The final concentration of the volatile organic compound was evaluated by using a UV-Visible spectrophotometer, GC-MS and GC-FID. The characterization of the adsorbents was done by using Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) to analyze the role of various functional groups in coconut shell activated carbon, rice husks, and metal-organic framework (UiO66) that brings changes in the morphology and physiology of bacterial cell surface during biodegradation of volatile organic compounds. There is scope to conduct bioremediation studies and materials studies for the treatment of VOCs under diverse operating conditions and by using different types of agricultural products and microorganisms. This will help to ascertain the potential of different resources for removing organic compounds. Bioremediation (biodegradation) and hybrid materials studies will help in evaluating the desired parameters needed for designing more potential bioremediation methods and the use of hybrid materials on a large scale at the industrial level. However, more research is required for (1) a comprehensive identification and evaluation of malodors entering innumerable ways and (2) the application of various agricultural byproducts and microorganisms in diverse forms for the treatment of pollutants entering the environment.

CHAPTER 3- HYPOTHESIS

1. Kala Sangian Drain receives wastewater from various water-polluting industries such as leather and textile. Based on this information, it was hypothesized that wastewater from this water body would possibly be having a higher level of physicochemical parameters and VOCs as compared to the limit set by World Health Organization. It was further assumed that VOCs present in the water body will be toxic to human health and have the ability to deplete the environment.

2. Adsorption and biodegradation methods are very effective for the remediation of VOCs. It was hypothesized that remediation is possible by applying these two methods.

3. Hybrid materials could have higher degradation efficiency as compared to individual materials hence they are more employed for complex chemical entities. These assumptions could hold good for VOCs as well.

CHAPTER 4-OBJECTIVES

1. Identification of pollutants responsible for the unpleasant odor of the Kala Sanghian Drain.

2. Evaluation of the performance of conventional and bio remediation methods in the removal of odor released by the effluents.

3. Designing innovative hybrid materials for remediating the responsible compounds for the unpleasant odor of Kala Sanghian odor.

CHAPTER 5-MATERIALS AND METHODS

Objective 1*: Identification of pollutants responsible for the unpleasant odor of the Kala Sanghian Drain.*

5.0 Materials and Methods

(a) Physicochemical properties studies

5.1. Physiochemical properties of water samples

This chapter describes the method for sample collection and analytical procedure adopted for the analysis of wastewater from the Kala Sanghian Drain using different analytical methods. The water samples were collected from selected four sites of the Kala Sanghian Drain, in triplicates, to analyze and identify the volatile organic compounds.

5.1.1. Sampling methods

A sampling of wastewater was done from selected sites of the Kala Sanghian Drain. Poly-propylene bottles with two-liter capacity and narrow-mouth were used for the sampling purpose. The bottles have well screw-caps and washed with dilute nitric acid two times and thrice with deionized water. Before sampling, bottles were washed two times with water and afterward, samples were collected.

5.1.2. Sampling sites

Samples were collected from selected four sites of the Kala Sanghian Drain with geographical locations.

Sites of Kala Sanghian Drain	Geographical location
Site-1 (LD)-a	31.33°N and 75.32° E
Site-1 (LD)-b	31.33° N and 75.32° E
Site-1 (LD)-c	31.33° N and 75 .32°E
Site-2 (LO)-a	31.33° N and 75. 31°E

Site-2 (LO)-b	31.33° N and 75.31°E
Site-2 (LO)-c	31.33°N and 75.31° E
Site-3 (FD)-a	31.22°N and 75.34° E
Site-3 (FD)-b	31.22°N and 75.34° E
Site-3 (FD)-c	31.22°N and 75.34° E
Site-4 (BD)-a	31.22° N and 75.35° E
Site-4 (BD)-b	31.22°N and 75.34° E
Site-4 (BD)-c	31.22° N and 75.35° E

5.1.3. Collection of samples

Sample bottles were washed three times with the wastewater to be collected and then filled to the top to avoid any encroachment of air-bubbles. Proper care was taken to prevent photo-degradation and contamination. The collection of samples was done in amber bottles to prevent photo-oxidation and for the prevention of contamination of samples; bottles with tight Teflon capped were used. To prevent headspace, the bottles were filled to the top and capped tightly. The sampling bottles were escorted to the laboratory after being tightly capped. The samples were preserved at 4 degree Celsius. With the help of digital thermometer and pHmeter, temperature and pH analysis was carried out in the field.

5.1.4 Labeling of samples

Every sample was coded properly with the help of permanent markers in two places. The proper records were made by entering the information in a field book such as the date of sampling, code of sampling, sampling location, and source of samples to avoid any confusion and error.

5.1.5 Analysis of samples

To determine the physicochemical properties of wastewater and to identify the volatile organic compounds present in the wastewater of the Kala Sanghian Drain, samples from selected sites were analyzed.

i. pH
ii. Temperature
iii. Total dissolved solids
iv. Electrical conductivity
v. D.O (Dissolved Oxygen)
vi. B.O.D. (Biological Oxygen Demand)

5.1.5.1. Chemicals and reagents

Analytical graded chemicals and reagents, purchased from Merck, India were used for the experimental work Double distilled water was used throughout the analysis.

5.1.5.2. Procedure

The physicochemical properties of the wastewater were evaluated by applying standard methods as described by ‘American Public Health Association’ [25], ‘American Water Works Association , and ‘Water Pollution Control Federation’ [24].

(b). Identification of volatile organic compounds

5.2. Identification of VOCs in water samples

GC-MS/MS instrument was used to identify the volatile organic compounds. This instrument is simple, versatile, accurate, and free from major interference [110, 208, 406].

5.2.1. Filtration of samples

To prevent micro drop instability, 0.45 pore Whatman filter paper was used. After filtration, the filtrates were transferred into 40mL screw-cap vials (OI Analytical Company, USA) [110, 208, 406].

5.2.2. Preservation of samples

The preservation of water samples was done by keeping the samples at the temperature of 4 °C [110, 208, 406].

5.2.3. Sample preparation

The preparation of water samples was done according to US EPA (Method 5030 C/8260B) [110, 208, 406].

Analyte type: VOCs using MSD; **EPA Method:** 5030 C/8260B

Sample preparation technique

P & T, direct injection, Head space; **Detector type:** MSD (Mass Selective Detector): **Sample matrix:** wastewater

5.2.4. Extraction Conditions

Sample Volume: 5 ml; **Trap:** both Tenax and Tenax-silica Gel-Charcoal; **Purge gas:** Helium

Purge flow:40mL/Min; **Purge time**:11 minutes; **Purge temperature:** Ambient; **Desorb temperature:** 225 °C; **Desorb time:** 3 min; **Desorb flow:**200mL/ Min [110, 406].

Extraction instrument: P& T: [110, 406].

Chromatographic column: VF 624 ms column 60m 0.25mm, 1.8 Micron [236].

5.2.5. Procedure: GC-QqQ-MS/MS Analysis

The analysis was executed by a 7890A gas chromatograph joined with a 7000A triple quadrupole mass spectrometer system (Agilent Technologies, Palo Alto, CA, USA). By using a VF 624 ms column 60m 0.25mm, 1.8 microns fused–silica capillary column (J&W Scientific, Folsom, CA, USA), the separation of these compounds was executed during the detection process. The oven was first set at 35 °C (5 minutes) and afterward raised to 160°C at 5°C/min, hold for 1 min, and again raise to 210°C at 5°C/min. The final temperature was kept for 5 minutes. Carrier and purging gas used in this instrument was Helium. The electron ionization (EI) in mass spectrophotometer was set at 70 eV and the ionization source was kept stable at the temperature of 200°C Acquisition was performed in scan mode from 35-300 a.m.u. [110, 236, 366].

Purge and Trap Equipment

TenaxÒ and TenaxÒ-SilicaGel-Charcoal cartridges of Purge and Trap concentrator Tekmark 3100 were used for the analysis. 100 mV and 380 V were set for the emission current voltage of the detector respectively. In sample preparation, an Aquatek 100 Liquid Auto sampler (Tekmar-Dohrmann) was used. An aliquot of 5ml was extracted and released into a purge tube of the purge and trap concentrator. To purge compounds from sample matrices, 40mL/ min helium with high purity was used for 10-12 minutes. The trap used was Tenax-Silica-gel-charcoal. It was followed by the heating of the trap at the temperature of 225°C. Helium was used as a carrier gas during desorption. Desorption. The liquid used for the washing of the purge tube and sampling needle was high-performance liquid chromatography water. At 230°C, for 10 minutes, the trap was baked [110, 392, 406].

***Objective 2**: Evaluation of the performance of conventional and bio remediation methods in the removal of odor released by the effluents*

5.3. Conventional Method (Adsorption): Preparation and characterization of adsorbents

5.3.1. Preparation of adsorbate

The adsorbate-dichloromethane (DCM) was purchased from Merck, India. By applying standard methods, the solution with standerized concentration of adsorbate was prepared [14, 60, 349]. The chemicals and reagents used were of analytical grade.

5.3.2. Preparation of coconut shell activated carbon (CSAC)

For preparation of CSAC, deionized water was used to wash the coconut shell taken from the local market followed by sun drying at 3-4 days. Subsequent oven drying was carried out at 105°C for 48 hours before milling and desired size was obtained for further processing [199, 376]. The impregnation was done for 2 hours with **a** boiling solution of 30 % H_3PO_4 [43, 66]. Afterward, it was soaked in the same activating agent for 24 hours with an impregnation ratio of 1:3. Subsequently, the excess solution from the material was decanted off and dried. Activation at 700 degree Celsius was performed in a muffle furnace for one hour. After that, the material was washed with water until the pH stabilized at 6-7. Later, it was filtered with Whatman filter paper. There filtered carbon was dried at 102 °C for at least two hours. After drying, the sample was stored in a tightly closed container [120, 141, 385].

5.3.3. Preparation of adsorbents from Rice husk (RRH, RHN, RHH)

From the rice mill, rice husk was collected, for the preparation of adsorbents. The rice husk had to be cleaned several times using distilled water with an aim of removing any foreign material. After that, it has been dried thoroughly at 105 degree Celsius for 24 hours. Subsequently, these were grinded and selected to desirable size by passing through sieves. Three different types of adsorbents from rice husk were prepared (i) Raw rice husk (RRH) (ii) chemical treated with 0.5 M Sodium hydroxide (NaOH) (RHN) (iii) Raw rice husk heated at 300°C (RHH). To get the desirable size, raw rice husk (RRH) was undergone milling. Rice husk modified with Sodium hydroxide (RHN) was obtained after chemically treated the rice husks for 4 hours at room temperature with 0.5 M NaOH solution. Later on, it was rinsed with water to remove any extra NaOH. The wet material was dried at 40 °C. By applying the thermal treatment method, third variety of adsorbent was obtained. It takes three hours to burn rice husk 300°C for this type of adsorbent [138].

5.3.4. Characterization studies of adsorbents

For the characterization of the adsorbent, standard methods were applied [181]. Fourier Transform Infrared (FTIR) analysis of the samples was done by Agilent Cary 630 FTIR spectrophotometer. It was integrated with the software Microlab PC-5.6.1 [154, 309]. To get the FESEM images, Field Emission Scanning Electron Microscopy was used (Zeiss, Jena, Germany) [89, 181]. Yield percent has been calculated by applying the standard methods [106, 117, 166, 199, 226].

5.3.5. Adsorption studies

The adsorption experiments were performed by using four types of adsorbents: (i) raw rice husk (RRH) (ii) raw rice husk after pretreatment with NaOH (RHN) (iii) rice husk heated at 300°C (RHH) (iv) CSAC (Coconut shell activated carbon).

5.3.5.1 Effect of pH on DCM adsorption

The range of pH from 2.0-10.0 has been used for studying the effect of pH on DCM adsorption. By using adsorbate solution (25 mL) with an initial concentration of 200 mg/L of DCM, the impact of **pH** on adsorption process achieved. The 50 mL capped glass flasks were used for the experimental work. The adjustment in pH of the solution was done by applying standard methods. The other parameters namely, initial concentration, temperature, carbon dose were kept constant except pH. In a water bath shaker at 25°C, the adsorbate solution was allowed to equilibrate for 4 hours [215].

5.3.5.2. Effect of contact time on DCM adsorption

The contact time studies were done by taking contact time range of 20-240 minutes and keeping other parameters constant. The required quantity of adsorbate solution was taken for UV-visible spectrophotometer analysis [17, 215].

5.3.5.3. Effect of adsorbent dosages on DCM removal

The impact of adsorbent dosages on dichloromethane was studied by taking adsorbent dosages range of 0.2g-1.0g. The other parameters were kept constant. The required quantity of adsorbate solution was seized for UV-visible spectrophotometer analysis [17, 215].

5.3.5.4. Effect of initial concentration on DCM removal

The initial concentration affects the adsorption or biodegradation significantly. The study of impact of initial concentration of adsorbate on adsorption was done by taking varied concentration of adsorbate and keeping other parameters constant- adsorbent dosage (0.1g), pH (7), temperature (25°C) and agitation time(150rpm) was kept constant [215].

Analysis of Removal efficiency

The removal percentage and final concentration of dichloromethane for four adsorbents were analyzed by using a UV-visible spectrophotometer [196, 399, 403].

5.3.6. Isotherm modeling

Languimer and Freudlich isotherm models were used to study the adsorption equilibria [14, 109, 167, 266, 316, 402].

5.3.7. Kinetic modeling

Pseudo-first order and Pseudo-second order kinetic model was used for the adsorption of dichloromethane (DCM) adsorbents [14, 398, 402].

5.3.8. Thermodynamics Modeling

Three indicators come under thermodynamic parameters-entropy change, enthalpy change, and free energy change. It provides information about the mechanism, feasibility, and spontaneity of the adsorption process [14, 398, 402].

5.4. Bioremediation

5.4.1. Culture of *Pseudomonas aeruginosa*

Cultures of the microorganisms were collected from the departmental stock culture collection. All cultures were grown on nutrient agar plates, and nutrient broth (4.5 ml) for 24 hours at 37°C [342, 364]

5.4.1.1. Preparation of culture media

Cetrimide agar was used as a selective and differential medium for the purification and identification of *Pseudomonas aeruginosa*. It is also known as Pseudomonas cetrimide agar or Pseudosel agar [334].

5.4.2. Purification and maintenance

Pseudomonas sp. was revived and purified using a qualitative method, which was pre-enriched in NKS Cetrimide plates at 37°C for 24 h. All cultures were maintained at-20°C in 15% (w/v) glycerol [219, 342, 364].

5.4.3. Observations of colonial forms

An intensive examination of the cultures on agar plates has been carried out to observe colonial forms after overnight incubation [243, 327].

Observation of pigment production*: Fluorescence*

The observation of colonies on the agar plate was done in a dark room with UV light. The lamp was Model A409 made by P. W. Allen and Co., 253 Liverpool Rd, London [219, 243, 327].

5.4.4. Morphological characterization

All cultures were examined biochemically.

5.4.5. Biochemical Tests

For the identification of *Pseudomonas aeruginosa*, biochemical tests were conducted. The biochemical tests were followed according to Bergey’s Manual for Determinative

Bacteriology. The identification tests namely, the Gram staining test, catalase test, oxidase test, and pigmentation test were conducted according to standard methods. Subsequently, bacteria have been identified by matching the results obtained with authoritative guidance tables [129, 131, 219, 243, 327].

The identification at species level was done by 16s rRNA test. The identification was done by Yaazh Xenomics Coimbature, Tamil Nadu. The methodology mentioned by them is described as under:

16s rRNA Gene Sequencing Protocol

Genomic DNA isolation: DNA isolation from Microbial samples was done using the Biobee Spin EXpure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd.,

Protocol:

1. **Lysis/homogenization:** Cells grown in monolayer should be lysed by suspending 1-3 colonies aseptically and mixed with 500 µl of lysis buffer in a 2 ml micro centrifuge tube and lyse the cells by repeated pipetting.
2. Add 4 µl of RNAse and 500 µl of neutralization buffer into it.
3. Vortex the content and incubate the tubes for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules, mix DNA solutions by inversion.
4. Centrifuge the tubes for 10 minutes at 10,000 rpm.
5. Following centrifugation, transfer the resulting viscous supernatant into a fresh 2 ml micro centrifuge tube without disturbing the pellet.
6. Add 600 µl of Chloroform Isoamyl Alcohol and do hand mixing vigorously.
7. Centrifuge the tubes for 10 minutes at 10,000 rpm. Carefully, transfer 600 µl of aqueous phase into a fresh 2ml micro centrifuge tube.
8. **Binding:** Add 600 µl of binding buffer to the content and mix thoroughly by pipetting and incubate the content at room temperature for 5 minutes.
9. Transfer 600 µl of the contents to a spin column placed in 2 ml collection tube.
10. Centrifuge for 2 minutes at 10,000 rpm and discard flow-through.

11. Reassemble the spin column and the collection tube then transfer the remaining 600µl of the lysate.

12. Centrifuge for 2 minutes at 10,000 rpm and discard flow-through.

13. **Washing:** Add 500 µL washing buffer I to the spin column. Centrifuge at 10,000 rpm for 2 minutes and discard flow- through.

14. Reassemble the spin column and add 500µl washing buffer II and Centrifuge at 10,000 rpm for 2mins and discard flow-through. Dry spins the tube for 5 minutes at 10,000 rpm.

15. Transfer the spin column to a sterile 1.5-ml micro centrifuge tube.

16. **Elution:** Add 100 µl of Elution buffer at the middle of spin column. Care should be taken to avoid touch with the filtrate.

17. Incubate the tubes for 2 minutes at room temperature and Centrifuge at 10,000 rpm for 2 minutes. The buffer in the micro centrifuge tube contains the DNA.

18. DNA concentrations were measured by Qubit flurometer 3.0 or 1% Agarose Gel Electrophoresis.

6. Add 600 µl of Chloroform Isoamyl Alcohol and do hand mixing vigorously.

7. Centrifuge the tubes for 10 minutes at 10,000 rpm. Carefully, transfer 600 µl of aqueous phase into a fresh 2ml micro centrifuge tube.

PCR Protocol

Polymerase Chain Reaction (PCR) is a process that uses primers to amplify specific cloned or genomic DNA sequences with the help of a very unique enzyme. PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA.

Composition of the Taq Master Mix

•Taq DNA polymerase is supplied in 2X Taq buffer

•0.4mM dNTPs,

•3.2mM MgCl2 and

•0.02% bromophenol blue

PRIMER DETAILS

Primer Name	Sequence Details	Number of Base
27F	5' AGAGTTTGATCTGGC TCAG 3'	20
1492R	5' TACGGTACCTTGTTA CGACTT 3'	20

Add 5 µL of isolated DNA in 25 µL of PCR reaction solution (1.5 µL of Forward Primer and Reverse Primer, 5 µL of deionized water, and 12 µL of Taq Master Mix). Perform PCR using the following thermal cycling conditions.

1. Denaturation

The DNA template is heated to 95°C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA.

2. Annealing

The mixture is cooled to anywhere from 55˚C. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

3. Extension

The reaction is then heated to 72° C, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template.

PCR CONDITIONS:

STAGES	TEMPERTAURE	TIME	
Initial Denaturation	95° C	2 Min.	
Denaturation	95° C	30 sec.	

Annealing	50 °C	30 sec.	25 cycles
Extension	72 °C	2 min.	
Final extension	72 °C	10 min.	
Hold	4 °C	α	

Purification of PCR Production

Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).The PCR product was sequenced using the primers. Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

Sequencing protocol

Single-pass sequencing was performed on each template using below 16s rRNA universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

Bioinformatics protocol:

1. The 16s rRNA sequence was blast using NCBI blast similarity search tool. The phylogeny analysis of query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment.

2.The program MUSCLE 3.7 was used for multiple alignments of sequences [105]. The resulting aligned sequences were cured using the program Gblocks 0.91b.This Gblocks eliminates poorly aligned positions and divergent regions (removes alignmentnoise) [324].Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model.

3. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while

being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering [93].

The sequencing Report contains:

1. Individual Sequence Data in Different File format.a)*.ab1 – Sequence with Chromatogram (System File).b)*.Pdf – Sequence with Chromatogramc) .c)*.txt – Sequence Data (Sequence in FASTA format).
2. Blast Result of Aligned sequence data

*All reports in finding are present in result section.

5.4.6. Enumeration

Estimation of several viable microorganisms was done by using the plate counting method and expressed as CFU per milliliter. The total amount of microorganisms (viable and nonviable cells) was estimated with a hemocytometer [131, 217, 219].

5.4.7. Batch experiment

5.4.7.1. Analysis of DCM

The removal percentage and final concentration of dichloromethane after biodegradation by bacteria were analyzed by using GC-FID [55, 291].

5.4.7.2. Procedure

To test the biodegradation of DCM by *Pseudomonas aeruginosa*, bacteria were incubated for 30 min at 37°C by taking 50 mg/L and 200 mg/L concentration of dichloromethane.

Objective 3*: Designing innovative hybrid materials for remediating the responsible compounds for the unpleasant odor of Kala Sanghian Drain.*

5.5. Removal of DCM by hybrid materials

Hybrid materials were prepared by using coconut shells, rice husk, MOF (UiO66), and bacteria (*Pseudomonas aeruginosa).* Preparation and characterization of adsorbents and the culture of bacteria were done by following standard methods as mentioned above.

5.5.1. Preparation and characterization of hybrid materials

5.5.1.1. Preparation and characterization of coconut shell activated carbon

It is already mentioned above.

5.5.1.2. Preparation and characterization of adsorbent prepared from rice husk

It is already mentioned above.

5.5.1.3. Preparation of MOF (UiO66)

Preparation for MOF has been done using the hydrothermal method. Zirconium chloride (0.169g) was dissolved in DMF (50 ml) after a proper stir for 1 hour at 60 °C. In addition, 0.121 g terephthalic acid was poured stirred it for another 20 minutes. After that, a prepared mixture for the hydrothermal treatment was transferred into an autoclave at 120°C for 24 hours. Thereafter, at room temperature, the obtained product was cooled. Once the product has been obtained, it is washed with 50 ml of DMF and rinsed twice with anhydrous ethanol. For 24 h. the recovered product was dehydrated at 80 °C. The collected sample was modified with HAc [247, 350, 365].

5.5.1.4. Culture and identification of *Pseudomonas aeruginosa*

It is already mentioned above

5.5.2. Batch experiment for DCM adsorption by hybrid materials

5.5.2.1. Procedure

To test the biodegradation of DCM by *Pseudomonas aeruginosa* and the different hybrid materials; bacteria and different hybrid materials were incubated for 30 min at 37°C by taking dichloromethane concentrations of 50 mg/L and 200 mg/L The types of hybrid materials for the batch experiment were bacteria (*Pseudomonas aeruginosa)* plus rice husk, bacteria (*Pseudomonas aeruginosa)* plus coconut shell activated carbon, bacteria (*Pseudomonas aeruginosa)* plus MOF, bacteria *(Pseudomonas aeruginosa)* plus rice husk plus coconut shell activated carbon (CSAC), and bacteria (*Pseudomonas aeruginosa)* plus rice husk plus coconut shell activated carbon(CSAC) plus MOF.

CHAPTER 6- RESULT AND DISCUSSIONS

Objective 1*: Identification of pollutants responsible for the unpleasant odor of the Kala Sanghian Drain.*

(a) Physicochemical properties studies

6.1. Physicochemical assessment of industrial effluents of Kala Sanghian Drain

6.1.1. Physicochemical characteristics of wastewater

The result of the physicochemical characteristics of samples from different sites of the Kala Sanghian Drain is illustrated with the help of Tables and Figures. The permissible limit set by the authorized organizations has been depicted in ***Table 6.1.1.*** The higher value of physicochemical parameters marks the unsuitability of water and is worthless for any use.

pH is a key parameter and mark its effect on many chemical processes such as degradation, solvability, and toxicity of metals [2]. The pH value of wastewater is dependent on the chemicals present in it. The toxic waste dump is also responsible for the reduction in the pH of water bodies. The addition of these chemicals in the water by any means causes fluctuations in pH. This causes disturbances in the physiology of aquatic species and proves lethal for some tender and prone members of flora and fauna [356]. Toxic pollutants such as volatile organic compounds, ammonia, hydrogen sulfide, dyes, and heavy metals released into the environment from the tannery, textile, and electroplating industries have a pH-altering effect on water bodies [86, 164]. Low pH act as poison for zooplankton and fishes by impairing their physiological functions [78]. High pH promotes the solubilization of elements present in waste water and badly affects the aquatic life [389]. The estimated permissible pH value for the samples collected in this study is not within the range of limits as set by WHO ***(Table 6.1.1 and Table 6.1.2).*** The hazardous chemicals in the waste water of tannery industries such as oils, detergents, tannins, and chromium impose a noxious effect on the ecosystem. Ammonia and Sulfide are produced during various stages of the tanning process. pH less than 8.0 leads to the production of hydrogen sulfide [97]**.**

The temperature has a big ascendancy on water chemistry. It plays a prime role in bio-chemical processes and an increase in temperature creates a disturbance in these processes and turns, cast a deleterious impact on aquatic organisms. It declines total cell count, hindered cell viability, and eventually leads to DNA impairment and apoptosis of fishes [183]. Thermal stress can slow down metabolic activities and impose adverse effects on the immune defense system in fish [78, 212, 356].

Temperature observed in the samples of study under consideration does not conform to the standard set by the WHO and rather they crossed these values ***(Table 6.1.2 and Table 6.1.4).*** Henceforth we can conclude that it is not suitable for aquatic and terrestrial organisms.

Electrical conductivity is entirely related to the total solids present and it can be used to measure the total dissolved ions in the sample. In other words, total solids are directly proportional to the dissolve ions in water [79].

The total dissolved solids specify the degree of dissolved substances [79, 250]. It depicts the amount of chemicals present in a liquid. The conductivity and salinity values increased by a higher total dissolved solids. High salinity causes salt-stress [393]. Salt stress furthermore impairs the essential physiological processes of plants such as photosynthesis, germination, growth nutrient imbalance, water relation, and yield [283]. The estimated value of the total dissolved solid of samples under experimentation exceeds the permissible values set by the WHO ***(Table 6.1.1).*** The estimated value of the total dissolved solid of samples under experimentation exceeds the permissible values set by the WHO. Exceeded value of the total dissolved solids and turbidity culminate in the fact that the concerned wastewater is hazardous for flora and fauna.

Dissolved oxygen is sweepingly used as a parameter to specify the water quality [345]. It is the main requirement of aquatic organisms, and its value is vulnerable to slight changes around it. In limnology, it is considered an essential parametric factor to check the level of water quality and to give a decisive statement about the extent of organic pollution in the particular water body Optimum dissolved oxygen is a lifeline for the aquatic organism and therefore low dissolved oxygen is responsible for the death of aquatic organisms [347]. The levels of dissolved oxygen detected in the samples as part of this study were considerably below WHO's permissible limit ***(Table 6.1. 1 and Table 6.1.2)***. Therefore this water body is considered malodorous for aquatic environments**.** ''Biochemical oxygen demand is the index of the degradable organic matter present in the water. The high value of biochemical oxygen demand not only represses the growth of microbes but also obtrudes deadly effects on organs and the physiology of aquatic organisms [384]. The values of biochemical oxygen demand in the samples were cross the WHO standards and clearly confirm high contaminated condition of wastewater **(*Table 6.1.1 and Table 6.1.2).***

6.1.2. Correlation among different parameters

Pearson coefficient of correlation was also performed to find out whether a positive or negative correlation exists between the various physicochemical parameters.

Pearson coefficient of correlation among various parameters was found to be varying from parameter to parameter (***Table 6.1.4).*** The coefficient correlation between the different parameters has been analyzed and the type of correlation whether it is positive or negative has been represented in ***Table 6.1.4.***

Between the temperature and TDS, BOD, and EC, a very high positive and statistically significant correlation was observed. Alternatively, a very high negative and statistically significant correlation was seen between temperature and pH and DO ***(Table 6.1.4).***

Between pH and DO, a high positive and statistically significant correlation was seen. The correlation between pH and temperature, TDS, BOD, and EC was high negative and statistically significant. The relationships between TDS and temperature, BOD and EC has been found to be very high positive and statistically significant **(*Table 6.1.4).*** Conversely, a very high negative and statistically significant correlation has been observed between pH and TDS. The correlation between DO and pH has been observed to be highly positive and statistically significant. However, temperature, TDS, BOD, and EC are correlated negatively and statistically significantly. The correlation between BOD and temperature, TDS, and EC was very high positive and statistically significant. By contrast, a high negative and statistically significant correlation was observed between pH and DO **(*Table 6.1.4*).** The correlation between EC and temperature, TDS, and BOD *was* very high positive and statistically significant. In contrast, the correlation between EC, pH, and DO was high negative and statistically significant **(*Table 6.1.4*).**

6.1.3. Comparison of various physicochemical parameters in different sites of the Kala Sanghian Drain

Sampling and analysis have been performed by taking the water samples from the different sites of the Kala Sanghian Drain. The mean and standard deviation have been illustrated with the help of ***Table 6.1.4***. According to WHO and IS 10500:1991 guidelines, the pH value should be lies in between 6.5–8.5. pH of the water samples of the present study from the selected sites of the drain was measured and was found in the range between 5.71-6.81 which were above the standards set by WHO and IS 10500:1991 ***(Fig 6.1.1).*** Analysis of effluents from different sites showed variation in the values of pH. In order to monitor the quality of water, TDS is also an excellent parameter. At four sites of drain, TDS ranges from 2426.88 mg/ L to 2693.49 mg/ L. Its value was lowest in Site 1(LD) and highest in Site 4 (BD) ***(Table 6.1.2).***

BOD value confirms the presence of organic impurities in the samples. And it is considered an important parameter in the physicochemical parametric world. The values of BOD of the water samples are out of the range of values as set by WHO and IS 10500:1991 with a maximum of 259.4 mg/L for Site 4 (BD) and a minimum 190.6 mg/L for Site 1(LD) ***(Table 6.1.2)***.

Temperature of water controls physiological functions of aquatic organisms and the fertility of land. The temperature of water samples of the current study does not go according to guidelines set by WHO and IS 10500:1991. It is highest at 45.2°C for Site 4 (BD) and a minimum of 41.24 °C for Site 1(LD) ***(Table 6.1.2).***

Electrical conductivity also has a pivotal role in the water quality index. The samples were collected from the study sites has shown values of electrical conductivity above the WHO standards. An electrical conductivity is maximum of 3617.89 µS/cm and a minimum of 2449.75 µS/cm have been recorded ***(Fig. 6.1.11).***

The existence of aquatic organisms is positively influence by dissolved oxygen. The values found in the water samples taken from four sites were 2.32 mg/L for site 1(LD),1.75 mg/L for Site 2 (LO), 1.73 mg/L for Site 3 (FD), and 1.45 mg/L for Site 4 (BD) ***(Fig. 6.1.11).***

6.1.4. Comparison of present physicochemical parametric study with other studies

The wastewater of the present study receives effluents from various types of industries such as tannery, textile, and electroplating industries. The values of the parameters under consideration crossed the permissible limit set by WHO guidelines and other environment-concerned organizations. The temperature has a big primacy on water chemistry. It has a high impact on the physiology of organisms [183]. The value of temperature of water samples in this study was above 40 °C, that is not lies in the range set by the World health organization and Indian Standard for drinking water (BIS)IS: 10500 [54, 363]. A similar trend has been seen in some previous studies done by Bakar [37]. pH a high impact on bio-chemical processes namely, metal toxicity, degradation, and solubility. pH range analyzed in the previous studies was not in the range set by the concerned organizations. A similar trend has been shown by previous studies to cross the limit set by the WHO [85, 114, 123]. Electrical conductivity is also a very important parameter. Electrical conductivity and total dissolved solids are interrelated to each other and are used to analyze the total dissolved ions in the sample matrix [37]. According to guidelines set by WHO, the value of electrical conductivity should not be above 1000 µS/cm. The result found in the present study is 2372.397-3617.89µs/cm which is quite far away from the permissible limit. Previous studies also have shown similar types of higher values; 4150µs/cm in textile wastewater [153]. TDS is also a major parameter for physicochemical properties. Total dissolved solids unveil the degree of dissolved substances [2]. It explores the amount of organic and inorganic compounds present in a liquid. TDS concentration depends on domestic and agricultural runoff, effluents of industries, and products of leaching of soil [303]. In the current work, the TDS value was more than the permissible limit (2693.49 mg/L). In previous studies, it has been reported that effluents from tannery and textile industries also had TDS values above the permissible limit as set by the WHO [123, 160, 294]. BOD reveals the amount of degradation of organic matter in water. The excess value of BOD affects the growth of microbes and the physiology of aquatic organisms [384]. Values set by WHO is 30 mg/L. In the present studies, it lies between 196.58- 259.4 mg/L. Previous studies also show BOD values of wastewater samples above the limit set by WHO [85, 294].

Nirgudeet et al. found very high values of BOD of effluents collected from textile industries to be in between 117–272 mg/L [153]. Methneni et al., conducted the same type of work, on textile dying industries by collecting water samples from dying mills and measuring their BOD levels, which were also above the permissible limit as set by World Health Organization and Bureau of Indian Standards [228]. High TDS value changes the water chemistry by altering the physicochemical properties of water [153]. This water stream is unfit for irrigation purposes and depletes the fertility of the soil by closing the pores of soil particles.

6.1.5. Statistical analysis

For the statistical analysis, the SPSS software was used. The experimental data are presented as mean ± SD of the experiment at $*p \leq 0.05$ **(Table 6.1.2).** The observed values have been taken for subsequent statistical analysis and interpretation. To find out the statistical value and significance of population means at 0.05 levels**,** Analysis of variance (ANOVA) One-way test was performed (***Table 6.1.3 Fig.2 and Fig.13).*** The Anova result reveals that temperature, pH, TDS, BOD, DO and EC across the sites and control differs significantly ($F_{4, 10}$= **364.94** (temperature), **1034.47** (pH), 4528.11 (TDS), 3843.54 (DO), 27853.69 (BOD) and 3211.78 (EC), p<0.05 respectively ***(Table 6.1.3).***

Since Levene's statistic is significant, the equal variance was not assumed. To check the individual difference between sites, Post hoc comparisons were assessed using Tukey. One-way ANOVA with post hoc Tukey's test indicated that the concentrations of all the measured physicochemical parameters were not equal across the drain. The mean value of physicochemical parameters with standard error has been shown in **Fig 6.1.1 (a) and 6.1.1(b).**

There has been observed, in case of temperature, the mean value of control is significantly different from the mean value of four sites. The mean values of temperature of four sites are also significantly different from each other except Site 2 (LO) and Site 3 (FD) ***Table 6.1.2, Table 6.1.3, Fig.6.1.2).***

The same result has been seen in case of pH. The mean value of pH of control is significantly different from the pH of four sites and pH of four sites are also significantly different from each other Site 2 (LO) and Site 3 ***(Table 6.1.2, Table 6.1.3, Fig.6.1.3***.); among TDS and DO ***(Table 6.1.2, Table 6.1.3, and Fig. 6.1 and Table 6.1.2, Table 6.1.3, and Fig.6.1.5. respectively*)**; among BOD and EC, same pattern has been seen ***(Table 6.1.2, Table 6.1.3, and Fig.6.1.6*** and ***Table 6.1.2, Table 6.1.3, and Fig.6.1.7 respectively).***

6.1.6. Conclusion and policy implications

The protection of the environment has become a global issue from the past two decades. The unenthusiastic nature of people towards the Environment Protection Act leads to a massive load of pollutants in the aquatic and terrestrial community. There is continuous addition of organic and inorganic matter in the drain under study, due to processing of hides and skin in leather industries, dyeing of clothes in the textile industries, and use of heavy metals in electroplating industries. These activities give a colossal contribution towards the unacceptable addition of chemicals in the concerned water body. The various chemical processes in industries, manipulate the water quality and crosses the permissible limit as set by World Health Organization. These waste water having spiked values of various parameters, confirm that these water bodies are unsuitable for any kind of anthropogenic utilization. The outcome of the study reveals that industrial effluents are malodorous for both aquatic and terrestrial life. It is extremely toxic for human beings and animals living near the concerned site. The values of parameters unguardedly confirm the toxicity of the water body under current research work. The alarming situation in the environment protection compels the prohibition of anthropogenic activities near the water courses. The alteration in the purity of water by industrial effluents, clearly reminds us about our duty to treat the effluent properly before discharging it into the water bodies. It must be emphatically endorsed to make environmental protection rules and regulations sincerely and further implement them strictly with regular monitoring of various quality parameters. Quality regulation must be enforced and proper vigilance on industries to set up and regulate properly the effluent treatment plants. Honesty towards this direction assuredly armors the quality of water for successors. Industries should have a separate drainage system that pours its effluents into the non-residential areas away from humans and animals to safeguard them from being consumed. To prevent salt load, ecofriendly methods should be utilized in unhairing and liming in tannery industries. It has been suggested that the textile effluents should be checked for toxicity potential and also microbiologically before discharging them in water bodies. As electroplating industries are responsible for the addition of acids in the water, suitable metallurgical options should be used for the recovery of these acids from water bodies. The systematically and repeatedly monitoring and evaluation of the quality index of effluents before discharging them in water bodies will leads to toxins- free water bodies.

In addition, public awareness on the sources of water pollution must be raised urgently so as to avoid further increase in solid and liquid discharges in natural waters. In controlling the spread of adverse effects on water bodies and in ensuring life safety for generations to come, it is essential that people’s activities are monitored. The current study indicates that there is an urgent need to take environmental issues seriously and create awareness in human society about the sources of pollution. The work should be carried out for monitoring of waste water treatment plants, building and implementing the bioremediation strategies to reduce the pollution in the concerned water body. Environmental laws should strictly imply the industries for proper treatment, management, and disposal of waste water and finding alternative methods for remediation of pollutants that exist through wastewater.

Last but not least, a large extent of the scope to characterize different type of chemicals including heavy metals released into the environment by the tannery, leather, and electroplating industries.

Table 6.1.1:Range prescribed by World Health Oranization ,2008, Bureau of Indian Standards and IS:10500;1983 for industrial effluents discharge [54, 363]

Parameters	Inland surface water	Public sewers	Land of irrigation	Marine/ coastal areas
Water temperature (°C)	25-40°C	30-40°C	-	-
pH	5.5-9	5.5 to 9	5.5 to 9	5.5 to 9
Electrical conductivity (μS/cm)	2000	-	-	-
Dissolved oxygen (mg/L)	4.5-8	4.5-8	4.5-8	-
Total dissolved solids (mg/L)	2100	2100	2100	-
Biochemical oxygen demand (mg/L)	30	350	100	100

Table 6.1.2: Descriptive statistics of water quality parameters of wastewater of Kala Sanghian Drain

Parameters	**Sites**	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
						Lower Bound	Upper Bound
	Control	3	24.8133	0.574	0.331	23.38	26.24
Temp. (°C)	Site 1 (LD)	3	41.24	0.63	0.363	39.67	42.80
	Site 2 (LO)	3	43.67	0.85	0.49	41.55	45.79
	Site 3 (FD)	3	44.0367	0.7072	0.4083	42.27	45.79
	Site 4 (BD)	3	45.2	1.00	0.58	42.69	47.7011
	Total	15	39.79	7.89	2.03	35.42	44.16
	Control	3	7.58	0.05	0.031	7.44	7.72
pH	Site 1 (LD)	3	6.8133	0.05	0.031	6.67	6.95
	Site 2 (LO)	3	6.67	0.01	0.005	6.64	6.69
	Site 3 (FD)	3	6.56	0.01	0.008	6.52	6.60
	Site 4	3	5.71	0.01	0.006	5.68	5.74

	(BD)						
	Total	15	6.67	0.61	0.15	6.32	7.01
	Control	3	321.49	1.48	0.85	317.80	325.17
TDS (mg/L)	Site 1 (LD)	3	2426.883	2.005	1.15	2421.90	2431.86
	Site 2 (LO)	3	2559.143	19.63	11.33	2510.36	2607.92
	Site 3 (FD)	3	2587.467	54.26	31.33	2452.65	2722.27
	Site 4 (BD)	3	2693.493	5.74	3.31	2679.21	2707.77
	Total	15	2117.695	934.035	241.16	1600.44	2634.94
	Control	3	6.6233	0.09	0.05	6.38	6.862
DO(mg/L)	Site 1 (LD)	3	2.3267	0.09	0.05	2.0906	2.56
	Site 2 (LO)	3	1.75	0.01	0.00577	1.7252	1.7748
	Site 3 (FD)	3	1.73	0.005	0.003	1.71	1.74
	Site 4	3	1.45	0.005	0.003	1.43	1.46

	(BD)						
	Total	15	2.77	2.01	0.51	1.66	3.89
	Control	3	30.47	0.58	0.33	29.02	31.91
BOD(mg/L)	Site 1 (LD)	3	190.66	0.49	0.28	189.42	191.89
	Site 2 (LO)	3	250.56	1.50	0.86	246.82	254.29
	Site 3 (FD)	3	251.82	1.20	0.69	248.83	254.80
	Site 4 (BD)	3	259.4	0.87	0.50	257.21	261.58
	Total	15	196.58	89.69	23.15876	146.91	246.25
EC(µS/cm)	Control	3	401.25	0.85	0.49	399.12	403.37
	Site 1 (LD)	3	2449.75	57.90	33.43	2305.89	2593.60
	Site 2 (LO)	3	2676.33	1.087	0.62	2673.63	2679.03
	Site 3 (FD)	3	2716.75	0.87002	0.50	2714.59	2718.91
	Site 4	3	3617.89	56.96	32.88	3476.38	3759.39

	(BD)							
	Total	15	2372.39	1101.10	284.30	1762.62		2982.16

Table 6.1.3: Test of homogeneity of variance and ANOVA

Parameters	**Test of homogeneity of variance**		**ANOVA**	
	Levene' statistic	**Sig.**	**F**	**Sig.**
Temperature (°C)	**0.36**	**0.828**	**364.94**	**<0.05**
pH	**3.62**	**0.045**	**1034.47**	**<0.05**
TDS (mg/L)	**11.47**	**0.001**	**4528.11**	**<0.05**
DO(mg/L)	**3.44**	**0.051**	**3843.54**	**<0.05**
BOD(mg/L)	**1.93**	**0.182**	**27853.69**	**<0.05**
EC(µS/cm)	**11.63**	**0.001**	**3211.78**	**<0.05**

Table 6.1.4: Parameters correlated and Results obtained

	Temperature	**pH**	**TDS**	**DO**	**BOD**	**EC**
Temperature	**1**					
pH	**-.817****	**1**				
TDS	**.994****	**-.791****	**1**			
DO	**-.995****	**.808****	**-.998****	**1**		
BOD	**.987****	**-.826****	**.978****	**-.988****	**1**	
EC	**.963****	**-0.937**	**954****	**-.960****	**.956****	**1**

AA

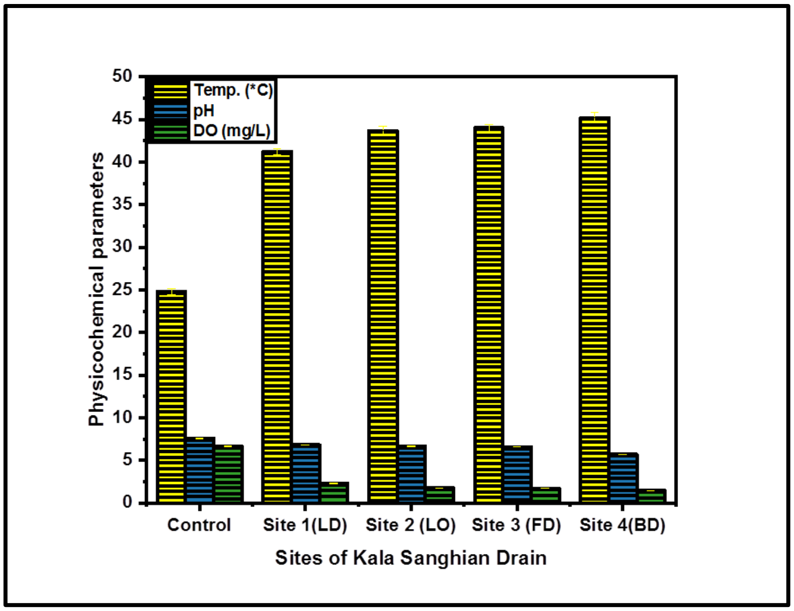


Fig.6.1.1 (a).Mean of physicochemical parameters (Temp., pH and DO) across the different sites of Kala Sanghian Drain

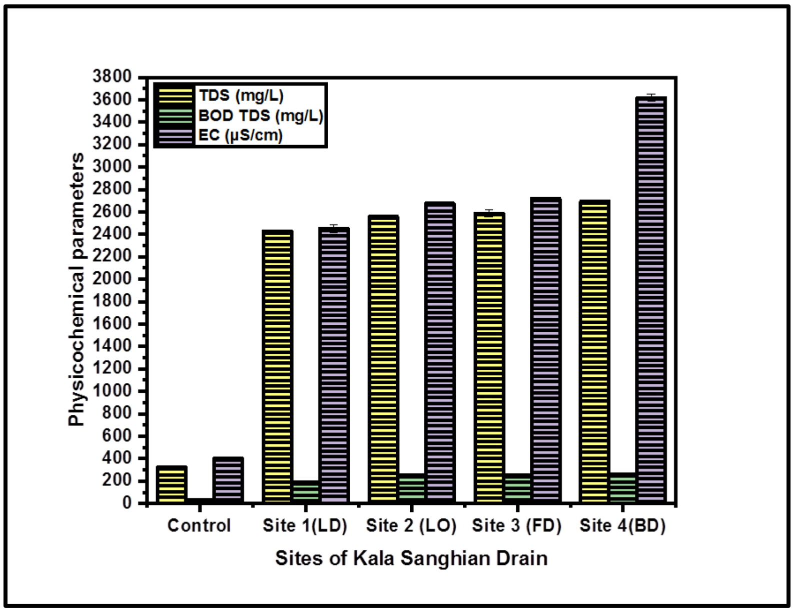


Fig.6.1.1 (b).Mean of physicochemical parameters (TDS, BOD and EC) across the different sites of Kala Sanghian Drain

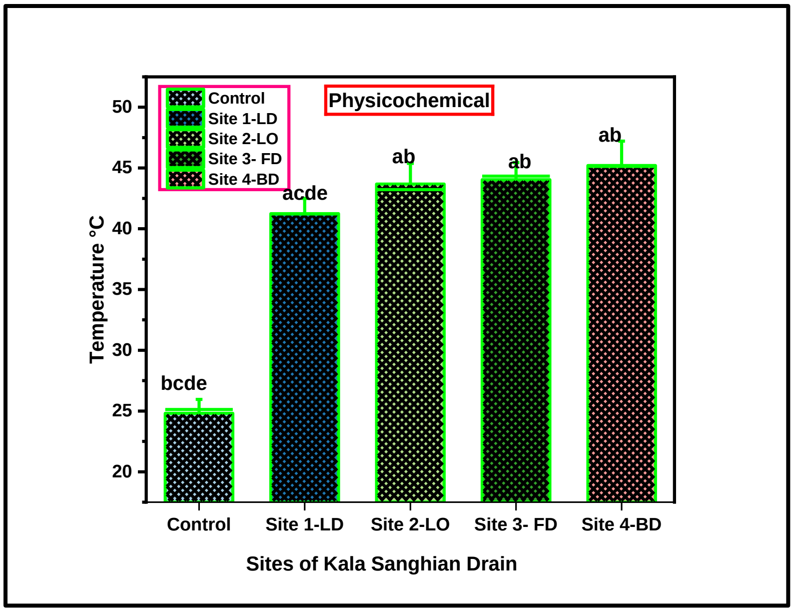


Fig.6.1.2. Comparisons of temperature in various sites of the Kala Sanghian Drain (p<0.05)

(*Control-a, Site 1-LD-b, Site2 -LO-c, Site 3-FD-d, Site 4-BD-e)

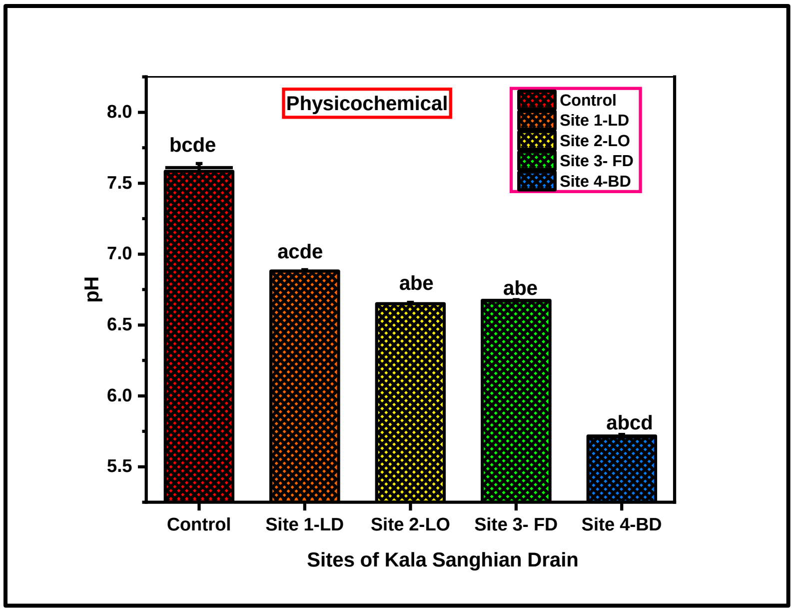


Fig. 6.1.3.Comparison of pH in various sites of the Kala Sanghian Drain (p<0.05)

(*Control-a, Site 1-LD-b, Site2 -LO, Site 3-FD, Site 4-BD)

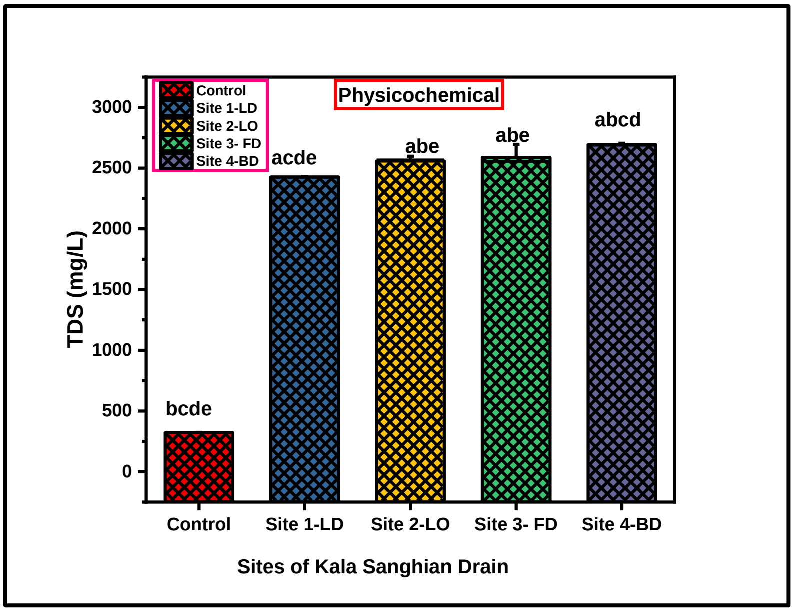


Fig.6.1.4. Comparison of TDS in various sites of the Kala Sanghian Drain ($p<0.05$)

(*Control-a, Site 1-LD-b, Site2 -LO-c, Site 3-FD-d, Site 4-BD-e)

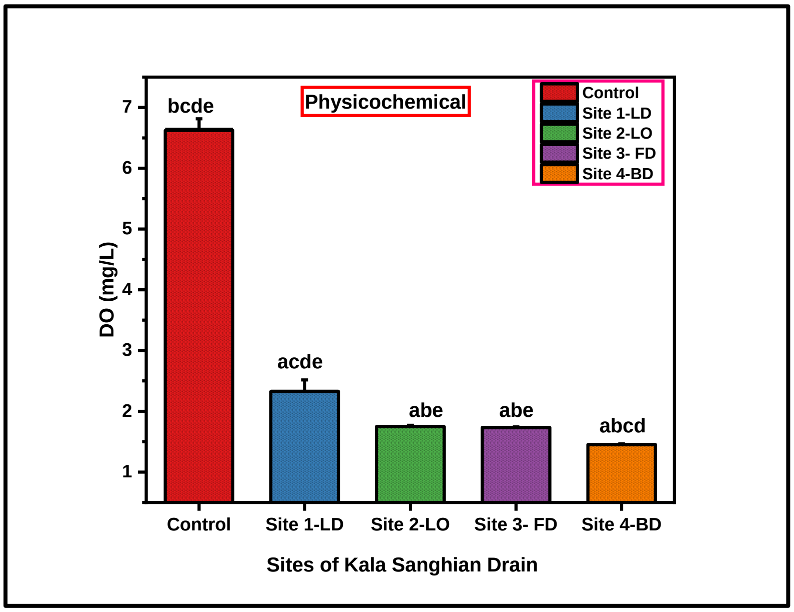


Fig. 6.1.5.Comparison of DO in various sites of the Kala Sanghian Drain ($p<0.05$)

(*Control-a, Site 1-LD-b, Site2 -LO-c, Site 3-FD-d, Site 4-BD-e)

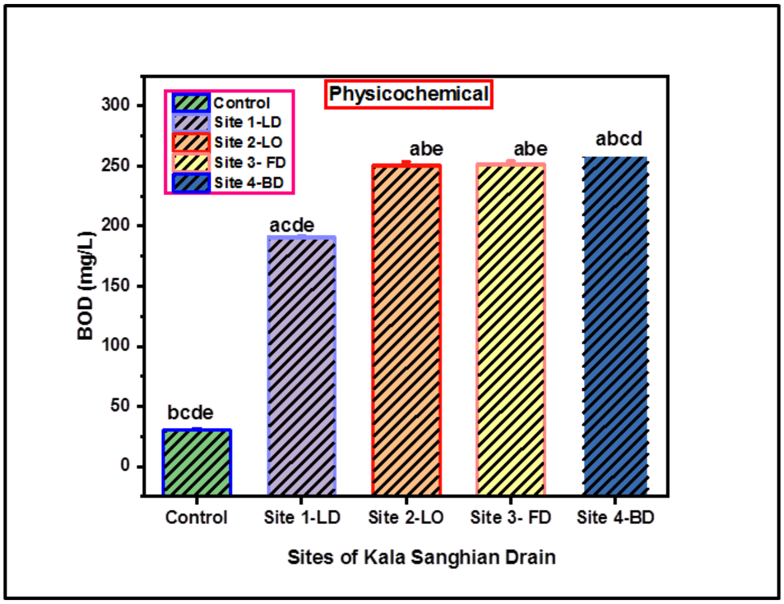


Fig.6.1.6 .Comparison of BOD in various sites of the Kala Sanghian Drain ($p<0.05$)

(*Control-a, Site 1-LD-b, Site2 -LO-c, Site 3-FD-d, Site 4-BD-e)

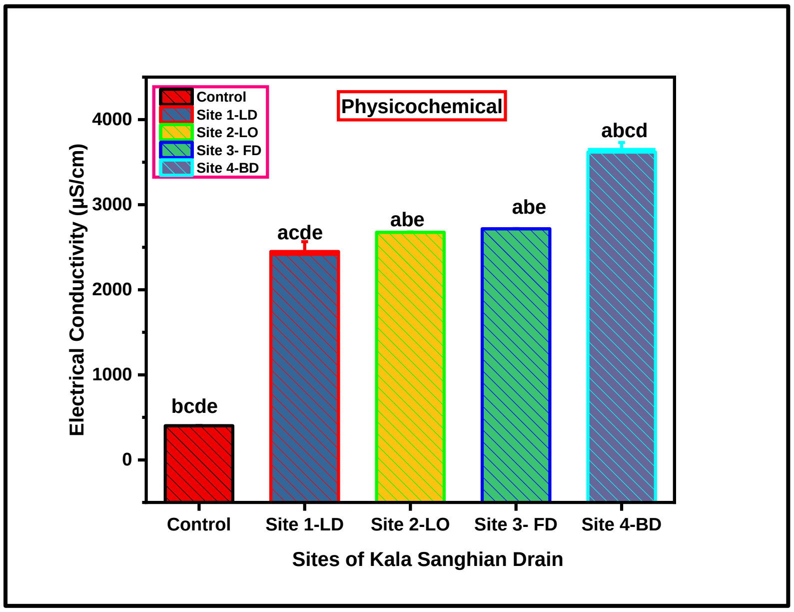


Fig. 6.1.7. Comparison of EC in various sites of the Kala Sanghian Drain ($p<0.05$).

(*Control-a, Site 1-LD-b, Site2 -LO-c, Site 3-FD-d, Site 4-BD-e)

(b). Identification of pollutants responsible for the unpleasant odor of the Kala Sanghian drain

6.2. Identification of toxic volatile organic compounds

6.2.1. Parameters important for purge and trap

In optimization of the purge and trap, among various parameters purge and bake are the prime ones. The volatile nature of solvents and volatile organic compounds holds the chances of contamination during the analysis [41]. To overcome this drawback, the cleanliness of all the glassware was also taken thoughtfully while undergoing analysis. Methanol was used as a solvent in the analysis of water samples for the current study. It is cheaper, less toxic, fully miscible with water, and highly compatible with buffers and mobile phase additives [279, 392].

6.2.2. Distribution of volatile organic compounds in four sites of Kala Sanghian Drain (KSD)

Samples have been collected from the four sites of the Kala Sanghian Drain (KSD) in triplicates. Eleven volatile organic compounds have been detected in these four sites and are represented in ***Table 6.2.1, Table 6.2.2, and Fig.6.2.1.***

The distribution of volatile organic compounds varies from site to site. From each site, the samples were collected in triplicates. In Site 1 (LD), three organic compounds, namely dichloromethane, cis-1, 2-Dichloroethene, and heptane have been detected In Site 1 (LD), dichloromethane, cis-1, 2-Dichloroethene, and heptane; have the mean concentration of7.19 ng/ml, 44.45ng/ml and 3.8ng/ml. There is complete absence of others compounds namely, 1, 3, 5-Trimethylbenzene, 2-Chlorotoluene, Bromobenzene, Cumene, Ethylbenzene, p-Isopropyltoluene, 1, 1, 1-Trichloroethane and Tetrachloroethane in this site ***(Tables 6.2.6 to 6.2.28 and Fig.6.2.3)***

In Site 2 (LO), two compounds heptane and cis-1, 2- Dichloroethene were detected with a mean concentration of 4.97ng/ml and 101.51ng/ml. There has been found a complete absence of other volatile organic compounds, namely, dichloromethane, 1, 3, 5-Trimethylbenzene, 2-Chlorotoluene, Bromobenzene, Cumene, Ethylbenzene, p-Isopropyltoluene, 1,1,1-Trichloroethane and Tetrachloroethane in this site in this site ***(Tables 6.2.6 to 6.2.28 and Fig.6.2.4)***

Site 3(FD) acted as a surprising site with a cluster of eight compounds. These compounds namely, heptane,1, 3, 5-trimethylbenzene, 2-chlorotoluene, bromobenzene, cis-1, 2-Dichloroethene, cumene, ethyl benzene, and p-Isopropyltoluene were detected with a mean concentration of 5.19 ng/ml, 29.46 ng/ml, 8.74 ng/ml, 26.41 ng/ml, 3.37 ng/ml, 7.57 ng/ml, and 148.54 ng/ml respectively. There is absence of other compounds such as dichloromethane, cis-1, 2- Dichloroethene, 1, 1, 1-Trichloroethane and Tetrachloroethane ***(Tables 6.2.6 to 6.2.28 and Fig.6.2.5)***

In the Site 4 (BD), four compounds were detected namely, Heptane, cis-1, 2-Dichloroethene, 1, 1, 1- Trichloroethane, and Tetrachloromethane with a mean concentration of 8.63 ng/ml and 4.09 ng/ml, 7.63 ng/ml and 3.57 ng/ml respectively ***(Tables 6.2.6 to 6.2.27 and Fig.6.2.6)***

On analysis of all volatile organic compounds, dichloromethane, heptane, and cis-1, 2-Dicholoroethene has been observed as the most frequently occurring VOCs in wastewater samples. On the evaluation of the concentration of compounds, heptane was detected at higher concentrations. Contrary to this, cumene was detected in very less concentration and restricted itself in Site 3 (FD). The total amount of VOCs emissions from four sites (1, 2, 3, 4) were 251.3802ng/ml, 319.468 ng/ml, 758.38 ng/ml, and 71.83 ng/ml respectively ***Table 6.2.2***.

6.2.3. Chemical Composition of VOCs

Eleven volatile organic compounds detected includes five species of a benzene series (1, 3, 5-trimethylbenzene, p-Isopropyltoluene, bromobenzene, cumene, and ethylbenzene) four chlorinated hydrocarbons (carbon tetrachloride, cis-1, 2-Dichloroethene, 1, 1, 1-Trichloroethane, tetrachloromethane) one chlorinated benzene compound (2-

chlorotoluene) and one species of alkane (heptane). Chemical composition has been shown with the help of ***Table 6.2.3, and Fig 6.2.2***.

The highest number of volatile organic compounds was observed in Site 3 (FD) with eight organic compounds. Benzene series shows maximum number of compounds including (1, 3, 5-Trimethylbenzene, p-Isopropyltoluene, bromobenzene, cumene, and ethylbenzene). It was followed by alkane incorporating a single species of heptane with total concentrations of 619.3 ng/ml inclusion of all the sites. Single species namely, 2-chlorotoluene belonging to the category of benzene-chlorinated compounds were detected. Four species namely, tetrachloromethane, dichloromethane, cis-1, 2-Dichloroethene and 1, 1-Trichloroethane belongs to the category chlorinated hydrocarbons were detected ***in Table 6.2.3, and Fig 6.2.2***.

6.2.4. Comparison of volatile organic compounds with industries situated near the sample sites of the Kala Sanghian Drain

The assessment of the number of compounds and occurrence of volatile organic compounds suggests that the discharged chemicals are specifically related to type of industries situated near the length of the Kala Sanghian Drain. To evaluate the differences, site by site comparison was carried out. As the drain is flowing, the effluents of one industry get mixed with other industry effluents. The volatile organic compounds detected in this drain revealed the type of industries that discharge these effluents. The common industries were leather and tannery, rubber, tire manufacturing, automotive parts manufacturing, textile industries, electroplating industries, pipe fitting, and motor parts manufacturing industries. A total of eleven volatile organic compounds have been detected from the four sites selected for analysis. The most repeated volatile organic compounds were heptane, cis-1, 2-Dichloroethene, and dichloromethane. Heptane and cis-1, 2-Dichloroethene were detected in all four sites, but dichloromethane has shown its presence in Site-1 (LD), Site 3 (FD), and Site 4 (BD) which receives the effluents from the leather and tannery industries. Heptane, cis-1, 2-Dichloroethene, and dichloromethane are used as a solvent in these industries. Their presence on this site is justified by the presence of textile and leather industries [19, 62, 87, 102, 268, 278, 323]. In Site 2 (LO), heptane and cis-1, 2-Dichloroethene again repeated themselves as this site receives the effluents from the same type of industries but dichloromethane was not detected at this site. Site 3 (FD) receives the effluents from tire manufacturing, automotive parts manufacturing, agricultural equipment parts manufacturing, and textile industries. Site 3 (FD) has shown the cluster of eight volatile organic compounds heptane, 1, 3, 5-Trimethylbenzene, 2-chlorotoluene, bromobenzene, cis-1, 2-Dichloroethene, cumene, ethyl benzene, and p-Isopropyltoluene. These volatile organic compounds complement the industries situated near this site [65, 286, 308, 396]. Site 4 (BD) receives the effluents from motor parts manufacturing industries, pipe fitting parts, and tire industries. The compounds detected over here were heptane, cis-1, 2-Dichloroethene, tetra chloromethane, and 1, 1, 1-trichloroethane.

The most abundant volatile organic compound-heptane has given a major contribution of 95.33% in Site 2 (LO).and cumene of 1.33% to the total emission of VOCs in site 3 (FD). After heptane, the species that attain the second position with a contribution of 58.76 % was p-Isopropyl toluene in site 3 (FD). Tetracholromethane was the third species found in a higher quantity of 38% in Site 4(BD). 2-chlorotoluene and ethyl benzene have given almost equal contributions of 3.46% and 3% respectively in Site 3 (FD) ***Table 6.2.4***

Volatile organic compounds were identified at different retention times (RT), viz. dichloromethane (5.409), heptane (7.544), cis-1, 2-Dichloroethene (6.466), 1, 3, 5-trimethylbenzene (13.896), 2-chloro toluene (13.448), bromobenzene (12.487), cumene (12.487), ethyl benzene (11.259), p-Isopropyl toluene (14.270) 1, 1, 1-Trichloroethane (6.242), and tetrachloromethane (6.349) respectively. It has been shown in ***Table 6.2.5.***

6.2.5. Toxicity of volatile organic compounds detected in Kala Sanghian Drain (KSD)

The volatile organic compounds are the health hazards for the living organisms. Acute exposure to heptane causes unconsciousness, feasible peripheral neuropathy, and neuroticism in the human nervous system. It has been reported to cause DNA damage in bacterial cells [47, 162]. It is used as a solvent in chemical processes and automotive parts-making industries [19, 268, 396]. Dichloromethane impairs visual, auditory, and motor functions [209]. It is enlisted by the health organization as a human carcinogen [272]. Due to its bioaccumulation potential, it is very toxic to the environment. cis-1,2-Dichloroethene is toxic for human beings and responsible for the dysfunction of the central nervous system and respiratory system [62, 272]. This chlorinated hydrocarbon is considered a human carcinogen and harms the environment due to its non-degradable nature. A high concentration of cis-1, 2-Dichloroethene affects human females during pregnancy and causes birth defects. It is used as a colorant in the textile industry and in hair dying, as intermediates for producing plastic, industrial solvents, degreasing agents, and textile and chemical industries [62, 102, 272]. Trimethylbenzene has been reported in human cord blood and causes a reduction in fetal body weight. It is a known carcinogen [23] and toxic for aquatic organisms too [256]. 2-chlorotoluene causes eye, skin, and respiratory problems in human beings. It acts as a teratogen and adds excessive toxicity to the aquatic ecosystem too [98, 249]. It is used in the form of solvent, intermediate in the production of pharmaceuticals, industrial cleaners, agrochemicals, optical brighteners, and pigments [98, 308]. Bromobenzene is reported as an environmental toxin that causes liver necrosis and massive hemorrhage in mice and acts as a hepatotoxic and neurotoxic agent [10]. It is used as an additive in motor oil as well as an industrial solvent [231]. Cumene is reported as a mutagenic agent in some bacterial strains [286, 315]. It is used as a solvent, catalyst, and curing agent in various chemical processes and enters into the environment as textile effluent [286]. Ethyl benzene is also a toxic compound. Short-term exposure causes fatigue, headache, and drowsiness. While long-term exposure causes defects in the kidneys, liver, and nervous system. It is also responsible for pulmonary and cardio vascular disease. It is produced during the production of pharmaceuticals, rubbers, thinners, adhesives, cosmetics, and more importantly during the production of BTEX compounds [58, 61, 239]. p-Isopropyltoluene has the ability to exert a genotoxic effect in drosophila [128]. It also causes ozone depletion [397]. 1,1,1-Trichloroethane impairs the physiology of liver, nervous and circulatory system [271, 360]. Tetrachloromethane on acute exposure manipulates the physiological functions in human beings and boosts the chances of liver cancer and damages the kidneys in animals. Its exposure for a short duration leads to irritation of the eyes, throat, impairs the physiology of the nervous system in humans [271, 301, 328]. It also acts as an ozone depleting agent. It is used as a solvent, paint thinner and motor fuel component. It is also used as an intermediary in organic chemicals synthesis and in chlorination procedures [301].

6.2.6. Method validation

a) Linearity and sensitivity

The method demonstrated good linearity with the coefficient of correlation value ranging between 0.98-0.99. LOD was discovered to be 0.002-0.1ng/mL ng/ml.

b) Method precision

A very good precision between 2% -5% was obtained.

6.2.7. Statistical analysis

The experimental data for eleven identified volatile organic compounds are presented as mean ± SD of the experiment at $*p \leq 0.05$ ***(Tables 6.2.2, 6.2.4, 6.2.6.,6.2.8, 6.2.10, 6, 6.2.12, 6.2.14, 6.2.16, 6.2.18, 6.2.20, 6.2.22, 6.2.24, Fig. 6.2.2.6, 6.2.17).*** The observed values have been taken for subsequent statistical analysis and interpretation. Analysis of variance (ANOVA) one-way test was also performed to find out the statistical value and significance of population means at 0.05 level **.**The ANOVA result suggests that there is the least significant difference in the mean of Dichloromethane between Control-Site 1(LD); Site-1(LD)-Control, Site 2(LO), Site 3(FD), and Site 4 (BD);Site-2(LO)-Site 1(LD); Site 1 (LD)-Site 3(FD) and Site-1(LD)-Site 4(BO) ($F_{4,10}=3.993$ $p<0.05$) ***(Table 6.2.7 to Table 6.2.17 and Fig 6.2.7)*** In case of Heptane, there is seen a significant difference in the mean of Heptane between Control-Site 2 (LO); Site 2 (LO)- Control, Site 3 (FD) and Site 4 (BD)and Site 2 (LO)-Site 4 (BD) ($F_{4,10}=6.194$ $p<0.05$ ***(Tables 6.2.7 to 6.2.17 and Fig 6.2. 8).***

There is no significant difference in the mean of cis- 1, 2-Dichloroethene between the different sites of the drain ($F_{4, 10}= 0.266$, $p>0.05$) (***Tables 6.2.7 to 6.2.17 and Fig***

6.2.9).The same result seen in case of 1, 3, 5-Trimethylbenzene(F 4, 10= 1.000, p>0.05) ***(Tables 6.2.7 to 6.2.17 and Fig 6.2.10*** and ***2-*** Chlorotoluene (F 4, 10= 1.000, p>0.05) ***Table 6.2.7-Table 6.2.17 and Fig 6.2.11 respectively):*** *among* Bromobenzene (F 4, 10= 1.000, p>0.05) ***(Tables 6.2.7 to*** *6.2.17* ***and Fig 6.2.12 and*** Cumene (F 4, 10= 1.000 p>0.05) ***Tables 6.2.7 to 6.2.17 and Fig 6.2.13 respectively);*** among Ethylbenzene (F 4, 10= 1.000 p>0.05) ***(Tables 6.2.7-6.2.17 and Fig 6.2.14)*** Isopropyltoluene (F 4, 10= 1.000, p>0.05) (***Table 6.2.7-Table 6.2.17 and Fig 6.2.15);***among 1,1,1-Trichloroethane (F 4, 10= 1.000 p>0.05) ***(Tables 6.2.7 to 6.2.17 and Fig 6.2.16*** *)* and Tetrachloroethane (F 4, 10= 1.000, p>0.05) (***Table 6.2.7-Table 6.2.27 and Fig 6.2.17).***

Six compounds namely1, 3, 5-Trimetylbenzene, 2-Chlorotoluene, Bromobenzene, Cumene, Ethylbenzene, and p-Isopropyltoluene are detected in the same Site 3(FD) of the drain. To check the significant difference among them, an independent T-test was done. T-value (Control-0.374, 1,3,5-Trimetylbenzene-0.423, 2-Chlorotoluene-0.423, Bromobenzene-0.423,Cumene-0.423, Ethylbenzene -0.423 and p-Isopropyltoluene -0.423 (F 4,10= 16, p>0.05 is showing that there is no significant difference in the mean of 1,3,5-Trimetylbenzene, 2-Chlorotoluene, Bromobenzene, Cumene, Ethylbenzene and p-Isopropyltoluene between the different sites of the drain **(*Tables 6.28 and 6.29 (a) (b).***

6. 2.8. Conclusion

The GC-QqQ-MS/MS analytical method has shown decent chromatographic resolution in the present study. It is an effortless, simple, and sensitive method generally used in the analysis of the torrent of volatile organic compounds. This method has given satisfactory results on the spectrum of compounds with different polarity, and volatility as well as having different retention times. The compounds detected in wastewater from the drain are hazardous to the environment. These are acting as carcinogens and mutagens and therefore lethal for the aquatic and terrestrial community. Volatile organic compounds detected from the wastewater are also responsible for the deteriorated taste and odor of the drain. There is a scope for more extensive study to remediate these volatile organic compounds that are very toxic and a big health hazard for the people living near this territory. In the future, there is high scope for using a variety of methods to reduce the pollutants responsible for bad odor as well as for the transmission of lethal diseases. There is a need of the hour for strict implementation of industrial guidelines and their proper monitoring. There should be a creation of a bridge between researchers, industries, and the common man for proper collection of samples, transportation, and tackling of toxicity of these compounds to prevent human beings and the environment from their lethal impact at a local and national level and deterioration of water quality index at the international level.

Table 6.2.1: Volatile organic compounds detected in Site 1 (LD), Site 2 (LO), Site3 (FD), and Site 4 (BD) of Kala Sanghian Drain (KSD)

Sites of Kala Sanghian Drain	Compound detected
Site-1 (LD)	Dichloromethane
	Heptane
	cis-1,2-Dichloroethene
Site 2 (LO)	cis-1,2-Dichloroethene
	Heptane
Site 3 (FD)	Heptane
	1,3,5-Trimethylbenzene
	2-Chlorotoluene
	Bromobenzene
	cis-1,2-Dichloroethene
	Cumene

	Ethylbenzene
	p-Isopropyltoluene
Site 4 (BD)	cis-1,2-Dichloroethene
	Heptane
	1,1,1-Trichloroethane
	Tetrachloromethane

Table 6.2.2: Concentration of volatile organic compounds in four sites of Kala Sanghian Drain (KSD)

Site of Kala Sanghian drain	Volatile organic compounds detected	Quantity(ng/ml)	Total concentration of VOC per /Site (ng/ml)
Site-1(LD)	Dichloromethane	21.5851	251.38
	Heptane	218.3641	
	cis-1,2-Dichloroethene	11.431	
Site-2 (LO)	cis-1,2-Dichloroethene	14.912	319.46
	Heptane	304.556	
Site-3 (FD)	1,3,5-Trimethylbenzene	88.375	758.38
	2-Chlorotoluene	26.222	
	Bromobenzene	79.228	
	cis-1,2-Dichloroethene	15.586	
	Cumene	10.112	
	Ethylbenzene	22.715	
	p-Isopropyltoluene	445.618	
	Heptane	70.524	

Site-4 (BD)	cis-1,2-Dichloroethene	12.275	71.81
	Heptane	25.899	
	1,1,1-Trichloroethane	10.728	
	Tetrachloromethane	22.913	

Table 6.2.3: Chemical composition of Volatile organic compounds detected in Kala Sanghian Drain (KSD)

Category of Volatile Organic Compounds	Volatile Organic Compound	Concentration of VOCs(ng/ml)
Benzene Series	1,3,5 -Trimethyl benzene	88.37
	p-isopropyl toluene,	445.61
	Bromobenzene	79.22
	Cumene	10.11
	Ethylbenzene	22.71
Chlorinated hydrocarbons	Tetrachloromethane	22.91
	Dichloro methane	34.6
	cis-1,2-Dichloroethene	54.19
	1,1,1-Trichloroethane	10.72
Chlorinated benzene compounds	2-Chlorotoluene	26.22
Alkane	Heptane	619.3

Table 6.2.4: Percentage contribution of Volatile organic compounds detected in wastewater of Kala Sanghian Drain (KSD)

Sample name	Volatile organic compounds	Percentage (%)
Site-1 (LD)	Dichloromethane	8.59
	Heptane	86.87
	cis-1,2-Dichloroethene	4.55
Site -2 (LO)	cis-1,2-Dichloroethene	6.79
	Heptane	95.33
Site-3 (FD)	Heptane	9.3
	1,3,5-Trimethylbenzene	11.65
	2-Chlorotoluene	3.46
	Bromobenzene	10.45
	cis-1,2-Dichloroethene	2.06
	Cumene	1.33
	Ethylbenzene	3
	p-Isopropyltoluene	58.76
Site-4 (BD)	cis-1,2-Dichloroethene	14.47
	Dichloromethane	15.35
	Heptane	30.54
	1,1,1-Trichloroethane	12.64
	Tetrachloromethane	27

Table 6.2.5: Volatile organic compounds identified in wastewater of Kala Sanghian Drain with its retention time (minutes)

Volatile organic compounds	Retention time (Min.)
Dichloromethane	5.409
Heptane	7.544
cis-1,2-Dichloroethene	6.466
1,3,5-Trimethylbenzene	13.896

2-Chlorotoluene	13.448
Bromobenzene	12.487
Cumene	12.487
Ethylbenzene	11.259
p-Isopropyltoluene	14.27
1,1,1-Trichloroethane	6.242
Tetrachloromethane	6.349

Table 6.2.6: Descriptive statistics (Dichloromethane)

Sites of Kala Sanghian Drain	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Control	**3**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Site 1(LD)	**3**	**7.19**	**6.23**	**3.60**	**-8.29**	**22.69**
Site 2(LO)	**3**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Site 3(FD)	**3**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Site 4(BD)	**3**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Total	**15**	**1.43**	**3.79**	**0.98**	**-0.66**	**3.54**

Table 6.2.7: Test of homogeneity of variance and ANOVA (Dichloromethane)

				Test of homogeneity of variance		ANOVA	
Sites	**N**	**Mean**	**S.D**	**Levene's**	**Sig.**	**F**	**Sig.**

				Statistic			
				15.76	0.000	3.993	0.034
Control	3	0	0				
Site 1 (LD)	3	7.19	**6.23**				
Site 2 (LO)	3	0	**0**				
Site 3(FD)	3	0	**0**				
Site 4 (BD)	3	0	0				
Total	**15**	**1.43**	3.79				

Table 6.2.8: Descriptive statistics (Heptane)

Sites of KSD	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	**3**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Site 1(LD)	**3**	**44.45**	**56.18**	**32.43**	**-95.10**	**184.01**
Site 2(LO)	**3**	**101.51**	**31.611**	**18.25**	**22.99**	**180.04**
Site 3(FD)	**3**	**5.195**	**8.99**	**5.19**	**-17.15**	**27.55**
Site 4(BD)	**3**	**8.63**	**12.98**	**7.49**	**-23.61**	**40.88**
Total	**15**	**31.96**	**46.78**	**12.07**	**6.054**	**57.86**

Table 6.2.9: Test of homogeneity of variance and ANOVA (Heptane)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**

				8.46	**0.003**	**6.19**	**0.009**
Control	3	0.00	0.00				
Site 1 (LD)	3	44.45	**56.18**				
Site 2 (LO)	3	101.51	**31.611**				
Site 3(FD)	3	5.195	**8.99**				
Site 4 (BD)	3	8.63	12.98				
Total	**15**	**31.96**	46.78				

Table 6.2.10: Descriptive statistics (cis-1, 2-Dichloroethene)

	N	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	**3**	**0.00**	**0.00**	**0.00**	0.00	0.00
Site 1(LD)	**3**	**3.81**	**6.59**	**3.8**	**-12.58**	**20.20**
Site 2(LO)	**3**	**4.97**	**8.60**	**4.97**	**-16.41**	**26.35**
Site 3(FD)	**3**	**5.19**	**8.99**	**5.19**	**-17.15**	**27.55**
Site 4(BD)	**3**	**4.09**	**7.08**	**4.09**	**-13.51**	**21.69**
Total	**15**	**3.61**	**6.27**	**1.61**	**0.14**	**7.08**

Table 6.2.11: Test of homogeneity of variance and ANOVA (cis-1, 2-Dichloroethene)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**S.D**	**Levene**	**Sig.**	**F**	**Sig.**

				Statistic			
				4.26	**0.029**	**0.26**	**0.89**
Control	3	0	0				
Site 1 (LD)	3	3.81	**6.59**				
Site 2 (LO)	3	4.97	**8.60**				
Site 3(FD)	3	5.19	**8.99**				
Site 4 (BD)	3	4.09	7.08				
Total	**15**	**3.61**	6.27				

Table 6.2.12: Descriptive statistics (1, 3, 5-Trimethylbenzene)

Sites of KSD	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	29.46	51.02	29.46	-97.29	156.21
Site 4 (BD)	3	0.00	0.00	0.00	0.00	0.00
Total	15	5.89	22.81	5.89	-6.74	18.52

Table 6.2.13: Test of homogeneity of variance and ANOVA (1, 3, 5-Trimethylbenzene)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				16	**0.000**	**1**	**0.452**
Control	3	0	0				
Site 1 (LD)	3	0	**0**				
Site 2 (LO)	3	0	**0**				
Site 3(FD)	3	29.46	**51.02**				
Site 4 (BD)	3	0	0				
Total	**15**	**5.89**	22.81				

Table 6.2.14: Descriptive statistics (2-Chlorotoluene)

Sites	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.000	0.000	0.000
Site 1 (LD)	3	0.00	0.00	0.000	0.000	0.000
Site 2 (LO)	3	0.00	0.00	0.000	0.000	0.000
Site 3(FD)	3	8.74	15.13	8.74	-28.86	46.34
Site 4 (BD)	3	0.00	0.00	0.000	0.000	0.000
Total	15					

Table 6.2.15: Test of homogeneity of variance and ANOVA (2-Chlorotoluene)

				Test of homogeneity of variance		**ANOVA**	

Sites	N	Mean	S.D	Levene Statistic	Sig.	F	Sig.
				16	**0.000**	1	0.45
Control	3	0.000	0.000				
Site 1 (LD)	3	0.000	**0.000**				
Site 2 (LO)	3	0.000	**00.00**				
Site 3(FD)	3	8.74	**15.13**				
Site 4 (BD)	3	0	0				
Total	**15**						

Table 6.2.16: Descriptive statistics (Bromobenzene)

Sites	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	26.41	45.74	26.41	-87.22	140.04
Site 4 (BD)	3	0.00	0.00	0.00	0.00	0.00
Total	15	5.282	20.45	5.28	-6.04	16.61

Table 6.2.17: Test of homogeneity of variance and ANOVA (Bromobenzene)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				16	**0.000**	1	0.452

Control	3	0.00	0.00
Site 1 (LD)	3	0.00	**0.00**
Site 2 (LO)	3	0.00	**0.00**
Site 3(FD)	3	26.41	**45.743**
Site 4 (BD)	3	0.00	0.00
Total	**15**	**5.282**	20.45

Table 6.2.18: Descriptive statistics (Cumene)

Sites	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	3.37	5.83	3.37	-11.12	17.86
Site 4 (BD)	3	0.00	0.00	0.00	0.00	0.00
Total	15	0.674	2.61	0.67	-0.77	2.11

Table 6.2.19: Test of homogeneity of variance and ANOVA (Cumene)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**

				16	**0.000**	1	0.452
Control	3	0.00	0.00				
Site 1 (LD)	3	0.00	**0.00**				
Site 2 (LO)	3	0.00	**0.00**				
Site 3(FD)	3	3.37	**5.83**				
Site 4 (BD)	3	0.00	0.00				
Total	**15**	**0.674**	2.61				

Table 6.2.20: Descriptive statistics (Ethylbenzene)

Sites	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	7.57	13.11	7.57	-25.00	40.14
Site 4 (BD)	3	0.00	0.00	0.00	0.00	0.00
Total	15	1.51	5.86	1.51	-1.73	4.76

Table 6.2.21: Test of homogeneity of variance and ANOVA (Ethylbenzene)

Sites	**N**	**Mean**	**S.D**	**Test of homogeneity of variance**		**ANOVA**	
				Levene Statistic	Sig.	F	Sig.
Control	3	0.00	0.00	**16**	0.000	1	0.452
Site 1 (LD)	3	0.00	**0.00**				
Site 2 (LO)	3	0.00	**0.00**				
Site 3(FD)	3	7.57	**13.11**				
Site 4 (BD)	3	0.00	0.00				
Total	**15**	**1.51**	5.86				

Table 6.2.22: Descriptive statistics (p-Isopropyltoluene)

Sites	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					LL	**UL**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	148.54	257.27	148.54	-490.57	787.65
Site 4 (BD)	3	0.00	0.00	0.00	0.00	0.00
Total	15	29.70	115.058	29.70	-34.00	93.42

Table 6.2.23: Test of homogeneity of variance and ANOVA (p-Isopropyltoluene)

Sites	**N**	**Mean**	**S.D**	**Test of homogeneity of variance**		**ANOVA**	
				Levene Statistic	**Sig.**	**F**	**Sig.**
Control	3	0.00	0.00	**16**	**0.000**	**1**	**0.45**
Site 1 (LD)	3	0.00	**0.00**				
Site 2 (LO)	3	0.00	**0.00**				
Site 3(FD)	3	148.54	**257.27**				
Site 4 (BD)	3	0.00	0.00				
Total	**15**	**29.70**	115.05				

Table 6.2.24: Descriptive statistics (1, 1, 1-Trichloroethane)

Sites	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					LL	UL
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	0.00	0.00	0.00	0.00	0.00
Site 4 (BD)	3	3.57	6.19	3.57	-11.81	18.96
Total	15	0.71	2.77	0.71	-0.81	2.24

Table 6.2.25: Test of homogeneity of variance and ANOVA (1, 1, 1-Trichloroethane)

	Test of homogeneity of variance	**ANOVA**

Sites	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				16	**0**	**1**	**0.45**
Control	3	0.00	0.00				
Site 1 (LD)	3	0.00	**0.00**				
Site 2 (LO)	3	0.00	**0.00**				
Site 3(FD)	3	0.00	**0.00**				
Site 4 (BD)	3	3.57	6.19				
Total	**15**	**0.71**	2.77				

Table 6.2.26: Descriptive statistics (Tetrachloroethane)

Sites	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					LL	**UL**
Control	3	0.00	0.00	0.00	0.00	0.00
Site 1 (LD)	3	0.00	0.00	0.00	0.00	0.00
Site 2 (LO)	3	0.00	0.00	0.00	0.00	0.00
Site 3(FD)	3	0.00	0.00	0.00	0.00	0.00
Site 4 (BD)	3	7.63	13.22	7.63	-25.22	40.49
Total	15	1.52	5.91	1.52	-1.74	4.80

Table 6.2.27: Test of homogeneity of variance and ANOVA (Tetrachloroethane)

				Test of homogeneity of variance		**ANOVA**	
Sites	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				16	**0.000**	**1**	**0.45**
Control	3	3	0.00	**0.00**			

Site 1 (LD)	3	3	**0.00**	**0.00**
Site 2 (LO)	3	3	**0.00**	**0.00**
Site 3(FD)	3	3	**0.00**	**0.00**
Site 4 (BD)	3	3	7.63	13.22
Total	**15**	**15**	1.52	5.91

Table 6.2.28: Descriptive statistics -Independent T-Test (VOCs identified in the common site of Kala Saghnian Drain)

Volatile organic compounds	Sites	N	Mean	Std. Deviation	Std. Error Mean
1,3,5-Trimetylbenzene	**Control**	**3**	**0**	**0**	**0.00**
	Site 3 (FD)	3	29.45	51.02	29.45
2-Chlorotoluene	Control	3	0.00	0.00	0.00
	Site 3 (FD)	3	8.74	15.13	8.74
Bromobenzene	Control	3	0.00	0.00	0.00
	Site 3 (FD)	3	26.40	45.74	26.40
Cumene	control	3	0.00	0.00	0.00
	Site 3 (FD)	3	3.37	5.83	3.37
Ethylbenzene	Control	3	0.00	0.00	0.00
	Site 3 (FD)	3	7.57	13.11	7.57
p-Isopropyltoluene	Control	3	0.00	0.00	0.00
	Site 3 (FD)	3	148.53	257.27	148.53

Table 6.2.29 (a): Independent T-Test (VOCs identified in the common site of Kala Sanghian Drain)

	Levene's Test for

						Equality of Variances	
Volatile organic compounds	**Sites**	**N**	**Mean**	**S.D**	**Std. Error Mean**	**F**	**Sig.**
1,3,5-Trimetylbenzene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	29.45	51.02	29.45	16	0.016
Chlorotoluene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	8.74	15.13	8.74	16	0.016
Bromobenzene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	26.40	45.74	26.40	16	0.016
Cumene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	3.37	5.83	3.37	16	0.016
Ethylbenzene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	7.57	13.11	7.57	16	0.016
Toluene	Control	3	0.00	0.00	0.00		
	Site-3 (FD)	3	148.54	257.27	148.53	16	0.016

Table 6.2.29 (b): Independent T-Test (VOCs identified in the common site of Kala Sanghian Drain)

t-test for Equality of Means

t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Confidence Interval of the Difference**	
					Lower Bond	**Higher bound**
-1	4	0.37	-29.45	29.45	**-111.24**	**52.33**
-1	2	0.42	-29.45	29.45	-156.20	97.29
-1	4	0.37	-8.74	8.74069	-33.00	15.52
-1	2	0.42	-8.74	8.74	-46.34	28.86
-1	4	0.37	-26.40	26.40	-99.73	46.91
-1	2	0.42	-26.40	26.40	-140.04	87.22
-1	4	0.37	-3.37	3.37	-12.72	5.98
-1	2	0.42	-3.37	3.37	-17.87	11.13
-1	4	0.37	-7.57	7.57	-28.59	13.45
-1	2	0.42	-7.57	7.57	-40.14	25.00
-1	4	0.37	-148.53	148.53	-560.95	263.87
-1	2	0.42	-148.53	148.53	-787.65	490.57

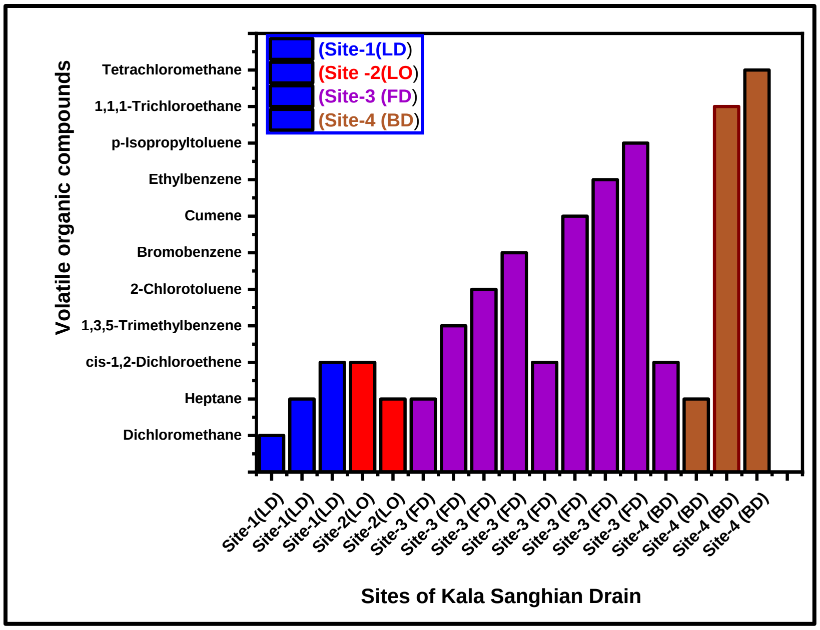


Fig.6.2.1. Volatile organic compounds detected in Site 1(LD), Site 2(LO), Site 3(FD) and Site 4 (BD) of Kala Sanghian Drain

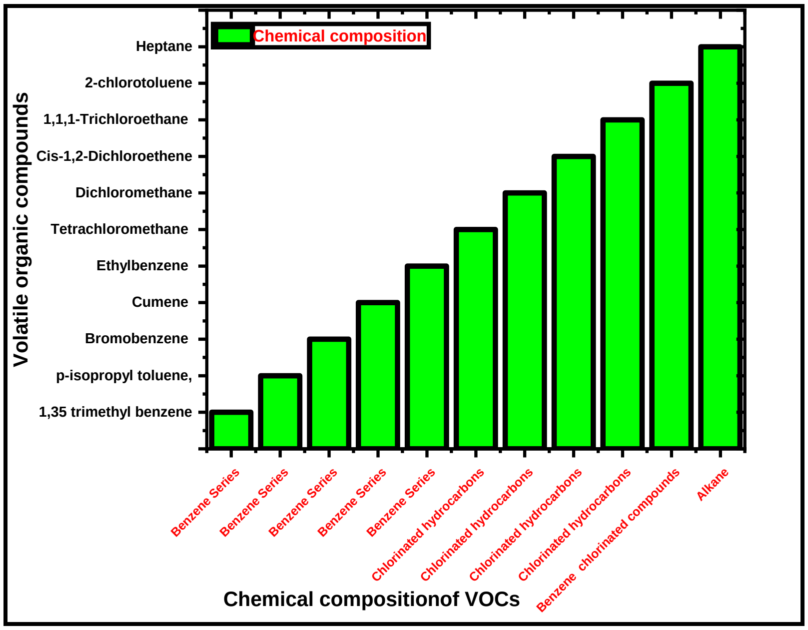


Fig.6.2.2. Chemical composition of volatile organic compounds detected in Kala Sanghian Drain (KSD)

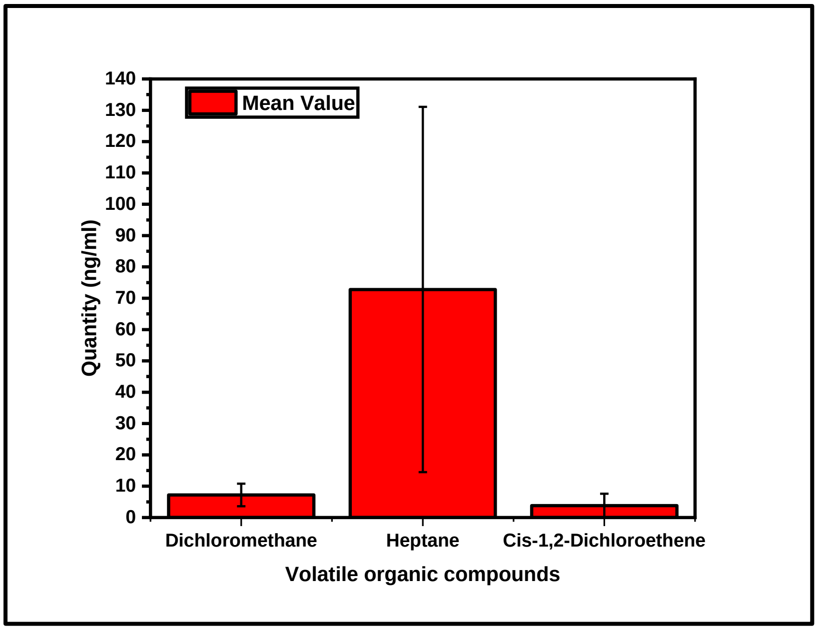


Fig. 6.2.3.Mean values of quantity of volatile organic compounds present in Site 1(LD)

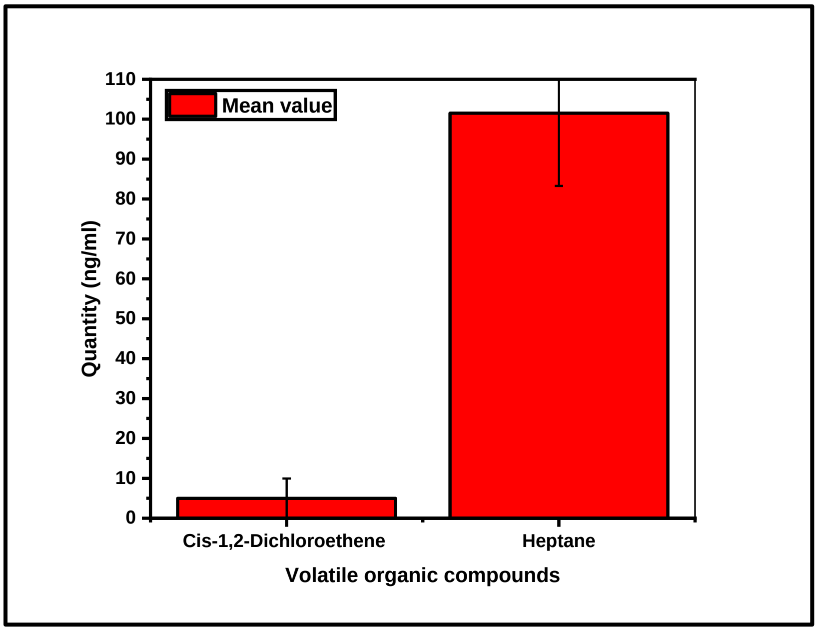


Fig.6.2.4. Mean values of quantity of volatile organic compounds present in Site 2(LO)

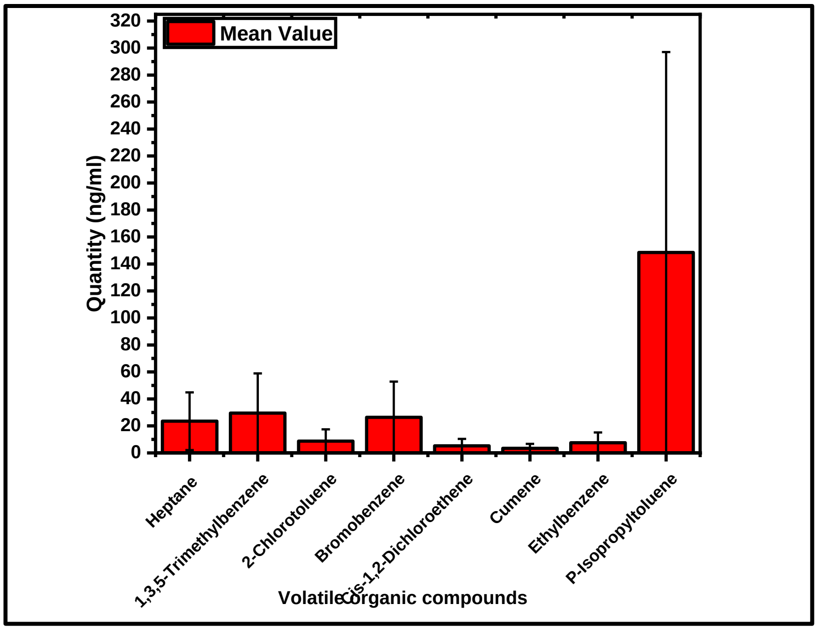


Fig. 6.2.5.Mean values of quantity of volatile organic compounds present in Site 3(FD)

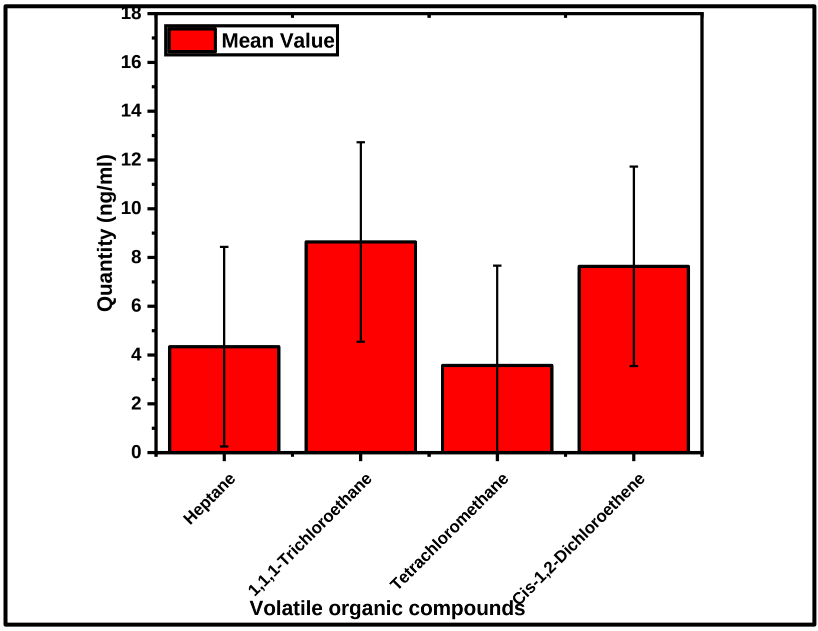


Fig. 6.2.6.Mean values of quantity of volatile organic compounds present in Site 4(BD)

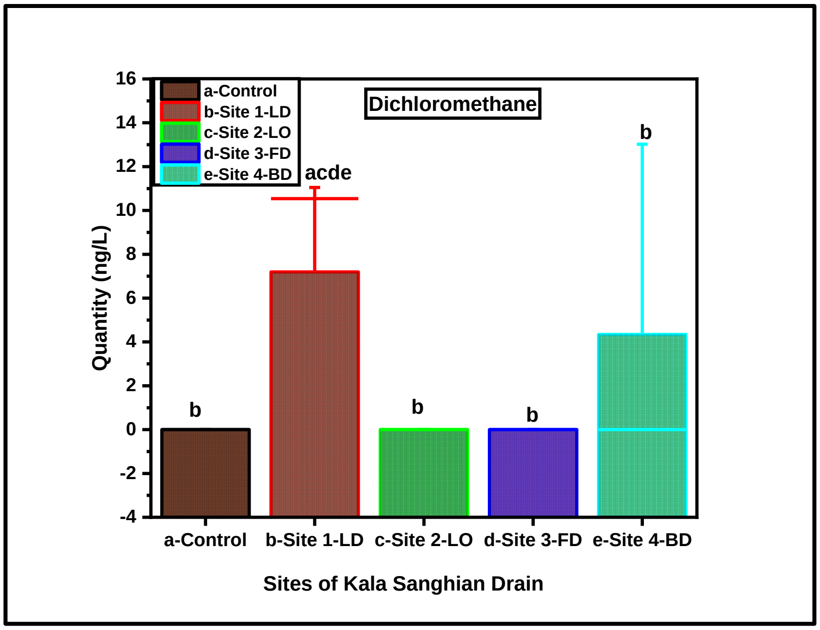


Fig.6.2.7. Comparison of the quantity of Dichloromethane in various sites of the Kala Sanghian Drain (p<0.05)

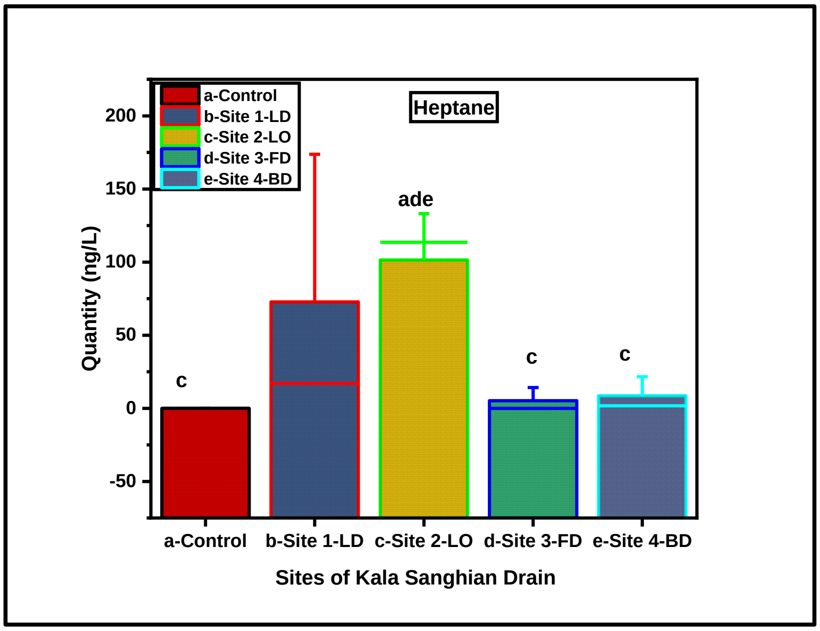


Fig.6.2.8. Comparison of the quantity of Heptane in various sites of the Kala Sanghian Drain (p<0.05)

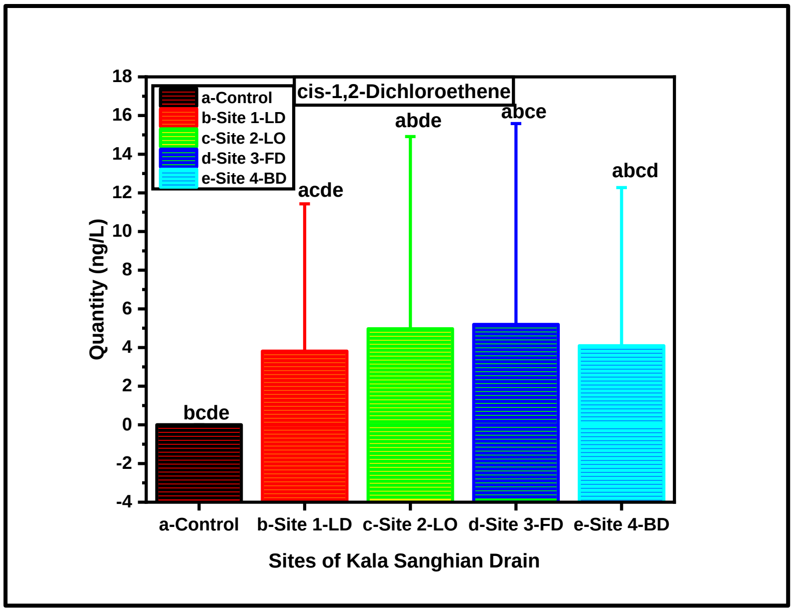


Fig.6.2.9. Comparison of the quantity of cis-1, 2 Dichloroethene in various sites of the Kala Sanghian Drain (p>0.05)

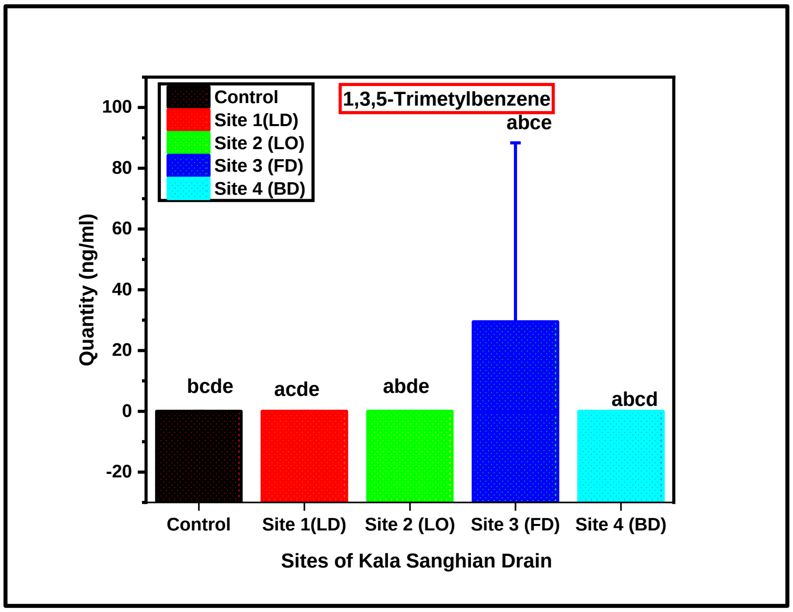


Fig.6.2.10. Comparison of the quantity of 1, 3, 5-Trimetylbenzene) in various sites of the Kala Sanghian Drain (p>0.05)

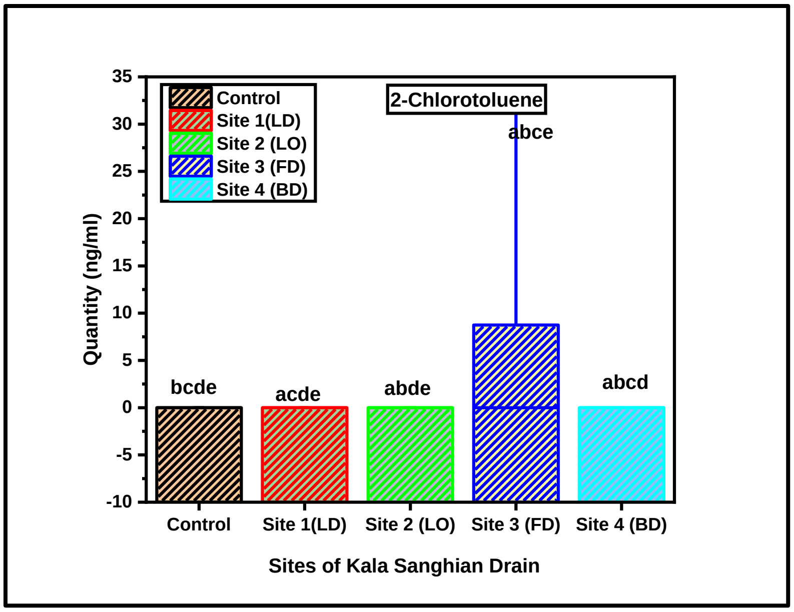


Fig.6.2.11. Comparison of the quantity of 2-Chlorotoluene in various sites of the Kala Sanghian Drain (p>0.05)

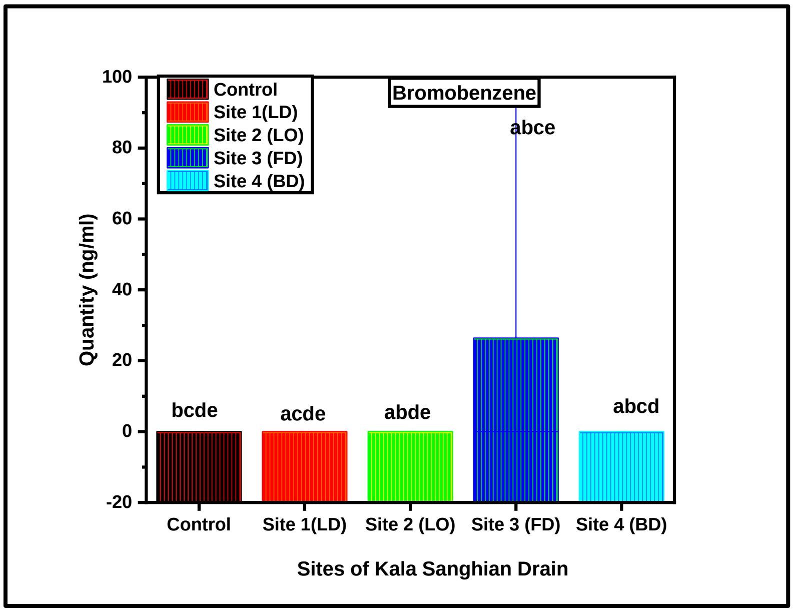


Fig. 6.2.12.Comparison of the quantity of Bromobenzene in various sites of the Kala Sanghian Drain (p>0.05)

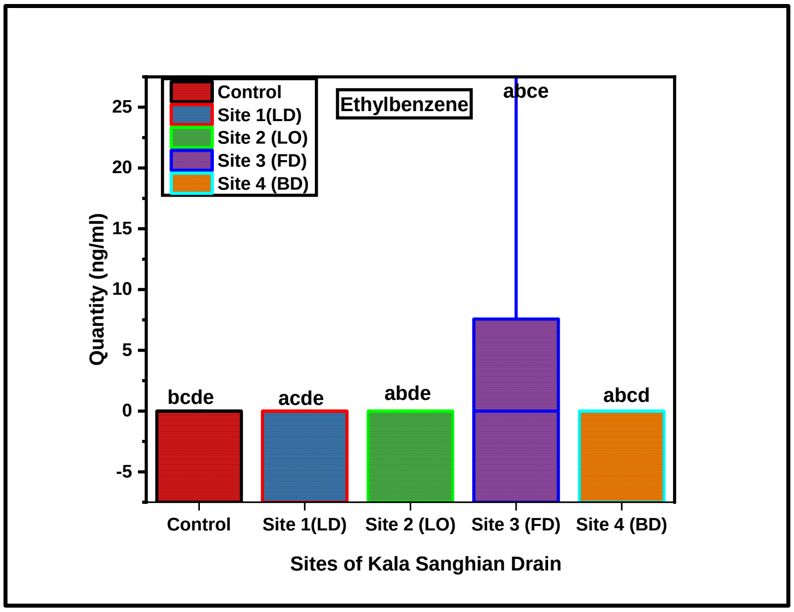


Fig.6.2.13. **Comparison of the quantity of Cumene in various sites of the Kala Sanghian Drain (p>0.05)**

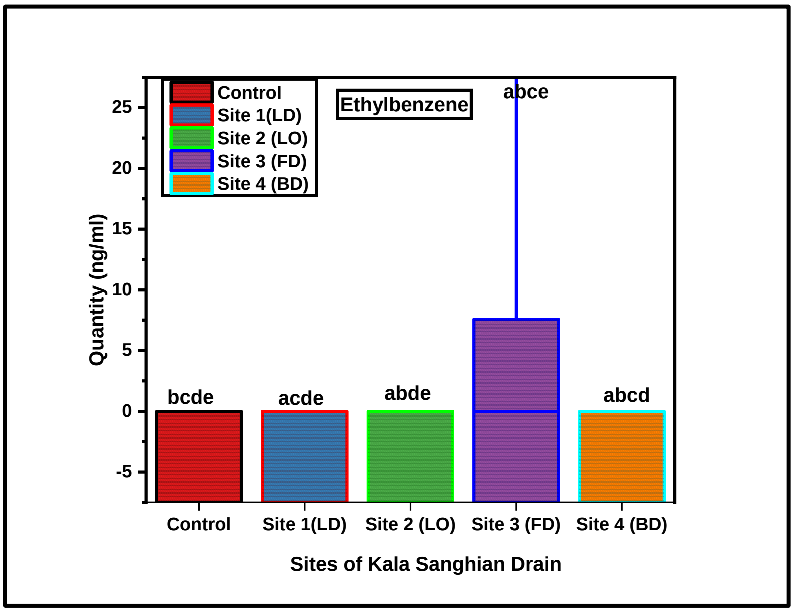


Fig.6.2.14. Comparison of Ethylbenzene in various sites of the Kala Sanghian Drain (p>0.05)

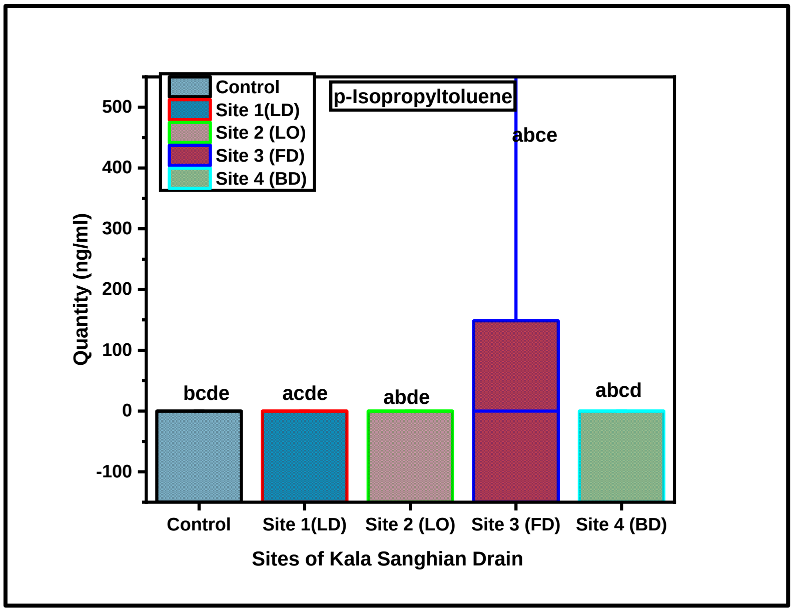


Fig.6.2.15. Comparison of the quantity of p-Isopropyltoluene in various sites of the Kala Sanghian Drain (p>0.05)

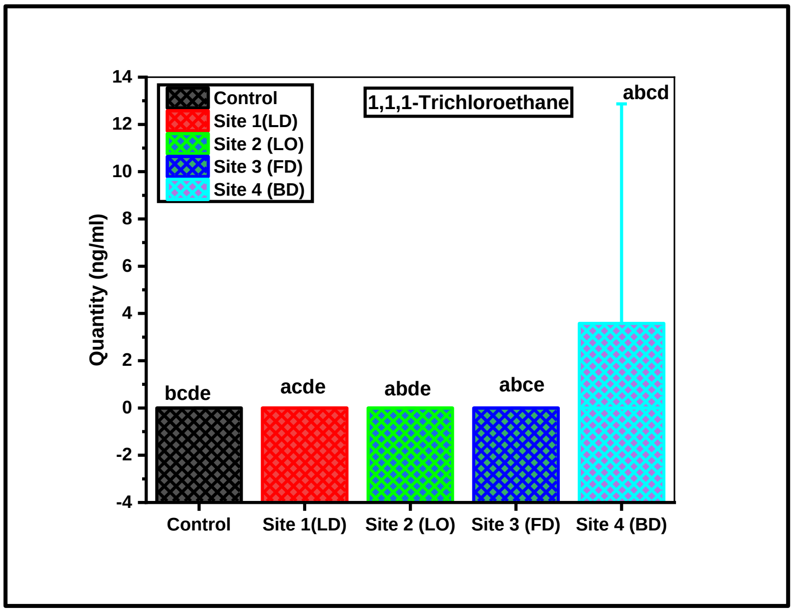


Fig.6.2.16. Comparison of the quantity of 1, 1, 1-Trichloroethane in various sites of the Kala Sanghian Drain (p>0.05)

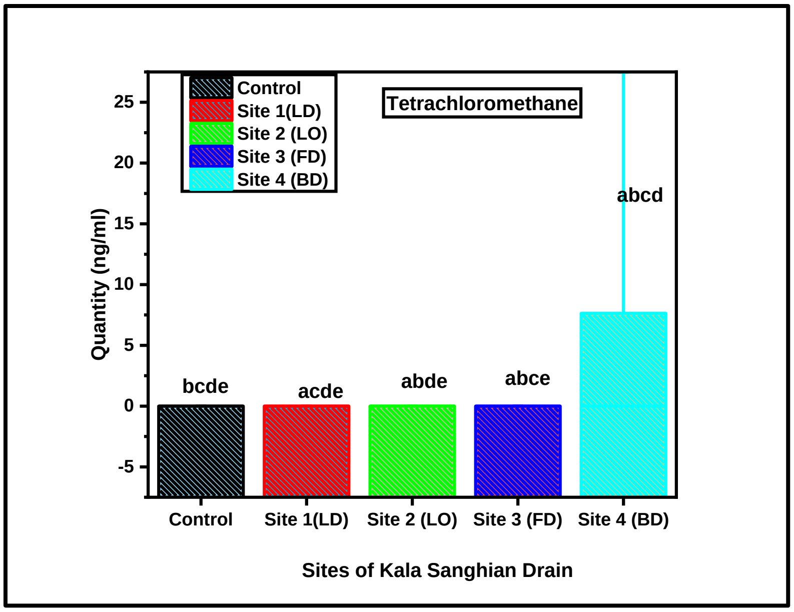


Fig.6.2.17. Comparison of the quantity of Tetrachloromethane in various sites of the Kala Sanghian Drain ($p>0.05$)

Objective 2*: Evaluation of the performance of conventional and bio remediation methods in the removal of odor released by the effluents*

6.3. Preparation, Characterization, and Assessment of Adsorbent Prepared from Coconut Shell for Removal of Toxic Dichloromethane

6.3.1. Physicochemical characteristics of coconut shell activated carbon (CSAC)

In the concerned study, a carbonization temperature of 700 $^{\circ}$C, with an activation time of 1 hour, and activating agent of phosphoric acid were used to obtain the desirable activated carbon. The estimated value of ash, moisture content, and density has been illustrated in ***Fig.6.3.1***. The physicochemical properties of DCM have also been shown with the help of ***Table 6.3.1***.

Yield percent

To analyze the yield percent of carbon, masses of carbon before and after activation were calculated. The resulting masses were 115 g and 71.5 g respectively. The yield percent was 73.1 % and the overall process yield percent of activated carbon obtained was 63.5

%. Yield percentage with an activation agent at an activation temperature of 700°C is interrelated to form a specific activated carbon. Phosphoric acid as activating agent, impregnation ratio 1:3, activation time 1 hour, and activation temperature 700 ° C were used in the preparation of activated carbon.

6.3.2. Characterization of coconut shell activated carbon

FTIR analysis

FTIR spectrum analysis helps to identify various functional groups present on the adsorbent's (CSAC). In ***Fig.6.3.2,*** the spectrum shows the existence of some specific bands of coconut shells belonging to functional groups. The broad band at 1160cm-1 shows the presence of a P=O bond. The band at 2100 cm-1, 2111 cm-1 2400 cm-1, and 2001 cm-1 shows the presence of C≡C, alkyne functional group. The band at 2656 cm-1 may be due to O-H stretching. The bond at 2224 cm-1 shows the presence of C≡N stretching. The bond at 3400 cm-1 shows the presence of an O-H bond. The bond 900-700 cm-1 can be assigned to the aromatic structures.

FESEM analysis

FESEM samples of activated coconut shells have been illustrated in ***Fig 6.3 3 (i), (ii), and (ii).*** The samples have cracked structures indicating the pyrolysis and activation of samples.

6.3.3. Adsorption studies

6.3.3.1. Effect of initial pH

The impact of pH on the adsorption was analyzed by taking an extensive range of initial pH (2 - 10). The repercussion of initial pH on adsorption is shown in ***Fig. 6.3.4***. The highest adsorption of DCM on CSAC was observed between pH 6.0 and 8

6.3.3.2. Effect of adsorbent dosages

There is an impact of different doses on the adsorption process. This has been depicted with the help of figures-***Fig. 6.3.5 (a) and Fig. 6.3.5 (b).*** Adsorption doses were found to be directly proportional to percent removal. The adsorption percentage increases with increasing adsorbent doses due to the availability of more sites of adsorption [196, 399].

6.3.3.3. Effect of initial DCM concentration

Adsorption increased with an increase in initial DCM concentration. The concentration study was performed at 25 degree Celsius with a various amount of initial dichloromethane concentrations (20-300 mg/L). The adsorption capacity of DCM was highest on CSAC (50.325 mg/g) at 300 mg/L at 25°C. It has been observed that the adsorption process was very speedy at low adsorbate concentrations. The large pore size of CSAC was responsible for obtaining high adsorption capacity at the high initial concentration of the dichloromethane. As the concentration of the DCM increases, its movement on the surface of the adsorbent speeds up and finally, it increases the adsorption capacity of the adsorbent [46]. On CSAC, dichloromethane (DCM) showed maximum adsorption capacity at 50.32mg/g.

6.3.3.4. Effect of contact time

Contact time has significant repercussions in the adsorption process. The contact time studies on CSAC were conducted with 200 mg/L initial adsorbate concentration with constant pH at 25°C. It was observed that adsorption capacity increases with the increase in contact time. The maximum adsorption capacity on CSAC was 33 mg/g to 200 mg/L at 25°C ***(Fig 6.3.6).***

6.3.4. Adsorption isotherms models

Experimental data in this study has been analyzed by Langmuir and Freundlich isotherm models. By plotting the linear plot between Ce/qe vs. Ce, slope, intercept, and qm values were calculated. The monolayer adsorption capacity value qm was highest for CSAC (48.42mg/g) ***(Table 6.3.2).*** Slope and intercept were used for the calculation of K_f and n after plotting a linear plot of log qe vs. log Ce (Table 6.3.2). In the present study, the values of n were found to be greater than 1 (n>1) for the sorption of dichloromethane on CSAC indicative of favorable adsorption. The regression coefficient (R^2) value has shown better applicability of the Freudlich model on CSAC [196, 399].

6.3.5. Adsorption kinetics

The mechanism of adsorption was well elucidated by the pseudo-first-order and pseudo-second-order rate equation. The values of pseudo-first order were calculated from the slope and intercept of the linear plots. In pseudo-second-order kinetics, the values of K_2 and qe (exp) were computed from the slope and intercept of the linear plots of t/qt vs. t *(Table 6.3.3)*. The correlation coefficient (R^2) value obtained for the pseudo-first-order model did not represent a good fit with experimental data ***(Table 6.3.3).*** On the contrary; it followed the pseudo-second-order kinetics model ***(Table 6.3.3)*** as evident from the R^2 value calculated from the experimental data.

6.3.6. Thermodynamic modeling

The parameters of thermodynamics for the adsorption process have been illustrated in ***Table 6.3.4.*** The positive values of standard enthalpy change (ΔH°), predicted the spontaneity and endothermic nature of adsorption. The positive values of standard entropy change (Δ*S*°), explored the randomness in the adsorption process at the solid or solution interface. The values of ΔS° were negative and demonstrated that the adsorption process was a spontaneous endothermic reaction [371, 373].

6.3.7. Statistical analysis

To analyze the adsorption studies and to check the effect of pH, carbon dose, and contact time on the adsorption of DCM statistical tools were applied. The experimental data are presented as mean ± SD of the experiment at $^*p \leq 0.05$. The observed values have been taken for subsequent statistical analysis and interpretation ***(Tables 6.3.5, 6.3.7, and 6.3.9).*** Analysis of variance (ANOVA) one-way test was also performed to find out statistical value and significance of population means at 0.05 level (***Tables 6.3.6, 6.3.8, 6.3.10, Fig 6.3.7, to 6.3.11)***.

The ANOVA result suggests that various groups of pH, carbon dose (g), and contact time (minutes) differ significantly ($F_{8, 18}$= (14.504) (pH), 1625.115 (carbon dose (g) and 6.385 (contact time (minutes).

Since Levene's statistic is significant, the equal variance was not assumed. To check the individual difference between different parameters post hoc comparisons were assessed using Tukey. One-way ANOVA with post hoc Tukey's HSD test indicated that there is a significant difference occurs in the mean value of various groups of pH, carbon dose, and contact time (***Tables 6.3.6, 6.3.8, 6.3.10, Fig 6.3.7, to Fig 6.3.11)***.

In case of CSAC and parameter pH, there exists a significant difference in the mean of value of removal (%) between pH 2 and pH 4,5, 6 and 7; between pH 3 and pH 6, 7; between pH 4 and pH 2, 7, 10; between pH 5 and pH 2; between pH 5 and pH 7; between pH 5 and pH 10; between pH 6 and pH 2; pH 6 and pH 3; pH 6 and pH 10; between pH 7 and pH 2; between pH 7 and pH 3; between pH 7 and pH 4; between pH 7 and pH 5; between pH 7 and pH 8; between pH 7 and pH 9; between pH 7 and pH 10 (***Table 6.3.6, Fig 6.3.7).***Similarly in case of carbon doses, there exists a significant differences in the mean value of removal (%) among different carbon doses (***Table 6.3.8 and Fig 6.3.8,)*** and among various parameter contact time (***Table 6.3.10 and Fig 6.3.9).***

6.3.8. Conclusion

The positive attributes such as physical resistance, adsorption properties, surface chemistry, reasonable cost, and recovery of the activating agent, made the carbon prepared from the plant's origin, the material of choice in the adsorbent world. Coconut shell-activated carbon is produced with an impregnation ratio of 1:3, activation temperature of 700 °C, and activation time of 1 hour. The present study has shown that there is effect of independent variables (pH, temperature, initial DCM concentration, quantity of adsorbent, and contact time on dichloromethane removal efficiency. The adsorption was detected higher (50.5 mg/g) for CSAC between pH of 6.0 and 8.0. On increasing contact time and temperature, the adsorption increases The Langmuir model didn't fit well on CSAC for the adsorption of dichloromethane. Rather it went well with the Freundlich model. Kinetic data has shown the applicability of the pseudo-second-order. The R_L value between 0 and 1 for the adsorption of dichloromethane specified favorable adsorption. The adsorption of dichloromethane on CSAC was considered spontaneous. Standard entropy changes with positive values showed the increase in entropy; therefore it confirmed the randomness of the system. This study reveals that coconut shell activated carbon (CSAC) has the capability of giving satisfactory removal efficiency for dichloromethane.

Table 6.3.1: Physicochemical properties of DCM

	Unit	**Dichloromethane**
Formula		CH_2Cl_2
Molecular weight MW	**g mol^{-1}**	**84.93**
Normal boiling point	**°C**	**39.8**
liquid density at 20°C	**g/cm^{-3}**	**1.31**
Solubility in water	**Mass%**	**1.32**
Vapor pressure at 293 K	**mm Hg**	**350**

Table 6.3.2: Langmuir and Freundlich constants for the adsorption of DCM on CSAC.

Adsorbent	**Langmuir constants**			
CSAC	**q max**	**K_L**	**R_L**	**R^2**
	30.18	**0.050**	**0.49**	**0.84**
Adsorbents	**Freundlich constants**			
CSAC	**K_F**	**n**	**R^2**	
	1.59	**1.37**	**0.97**	

Table 6.3.3: Kinetic parameter for the adsorption of DCM on CSAC at 25 °C (Co=200 mg/L)

Adsorbent	**Pseudo first order**			**Pseudo second order**	
CSAC	qe	K_1	R^2	K_2	R^2
	6.17	-0.0001	0.87	0.008	0.99

Table 6.3.4: Effect of concentration on the thermodynamics parameters for the adsorption of DCM at different temperatures on CSAC

Concentration (mg/L)	**ΔS° (J/K mol)**	**ΔH° (kJ/mol)**	**ΔG° (kJ/mol)**
100			**-17.30**
	61.20	**19.66**	**-18.22**
			-18.83
200			**-11.63**
	41.14	**14.21**	**-12.24**
			-12.65
300			**-7.47**
	26.45	**14.21**	**-7.87**
			-8.138

Table 6.3.5: Descriptive statistics (Effect of pH on DCM adsorption)

pH	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	Upper Bound	
PH 2	3	58.50	0.98	0.56	56.06	60.94	57.55
PH 3	3	60.75	1.50	0.86	57.01	64.49	59.41
PH 4	3	62.52	1.45	0.84	58.89	66.14	61.26
PH 5	3	63.25	0.99	0.57	60.79	65.71	62.25
PH 6	3	64.75	1.58	0.91	60.82	68.68	63.44
PH 7	3	65.35	2.05	1.18	60.24	70.45	63.31
PH 8	3	61.18	1.52	0.88	57.38	64.97	59.51
PH 9	3	62.35	1.14	0.65	59.52	65.18	61.23
9	3	58.38	1.19	0.69	55.42	61.35	57.13
Total	27	61.89	2.63	0.50	60.85	62.93	57.13

Table 6.3.6: Test of homogeneity of variance and ANOVA (Effect of pH on DCM adsorption)

				Test of homogeneity of variance		**ANOVA**	
PH	**N**	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				0.30	0.95	**14.50**	**<0.05**
2	3	58.50	0.98				
3	3	60.75	1.50				
4	3	62.52	1.45				
5	3	63.25	0.99				

6	3	64.75	1.58
7	3	65.35	2.05
8	3	61.18	1.52
9	3	62.35	1.140
10	3	58.38	1.19
Total	27	61.89	2.63

Table 6.3.7: Descriptive statistics (Effect of carbon doses on DCM)

Carbon doses	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	**Upper Bound**	
0.2	3	35.12	1.005	0.58	32.62	37.62	34.11
0.4	3	60.25	1.005	0.58	57.75	62.75	59.23
0.6	3	76.21	0.96	0.55	73.83	78.60	75.24
0.8	3	82.51	0.91	0.52	80.25	84.77	81.59
1	3	87.5	0.61	0.35	85.97	89.02	87.13
Total	15	68.32	19.64	5.07	57.44	79.20	34.11

Table 6.3.8: Test of homogeneity of variance and ANOVA (Effect of carbon doses on DCM adsorption carbon doses)

				Test of homogeneity of variance		**ANOVA**	
Carbon doses (g)	**N**	**Mean**	**Std. Deviation**	**Levene's Statistic**	**Sig.**	**F**	**Sig.**
0.2	3	35.12	1.005	0.09	0.98	1625.1	**<0.05**

0.4	3	60.25	1.005
0.6	3	76.21	0.96
0.8	3	82.51	0.91
1	3	87.5	0.61
Total	15	68.32	19.64

Table 6.3.9: Descriptive statistics (Effect of Contact Time on DCM adsorption)

Contact Time (minutes)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
20	3	28.31	1.18	0.68	25.38	31.24	27.16
40	3	29.96	1.51	0.87	26.19	33.72	28.32
60	3	31.45	0.95	0.55	29.08	33.82	30.55
80	3	31.23	0.90	0.51	28.99	33.46	30.33
100	3	32.36	1.01	0.58	29.84	34.89	31.38
120	3	32.45	0.92	0.53	30.16	34.75	31.58
140	3	33.13	0.99	0.57	30.65	35.61	32.14
160	3	33.14	0.99	0.57	30.67	35.61	32.15
180	3	33.15	1.001	0.57	30.66	35.64	32.16
200	3	33.16	0.99	0.57	30.69	35.63	32.17
210	3	33.17	1.003	0.57	30.68	35.66	32.18
220	3	33.18	1.01	0.58	30.66	35.70	32.15
240	3	33.19	1.009	0.58	30.68	35.70	32.17
Total	39	32.14	1.72	0.27	31.58	32.70	27.16

Table 6.3.10: Test of homogeneity of variance and ANOVA (Effect of Contact Time on DCM adsorption)

				Test of homogeneity of variance		**ANOVA**	
Contact Time (minutes)	**N**	**Mean**	**Std. Deviation**	**Levene's Statistic**	**Sig.**	**F**	**Sig.**
				0.14	0.99	6.38	<**0.05**
20	3	28.31	1.180				
40	3	29.96	1.51				
60	3	31.45	0.95				
80	3	31.23	0.900				
100	3	32.36	1.016				
120	3	32.45	0.92				
140	3	33.13	0.99				
160	3	33.14	0.99				
180	3	33.15	1.001				
200	3	33.16	0.99				
210	3	33.17	1.003				
220	3	33.18	1.014				
240	3	33.19	1.009				
Total	39	32.14	1.72				

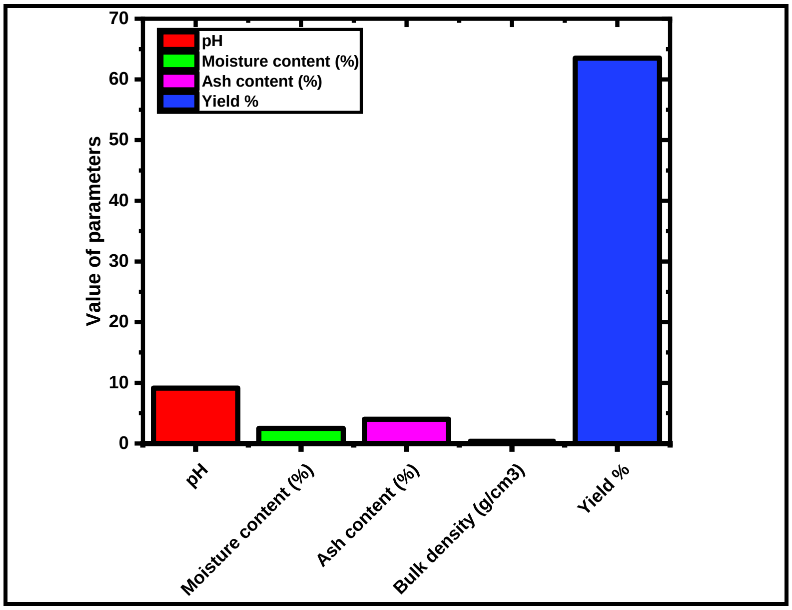


Fig.6.3.1: Physicochemical characteristics of coconut shell derived activated carbon

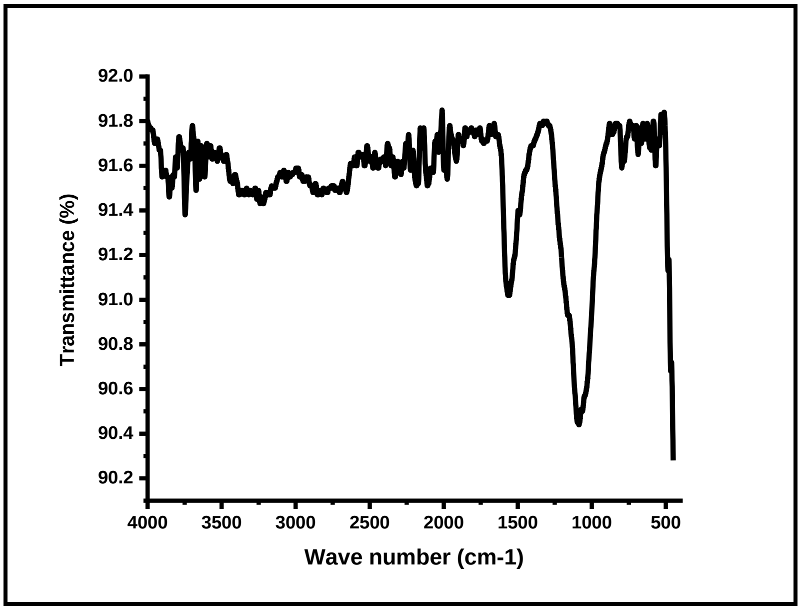


Fig.6.3.2. FTIR spectra of coconut shell-derived activated carbon

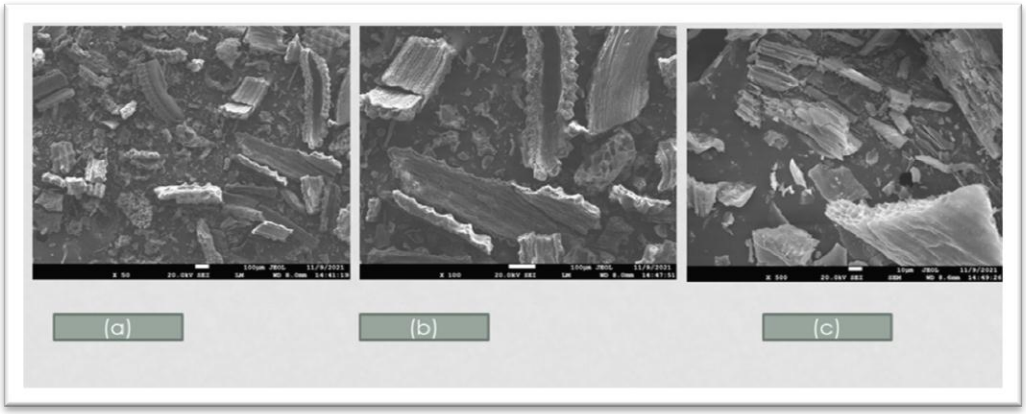


Fig.6.3.3 (i).FESEM Images of coconut shell activated carbon (a-c)

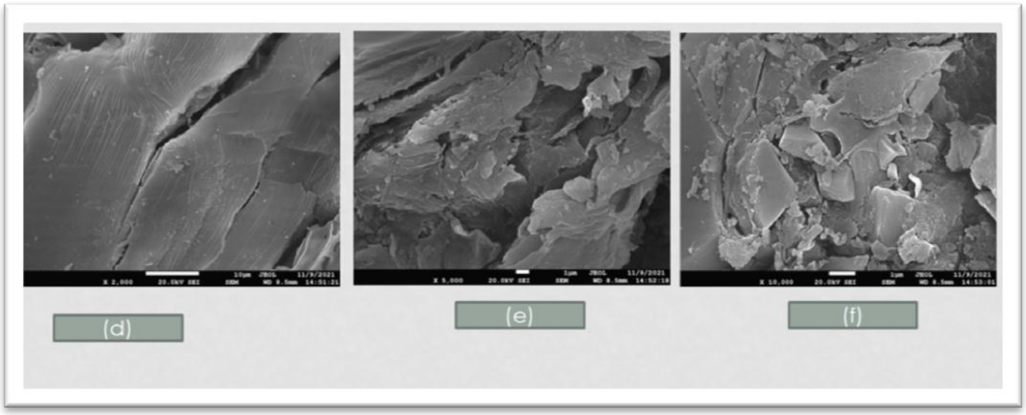


Fig. 6.3.3 (ii).FESEM Images of coconut shell activated carbon (d-f)

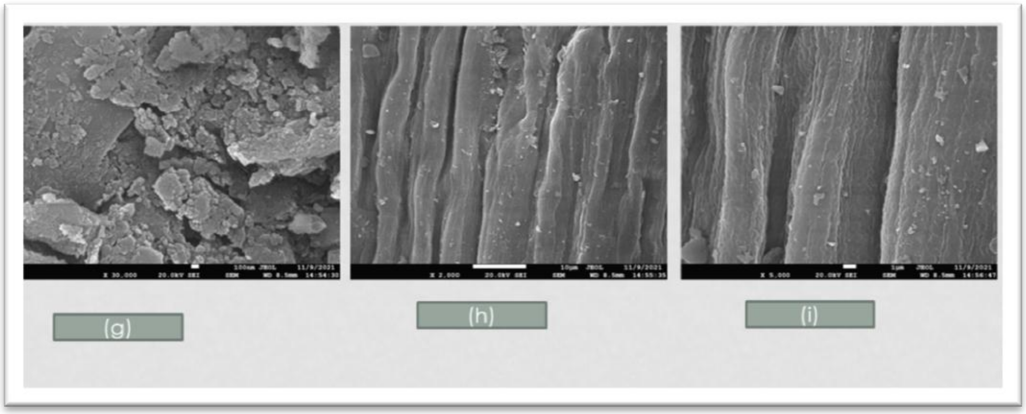


Fig. 6.3.3 (iii).FESEM Images of coconut shell activated carbon (g-i)

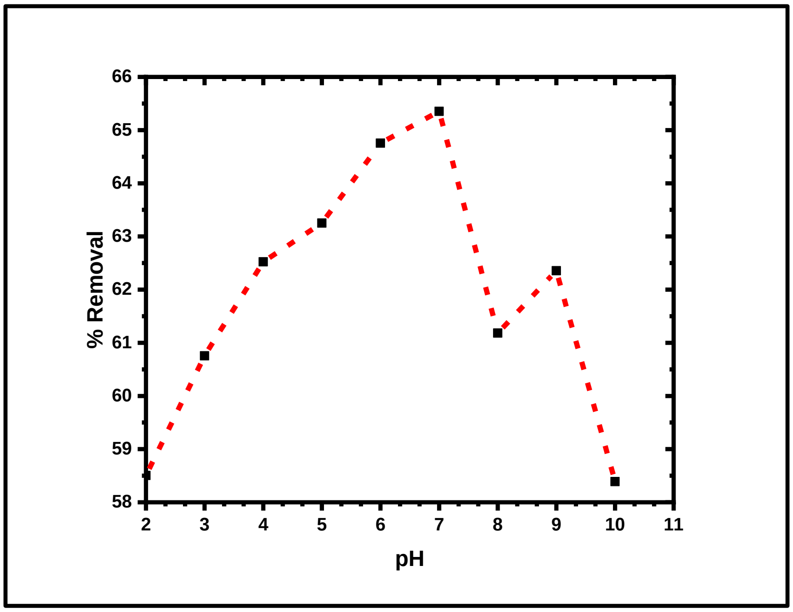


Fig.6.3.4.Effect of initial pH on the removal percentage of DCM on CSAC

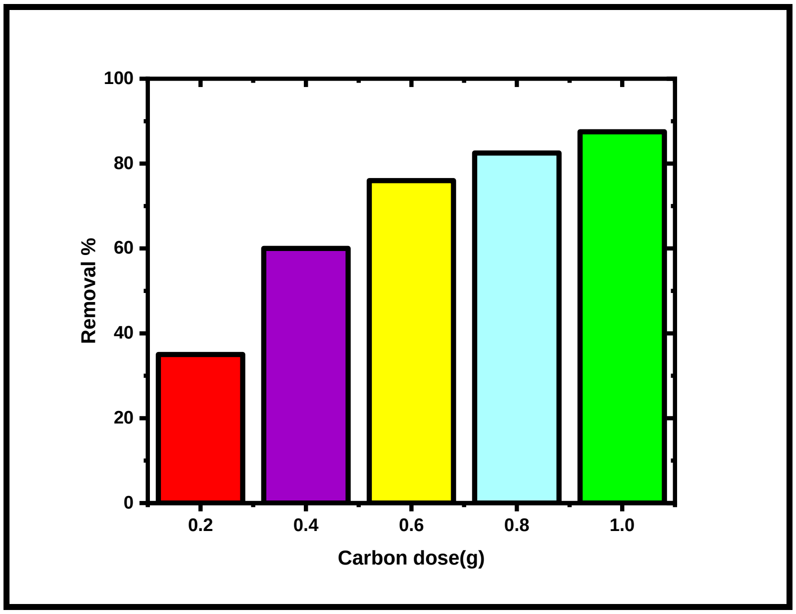


Fig. 6.3.5 (a).Effect of carbon dose on the removal percentage of DCM on CSAC

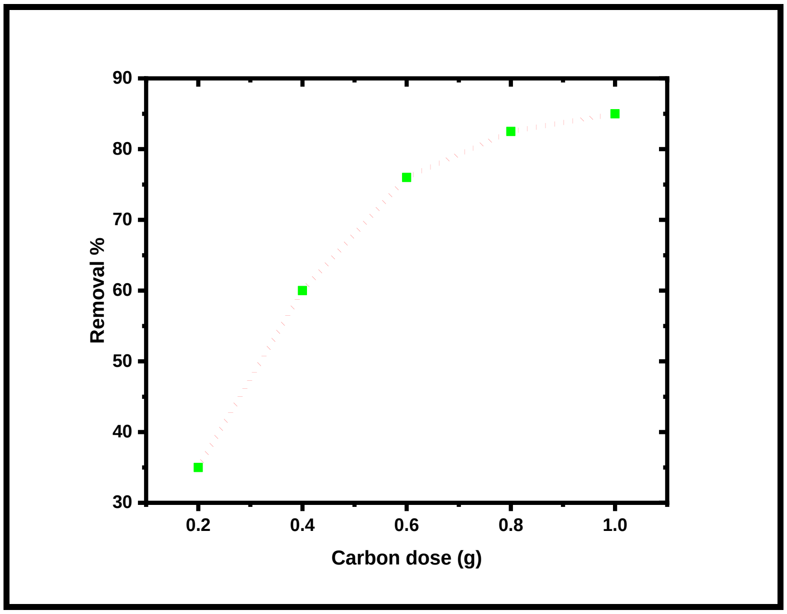


Fig.6.3.5 (b).Effect of carbon dose on the removal percentage of DCM on CSAC

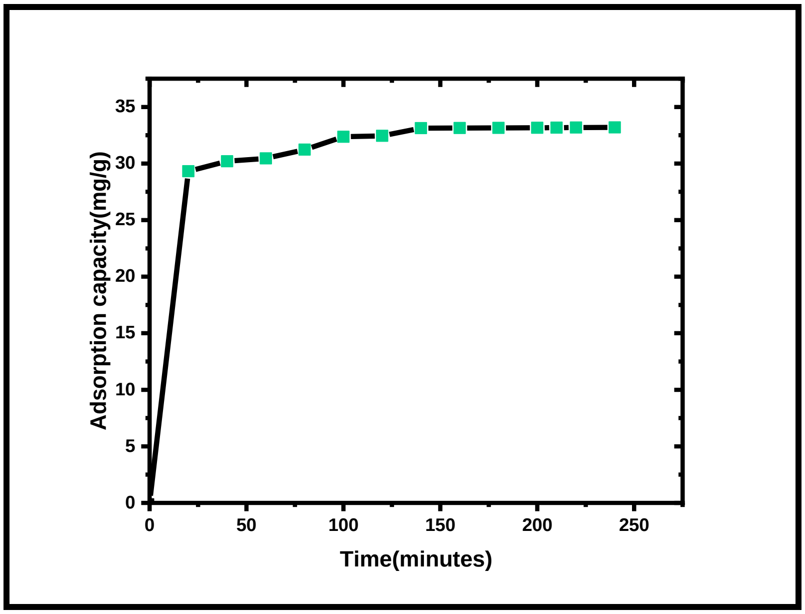


Fig.6.3.6. Effect of contact time on the adsorption capacity of DCM on CSAC

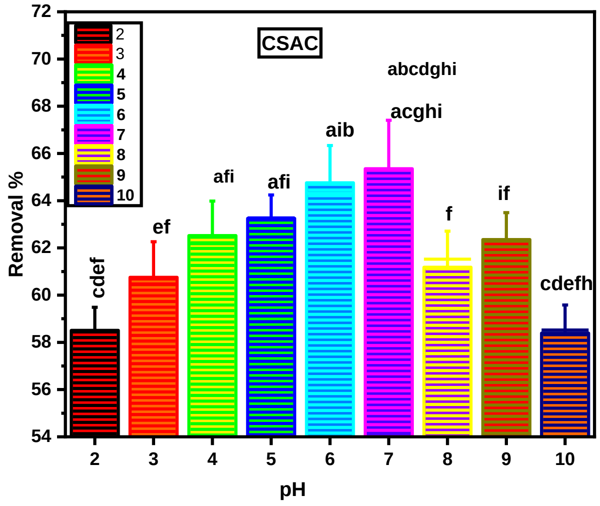


Fig.6.3.7. Comparison of different range of pH on removal percentage of DCM on CSAC (p<0.05)

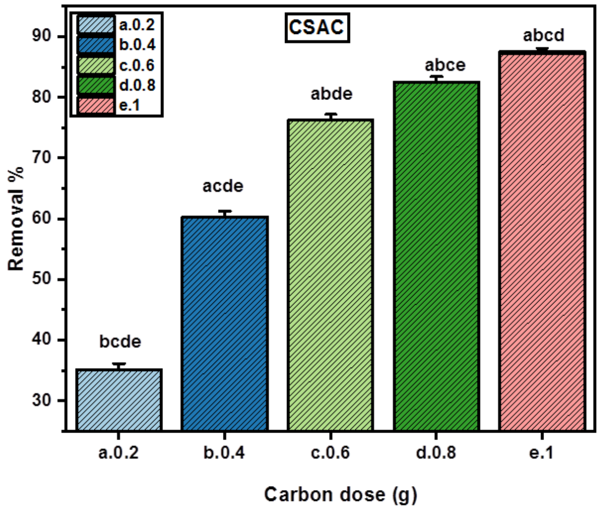


Fig.6.3.8. Comparison of different carbon doses on removal percentage of DCM on CSAC (p<0.05)

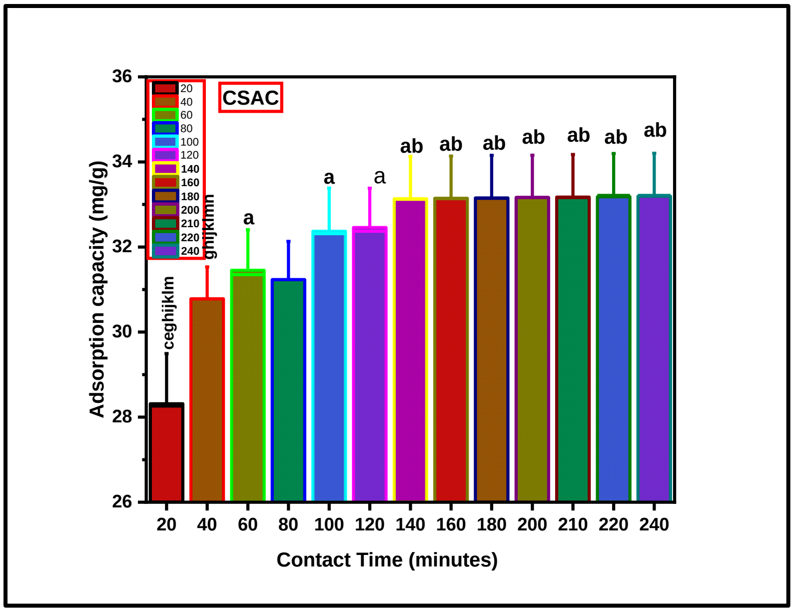


Fig.6.3.9. Comparison of different ranges of contact time on removal percentage of DCM on CSAC ($p<0.05$)

6.4. Preparation, Characterization, and assessment of adsorbent prepared from Rice husks for removal of Toxic Dichloromethane

Three different types of adsorbents from rice husk were prepared (i) Raw rice husk (RRH) (ii) chemically treated rice husk using 0.5 M NaOH (RHN) (iii) Thermally treated rice husk heated at 300°C (RHH).

6.4.1. Effect of burning time

Silica is one of the main content of rice husks and has better thermal and chemical stability. On the contrary, carbon is vulnerable to temperature and gets decreases with an increase in temperature. Compared to untreated and chemically treated rice husk, thermally treat rice husk is more porous with large specific area. The key factor for determining the content of carbon and silica in rice husk is its burning time. High burning time leads to an increase in the silica content and deteriorates the carbon content. The adsorbent with less carbon content does have not good porosity. Thermally and chemically modified rice husk causes reduction in hemicellulose, lignin, and cellulose.

As these components are reduced, the specific area of the adsorbents is increased. Untreated raw rice husk has not large surface area as compared to thermally treated and chemically treated rice husk. In the present study, the effect of chemical and thermal treatment on the surface chemistry of rice husk.

6.4.2. Characterization of adsorbents

The values of physiochemical properties of raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN) and raw rice husk heated at 300°C (RHH) have been illustrated in the tabulated form ***(Tables 6.4.1, 6.4.2 and 6.4.3).*** Among many parameters, low ash content and moisture content revealed that the particular raw material can act as a good adsorbent. From ***Tables 6.4.1, 6.4.2, and 6.4.3***, it can be analyzed that raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN) and raw rice heated at 300°C (RHH) have less amount of moisture content with value of 15%, 10.9% and 10% respectively. In the current study, the low ash content of three adsorbents was investigated ***(Tables 6.4.1, 6.4.2, and 6.4.3).*** To calculate the yield percent of adsorbents, masses of carbon before and after activation has been investigated. Yield percent of 71%, 40.99 %, and 14% were estimated for RRH, RHN, and RHH adsorbents respectively. It has been illustrated with the help of ***Tables 6.4.1, 6.4.2, and 6.4.3.***

FTIR Analysis

The C-H stretching vibration near 2924.81 cm^{-1}, in FESEM images of raw rice husk (RRH), specifies that an alkane functional group is present. The C=O stretching near 1638 cm-1 signifies the hemicelluloses and lignin aromatic groups. [233]. The presence of CH_2 and CH_3 groups and CH_3 group unveil by the presence of peaks near 1456.94 cm-1 and 1359 cm-1 [230]. The Si-O-Si stretching is confirms by the appearance of the peak near 1026.06 cm-1. The peak appears near 709 cm-1 shows the presence of the Si-H group. The small peak at 1646 characterized the C-OH group of water molecules and the existence of C=C in the organic components ***Fig.6.4.1(a)*** [165].

Rice husk with chemical and thermal treatment has shown a shift at lower and higher wave numbers. The surface chemistry of rice husk was found to change as a result of the chemical treatment, and so also its functional groups were changed. In the case of rice husk modified with 0.5 M NaOH, peaks are seen near 3100-2800 cm^{-1} ***Fig. 6.4.1 (b).*** There has been a change in the FTIR graph of rice husk heated at 300 °C on comparing it with raw rice husk. The thermal treatment leads to disappearance of the bands such as C-H, C-O, and C-C ***(Fig 6.4.1 (c)*** [165, 230].

FESEM Analysis

FESEM images of raw rice husk (RRH) have shown well-organized features, such as tall and wide creases. These creases with tissues adhered themselves to the inner epidermis ***(Fig.6.4.2 (a).*** FESEM images, illustrate the morphology of treated rice husk with 0.5 M NaOH. Silica is a key component of rice husk, and forms sodium silicate when it reacts with 0.5 M NaOH.. Sodium silicate removal by water-washing is responsible for the formation of large holes on the epidermis ***(Fig 6.4. 2 (b1)-1-9, Fig. 6.4.2 (b2)10-15)*** [411]. In FESEM images of rice husk heated at 300°C (RHH), the pores with variation in sizes and shapes could be observed. The formation of pores was due to the thermal treatment of rice husk. On receiving heat, raw rice husk gets decomposed into small particles. These particles have a large surface area [411]. The ridges are seen but these are not fully straight and high. The thermal cracking of rice husk occurs due to increase in temperature ***Fig 6.4.2 (c1)1-9; Fig: 6.4.2 (c2)10-15.***

6. 4.3. Adsorption Studies

6.4.3.1. Effect of initial pH

The pH performs a pivot role in the adsorption process. It has a significant impact on the surface of the adsorbents. As H^+ and OH^- ions energetically adsorbed and, therefore, adsorption is affected by the pH of the adsorbate [203, 273]. A temperature (25°C), initial concentration (200 mg/L), agitation (150 rpm) constant, and pH in the range of 2-10 has been conducted in order to study the effect of initial pH on adsorption. At pH 7, the highest adsorption was detected. On increase or decrease of the pH, a decline in the adsorption efficiency was observed. The reason behind this deterioration is due to the decreasing electrostatic forces between the adsorbent and adsorbate [255, 262]. The experimental results of this parameter are presented in ***(Fig. 6.4.3 (a), Fig 6.4.3 (b)* and *Fig. 6.4.3(c).*** In the case of raw rice husk, the removal percentage was 63%, rice husk modified with 0.5 M NaOH (RHN) 79.4% and thermally treated rice husk heated at 300°C (RHH) was 81.35%. The maximum adsorption has been observed between pH 6.0 and 8, the maximum adsorption was detected.

6.4.3.2. Effect of adsorbent dosages

The adsorbent-to-adsorbate ratio has a massive impact on the overall adsorption process and optimization of adsorption is usually done about adsorbent dose in a fixed volume of adsorbate solution, initially, several adsorption sites were accessible for the adsorbate but at the later stage these sites were reduced and repellent forces started between the adsorbate molecules [306]. The impact of quantity of adsorbent on removal efficiency is revealed in ***Fig. 6.4.4 (a) Fig. 6.4.4 (b) and Fig.6.4.4 (c).*** It has been observed that on increasing the adsorbent dosages, there is a decrease in the adsorbent capacity. The adsorption is based on behavior adsorbent with adsorbate and is regulated by the number of adsorbent sites in an adsorbent. The adsorbent of a particular mass can absorb a specific concentration of adsorbate. When the adsorbent dosages increase, it rapidly increases the number of adsorption sites and increases the surface area, and speeds up the adsorption percentage [306]. The result of the percentage removal of raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN) and rice husk heated at 300°C (RHH) was 81%, 92%, and 94% respectively.

6.4.3.3. Effect of initial DCM concentration

The initial concentration occupies a pivot position in the process of adsorption and effect the adsorption efficiency significantly. The adsorption was observed to be directly related to the initial DCM concentration. On increasing the DCM concentration, the molecules of adsorbate speedily moved toward the surface of the adsorbents. [147].The concentration studies were accomplished at 25°C on an extensive range of initial dichloromethane concentrations (20-300 mg/L). The adsorption was speedy and efficient on the lower initial concentration of adsorbate but its efficiency decreases on increasing the adsorbate concentration. Hydrophobicity and pore size of adsorbents and the availability of adsorption sites decide the speed and efficiency of the adsorption process. In higher concentrations, the adsorption sites left few. As a result of it, there is a decrease in the efficiency of adsorption. It was evaluated in the current study that the adsorption graph moves from higher to lower on increasing the initial dichloromethane concentration on proceeding toward the equilibrium [306]. On comparing the performance of three adsorbents-RRH, RHN, and RHH, it was observed that the adsorption capacity of raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN) and rice husk heated at 300°C (RHH) was 45.25 mg/g, 52.25 mg/g and 53.85 mg/g (RHH) respectively.

6.4.3.4. Effect of contact time

In the adsorption process, contact time is one of the most decisive factors. To perform this study, all the parameters were kept constant except the contact time (20-240 minutes). The adsorption increases with the increase in contact time. The adsorption process was completed in two steps. The first step is faster and is designated as external surface adsorption and the second step is slower and known as internal surface adsorption. On analyzing the adsorption capacity of raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN) and rice husk heated at 300°C (RHH), it was 31.39mg/g, 39.58 mg/g, and 40.58 respectively ***(Fig 6.5. 5 (a), Fig 6.5.5 (b) and Fig 6.5.5 (c).***

6.4.4. Adsorption isotherms

To understand the relation between adsorbate and the adsorbent, adsorption isotherms are very helpful [156, 355]. Langmuir and Freundlich's models are the most frequently used isotherm models. For the analysis of experimental data, isotherm models were taken into account in the current study. The Languimer model explains the adsorption on the monolayer surface and the Freundlich model illustrates the adsorption on heterogeneous surface of adsorbents.

Languimer and Freundlich model

The Freundlich model deals with adsorption on the surface having heterogeneity whereas the Languimer model is concerned with adsorption on a monolayer adsorbent surface. The experimental results of the adsorption studies have been illustrated in ***Table 6.4.4, Table 6.4.6, and Table 6.4.8.*** Regression coefficient (R^2) value for the adsorption of dichloromethane (DCM) on raw rice husk (RRH), rice husk heated at 300°C (RHH), and rice husk modified with 0.5 M NaOH (RHN) suggested that Freudlich fits much better on raw rice husk (RRH), raw husk heated at 300 °C (RHH) and rice husk modified with 0.5 M NaOH (RRN). The favorability in adsorption has been shown by R_L values that appears between 0 and 1 specifying the favorability in adsorption [184]. The values of constant (n) were detected to be greater than 1. It predicted the favorable adsorption of dichloromethane (DCM) on four adsorbents.

6.4.5. Adsorption kinetics

To calculate the rate constants, Pseudo-first-order kinetics and Pseudo second order kinetics models were used.

Pseudo-first-order kinetics

The regression coefficient (R^2) values and rate constants values for raw rice husk (RRH), rice husk heated at 300°C (RHH), and rice husk modified with 0.5 M NaOH (RHN) are reported in ***Table 6.4.5, Table 6.4.7, and Table 6.4.9.***

Pseudo-second-order kinetics

By plotting the linear plot of t/qt vs t, the applicability of this model was investigated. The value of K_2 and qe (exp) is calculated, by using the value of slope and intercept from the linear plot. The correlation coefficient (R^2) and other constants for pseudo-second-order have been illustrated in ***Table 6.4.5, Table 6.4.7, and Table 6.4.9.*** By calculating the value of correlation coefficients (R^2), the quantification of the applicability of Pseudo first order and Pseudo second order was done. However, correlation coefficients (R^2) showed that Pseudo second order fits better with the experimental data as compared to the Pseudo-first order. So the adsorption kinetics is fitted well with Pseudo second order reaction.

6. 4.6. Comparison of Efficiency of Rice husk as per previous work

Sahu has observed adsorption capacity of 0.078 to 0.166 mg/g for pesticides in wastewater by using rice husk. Iraqi rice husk also proved as a potent adsorbent for the removal of Cr^{+6} ions from the wastewater and gained good appreciation as an adsorbent due to cheap, economical, easy, and simple to use [292]. Rice husk works well as an adsorbent for the removal of 2-chlorophenol [18]. Rice husk have high surface area and have the potency to remove gasoline due [53, 258]. These have the adsorption capacity of 6.61 mg/g, 7.28 mg/g, and 11.22 mg /g, for ammonia [36]. For the removal of malachite green dye, rice husk acts as a substantial adsorbent [285, 337, 388]. Dhaki has investigated a removal efficiency of about 84.97% for rice husk as an adsorbent under optimum conditions [84]. Rice husk appears to be a reliable adsorbent in the case of volatile organic compounds too, such as ammonia, methyl mercaptan, and benzene [36]. Eventually, its application spread to diverse organic compounds [132]. The adsorption efficiency of 373.02 mg /g has been demonstrated using the modified rice husks [337]. Using rice husk, the efficiency of adsorption of 19.19 mg/g and 201 mg/g for phenol has been detected [300]. The removal percentage of 98% has been noticed for pesticide triphosphate by using rice husks as adsorbents [13]. Rice husks also act as good adsorbents for heavy metals [13]. Using activated rice husk, removal percentage of 99%, 95%, 95% and 97% of for Pb, Cu, Zn and Cd has been detected [13]. The percentage removal of 93.36%, 94.8%, 96.72%, and 99.35% has been observed for lead (II) ions by using chemical and thermal treated adsorbents prepared by rice husk. It was also exhibited by Alam that rice husk derived ash could remove fluoride ions (F-) up to 42.5% [13].Using rice husk as adsorbent, approximately six and three mg/g, of adsorption capacity were found for phosphate and nitrate [313]. The rice husk char were also have the potential to adsorb nitrates and nitrites [173].

6.4.7. Comparison of the efficiency of other adsorbents for the adsorption of dichloromethane

In the previous study, researchers have used many materials for the adsorption of dichloromethane such as cerium oxide activated carbon, bituminous granular activated carbon, coconut granulated activated carbon, wood granulated activated carbon, high-silica zeolites, Pd and Pt co-impregnated activated carbon, pitch-based activated carbon fiber, nitrogen-doped hierarchical porous bio-char (NHWC), activated carbon monoliths (ACM) and Cu doped cerium-titanium binary oxides (Deng et al.,2020; Yang et al.,2019). Alhooshani found removal percentage of 32.04 % to 82.72 % using cerium oxide activated carbon at temperature 25 °C for dichloromethane [14]. Some other adsorbents such as high-silica zeolites also have shown remarkable adsorption capacity on dichloromethane [90]. In the oxidation of dichloromethane, Cu-doped cerium-titanium binary oxides play a significant role [383]. As compared to all adsorbents mentioned, the rice husks act as satisfactory adsorbents on dichloromethane with good adsorption capacity and removal percentage.

6.4.8. Statistical analysis

To analyze the adsorption studies and to check the effect of pH, carbon doses and, contact time on the adsorption of DCM by various adsorbents prepared by rice husk (RRH, RHN, and RHH), statistical tools were applied separately for each adsorbent. The second part of this study is done by comparing the efficiency of three adsorbents with each other. The experimental data are presented as mean ± SD of the experiment at $^{*}p \leq 0.05$. The observed values have been taken for subsequent statistical analysis and interpretation. Analysis of variance (ANOVA) one-way test was also performed to find out the statistical value and significance of population means at 0.05 level ***(Tables 6.4.12, 6.4.14, 6.4.16, 6. 4.18, 6. 4.20, 6. 4.22, 6.4.24- 6.4.26- 6.4.28,).*** Descriptive statistics have been illustrated with the help of tables ***(Tables 6. 4.11, 6. 4.13, 6.4.15, 6. 4.17, 6.***

4.19, 6. 4.21, 6.4.23, 6.4.25- 6.4.27, Fig. 6.4.6 to Fig. 6.4.14). The ANOVA result suggests that various groups of pH, carbon dose (g), and contact time (minutes) differs significantly for three adsorbents (RRH, RHN, and RHH) ($F_{8,\ 18}$= (33.688) (pH), 424.088 (carbon dose) and 5.82 (contact time) for RRH. (F 8, 18= (24.78) (pH), 418.85 (carbon dose), and 6.39 (contact time) for RHN. (F 8, 18= (17.589) (pH), 378.516 (carbon dose), and 4.859 (contact time) for RHH.

Since Levene's statistic is significant, the equal variance was not assumed. To check the individual difference between sites post hoc comparison were assessed using Tukey. One-way ANOVA with post hoc Tukey's HSD test showed that there is significant difference occurs among the various groups of pH, carbon dose, and contact time.

In case of RRH and parameter pH, there exists a significant difference in the mean of value of removal (%) among different pH (***Fig. 6.4.6),*** among carbon doses ***(Fig. 6.4.9),*** among various parameter contact time (Fig. 6.4.12). Similarly in case of RHN as well as RHH, there exists a significant differences in the mean value of removal (%) among different pH ***(Fig. 6.4.7 and Fig.6.4.8 respectively) ;*** among different carbon doses ***(Fig. 6.4.10 and Fig. 6.4.11***, ***respectively)*** and among various parameter contact time ***(Fig. 6.4.13 and Fig. 6.4.14).***

Comparison between three adsorbents prepared by rice husk

To compare the performance of three adsorbents prepared from rice husk (RRH, RHN, and RHH), three parameters (pH, carbon dose, and contact time) on the adsorption of DCM, statistical tools were applied. The ANOVA result illustrates that various groups of pH, carbon dose (g), and contact differ significantly for three adsorbents (RRH, RHN, and RHH) ($F_{3\ 8}$= (5733.592) (pH), 8116.348 (carbon dose) and 1438.968 (contact time) One-way ANOVA with post hoc Tukey's HSD test showed that there is significant difference occurs among the various groups of pH, carbon dose, and contact time ***(Table 6.4.30, Table 6.4.32, Table 6.4.34, Fig. 6.4.15, Fig. 6.4.16, and Fig. 6.4.17).***

In case of parameter pH, there exists a significant difference in the mean value of removal % of Control and RRH, Control and RHN and Control and RHH. There is significant difference in the mean value of removal % of RRH and RHN and RRH and RHH ***(Table 6.4.30, and Fig. 6.4 . 15).***In the case of parameter carbon dose (g), there exists a significant difference in removal % between control and RRH; Control and RHN, and Control and RHH; RRH and RHN; RRH and RHH ***(Table 6.4.32, Fig. 6.4.16).*** In case of parameter contact time (min), there exist a significant difference in removal % between control and RRH; Control and RHN; Control and RHH; RRH and RHN and, between RRH and RHH (***Table 6.4.34, and Fig. 6.4.17).***

6.4.9. Conclusion

Industry is an asset for the economic development of countries. Releasing malodorous compounds into the environment is the consequence associated with it. It attracts the research community to use the adsorbents for the removal of these toxic compounds that are manipulating the ecosystem and becoming health hazards for the living world. The treatment of adsorbents thermally or chemically and functional groups related to them is a crucial factor for the change in surface area and subsequent surface chemistry. Alteration in the functional groups increases the specific surface area of the adsorbents. The relation between the functional group and specific surface area has been seen in the present study. The carbon and silica are the main constituents of rice husks. High temperature doesn’t favor the abundance of carbon, and it depletes by speeding up the temperature. On the other hand, on increasing the temperature, silica content increases. High temperature enhances the porosity and bigger surface area of the adsorbent. It has been observed that thermal treated adsorbent heated at 300°C have high porosity and high specific surface area as compared to chemically raw rice husk with 0.5 M NaOH and raw rice husk.

1. The adsorption capacities of thermally treated rice husk is more as compared to raw rice husk and chemically treated rice husk.

2. There is upliftment in the adsorption capacities of rice husk after treated thermally and chemically.

3. The three adsorbents follow pseudo second order kinetics.

4. pH control the efficiency of the adsorbents.

5. Thermal treated Rice husk heated at 300°C (RHH) have more efficiency among the adsorbents under study.

6. Adsorption capacity of thermally treated rice husk is more wit 53.85 mg/g as compared to chemically treated Rice husk treated with 0.5 M NaOH with 52.25, mg/g and Raw rice husk with 45.25 mg/g.

The present study opens up the scope of exploration of various adsorbents from the agricultural background for the adsorption of organic compounds that enter the environment from numerous chemical processes. Although easily accessible, these are economically reasonable and environmentally friendly too. There is a wide range of adsorbents that still need to explore and use for treating the organic or inorganic compounds before draining into the environment or after they become a part and parcel of the aquatic and terrestrial ecosystem.

Table 6.4 .1: Physicochemical properties of Raw rice husk (RRH)

Parameters	value
pH	7.27
Moisture content	5%
Ash content	15%
Yield %	71%

Table 6.4.2: Physicochemical properties of Rice husk modified with 0.5 M NaOH (RHN)

Parameters	value
pH	8-9
Moisture content	7.3
Ash content	10.9
Yield %	40.99

Table 6.4.3: Physicochemical properties of Rice husk heating at 300° C (RHH)

Parameters	value
pH	8-9
Moisture content	10%
Ash content	18%
Yield %	14%

Table 6.4.4: Languimer and Freundlich constants for the adsorption of dichloromethane (DCM) on Raw rice husk (RRH)

Adsorbents	Langmuir constants			
RRH	q max	K_L	R_L	R^2
	63.131	0.011	0.814	*0.95*

Adsorbents	Freundlich constants		
RRH	**K_F**	**n**	**R^2**
	2.303	**2.758**	**0.98**

Table 6.4.5: Kinetics parameters for the adsorption of dichloromethane (DCM) on Raw rice husk (RRH)

Adsorbents	Pseudo first order			Pseudo second order	
RRH	**qe**	**K_1**	**R^2**	**K_2**	**R^2**
	4.66	**-7.08333E-05**	**0.83**	**0.0070**	**0.99**

Table 6.4.6: Langmuir and Freundlich constants for the adsorption of dichloromethane (DCM) on Rice husk modified with 0.5 M NaOH (RHN)

Adsorbents		Langmuir constants		
RH modified with 0.5 M NaOH	**q max**	**K_L**	**R_L**	**R^2**
	72.99	**0.015**	**0.76**	**0.95**
Adsorbents	**Freundlich constants**			
RH modified with 0.5 M NaOH	**K_F**	**n**	**R^2**	
	1.31	**1.192**	**0.97**	

Table 6.4.7: Kinetics parameters for the adsorption of dichloromethane (DCM) on Rice husk modified with 0.5 M NaOH at 25 °C (Co=200 mg/L)(RHN)

Adsorbents	Pseudo first order			Pseudo second order	
RH modified with 0.5 M NaOH	qe	K_1	R^2	K_2	R^2
	4.18	-5.41667E-05	0.79	0.0069	0.99

Table 6.4.8: Langmuir and Freundlich constants for the adsorption of dichloromethane (DCM) on Rice husk heated at 300 °C (RHH)

Adsorbents	Langmuir constants			
RH heated at 300 °C	q max	K_L	R_L	R^2
	80	0.014	0.01	0.96
Adsorbents	Freundlich constants			
RH heated at 300° C	K_F	n	R^2	
	1.27	0.87	0.97	

Table 6.4.9: Kinetics parameters for the adsorption of dichloromethane (DCM) on Rice husk heated at 300 °C (RHH) at 25 °C (Co=200 mg/L)

Adsorbents	Pseudo first order			Pseudo second order	
RH heated at 300	qe	K_1	R^2	K_2	R^2

°C					
	4.38	**-5.41667E-05**	**0.76**	**0.006**	**0.99**

Table 6.4.10: Code for developed adsorbents

Number	**Type of treatment**	**Adsorbent name**	Code
1	Raw	Raw Rice Husk	RRH
2	Chemical	Raw Rice Husk+ 0.5 M NaOH (chemical treated rice husk)	RHN
3	Thermal	Raw Rice Husk 300 °C, 3hrs (Thermal treated rice husk)	RHH

Table 6.4.11: Descriptive statistics (Effect of pH on adsorption by RRH)

pH	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	**Upper Bound**	
pH-2	3	54.54	1.09	0.63	51.81	57.26	53.42
pH-3	3	56.77	0.58	0.33	55.32	58.21	56.1
pH-4	3	58.57	1.60	0.92	54.57	62.57	57.15
pH-5	3	60.46	1.02	0.59	57.92	63.00	59.49
pH-6	3	62.81	0.51	0.29	61.52	64.10	62.47
pH-7	3	63.12	0.99	0.57	60.66	65.58	62.13
pH-8	3	56.42	0.99	0.57	53.95	58.89	55.42
pH-9	3	56.76	0.99	0.57	54.30	59.22	55.79
pH-10	3	53.45	1.00	0.58	50.95	55.94	52.44

Total	27	58.10	3.39	0.65	56.75	59.44	52.44

Table 6.4.12: Test of homogeneity of variance and ANOVA (Effect of pH on adsorption by RRH)

				Test of homogeneity of variance		**ANOVA**	
pH	**N**	**Mean**	**Std. Deviation**	**Levene's Statistic**	**Sig.**	**F**	**Sig.**
				0.49	0.84	33.68	**<0.05**
2	3	54.54	1.09				
3	3	56.77	0.58				
4	3	58.57	1.60				
5	3	60.46	1.023				
6	3	62.81	0.51				
7	3	63.12	0.99				
8	3	56.42	0.99				
9	3	56.76	0.99				
10	3	53.45	1.005				
Total	27	58.10	3.39				

Table 6.4.13: Descriptive statistics (Effect of pH on adsorption by RHN)

pH	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	**Upper Bound**	
pH-2	3	72.39	0.9	0.51	70.15	74.63	71.5
pH-3	3	73.42	0.95	0.55	71.04	75.80	72.52
pH-4	3	74.40	0.97	0.56	71.98	76.82	73.43

pH-5	3	76.35	1.1	0.63	73.62	79.08	75.26
pH-6	3	77.44	1.10	0.63	74.70	80.17	76.34
pH-7	3	78.45	0.10	0.058	78.20	78.70	78.37
pH-8	3	75.84	0.59	0.34	74.36	77.32	75.47
pH-9	3	73.42	0.97	0.56	71.008	75.84	72.51
pH-10	3	71.38	0.93	0.53	69.06	73.69	70.44
Total	27	74.90	2.59	0.49	73.87	75.92	70.44

Table 6.4.14: Test of homogeneity of variance and ANOVA (Effect of pH on adsorption by RHN)

				Test of homogeneity of variance		**ANOVA**	
pH	**N**	**Mean**	**Std. Deviation**	**Levene's Statistic**	**Sig.**	**F**	**Sig.**
				0.52	0.82	24.78	**<0.05**
2	3	72.39	0.9				
3	3	73.42	0.95				
4	3	74.40	0.97				
5	3	76.35	1.1				
6	3	77.44	1.10				
7	3	78.45	0.10				
8	3	75.84	0.59				
9	3	73.42	0.97				
10	3	71.38	0.93				
Total	27	74.90	2.59				

Table: 6.4.15: Descriptive statistics (Effect of pH on adsorption by RHH)

pH	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
pH-2	3	75.60	0.61	0.35	74.08	77.12	75.13
pH-3	3	75.35	1.006	0.58	72.85	77.85	74.43
pH-4	3	77.40	0.96	0.55	74.99	79.81	76.44
pH-5	3	78.85	0	0	78.85	78.85	78.86
pH-6	3	80.94	1.09	0.63	78.23	83.66	80.31
pH-7	3	81.35	0.98	0.56	78.90	83.79	80.36
pH-8	3	79.84	0.99	0.57	77.38	82.30	78.86
pH-9	3	77.33	0.94	0.54	74.99	79.67	76.35
pH-10	3	79.38	0.92	0.53	77.08	81.67	78.47
Total	27	78.45	2.21	0.42	77.57	79.32	74.43

Table 6.4.16: Test of homogeneity of variance and ANOVA (Effect of pH on adsorption by RHH

				Test of homogeneity of variance		ANOVA	
pH	N	Mean	Std. Deviation	Levene Statistic	Sig.	F	Sig.
				0.82	0.59	17.58	**<0.05**
pH-2	3	3	75.60	0.61			
pH-3	3	3	75.35	1.006			
pH-4	3	3	77.40	0.96			

pH-5	3	3	78.85	0
pH-6	3	3	80.94	1.09
pH-7	3	3	81.35	0.98
pH-8	3	3	79.84	0.99
pH-9	3	3	77.33	0.94
pH-10	3	3	79.38	0.92
Total	27	27	78.45	2.21

Table 6.4.17: Descriptive statistics (Effect of carbon dose on adsorption by RRH)

Carbon doses (g)	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	Upper Bound	
0.2	3	50.12	1.005	0.58	47.62	52.61	49.12
0.4	3	65.25	1.00	0.57	62.77	67.74	64.24
0.6	3	74.26	0.99	0.57	71.79	76.73	73.27
0.8	3	76.45	1.04	0.60	73.86	79.03	75.41
1	3	80.17	1.01	0.58	77.65	82.69	79.15
Total	15	69.25	11.16	2.88	63.07	75.43	49.12

Table 6.4.18: Test of homogeneity of variance and ANOVA (Effect of carbon dose on adsorption by RRH)

				Test of homogeneity of variance		**ANOVA**	
Carbon doses	**N**	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**

0.2	3	50.12	1.00	0.001	1	424.08	<**0.05**
0.4	3	65.25	1.00				
0.6	3	74.26	0.99				
0.8	3	76.45	1.04				
1	3	80.17	1.01				
Total	15	69.25	11.16				

Table 6.4.19: Descriptive statistics (Effect of carbon dose on adsorption by RHN)

Carbon dose (g)	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	Upper Bound	
0.2	3	62.12	1.005	0.58	59.62	64.61	61.12
0.4	3	77.26	0.99	0.57	74.79	79.73	76.28
0.6	3	85.31	0.96	0.55	82.91	87.71	84.36
0.8	3	86.47	1.02	0.59	83.93	89.00	85.41
1	3	92.21	0.94	0.54	89.86	94.56	91.29
Total	15	80.67	10.83	2.79	74.67	86.67	61.12

Table 6.4.20: Test of homogeneity of variance and ANOVA (Effect of carbon dose on adsorption by RHN)

				Test of homogeneity of variance		**ANOVA**	
Carbon dose (g)	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
0.2	3	62.12	1.00	0.006	1	418.85	<**0.05**
0.4	3	77.26	0.99				

0.6	3	85.31	0.96
0.8	3	86.47	1.02
1	3	92.21	0.94
Total	15	80.67	10.83

Table 6.4.21: Descriptive Statistics (Effect of carbon dose on adsorption by RHH)

Carbon dose (g)	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**
					Lower Bound	Upper Bound	
0.2	3	64.13	0.99	0.57	61.67	66.58	63.13
0.4	3	79.32	0.90	0.52	77.08	81.56	78.45
0.6	3	88.29	0.94	0.54	85.94	90.63	87.36
0.8	3	90.33	0.87	0.50	88.16	92.49	89.41
1	3	94.04	1.49	0.86	90.33	97.75	92.49
Total	15	83.22	11.11	2.87	77.06	89.38	63.13

Table 6.4.22: Test of homogeneity of variance and ANOVA (Effect of carbon dose on adsorption by RHH)

				Test of homogeneity of variance		**ANOVA**	
Carbon doses	**N**	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
0.2	3	64.13	0.99	0.30	0.87	378.51	<**0.05**
0.4	3	79.32	0.90				

0.6	3	88.29	0.94
0.8	3	90.33	0.87
1	3	94.04	1.49
Total	15	83.22	11.11

Table 6.4.23: Descriptive statistics (Effect of contact time on adsorption by RRH)

Contact Time (minutes)	N	Mean	S.D	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	**Upper Bound**	
20	3	27.21	1.05	0.61	24.58	29.84	26.21
40	3	29.26	0.99	0.57	26.80	31.72	28.25
60	3	28.70	0.97	0.56	26.28	31.12	27.73
80	3	29.23	0.90	0.52	26.99	31.47	28.32
100	3	31.31	0.89	0.51	29.09	33.54	30.42
120	3	30.45	1.10	0.63	27.71	33.1848	29.35
140	3	31.15	1.01	0.58	28.64	33.661	30.14
160	3	31.25	0.95	0.55	28.87	33.6338	30.32
180	3	31.39	0.97	0.56	28.97	33.8107	30.37
200	3	31.39	0.95	0.55	29.02	33.7632	30.39
210	3	31.39	0.95	0.55	29.02	33.7652	30.5
220	3	31.39	0.94	0.54	29.06	33.732	30.45
240	3	31.39	1.1	0.63	28.66	34.1301	30.3
Total	39	30.42	1.56	0.25	29.92	30.9374	26.21

Table 6.4.24: Test of homogeneity of variance and ANOVA (Effect of contact time on adsorption by RRH)

	Test of homogeneity of variance	ANOVA

Contact time (minutes)	N	Mean	S.D	Levene Statistic	Sig.	F	Sig.
				0.02	1	5.82	**<0.05**
20	3	27.21	1.05				
40	3	29.26	0.99				
60	3	28.70	0.97				
80	3	29.23	0.90				
100	3	31.31	0.89				
120	3	30.45	1.10				
140	3	31.15	1.01				
160	3	31.25	0.95				
180	3	31.39	0.97				
200	3	31.39	0.95				
210	3	31.39	0.95				
220	3	31.39	0.94				
240	3	31.39	1.1				
Total	39	30.42	1.56				

Table 6.4.25: Descriptive statistics (Effect of contact time on adsorption by RHN)

Contact time (minutes)	N	Mean	S.D	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
20	3	35.15	1.00	0.57	32.67	37.64	34.13
40	3	36.25	1.05	0.61	33.62	38.88	35.21
60	3	36.75	0.95	0.54	34.39	39.11	35.8
80	3	37.25	1.01	0.58	34.73	39.78	36.25
100	3	38.02	1.01	0.58	35.51	40.53	37.02

120	3	38.57	0.99	0.57	36.10	41.045	37.58
140	3	39.37	0.98	0.56	36.92	41.82	38.39
160	3	39.37	0.99	0.57	36.89	41.85	38.38
180	3	39.37	1.00	0.57	36.89	41.85	38.38
200	3	39.37	0.9	0.51	37.13	41.61	38.48
210	3	39.37	1.00	0.57	36.89	41.85	38.38
220	3	39.37	1.10	0.63	36.64	42.10	38.28
240	3	39.51	1.10	0.63	36.78	42.24	38.41
Total	39	38.29	1.66	0.26	37.75	38.83	34.13

Table 6.4.26: Test of homogeneity of variance and ANOVA Descriptive statistics (Effect of contact time on adsorption by RHN)

				Test of homogeneity of variance		**ANOVA**	
Contact time (minutes)	**N**	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
				0.013	1	6.39	<**0.05**
20	3	35.15	1.00				
40	3	36.25	1.05				
60	3	36.75	0.95				
80	3	37.25	1.01				
100	3	38.02	1.01				
120	3	38.57	0.99				
140	3	39.37	0.98				
160	3	39.37	0.99				
180	3	39.37	1.00				
200	3	39.37	0.9				
210	3	39.37	1.00				
220	3	39.37	1.10				

240	3	39.51	1.10
Total	39	38.29	1.66

Table 6.4.27: Descriptive statistics (Effect of contact time on adsorption by RHH)

Contact time (minutes)	N	Mean	S.D	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
20	3	37.49	1.091	0.63	34.78	40.20	36.23
40	3	37.23	0.97	0.56	34.81	39.65	36.21
60	3	37.71	1.03	0.59	35.14	40.27	36.67
80	3	38.24	1.00	0.58	35.74	40.74	37.25
100	3	39.32	0.99	0.57	36.86	41.78	38.33
120	3	39.45	1.00	0.58	36.95	41.95	38.46
140	3	40.15	0.99	0.57	37.67	42.63	39.16
160	3	40.25	0.87	0.50	38.07	42.42	39.43
180	3	40.39	0.89	0.51	38.15	42.62	39.49
200	3	40.39	1.00	0.58	37.89	42.88	39.38
210	3	40.39	1.01	0.58	37.88	42.90	39.37
220	3	40.39	0.89	0.51	38.16	42.63	39.5
240	3	40.58	1.1	0.63	37.85	43.32	39.49
Total	39	39.38	1.47	0.23	38.90	39.86	36.21

Table 6.4.28: Test of homogeneity of variance and ANOVA (Effect of contact time on adsorption by RHH)

	Test of homogeneity of variance	ANOVA

Contact time (minutes)	N	Mean	S.D	Levene Statistic	Sig.	F	Sig.
				0.04	1	4.85	**<0.05**
20	3	37.49	1.09				
40	3	37.23	0.97				
60	3	37.71	1.03				
80	3	38.24	1.00				
100	3	39.32	0.99				
120	3	39.45	1.00				
140	3	40.15	0.99				
160	3	40.25	0.87				
180	3	40.39	0.89				
200	3	40.39	1.00				
210	3	40.39	1.01				
220	3	40.39	0.89				
240	3	40.58	1.1				
Total	39	39.38	1.47				

Comparison of three adsorbents-RRH, RHN, and RHH

Table 6.4.29: Descriptive statistics (Comparatively effect of pH in adsorption by RRH, RHN, and RHH)

Adsorbents	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00

RRH	3	63.12	0.99	0.57	60.66	65.58
RHN	3	79.45	1.00	0.57	76.96	81.94
RHH	3	81.35	1.03	0.59	78.78	83.92
Total	12	55.98	34.56	9.97	34.01	77.94

Table 6.4.30: Test of homogeneity of variance and ANOVA (Comparatively effect of pH in adsorption by RRH, RHN, and RHH)

			Test of homogeneity of variance		**ANOVA**	
Adsorbents	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
			1.54	**0.27**	**5733.59**	**<0.05**
Control	0	0				
RRH	63.12	**0.99**				
RHN	79.45	**1.00**				
RHH	81.35	**1.03**				

Table 6.4.31: Descriptive statistics (Comparatively effect of carbon dose in adsorption by RRH, RHN, and RHH)

Adsorbent	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
RRH	3	81.17	1.00	0.58	78.67	83.67
RHN	3	92.16	1.03	0.59	89.59	94.73
RHH	3	94.18	0.95	0.54	91.82	96.54

Total	12	66.88	40.66	11.73	41.04	92.72

Table 6.4.32: Test of homogeneity of variance and ANOVA (Comparatively effect of carbon dose in adsorption by RRH, RHN, and RHH)

			Test of homogeneity of variance		**ANOVA**	
Adsorbents	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
Control	**0**	**0**	**1.51**	**0.28**	**8116.34**	**<0.05**
RRH	**81.17**	**1.00**				
RHN	**92.16**	**1.03**				
RHH	**94.18**	**0.95**				
Total	**66.88**	**40.66**				

Table 6.4.33: Descriptive statistics (Comparatively effect of contact time in adsorption by RRH, RHN, and RHH)

Adsorbents	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0.00	0.00	0.00	0.00	0.00
RRH	3	31.39	1.03	0.59	28.83	33.95
RHN	3	39.58	0.95	0.55	37.20	41.95
RHH	3	40.58	1.02	0.59	38.037	43.12
Total	12	27.89	17.24	4.97	16.93	38.84

Table 6.4.34: Test of homogeneity of variance and ANOVA (Comparatively effect of contact time in adsorption by RRH, RHN, and RHH)

			Test of homogeneity of variance		**ANOVA**	

Adsorbents	Mean	S.D	Levene Statistic	Sig.	F	Sig.
			1.572	**0.27**	**1438.968**	**<0.05**
Control	0	**0**				
RRH	31.39	1.03				
RHN	39.58	0.95				
RHH	40.58	1.02				

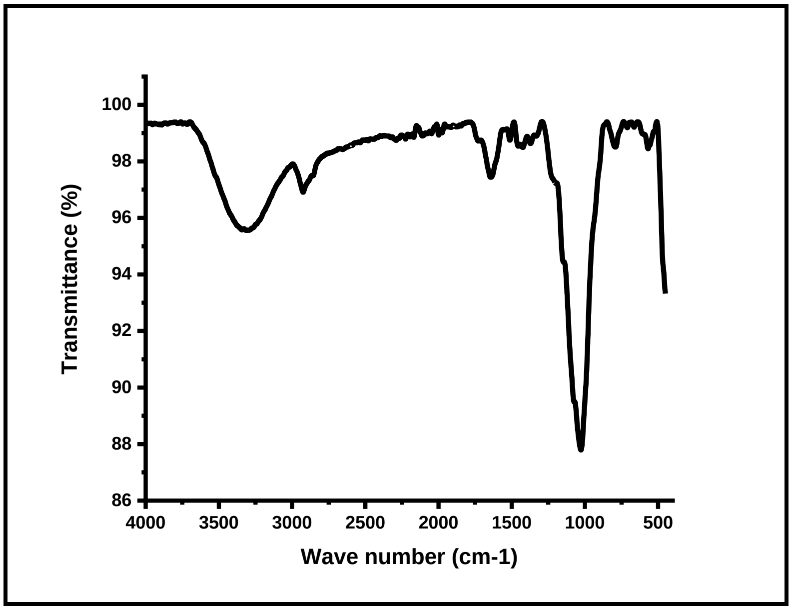


Fig. 6.4.1 (a).FTIR Spectra of Raw rice husk (RRH)

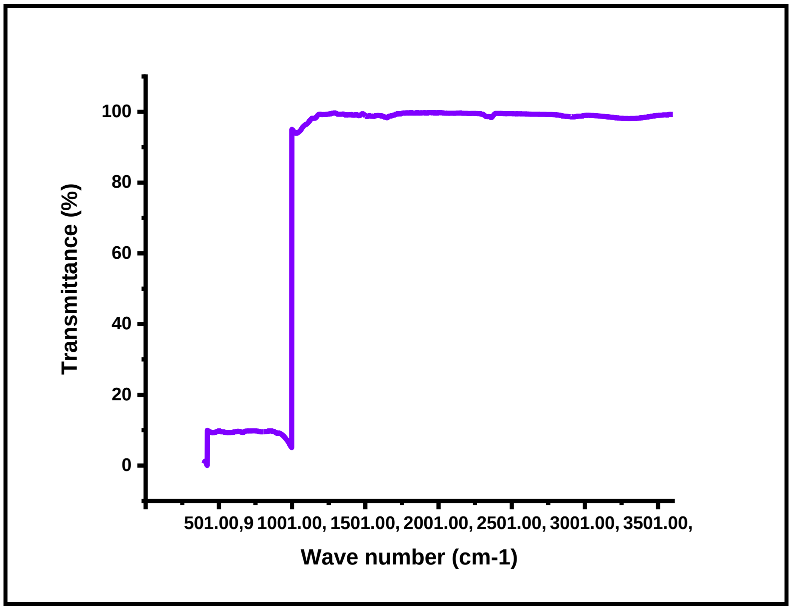


Fig.6.4.1 (b).FTIR Spectra of Rice husk modified with 0.5 M NaOH (RHN)

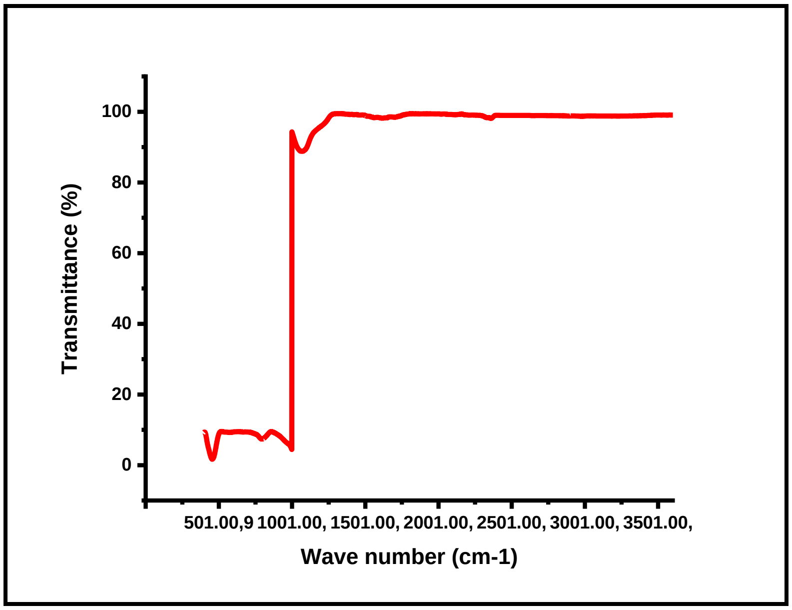


Fig.6.4.1 (c).FTIR Spectra of Rice husk heated at 300°C (RRH)

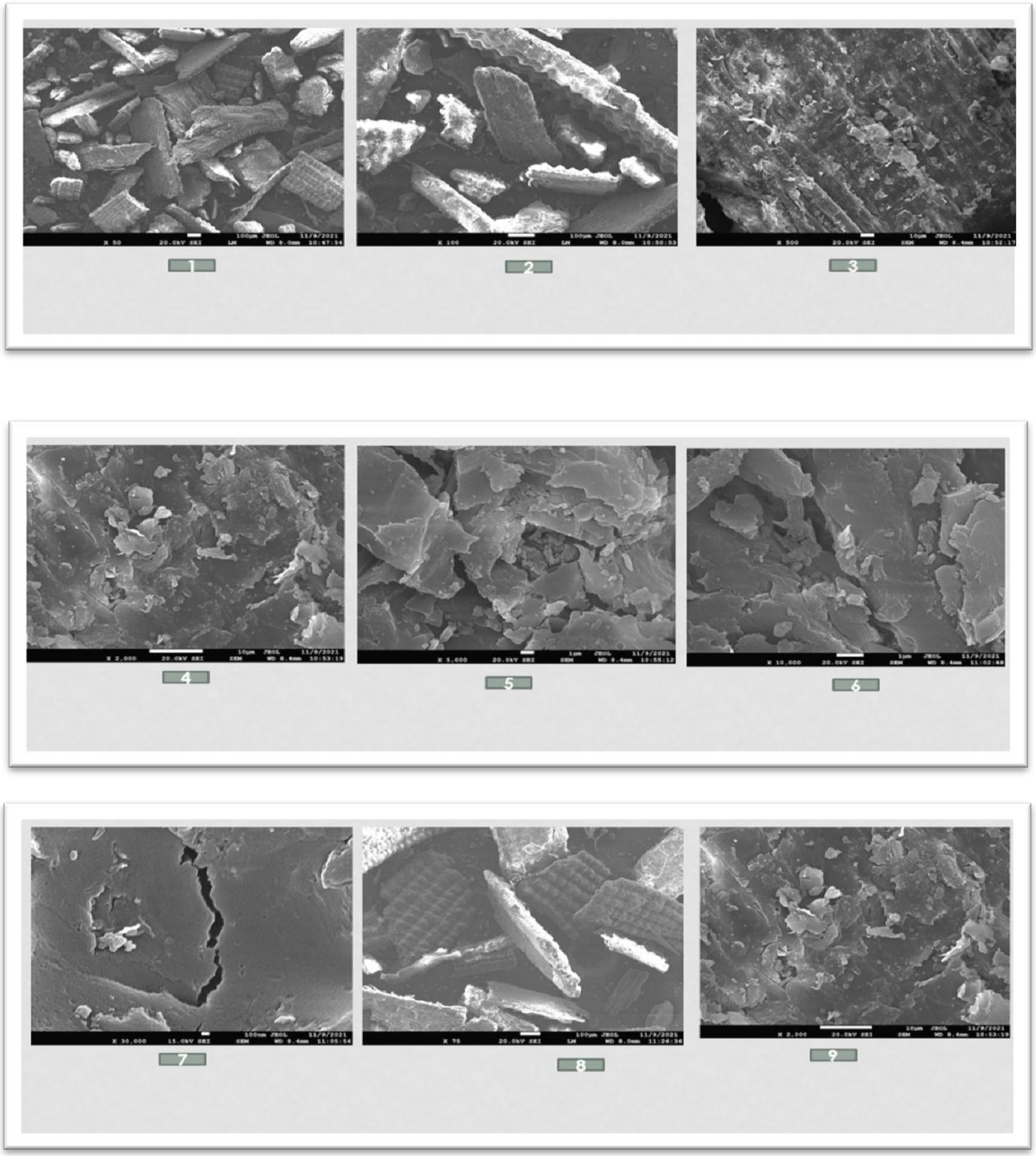
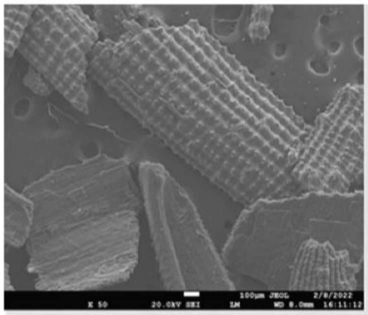


Fig.6.4.2 (a). FESEM images of Raw rice husk (RRH) (1-9)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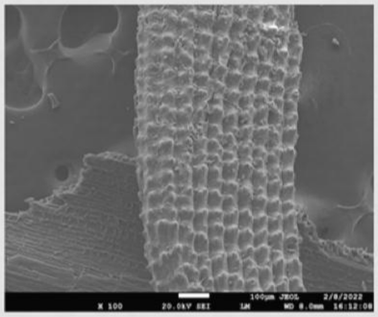
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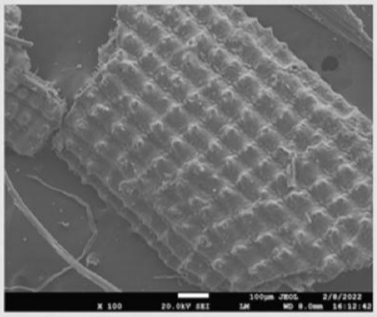
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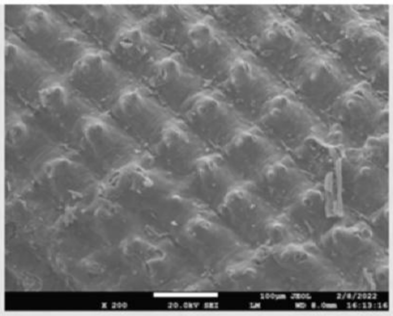
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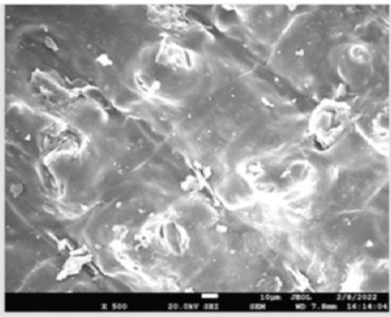
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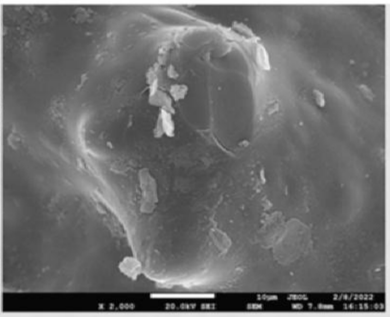
5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6

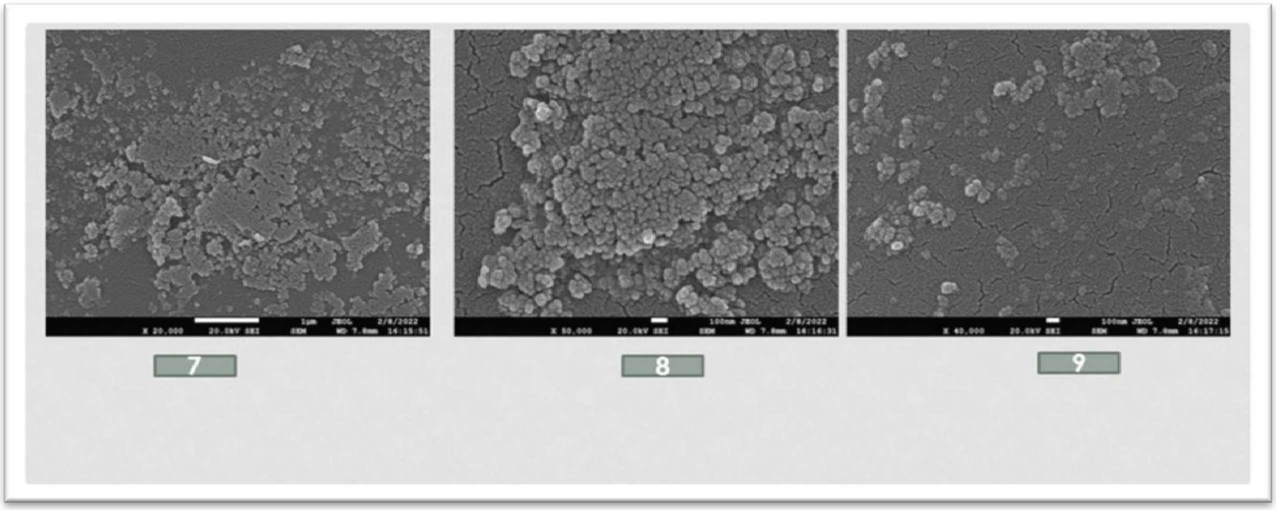
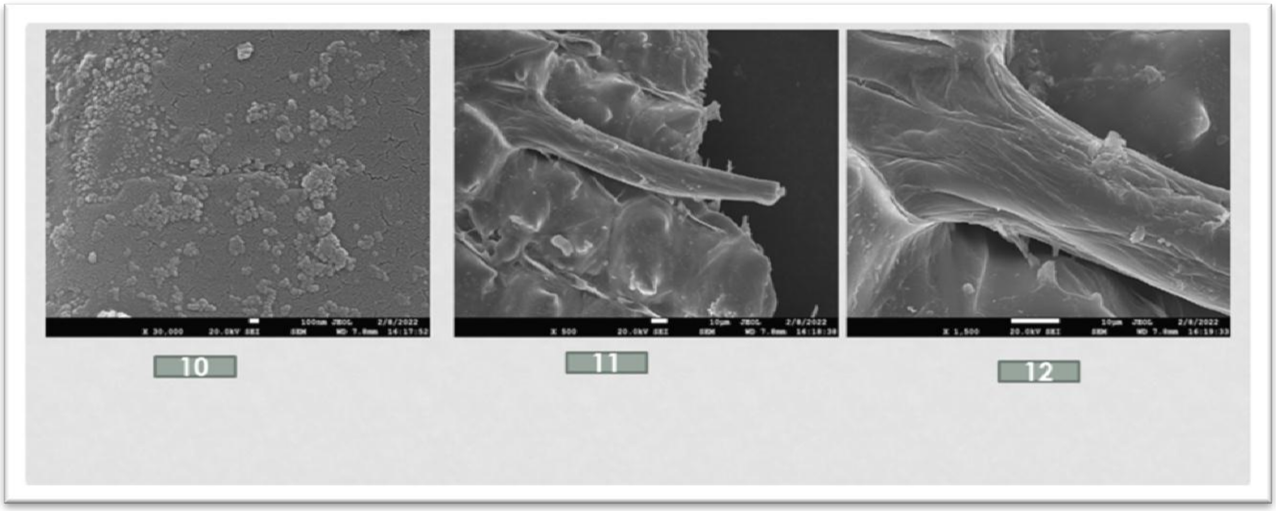


Fig. 6.4.2 (b1).FESEM images of Rice husk modified with 0.5 M NaOH (RHN) (1-9)



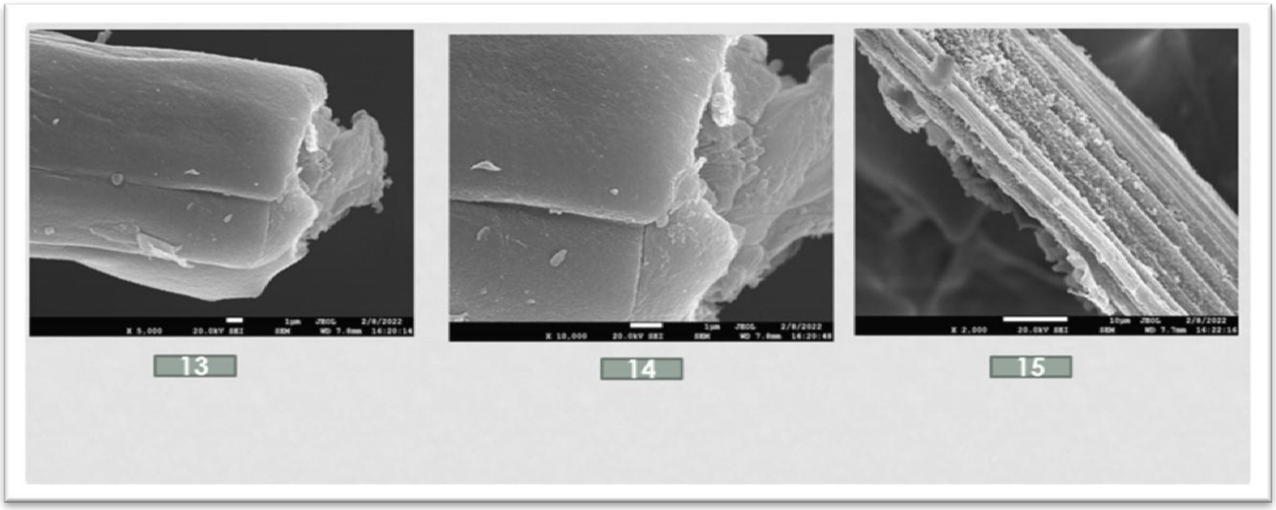
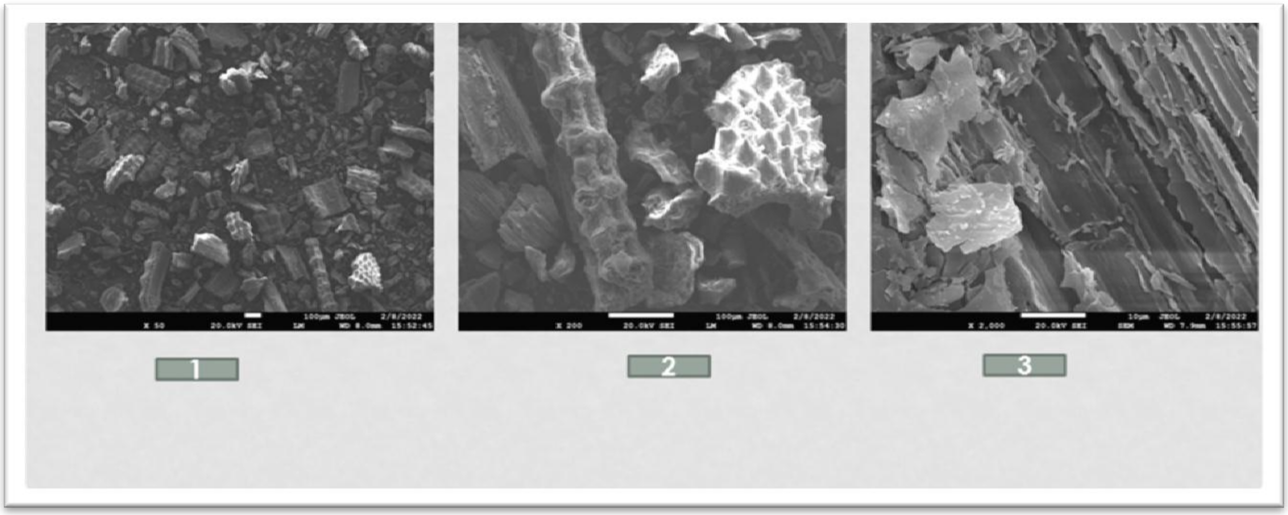


Fig.6.4.2. (b2).FESEM images of Rice husk modified with 0.5 M NaOH (RHN) (10-15)



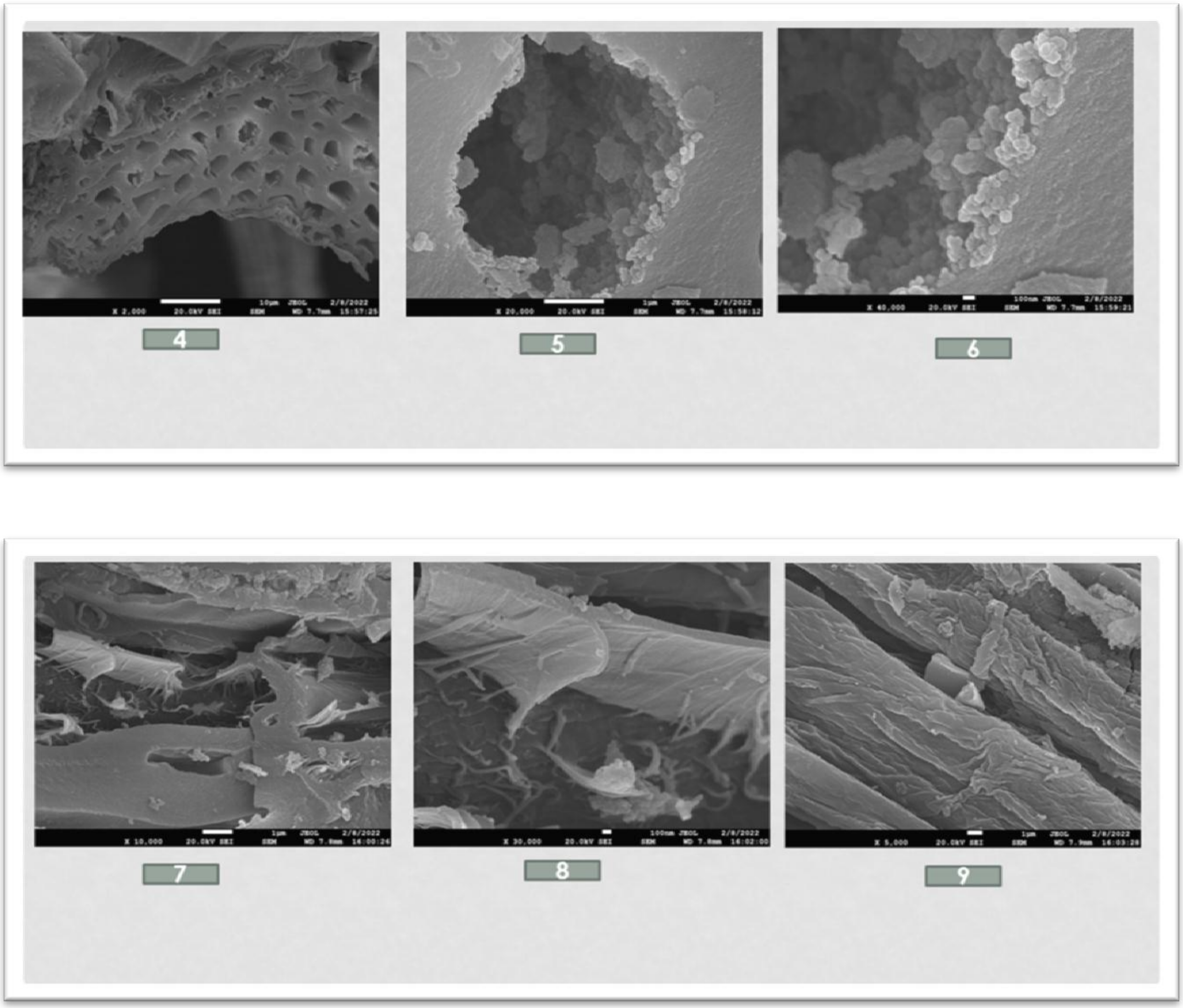
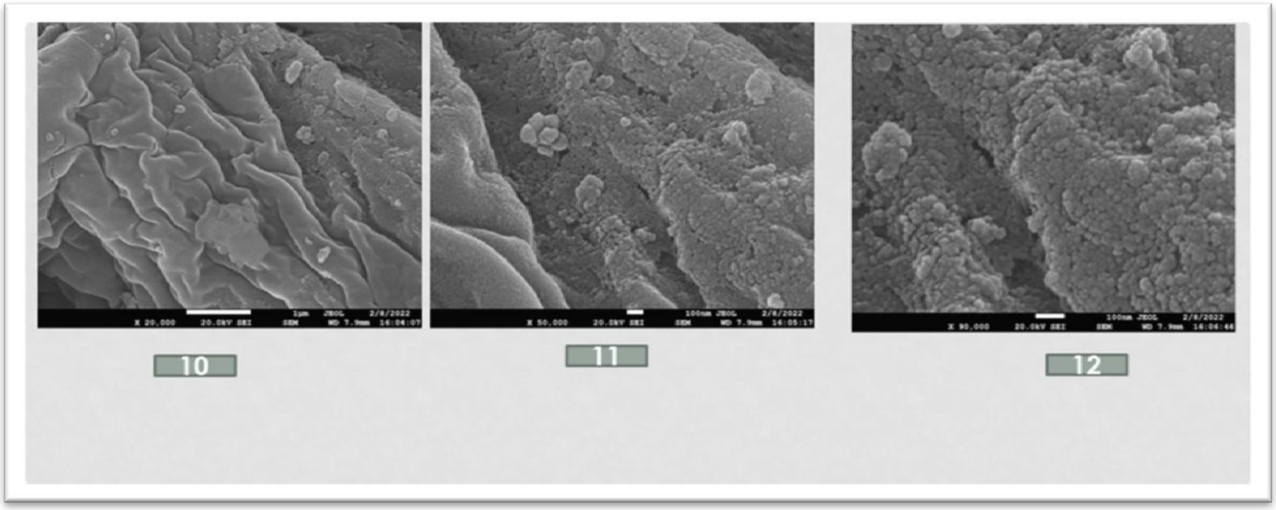


Fig.6.4.2. (c1).FESEM images of Rice husk heated at 300°C (1-9) (RHH)



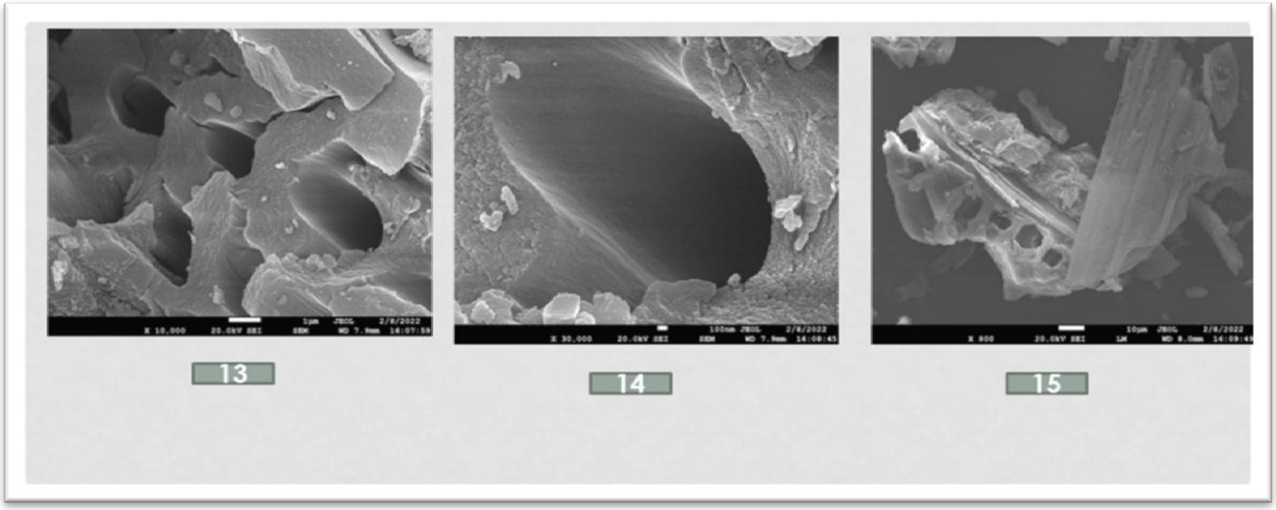


Fig.6.4.2. (c2).FESEM images of Rice husk heated at 300°C (10-15) (RHH)

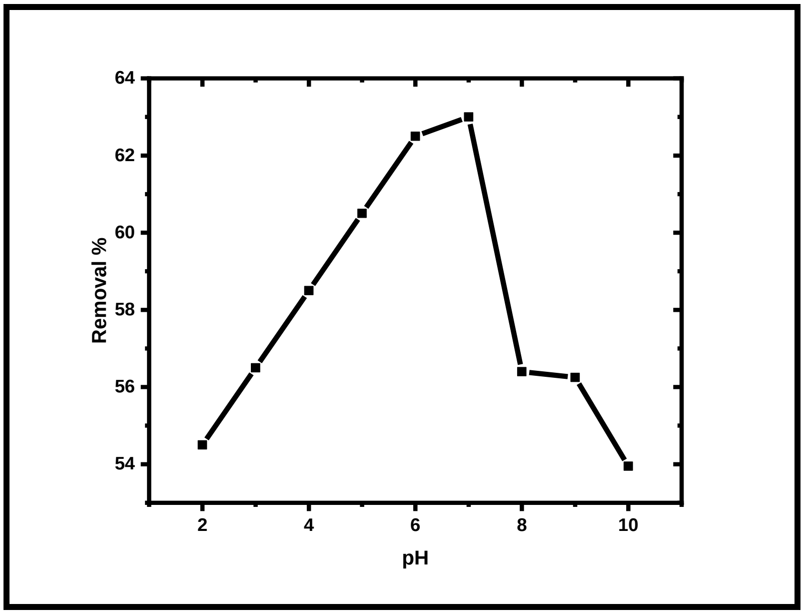


Fig.6.4.3 (a).Effect of initial pH on removal percentage of dichloromethane (DCM) on Raw rice Husk (RRH)

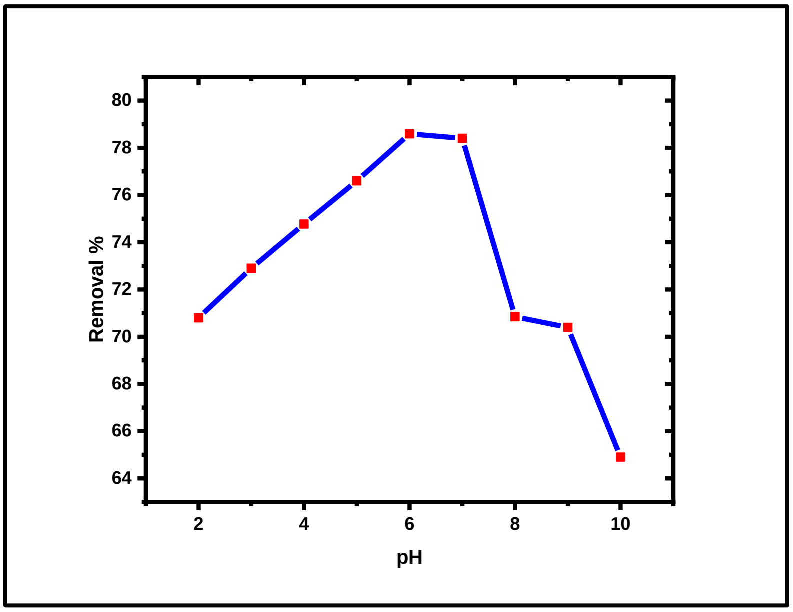


Fig.6.4.3. (b).Effect of initial pH on removal percentage of dichloromethane (DCM) on Rice husk modified 0.5 M NaOH (RHN).

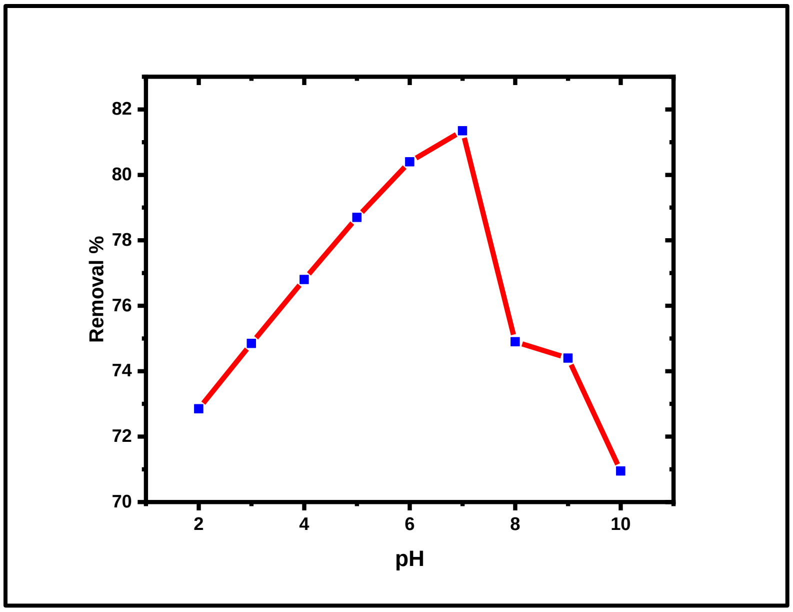


Fig.6.4.3(c).Effect of initial pH on removal percentage of dichloromethane (DCM) on Rice husk heated at 300 °C (RHH)

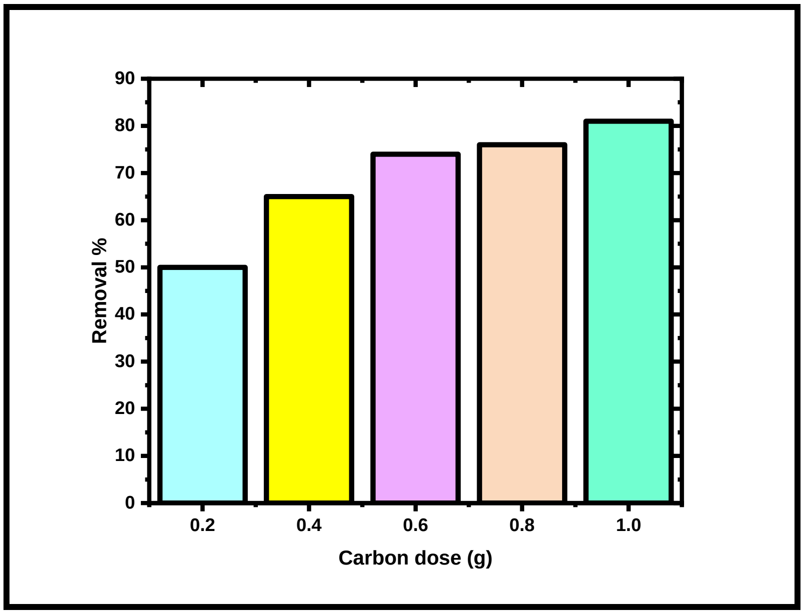


Fig.6.4.4 (a).Effect of carbon dose on removal percentage of dichloromethane (DCM) by Raw rice husk (RRH)

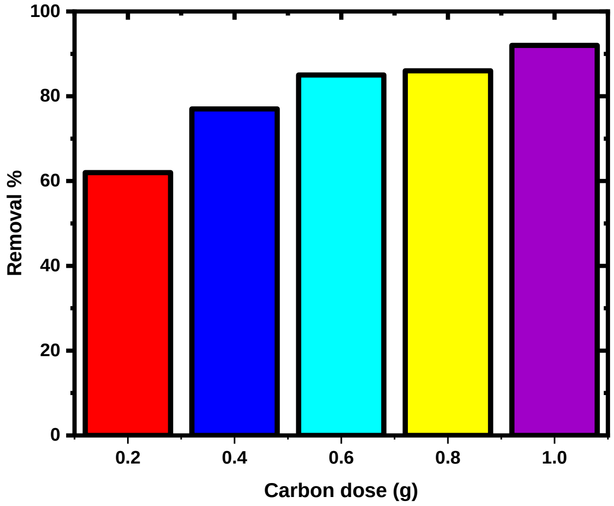


Fig.6.4.4 (b).Effect of carbon dose on removal percentage of dichloromethane (DCM) by Rice husk modified with 0.5 M NaOH (RHN)

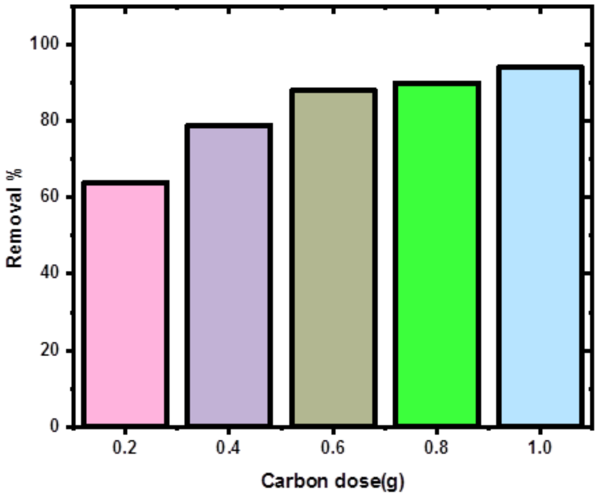


Fig.6.4.4 (c).Effect of carbon dose on removal percentage of dichloromethane (DCM) on Rice husk heated at 300 °C (RHH)

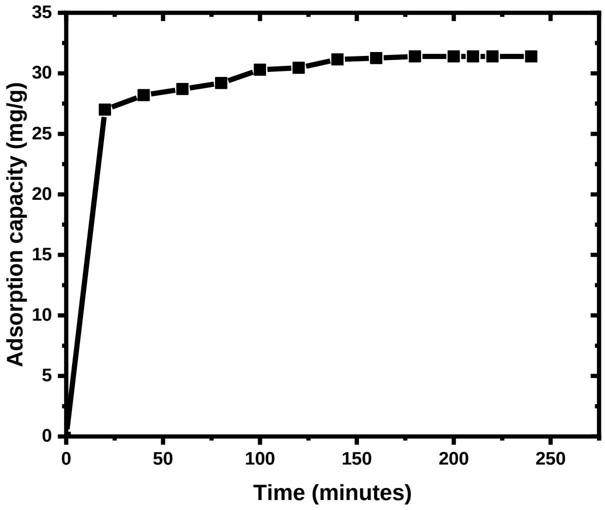


Fig.6.4.5 (a).Effect of contact time on adsorption capacity of dichloromethane (DCM) on Raw rice husk (RRH)

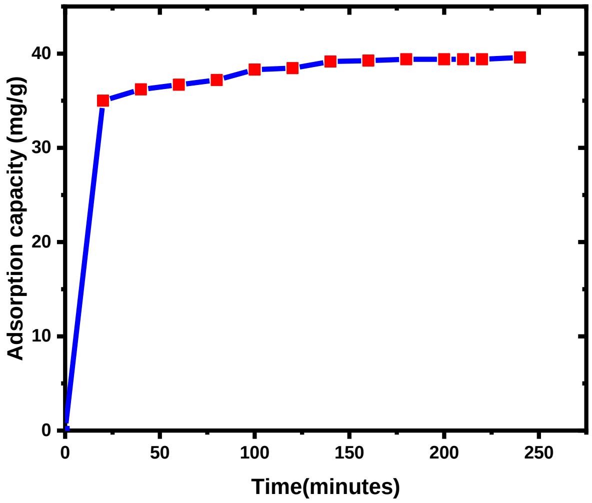


Fig. 6.4.5 (b).Effect of contact time on adsorption capacity of dichloromethane (DCM) on Rice husk modified with 0.5 M NaOH (RHN)

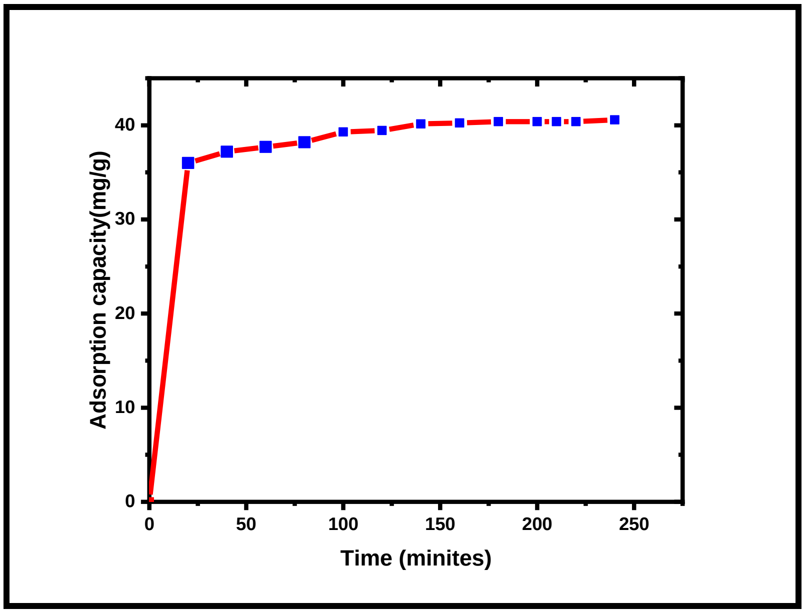


Fig.6.4.5 (c).Effect of contact time on adsorption capacity of dichloromethane (DCM) on Rice husk heated at 300°C (RRH)

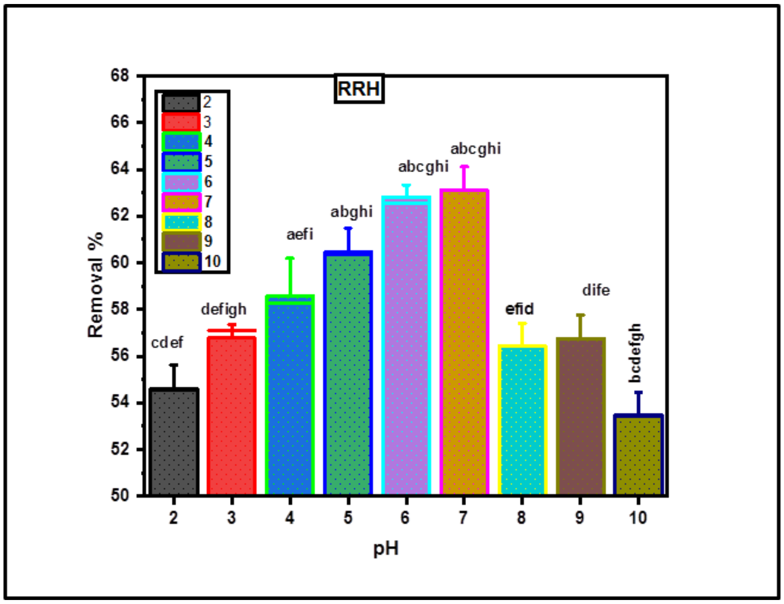


Fig. 6.4.6.Comparison of different range of pH on removal percentage of dichloromethane (DCM) on Raw Rice husk (RRH) (p<0.05)

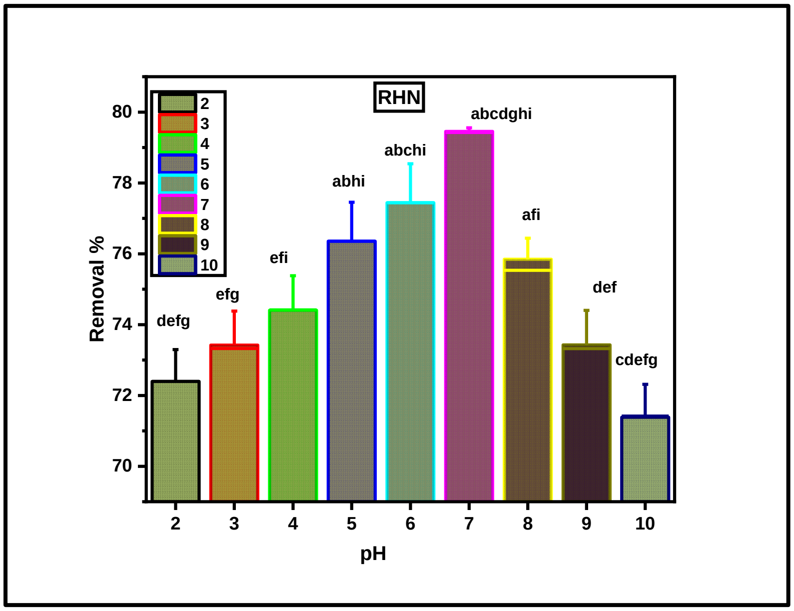


Fig.6.4.7. Comparison of different range of pH on removal percentage of dichloromethane (DCM) on RHN (p<0.05)

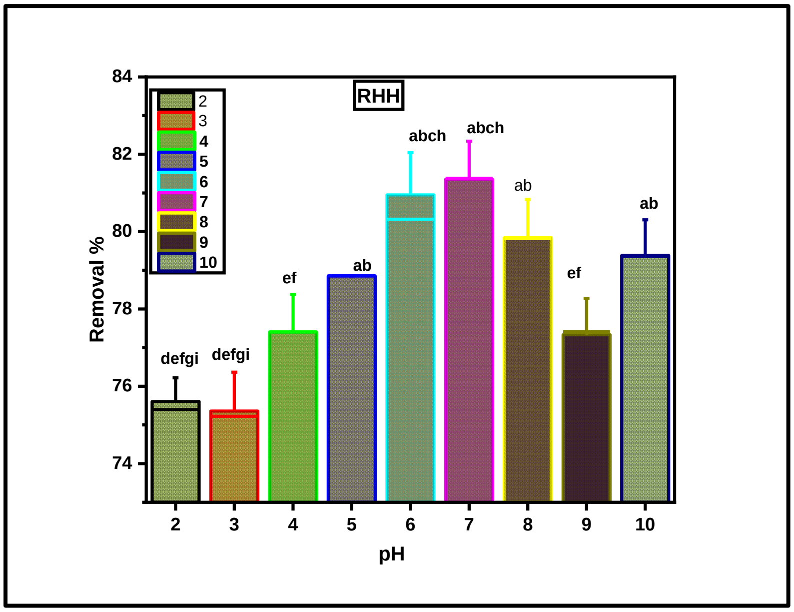


Fig. 6.4.8.Comparison of different range of pH on removal percentage of dichloromethane (DCM) on RHH ($p<0.05$)

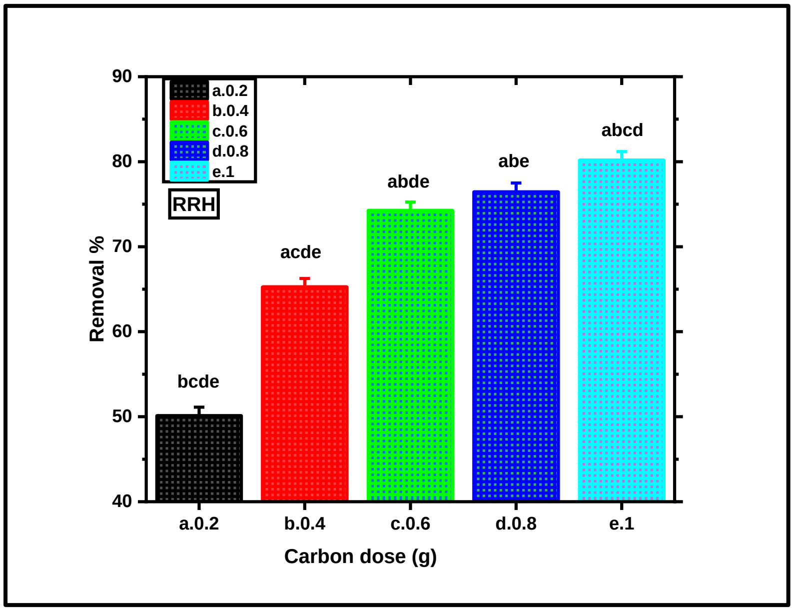


Fig.6.4.9. Comparison of different carbon doses on removal percentage of dichloromethane (DCM) on RRH ($p<0.05$)

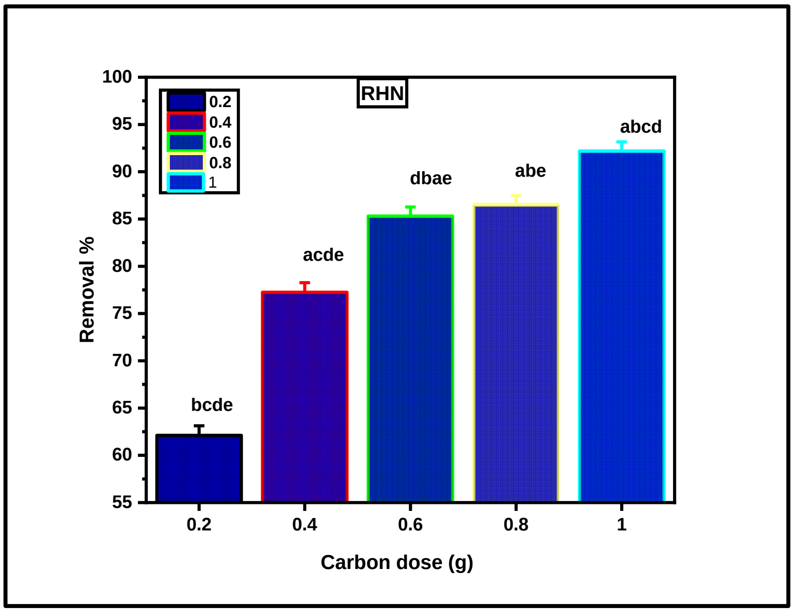


Fig. 6.4.10.Comparison of different carbon doses on removal percentage of dichloromethane on RHN (p<0.05)

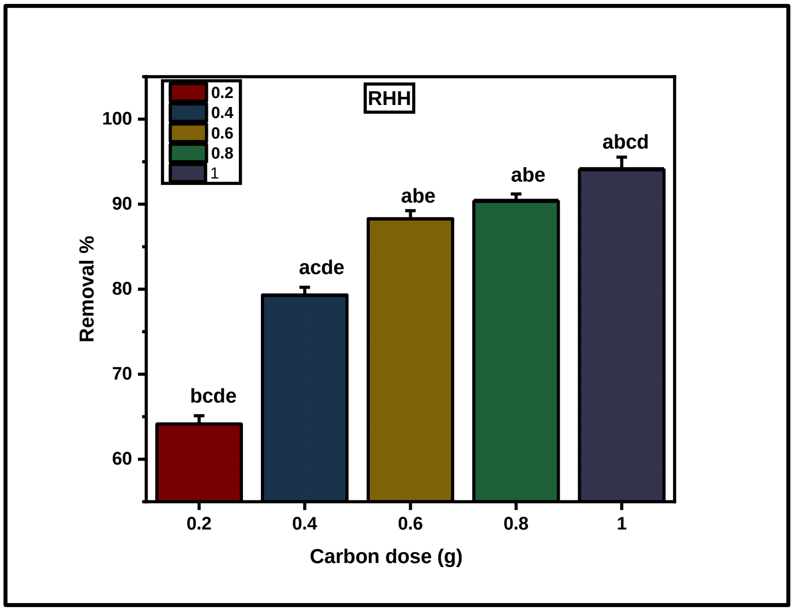


Fig.6.4. 11.Comparison of different carbon doses on removal percentage of dichloromethane on RHH (p<0.05)

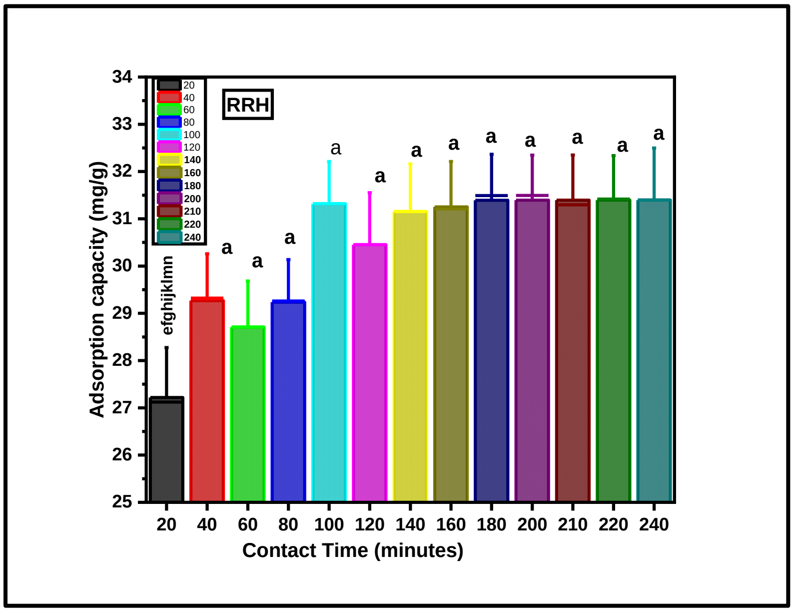


Fig.6.4. 1 2.Comparison of different contact time on removal percentage of dichloromethane on RRH (p<0.05)

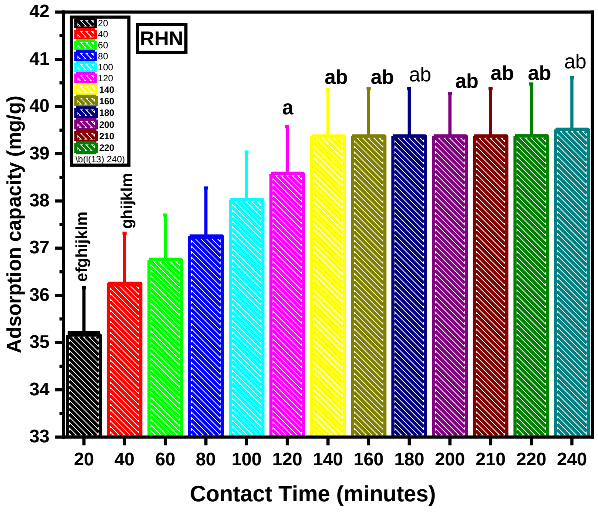


Fig. 6.4.13.Comparison of different contact time on removal percentage of dichloromethane RHN ($p<0.05$)

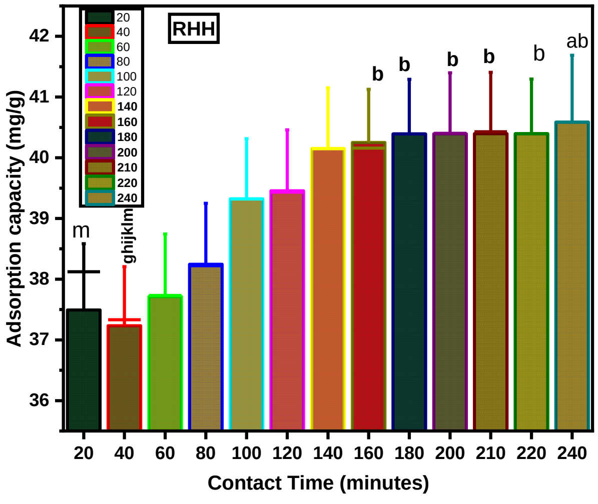


Fig. 6.4.14.Comparison of different contact time on removal percentage of dichloromethane on RHH ($p<0.05$)

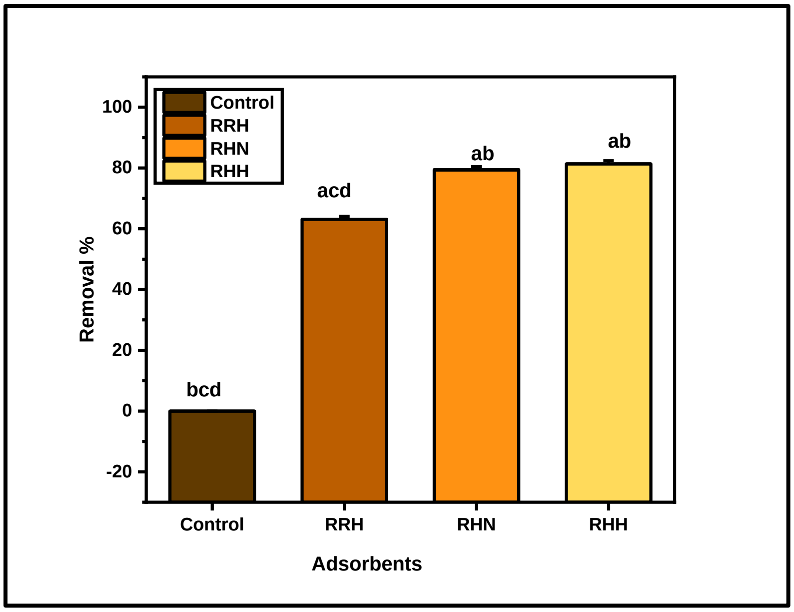


Fig. 6.4.15.Comparison of different range of pH on removal percentage of dichloromethane (DCM) on RRH, RHN, and RHH ($p<0.05$)

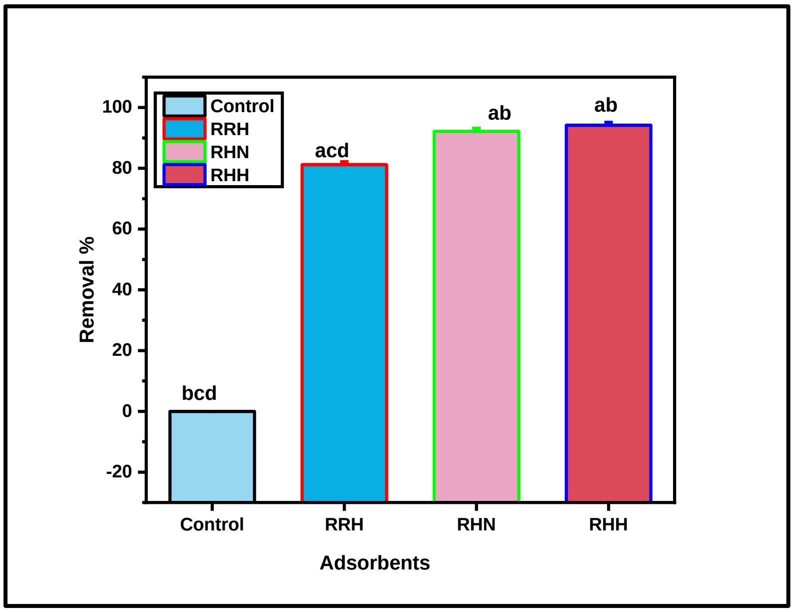


Fig. 6.4.16.Comparison of different carbon doses on removal percentage of dichloromethane (DCM) on RRH, RHN, and RHH) ($p<0.05$)

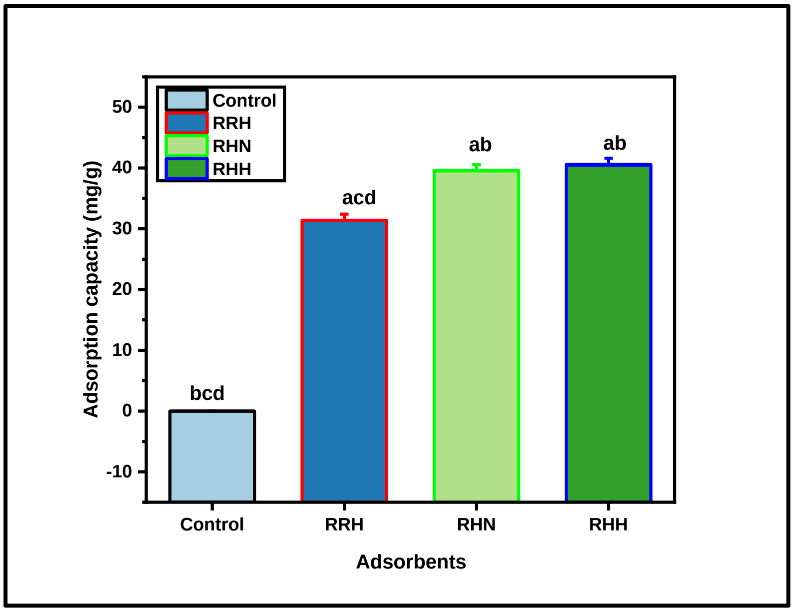


Fig. 6.4.17 .Comparison of different contact time on removal percentage of dichloromethane (DCM) on RRH, RHN, and RHH ($p<0.05$)

6.5. Bioremediation for the removal of dichloromethane

6. 5.1: Culture of *Pseudomonas aeruginosa*

6.5.1.1. Growth on cetrimide agar: Blue-green colored were colonies observed (fluorescence observed under UV) ***(Fig.6.5.1 and Fig.6.5.2).***

6.5.2. Identification tests of *Pseudomonas aeruginosa*

Pseudomonas is a rod-shaped, motile, non-capsulated, and non-spore forming bacterium. On testing for bacterial isolation, samples were found to be Gram-negative bacilli and considered positive for *Pseudomonas* sp. due to the appearance of bluish-green pigmentation on cetrimide agar.

Biochemical tests of these isolates marked them positive for oxidase and catalase tests ***(Table 6.5.3, Fig. 6.5.3, and Fig.6.5.4).*** Isolates were confirmed to be *Pseudomonas* *aeruginosa* showing a characteristic pyoverdin associated with blue-green fluorescence, as confirmed by the study done by Quinn *et al.* and Samanta [40, 265, 289].

The collected strain of bacteria was also identified by 16sRNA test.

The sequencing Report contains:

1. Individual Sequence Data in Different File format.a)*.ab1 – Sequence with Chromatogram (System File)b)*.Pdf – Sequence with Chromatogramc)*.txt – Sequence Data (Sequence in FASTA format). 2. Blast Result of Aligned sequence data.
*All reports in finding are attached in the end.

6.5.3. Enumeration

By using a hemocytometer, cell counts were done and about 1.7 X 10^9 CFU/ml cells were investigated [217, 219, 243, 327].

6.5.4. Bioremediation of dichloromethane by bacteria

6.5.4.1. Biodegradation Efficiency of *Pseudomonas aeruginosa*

In the present study, *Pseudomonas aeruginosa* was screened to check its potential to biodegrade the dichloromethane-a toxic volatile organic compound. The result obtained highlighted its biodegradation efficiency and its potential to biodegrade the dichloromethane. The *Pseudomonas aeruginosa* degraded 93.7% and 92.33 % by taking the initial concentration of 50 mg/L and 200 mg/L of dichloromethane. Further final concentration obtained were 3.11 mg/L and 15.33 mg/L with the initial concentration of 50 mg/L and 200 mg/L respectively [108] ***(Table 6.5.4, Table 6.5.5 , Fig. 6.5.5 Fig. 6.5.6)***

6.5.5 Mechanism for the adsorption of dichloromethane by bacteria

Pseudomonas aeruginosa has the efficiency to biodegrade dichloromethane. Biodegradation is a microbial-driven natural process and undergoes with the help of catalytic enzymes. The mechanism behind biodegradation is to convert the chemical or compound on which a particular microbe acts to a particular end product by passage through the number of intermediates between them. This process helps in the recycling of nutrients into the environment [108].

6.5.6. Comparison of the efficiency of other bacteria in biodegradation

The removal efficiency of 51.43%, 25.35%, and 8.08% was detected using Acidithiobacillus sp, Metallibacterium sp, and Thionomas sp. respectively [319]. The removal efficiency of 99.93% of methyl tert-butyl ether (MTBE) by using te enzymatic properties of mixture of two species of *Pseudomonas ,viz., aeruginosa and putida with Enterobacter oryzendophyticus* has been observed by Mahmoodsaleh, & Roayaei [214]. The consortium of *Pseudomonas aeruginosa* and *Pseudomonas citronellolis* degraded ethyl tertiary-butyl ether (ETBE) by about 92% [331]. The consortium of *Xanthomonas* sp, *Methylibium sp*, *Methylobacillus* sp,*Methylovorus* sp. degraded 42% of ethyl tertiary-butyl ether (ETBE) [331].

6.5.7. Statistical analysis

To analysis the performance of *Pseudomonas aeruginosa* to degrade DCM, statistical tool were applied. The experimental data are presented as mean ± SD of the experiment ***(Table 6.5.6, Table 6.5.8)***. The ANOVA result suggests that various groups Control, 50 mg/L, and 200 mg/L differ significantly ($F_{2,6}$= 295.103), $p<0.05$ for final concentration (***Table 6.5.7, Table 6.5.8 and Fig.6.5.7).***One-way ANOVA with post hoc Tukey's HSD test indicated that there is a significant difference in the mean value of final concentration of groups , namely, Control, 50 mg/L, and 200 mg/L DCM concentration (***Table 6.5.6, Table 6.5.7 and Fig.6.5.7).***

One way AONA for Removal percentage was also done. The ANOVA result for removal percentage revealed that the mean value of removal percentage of Control and 50 mg/L and Control and 200 mg/L differs significantly from each other ($F_{2,6}$=17783.252).$p<0.05$. The post hoc test revealed that there is significant difference in the mean value of removal percentage of Control and 50 mg/L, Control and 200 mg/L***(Table 6.58, Fig.6.5.8),***

6.5.8. Conclusion

The impact of VOCs on the environment is decided by the nature of VOCS, concentration of compound, and the source of emission of compound. The volatile organic compounds are a burning issue in the context of public health. Many VOCs have carcinogenic, teratogenic, and mutagenic properties. The present study demonstrates the biodegradative properties of *Pseudomonas aeruginosa* to biodegrade the toxic volatile organic compound-dichloromethane. The final concentration evaluated was 3.11 mg/L and 15.33 mg/L and the removal percentage was 93.78% and 92.33 % with initial concentrations of 50 mg/L and 200 mg/L of dichloromethane respectively. The potential for using these bio-materials to biodegrade the malodorous compounds released by the industrial processes into the environment is very promising. Since at the level of industries most of the contaminants and effluents of industries are released into the watercourses, therefore, there is a need for time for a transparent and strong bond between industries, researchers, and the scientific community. Further, there is a large scope of exploration of different microbial communities or living organisms along with the agricultural byproducts to use, for study in depth their adsorptive and biodegradative ability for removal of organic compounds that can act as carcinogen, teratogen, and harm the environment directly or indirectly.

Table 6.5.1: Scientific classification of *Pseudomonas aeruginosa*

Domain	**Bacteria (***Pseudomonas aeruginosa)*
Phylum	**Pseudomonadota**
Class	**Gammaproteobacteria**
Order	**Pseudomonadales**

Family	**Pseudomonadaceae**
Genus:	***Pseudomonas***
Species	***aeruginosa***

Table 6.5.2: Composition of cetrimide agar

ingredients	**gm/L**
Pancreatic Digest of Gelatin	**20 g**
Potassium Sulfate	**10 g**
Magnesium Chloride	**1.4 g**
Cetyltrimethylammonium Bromide	**0.3 g**
Glycerine	**10.0 ml**
Agar	**13.6 g**
Distilled Water	**1000 ml**

Table 6.5.3: Identification tests

Characteristics	***Pseudomonas aeruginosa***
Shape	**Rods**
Capsule	**Non-capsulated**
Motility	**Motile**

Spore	**non-sporing**
Gram-staining	**Negative**
Catalase	**Positive**
Oxidase	**Positive**

Table 6.5.4: Final concentration of DCM after biodegradation with bacteria *(Pseudomonas aeruginosa)*

Samples	**Final conc. (mg/L).**
DCM(50mg/L)+ culture	**3.11**
DCM (200 mg/L)+culture	**15.33**

Table 6.5.5: Removal percentage of DCM after bioremediation with bacteria (*Pseudomonas aeruginosa)*

Samples	**Removal %**
DCM(50mg/L)+ culture	**93.78**
DCM (200 mg/L)+culture	**92.335**

Table 6.5.6: Descriptive statistics (Final conc. of DCM after bioremediation by bacteria *(Pseudomonas aeruignosa*)

Test Samples	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper**

						Bound
Control	3	0	0	0	0	0
50 mg/L	3	3.11	1.00	0.57	0.63	5.60
200 mg/L	3	15.3	0.99	0.57	12.82	17.77
Total	9	6.13	7.037	2.34	0.72	11.54

Table 6.5.7: Test of Homogeneity of Variances and ANOVA (Final conc. of DCM after bioremediation by bacteria *(Pseudomonas aeruignosa)*

Test Samples	**N**	**Mean**	**Std. Deviation**	**Test of Homogeneity of Variances**		**ANOVA**	
				Levene Statistic		**F**	**Sig.**
Control	3	0	0	2.24	0.18	295.10	**<0.05**
50 mg/L	3	3.11	1.00				
200 mg/L	3	15.3	0.99				
Total	9	6.13	7.03				

Table 6.5.8: Descriptive statistics (Removal percentage of DCM after bioremediation by bacteria (*Pseudomonas aeruignosa)*

Test Samples	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
Control	3	0	0	0	0	0
50 mg/L	3	93.78	0.67	0.38	92.12	95.45
200 mg/L	3	92.32	1.00	0.58	89.82	94.82

Total	9	62.03	46.53	15.51	26.26	97.80

Table 6.5.9: Test of Homogeneity of Variances and ANOVA (Removal percentage of DCM after bioremediation by bacteria (*Pseudomonas aeruignosa)*

Test Samples	N	Mean	S.D	Test of Homogeneity of Variances		ANOVA	
				Levene Statistic	Sig.	F	Sig.
Control	3	0.00	0.00	4.76	0.05	17783.25	**<0.05**
50 mg/L	3	93.78	0.670				
200 mg/L	3	92.32	1.00				
Total	9	62.03	46.53				

Fig. 6.5.1.Growth on cetrimide agar under UV along with negative control (A-C)

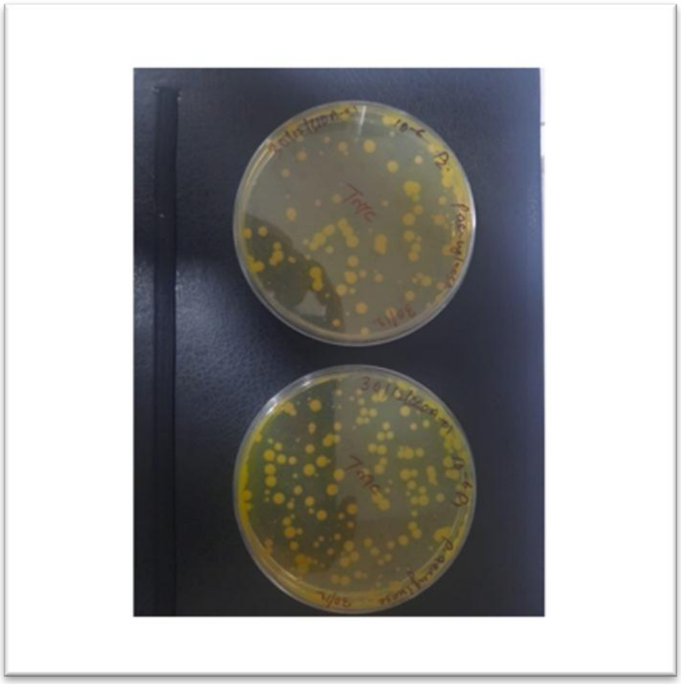


Fig.6.5.2. Enumeration plates

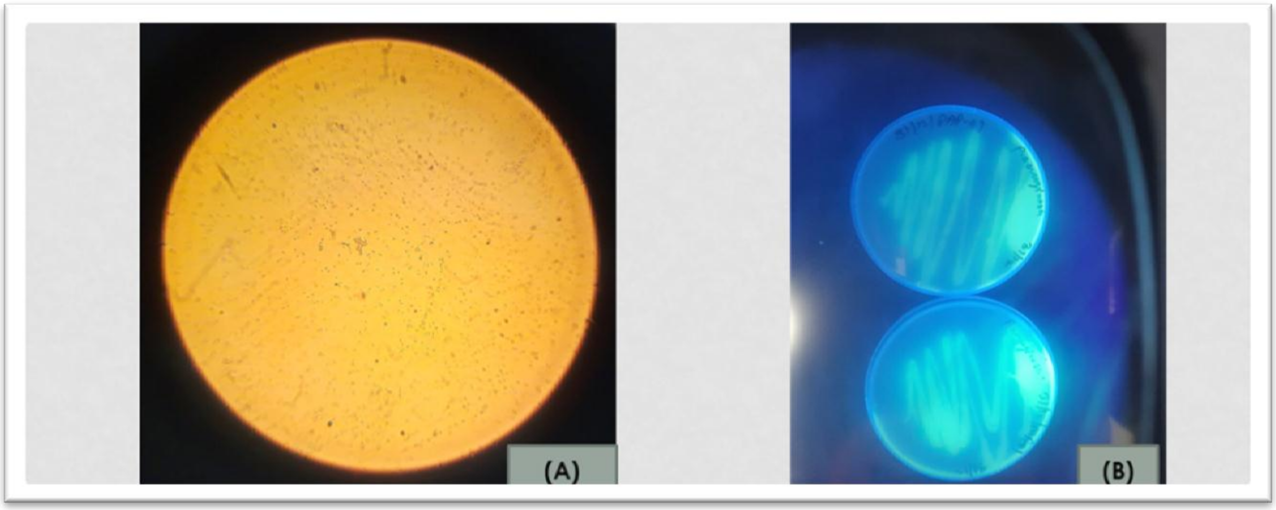


Fig.6.5.3. Identification Test: (A) Gram staining slide under microscope (B) Pigmentation test and growth on PAP Media plates

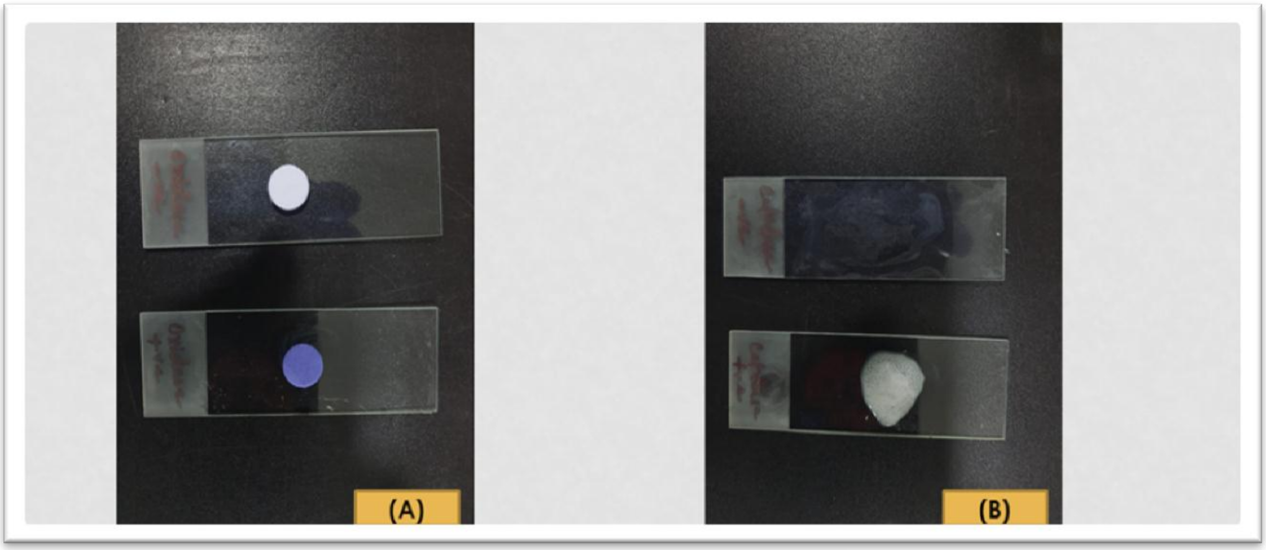


Fig.6.5.4. Identification Test: (A) Oxidase Test (B) Catalase Test

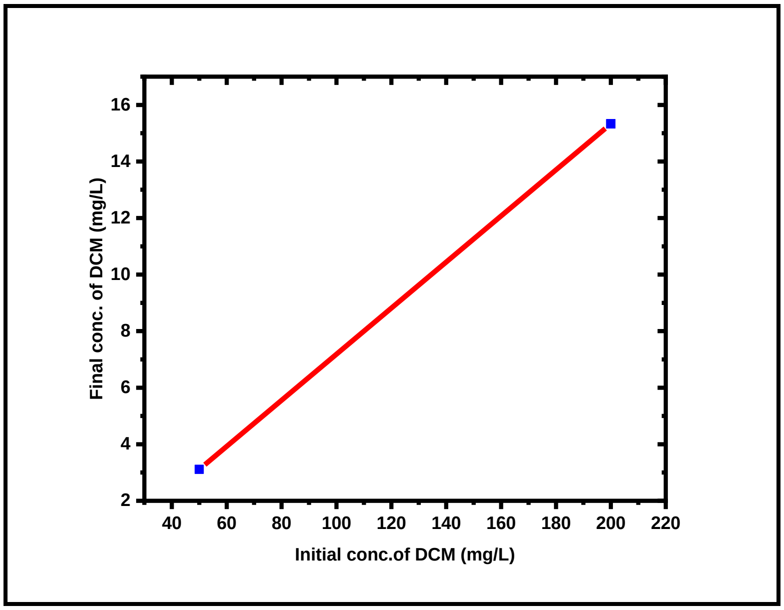


Fig.6.5.5. Final concentration of DCM after bioremediation by bacteria *(Pseudomonas aeruignosa)*

***(Conc. of Bacteria-8.2x10^9CFU/mL)**

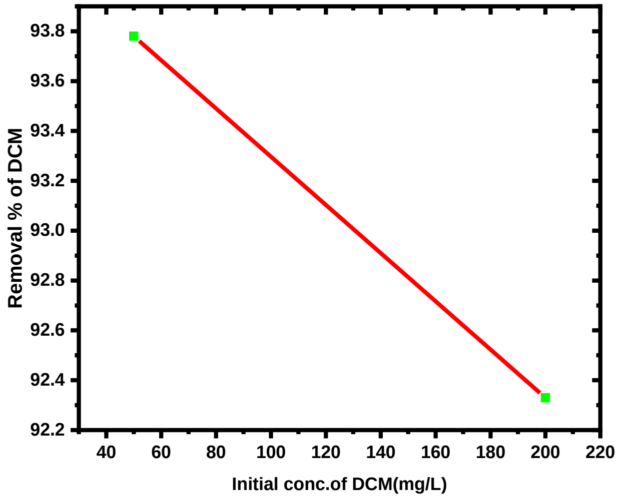


Fig.6.5.6. Removal percentage of DCM after bioremediation by bacteria *(Pseudomonas aeruignosa)* (Conc. of Bacteria-8.2x10^{9} CFU/mL)

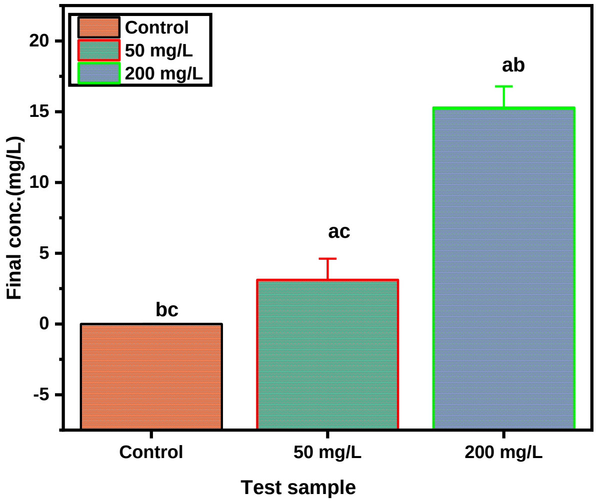


Fig.6.5.7. Comparison of final concentration of DCM after bioremediation by bacteria *(Pseudomonas aeruginosa)* on different initial conc. of DCM (p<0.05)

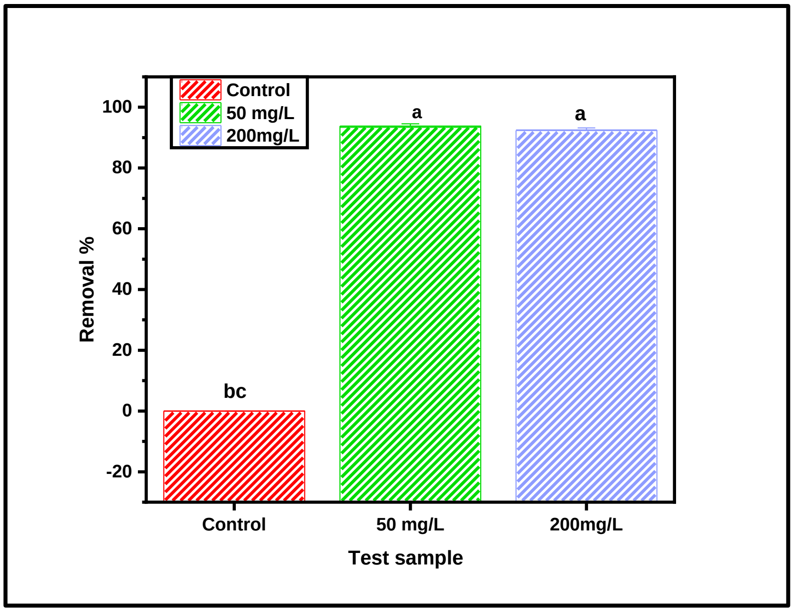


Fig.6.5.8. Comparison of removal percentage of DCM after bioremediation by bacteria (*Pseudomonas aeruginosa)* on different initial conc. of DCM ($p<0.05$)

6.6. Comparison of conventional method (Adsorption) and bioremediation method for removal of dichloromethane

6.6.1. Comparison of the final concentration of the conventional method and bioremediation method

The result of the final concentration of dichloromethane of four adsorbents-Coconut shells activated carbon (CSAC) Raw rice husk (RRH), Rice husk modified with 0.5 M NaOH (RHN), Rice husk heated at 300° C (RHH) (conventional method) and bacteria (bioremediation) were 69.3 mg/L, 74 mg/L, 41.2 mg/L, 37.3 mg/L, 15.33 mg/L respectively ***(Table 6.61 and Fig.6.6.1)***

6.6.2. Comparison of Removal percentage of conventional method and bioremediation method

The removal percentage of four adsorbents - Coconut shell activated carbon (CSAC), Raw rice husk (RRH), Rice husk modified with 0.5 M NaOH (RHN), Rice husk heated at 300° C (RHH), and Bacteria were 65.35 %, 63%,78.4%, 81.35% and 92.3% respectively **(*Table 6.6.2 and Fig.6.6.2).***

6.6.3. Statistical analysis

In order to do the comparative studies of adsorption of DCM by various adsorbents prepared by coconut shell and rice husk and degradation by bacteria (Pseudomonas aeuginosa, statistical tool were applied. Final conc. and Removal % of DCM was analyzed by using all four adsorbents-CSAC, RRH, RHN, RHH and bacteria (*Pseudomonas aeruginosa).*

Analysis of variance (ANOVA) one-way test was also conducted for final concentration and Removal % of DCM to find out the statistical value and significance of population means at 0.05 level *(**Table 6.6.4, and Table 6.6.6**)*. Descriptive statistics have been illustrated with the help of tables *(**Table 6.6.3, Table 6.6.5**)*. Th**e** Anova result suggests that groups of CSAC, RRH, RHN, RHH, and bacteria (*Pseudomonas aeruginosa)* differ significantly from each other for final conc. (F 4, 10= 1894.555) and removal % (F 4, 10=785.604) $p<0.05$.

One-way ANOVA with post hoc Tukey's HSD test showed a significant difference in the mean value of final conc. (Table 6.6.3 and 6.6.4; Fig 6.6.1 and 6.6.3)) and removal percentage***(Table 6.6.5 and 6.6.6 ; Fig 6.6.2 and 6.6.4)*** of DCM by all four adsorbents-CSAC, RRH, RHN, RHH and bacteria (*Pseudomonas aeruginosa)* **(*Table 6.6.3, 6.6.5, Fig 6.6.1 to Fig.6.6.4).***

6.6.4. Conclusion

Industrial effluents discharge many organic compounds into the environment. Many organic compounds act as carcinogens, mutagens, and teratogens. Dichloromethane can impair the genetic makeup of living organisms by causing mutation and interfering in the physiological function of the nervous system and is responsible for cancer of many vital organs. The comparative study of efficiency of removal of dichloromethane of all prepared adsorbents was done in the present work. The result of the final concentration of dichloromethane for four adsorbents- Coconut shell activated carbon (CSAC) Raw rice husk (RRH), Rice husk modified with 0.5 M NaOH (RHN), Rice husk heated at 300° C (RHH) (conventional method) and bacteria (bioremediation) was 69.3 mg/L, 74 mg/L, 41.2 mg/L, 37.3 mg/L, 15.33 mg/L respectively. The removal percentage of four adsorbents - Coconut shell activated carbon (CSAC), Raw rice husk (RRH), Rice husk modified with 0.5 M NaOH (RHN), Rice husk heated at 300° C (RHH), and bacteria were 65.35 %, 63%,78.4%, 81.35%, and 92.3% respectively. The present study gives a new vision to the use of indigenous or exotic microbes in the removal of malodorous compounds present in the industrial effluents. The toxic organic compounds become a threat to living beings and lead to the extinction of species. To add up support in this direction, the effluents should be treated properly before releasing in the environment.

Table 6.6.1: Comparison of final concentration (mg/L) of various adsorbents and bacteria (*Pseudomonas aeruginosa)*

S.NO	**Adsorbents/Bacteria**	**Final concentration (mg/L)**
1	Coconut shell-activated carbon	69.39
2	Raw rice husk	74.03
3	Rice husk modified with 0.5 M NaOH	41.29
4	Rice husk heated at 300° C	37.34
5	Bacteria (*Pseudomonas aeruginosa)*	15.33

Table 6.6.2: Comparison of Removal percentage of various adsorbents and bacteria (*Pseudomonas aeruginosa)*

S.NO	**Adsorbents**	**Removal %**
1	Coconut shell	65.35

2	Raw Rice husk	63.00
3	Rice husk modified with 0.5 M NaOH	78.48
4	Rice husk heated at 300° C	81.35
5	Bacteria (*Pseudomonas aeruginosa)*	92.38

Table 6.6.3: Descriptive statistics (Final concentration (mg/L) of various adsorbents and bacteria (*Pseudomonas aeruginosa*)

Adsorbents	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	Upper Bound
CSAC	3	69.39	1.10	0.63	66.64	72.13
RRH	3	74.03	0.64	0.37	72.42	75.64
RHN	3	41.29	1.12	0.64	38.50	44.08
RHH	3	37.34	0.90	0.52	35.08	39.59
Bacteria	3	15.33	0.97	0.56	12.92	17.74
Total	15	47.47	22.50	5.80	35.01	59.93

Table 6.6.4: Test of homogeneity of variance and ANOVA (Final concentration (mg/L) of various adsorbents and bacteria (*Pseudomonas aeruginosa*)

				Test of homogeneity of variance		**ANOVA**	
Adsorbents	**N**	**Mean**	**Std. Deviation**	**Levene's Statistic**	**Sig.**	**F**	**Sig.**

CSAC	3	69.39	1.10	0.169	0.94	1894.55	<**0.05**
RRH	3	74.03	0.64				
RHN	3	41.29	1.12				
RHH	3	37.34	0.90				
Bacteria	3	15.33	0.97				
Total	15	47.47	22.50				

Table 6.6.5: Descriptive statistics (Removal percentage (%) of various adsorbents and bacteria (*Pseudomonas aeruginosa*)

Adsorbents	**N**	**Mean**	**S.D**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
CSAC	3	65.35	0.68	0.39	63.64	67.06
RRH	3	63.00	0.61	0.35	61.47	64.53
RHN	3	78.48	0.82	0.47	76.44	80.52
RHH	3	81.03	0.72	0.41	79.22	82.83
Bacteria	3	92.38	0.75	0.43	89.50	93.26
Total	15	75.85	10.87	2.80	69.82	81.87

Table 6.6.6: Test of homogeneity of variance and ANOVA (Removal percentage (%) of various adsorbents and bacteria (*Pseudomonas aeruginosa*)

				Test of homogeneity of variance		**ANOVA**	
Adsorbents	**N**	**Mean**	**Std. Deviation**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
CSAC	3	65.35	0.68	0.04	0.99	785.60	<**0.05**
RRH	3	63.00	0.61				

RHN	3	78.48	0.82
RHH	3	81.03	0.72
Bacteria	3	91.38	0.75
Total	15	75.85	10.87

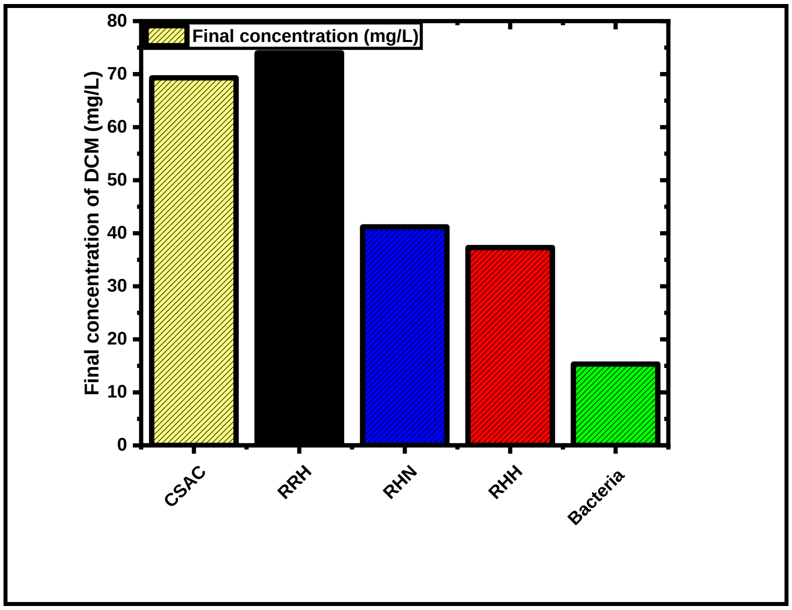


Fig.6.6.1. Final concentration of dichloromethane after adsorption/biodegradation by CSAC, RRH, RHN, RHH and bacteria (*Pseudomonas aeruignosa)*

CSAC-Coconut shell activated carbon; RRH-Raw rice Husk; RHN- Rice husk modified with 0.5 M NaOH; RHH-Rice husk heated at 300 °C; bacteria (*Pseudomonas aeruignosa)

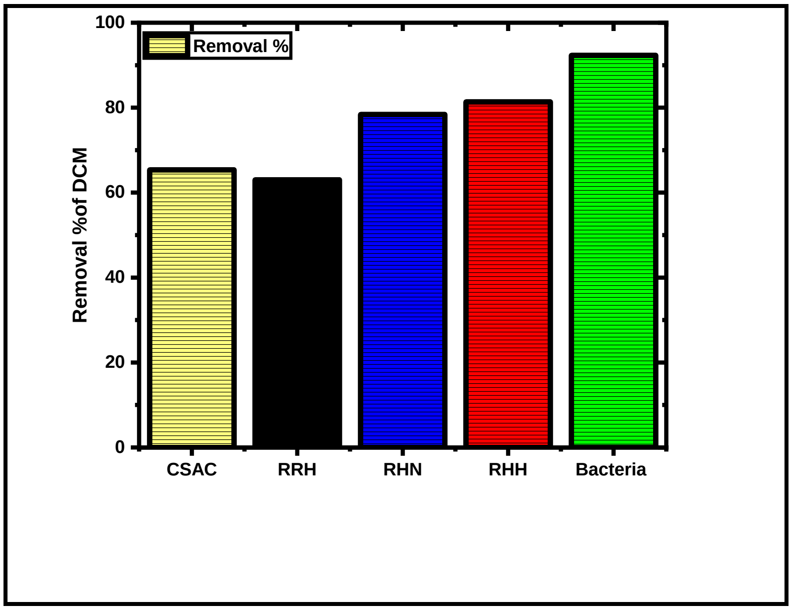


Fig.6.6.2. Removal percentage of dichloromethane after adsorption/biodegradation by CSAC, RRH, RHN, RHH and bacteria (*Pseudomonas aeruignosa)*

CSAC-Coconut shell activated carbon; RRH-Raw rice Husk; RHN- Rice husk modified with 0.5 M NaOH; RHH-Rice husk heated at 300 °C; bacteria (*Pseudomonas aeruignosa)

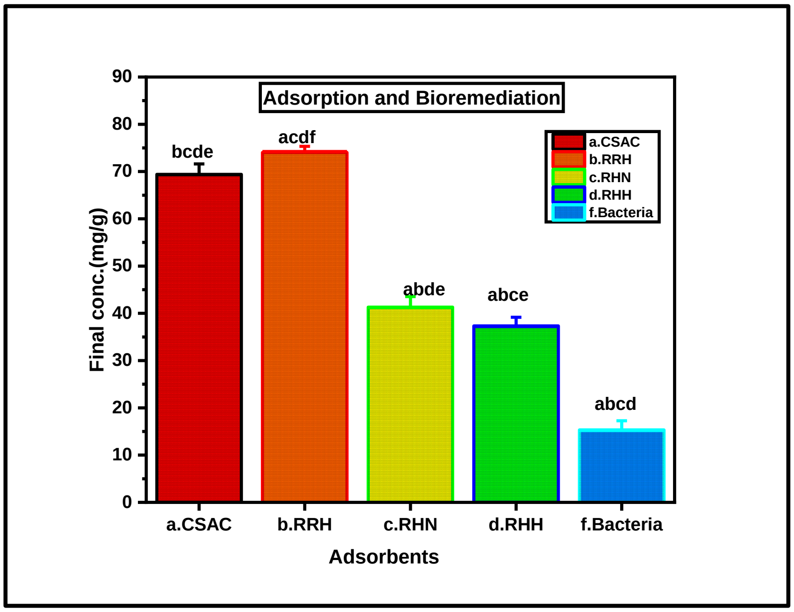


Fig.6.6.3. Comparison of final conc. of DCM by adsorption and bioremediation (p<0.05)

CSAC-Coconut shell activated carbon; RRH-Raw rice Husk; RHN- Rice husk modified with 0.5 M NaOH; RHH-Rice husk heated at 300 °C; bacteria (*Pseudomonas aeruignosa)

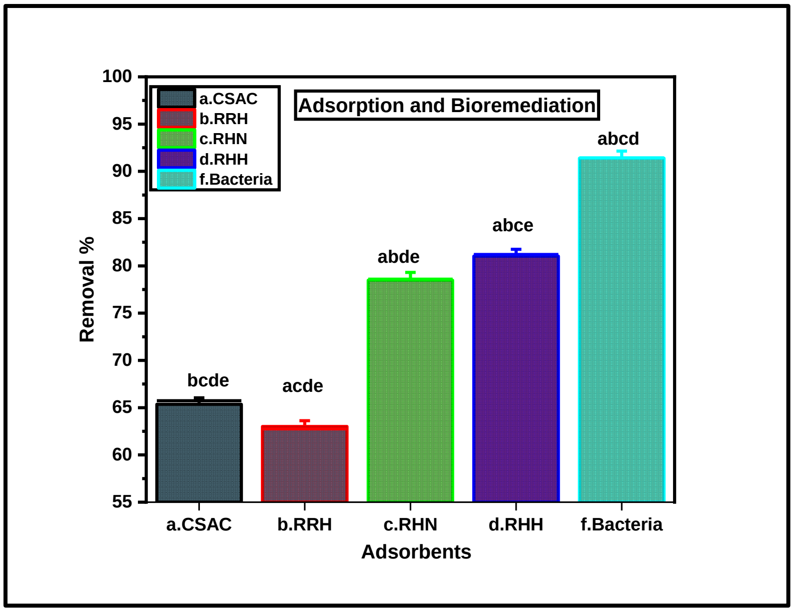


Fig.6.6.4. Comparison of removal percentage by adsorption and bioremediation (p<0.05)

CSAC-Coconut shell activated carbon; RRH-Raw rice Husk; RHN- Rice husk modified with 0.5 M NaOH; RHH-Rice husk heated at 300 °C; bacteria (*Pseudomonas aeruignosa)

Objective 3: *Designing innovative hybrid materials for remediating the responsible compounds for the unpleasant odor of Kala Sanghian odor*.

6.7. Preparation of hybrid material for removal of VOCs (dichloromethane)

6.7.1. Characterization of coconut shell activated carbon

Already it is done under heading 6.3 and objective 2.

6.7.2. Characterization of adsorbent prepared from Rice husk

Already it is done under heading 6.4 and under objective 2.

6.7. 3. Characterization of MOF

FTIR: FTIR graph of the prepared samples is illustrated with the help of ***Fig. 6.7.1***. The FTIR graph of MOF shows O–C–O moiety and Zr–O–C moiety at (1537 and 1366 cm -1) and (748 and 658cm $^{-1}$) respectively [63, 126, 155, 195, 348, 369].

XRD: The XRD graph is presented in ***Fig. 6.7.2*.** The peaks specify the particular MOF.

6.**7.4. Culture and Identification of *Pseudomonas aeruginosa***

Already it is done in heading 6.5 under objective 2

6.7.5. Efficiency of different hybrid materials in the biodegradation of dichloromethane (DCM)

6.7.5.1. Efficiency of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa acts as a capable microbial degrader for volatile organic compounds such as DCM. When *Pseudomonas aeruginosa* with numbers of cells *8.2x10^{9} CFU/ml* was used to biodegrade the 50 mg/L and 200 mg/L concentrations, out of which about 93 % of DCM was degraded**.** It shows the efficiency of *Pseudomonas aeruginosa* to biodegrade the DCM. The process demonstrates the transformation of a substrate into a product through microbial biochemical reactions. In this case, the pollutant acts as a substrate for energy generation for the specific microbe [108] **(*Table 6.7.1, Table 6.7.2, Fig.6 .7.3, Fig.6.7.4 (a) and Fig. 6.7.4 (b).***

6.7.5.2. Efficiency of *Pseudomonas aeruginosa* with rice husk

Rice husk is agricultural byproduct that contains cellulose and silica in its composition *[144, 222, 238, 241]*. The silica content present is highly porous in structure and is lightweight, with a specific surface area **[259]**. Rice husk enhance the biodegradation along with adsorption of DCM when the *Pseudomonas aeruginosa* was used in combination with a fixed known amount of raw rice husk. The utilization of both adsorbent and microbial culture led to increases in removal percentage from 94 % to 96 % ***(Table 6.7.1, Table 6.7.2, Fig.6 .7. 3, Fig.6.7.4 (a) and Fig. 6.7.4 (b).***

6.7.5.3. Efficiency of *Pseudomonas aeruginosa* with coconut shell activated carbon

It is well known that the porosity of adsorbent is the key to the adsorption of contaminants. Due to its large surface area, it is used for the elimination of contaminants from wastewater [201]. Activated carbon prepared from the coconut shell speeds up the adsorption of DCM to 99%. This show, coconut shell activated carbon with *Pseudomonas aeruginosa* act as a good adsorbent to biodegrade or adsorb organic compounds such as DCM *(**Table 6.7.1, Table 6.7.2, Fig.6 .7.3, Fig.6.7.4 (a) and Fig. 6.7.4 (b).***

6.7.5.4. Efficiency of *Pseudomonas aeruginosa* with MOF (UiO66)

MOFs have special diversity in structure as compared to other adsorbents. These frameworks have the attributes of tunable porosity, uniformity in pore structures, flexibility in geometry, and chemical functionality. These features give them a unique position in the ocean of porous materials [6, 42]. The removal efficiency *of Pseudomonas aeruginosa* along with MOF (UiO66) completely mirrors the combination of *Pseudomonas aeruginosa* along with rice husk. It showed a removal efficiency of 96% ***(Table 6.7.1, Table 6.7.2, Fig.6 .7.3, Fig.6.7.4 (a) and Fig. 6.7.4 (b).***

6.7.5.5. Efficiency of *Pseudomonas aeruginosa* with Rice husk, activated carbon, and MOF (UiO66) When *Pseudomonas aeruginosa is* combined with Rice husk, coconut shell activated carbon and MOF (UiO66); it speeds up the biodegradation ability of *the Pseudomonas aeruginosa* by 98% ***(Table 6. 7.1, Table 6.7.2, Fig.6 .7. 3, Fig.6.7.4 (a) and Fig. 6.7.4 (b)***

6.7.6. Statistical analysis

To do the comparative studies of adsorption/degradation of DCM by various hybrid materials prepared by coconut shell, rice husk, metallic organic framework, and bacteria (*Pseudomonas aeuginosa),* statistic tools were applied. The removal % of DCM was analyzed by taking two concentrations of DCM-50 mg/ L and 200 mg/L and using all hybrid materials.

Descriptive statistics has been illustrated with the help of tables ***(Table 6.7.3,and Table 6.7.5)*** Anova result suggests that mean value of removal % of Control, Pse, Pse+-RRH, Pse +CSAC, Pse+ MOF, Pse +RRH+CSAC and Pse +RRH+CSAC+MOF differ significantly for 200mg/L of initial conc. of DCM (F 6, 14= 6502.47) . There has been observed, the mean value of removal % of Control, Pse, and Pse +RRH+CSAC+MOF also differ significantly for 50mg/L of initial conc. of DCM (F 2, 6= 18948.723).$p<0.05$

One-way ANOVA with post hoc Tukey's HSD test showed that there is significant difference occurs among removal percentage of DCM by various hybrid materials ***(Table 6.7.4, Table 6.7.6 Fig 6.7.5, Fig 6.7.6, Fig 6.7.7 and Fig 6.7.8).*** In the case of 200 mg/L of DCM concentration, it has been observed that there exists a significant difference between the mean value of removal % of Control and Pse; Control and Pse+ RRH; Control and Pse +CSAC; Control and Pse+MOF; Control and Pse+ RRH+CSAC, and between Control and Pse +RRH+CSAC+MOF. It is also been observed that there exists a significant difference in the mean value of removal percentage of Pse and Pse+RRH; Pse-Pse +CSAC; Pse-Pse+MOF; Pse-Pse+RRH+CSAC, and among Pse and Pse+RRH+CSAC+MOF. The mean value of removal percentage of Pse+ RRH and Pse+CSAC; Pse+CSAC and Pse+MOF; and between Pse+CSAC and Pse+RRH+CSAC is also significantly different (***Table 6.7.4 and Fig 6.7.5).*** In the case of 50 mg/L of DCM concentration, there exists a significant difference in the mean value of removal percentage of Control and Pse; Control and Pse+RRH+CSAC+MOF; and Pse and Pse+RRH+ CSAC+MOF ***(Table 6.7.6 and Fig 6.7.7).***

6.7.7. Conclusion

An increase in interference in the environment has led environmental protection agencies to be strictly vigilant towards the standards set by them. Toxicity, volatility, persistence, and solubility are the properties of volatile organic compounds that can be used to manipulate the purity of the ecological environment. The impact of VOCs on the living organisms and the environment is determines by the nature of VOCs, their concentration, and their source of emission. These volatile compounds because of their toxicity, have gained tremendous attention worldwide in the field of water science. Volatile organic compounds in water are a burning issue in the context of public health. Some of these compounds, even in small quantities, contain a high number of organic substances and can prove lethal for living beings. Many VOCs have carcinogenic, teratogenic, and mutagenic properties. Adsorbents of agricultural origin have the capability of adsorption of VOCs. Rice husk shall be treated as an efficient material for the treatment of organic contaminants in industrial effluents. Rice husk helps *Pseudomonas aeruginosa* to speed up its capability to biodegrade the organic compound. The present study demonstrates this statement. Carbon adsorption is gaining more appraisals in this zone due to its positive attributes of the high surface area, easy availability, hydrophobicity, pore volume distribution, extensive pore size distribution, removal effectiveness at less concentration, reutilizing ability, low energy consumption and probability of product repossession The present study enhances, the adsorptive properties of *Pseudomonas aeruginosa* to biodegrade the organic compounds. Metal-organic frameworks have structural uniformity and flexibility in dimension and geometry. These characteristics make them a porous material with a high surface area. The present study proved the ability of UiO66 to speed up the capability of *Pseudomonas aeruginosa* to biodegrade organic compounds. As all the biomaterials used in the present study have adsorptive or biodegradation ability, therefore, it enhances the final reduction of organic compounds used in the present study**.** There is a bright prospect of using these bio-materials in biodegradation and the adsorption of malodorous compounds entering the environment. There is a need to make a transparent and strong bond between industries, researchers, and the scientific community to curb the manipulation of watercourses by the addition of effluents of industries. Before discharge of wastewater into the environment, a serious effort should be made to treat it. Further, there is a large scope of exploration of different microbial communities or living organisms along with the agricultural byproducts to use, for study in depth their adsorptive and biodegradative ability for removal of organic compounds that can act as carcinogen, teratogen, and harm the environment directly or indirectly.

Table 6.7.1: The final concentration of DCM after biodegradation with different hybrid materials

Samples	**Final conc. (mg/L).**
DCM(50mg/L)+ Culture (*Pseudomonas aeruginosa)*	3.11
DCM (200 mg/L)+Culture (*Pseudomonas aeruginosa)*	15.33
DCM (200 mg/L)+ Culture(*Pseudomonas aeruginosa)*+ Rice husk	7
DCM (200 mg/L)+ Culture (*Pseudomonas aeruginosa)*+ Coconut shell activated carbon	1
DCM (200 mg/L)+ Culture(*Pseudomonas aeruginosa)*+ MOF	7
DCM (200 mg/L)+ Culture (*Pseudomonas aeruginosa)* + Rice husk+ Coconut shell activated carbon	6
DCM (200 mg/L)+ Culture (*Pseudomonas aeruginosa)* + Rice husk+ Coconut shell activated carbon+ MOF	4
DCM(50 mg/L)+ Culture (*Pseudomonas aeruginosa)*+ +Rice husk+ Coconut shell activated carbon+ MOF	1

***Culture used-8.2x10^9 CFU/ml;*MOF-Metal organic framework used-UiO66**

Table 6.7.2: Removal percentage of dichloromethane (DCM) after biodegradation with different hybrid materials

Samples	Removal %
DCM(50mg/L)+ Culture (*Pseudomonas aeruginosa)*	93.78
DCM (200 mg/L)+Culture(*Pseudomonas aeruginosa)*	92.32
DCM (200 mg/L)+ Culture (*Pseudomonas aeruginosa)* + Rice husk	96.5
DCM(200 mg/L)+ Culture (*Pseudomonas aeruginosa)*+ Coconut shell activated carbon	99.5
DCM (200 mg/L)+ Culture (*Pseudomonas aeruginosa)*+ MOF	96.5
DCM (200 mg/L)+ Culture ((*Pseudomonas aeruginosa)*+ +Rice husk+ Coconut shell activated carbon	97.06
DCM (200 mg/L)+Culture(*Pseudomonas aeruginosa)*+ +Rice husk+ Coconut shell activated carbon+ MOF	98.09
DCM(50 mg/L)+ Culture (*Pseudomonas aeruginosa)* +Rice husk+ Coconut shell activated carbon + MOF	98.08

***Culture used-8.2x10^9 CFU/ml;*MOF-Metal organic framework used-UiO66**

Table 6.7.3: Descriptive statistics (Removal percentage of dichloromethane (DCM) after biodegradation with different hybrid materials at 200 mg/L of DCM)

Adsorbents	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
Control	3	0	0	0	0
Pse	3	92.32	1.00	0.58	89.82

Pse+ RRH	3	96.5	0.93	0.54	94.17
Pse+ CSAC	3	99.5	0.47	0.27	98.31
Pse +MOF	3	96.5	0.92	0.53	94.20
Pse +RRH+CSAC	3	97.06	0.63	0.36	95.48
Pse+ RRH+ CSAC+ MOF	3	98.09	0.97	0.56	95.66
Total	21	82.85	34.72	7.57	67.04

***Pse-*Pseudomonas aeruginosa*; CSAC-Coconut Shell activated carbon; RRH-Raw Rice husk; MOF-Metallic organic frame work MOF used-UiO66**

Table 6.7.4: Test of homogeneity of variance and ANOVA (Removal percentage of Dichloromethane (DCM) after biodegradation with different hybrid materials at 200 mg/L of DCM)

			Test of homogeneity of variance		**ANOVA**	
Adsorbents	**Mean**	**S.D**	**Levene Statistic**	**Sig.**	**F**	**Sig.**
			1.13	0.39	6502.47	**<0.05**
Control	0	0				
Pse	92.32	1.00				
Pse+-RRH	96.5	0.93				
Pse+ CSAC	99.5	0.47				
Pse+ MOF	96.5	0.92				
Pse+ RRH+CSAC	97.06	0.63				
Pse+ RRH+ CSAC+ MOF	98.09	0.97504				

***Pse-*Pseudomonas aeruginosa;* CSAC-Coconut Shell activated carbon; RRH-Raw Rice husk; MOF-Metallic organic frame work used- UiO66**

Table 6.7.5: Descriptive statistics (Removal percentage of dichloromethane (DCM) after biodegradation with different hybrid materials at 50 mg/L of DCM

Adsorbents	N	Mean	S.D	Std. Error	95% Confidence Interval for Mean
					Lower Bound
Control	3	0	0	0	0
PSE	3	93.78	0.67	0.38	92.12
Pse+ RRH+ CSAC+ MOF	3	98.08	1.00	0.58	95.58
Total	9	63.95	48.00	16.00	27.05

***Pse-*Pseudomonas aeruginosa*; CSAC-Coconut Shell activated carbon; RRH-Raw Rice husk;MOF-Metallic organic frame work used- UiO66**

Table 6.7.6: Test of homogeneity of variance and ANOVA ((Removal percentage of dichloromethane (DCM) after biodegradation with different hybrid materials at 50 mg/L of DCM)

			Test of homogeneity of variance		ANOVA	
Adsorbents	Mean	S.D	Levene 's Statistic	Sig.	F	Sig.
			3.034	0.123	18948.723	<0.05
Control	0.00	0.00				
Pse	93.7867	0.67002				
Pse + RRH+CSAC+MOF	98.0867	1.0052				

***Pse-*Pseudomonas aeruginosa*; CSAC-Coconut Shell activated carbon; RRH-Raw Rice husk; MOF-Metallic organic frame work used- UiO66**

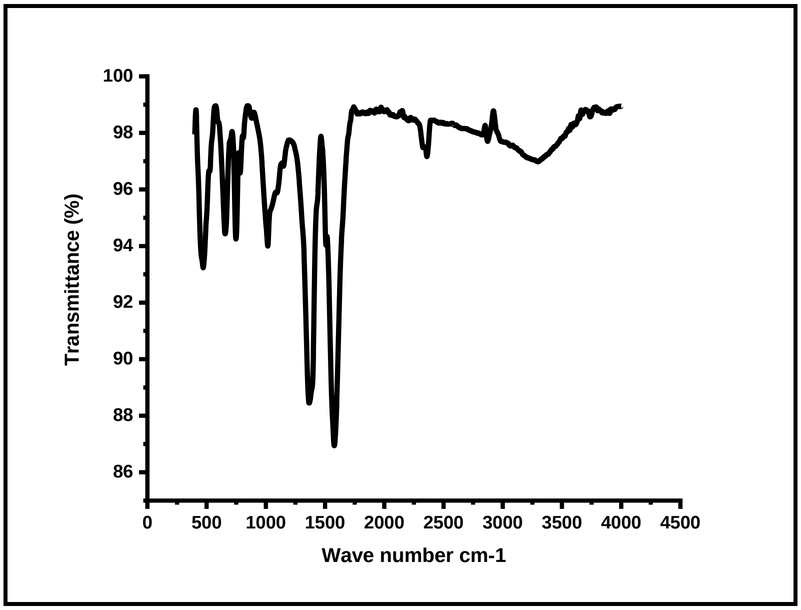


Fig.6.7.1. FTIR Spectra of MOF (UiO66)

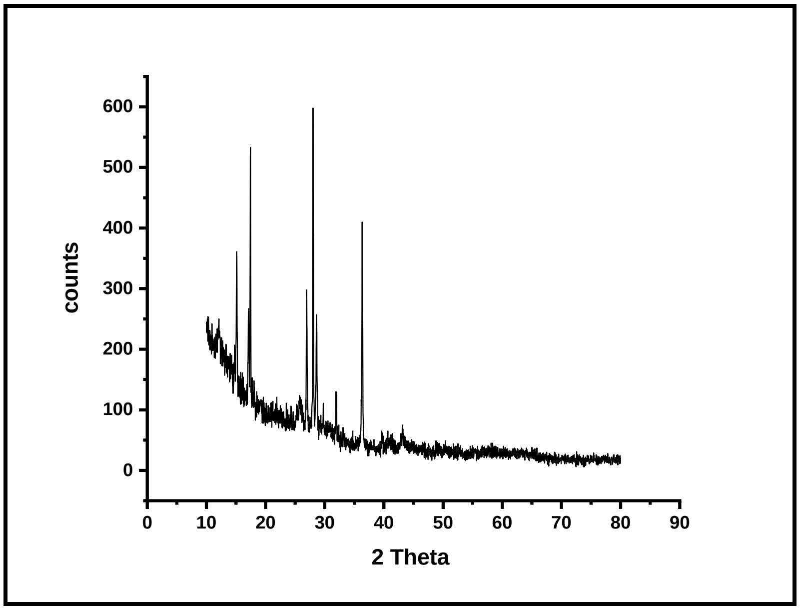


Fig.6 7.2.XRD of MOF (UiO66)

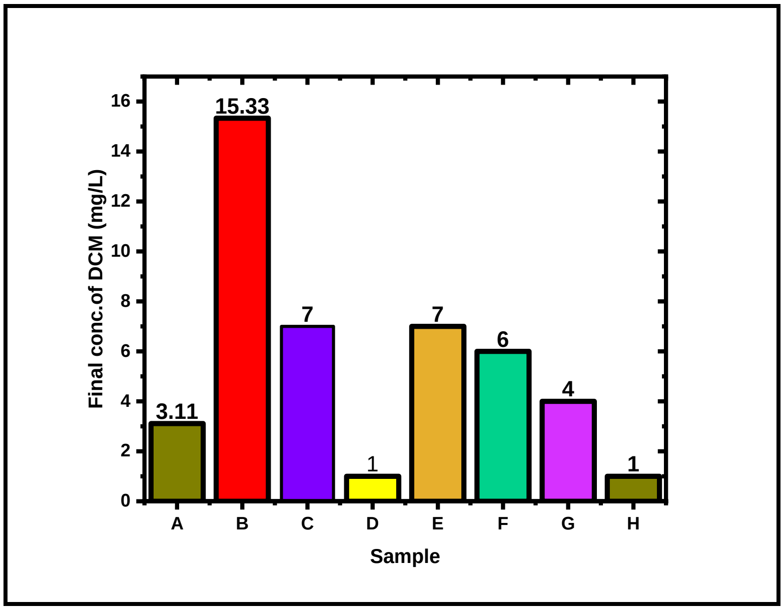


Fig.6.7.3. Final concentration of DCM after biodegradation with different hybrid materials

Sample Combination

A DCM (50mg/L) + Culture (*Pseudomonas aeruginosa)*

B DCM (200 mg/L) +Culture (*Pseudomonas aeruginosa)*

C DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk

D DCM (200 mg/L) + Culture (Pseudomonas aeruginosa) +Coconut shell activated carbon

E DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + MOF

F DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk+ Coconut shell activated carbon

G DCM (200 mg/L) +Culture *(Pseudomonas aeruginosa)* + Rice husk+ Coconut shell activated carbon+ MOF

H DCM (50 mg/L) + Culture *(Pseudomonas aeruginosa*) + Rice husk+ Coconut shell activated carbon+ MOF

***Culture used- *Pseudomonas aeroginosa*-8.2x10^{9} CFU/ml;* MOF-Metal organic framework used-UiO66**

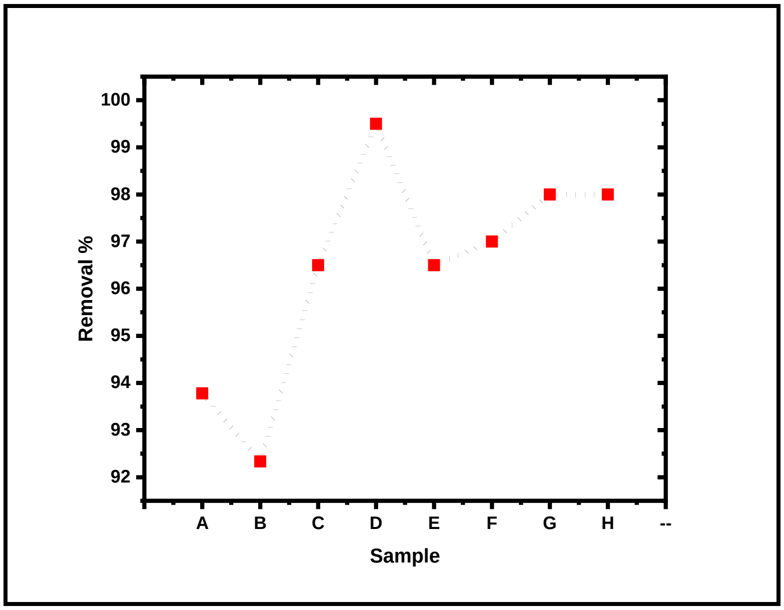


Fig 6.7.4 (a) Removal percentage of DCM after biodegradation with different hybrid materials

Sample	**Combination**
A	**DCM (50mg/L) + Culture *(Pseudomonas aeruginosa)***
B	**DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)***
C	**DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk**
D	**DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)*+ Coconut shell activated carbon**
E	**DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* +MOF**

F DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk+ Coconut shell activated carbon

G DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* +Rice husk + Coconut shell activated carbon+ MOF

H DCM (50 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk + Coconut shell activated carbon+ MOF

***Culture used-*Pseudomonas aeruginosa*-8.2x10^9 CFU/ml;*MOF-Metal organic frame work usd-UiO66**

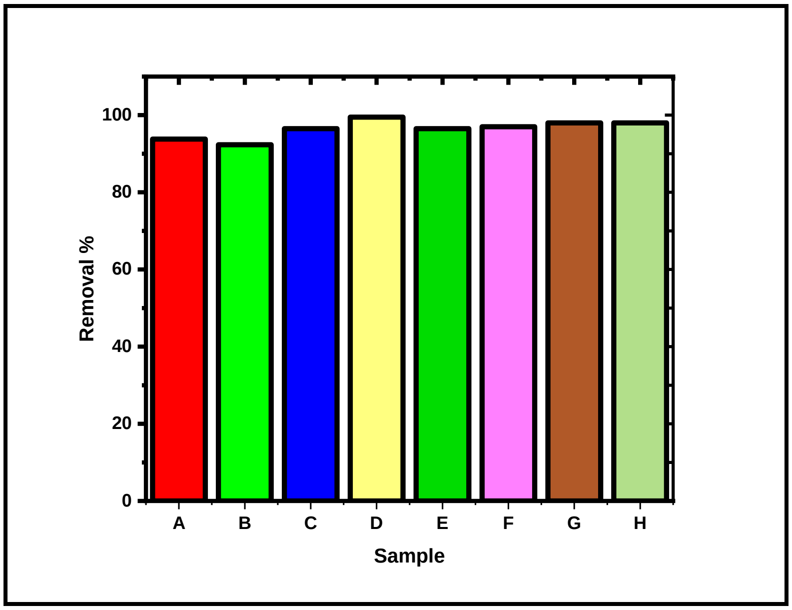


Fig 6.7.4 (b) Removal percentage of DCM after biodegradation with different hybrid materials

Sample Combination

A DCM (50mg/L) + Culture *(Pseudomonas aeruginosa)*

B DCM (200 mg/L) +Culture *(Pseudomonas aeruginosa)*

C DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)*+ Rice husk

D DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* + Coconut shell activated carbon

E DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)* +MOF

F DCM (200 mg/L) + Culture *(Pseudomonas aeruginosa)*+ Rice husk+ Coconut shell activated carbon

G DCM (200 mg/L) +Culture *(Pseudomonas aeruginosa)* +Rice husk + Coconut shell activated carbon+ MOF

H DCM (50 mg/L) + Culture *(Pseudomonas aeruginosa)* + Rice husk + Coconut shell activated carbon+ MOF

***Culture used- *Pseudomonas aeroginosa*-8.2x10^9 CFU/ml;*MOF-Metal organic framework used-UiO66**

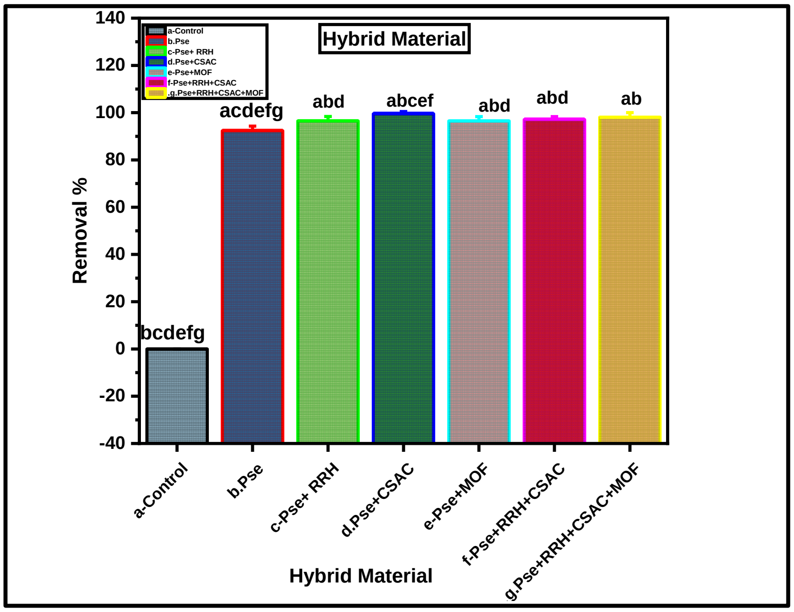


Fig. 6.7.5. Comparison of removal percentage by various hybrid materials at 200 mg/L of concent of DCM

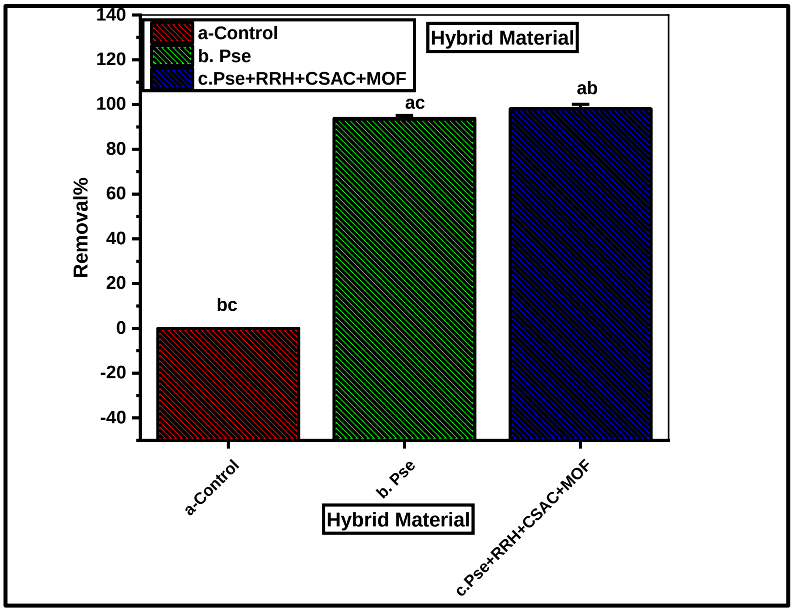


Fig. 6.7.6 Comparison of removal percentage by various hybrid materials at 50 mg/L of concentration of DCM

CHAPTER 7-SUMMARY AND CONCLUSIONS

The fast industrial and urbanization is deteriorating the environment in such a pace that the protection of the environment has become a global issue. The unenthusiastic nature of people towards a sustainable environment leads to a massive load of pollutants in the aquatic and terrestrial communities. The present study was carried out at the Kala Sanghian Drain that receives wastewater from many industries such as leather, textile, and electroplating units established over there. The raw materials used in various industries, namely, hides and skin, dyes and heavy metals in tannery, textiles and electroplating industries is seamlessly altering the water quality index of water bodies. The chemicals discharged by industries interferes the normal physicochemical properties of water bodies that cross the permissible limit set by World Health Organization. This wastewater contains spiked values of various contaminants parameters consents that the contaminated water from these aqueous bodies is unsuitable for any kind of anthropogenic application.

The permissible pH value should be lies between 6.5–8.5, according to WHO and IS 10500:1991 guidelines. The pH value of the samples of the current study from the selected sites of the drain was measured and was found in the range between 5.71-6.84 which were above the standards set by WHO and IS 10500:1991. Analysis of effluents from different sites showed variation in the values of pH. TDS was observed to be in the range of 2426.66mg/ L and 2690.33 mg/ L and it is considered an important parameter in the physicochemical parametric world. The value of BOD in the wastewater was not going as per guidelines of WHO and IS 10500:1991 with a maximum of 269.66 mg/L and a minimum of 190 mg/L. The temperature of the water samples of the current study deviates from the guidelines, as set by WHO and IS 10500:1991. It is highest at 45°C and lowest at 41.88 °C. The samples of water taken from the selected locations have the value of electrical conductivity above the prescribed levels with 4650.33µS/cm and 2616.6 µS/cm as the higher and lower values respectively. The values for dissolved oxygen recorded in the wastewater samples were a maximum of 4.17 mg/L and a minimum of 1.7 mg/L.

Besides this, experiments were also conducted to find out the volatile organic compounds emitted from this drain. GC-QqQ-MS/MS analytical method was used, to identify volatile organic compounds. The GC-QqQ-MS/MS analytical method has shown decent chromatographic resolution in the present study. This method has given satisfactory results on the spectrum of compounds with different polarity, and volatility as well as having different retention times. The volatile organic compounds detected in wastewater from the drain are hazardous to the environment.

The in-depth studies of literature clearly mark the toxic nature of the volatile organic compounds identified in the wastewater of the Kala Sanghian Drain. Some of these compounds have shown carcinogenicity and mutagenicity and therefore malodorous and lethal for the aquatic and terrestrial community. Volatile organic compounds detected from the wastewater are also responsible for the deteriorated taste and odor of the drain.

Eleven volatile organic compounds were detected in the drain. These were dichloromethane, cis-1, 2-Dichloroethene, heptane, 1, 3, 5-Trimethylbenzene, 2-Chlorotoluene, bromobenzene, cumene, ethyl benzene and p-Isopropyltoluene, 1, 1, 1, Trichloroethane and tetrachloromethane. Among these volatile organic compounds, dichloromethane was selected further for remediation. We have chosen dichloromethane for further remediation purposes because it impairs the genetic makeup of living organisms by causing mutation and interferes with the physiological function of the nervous system and is responsible for cancer of many vital organs. The methods adopted for remediation were adsorption and biosorption. The adsorption method is appreciable worldwide. The positive attributes such as physical resistance, adsorption properties, surface chemistry, reasonable cost, and recovery of the activating agent, makes the carbon prepared of the plant origin, the material of choice in the adsorbent world. In adsorption method employs two adsorbents namely coconut shells and rice husks which are agricultural byproducts. The adsorbent from the coconut shell was prepared by applying standard methods. Under various optimal conditions, the adsorption process was studied. The present study has shown that the adsorption process of dichloromethane on CSAC was influenced by temperature, pH, DCM concentration, the adsorbent dosage, and contact time. The adsorption was observed maximum of 50.5 mg/g for CSAC at a pH range of 6.0–8.0. The increasing of contact time and temperature increases the adsorption. The data obtained was also tried to fit with Languimer and Freundlich models. We analyzed that the Langmuir model didn't fit well on CSAC for the adsorption of dichloromethane. Rather it went well with the Freundlich model. The kinetic models such as Pseudo first order and Pseudo second order were also taken into account for the analysis of experimental data. After analysis, it is clear that kinetic data has shown better applicability of the pseudo-second-order. The R_L value between 0 and 1 for the adsorption of dichloromethane, predicts the favorable adsorption. The adsorption of dichloromethane on CSAC was considered spontaneous. In thermodynamic studies, standard entropy changes with positive values showed an increase in entropy, therefore it confirmed the randomness of the system.

The second adsorbent used for the removal of dichloromethane was rice husks. Three types of adsorbents namely RRH, RHN, and RHH were prepared by applying chemical and thermal treatment. The main constituents present in rice husks that play a significant role in adsorption are carbon and silica. High temperature doesn't favor the abundance of carbon, and it depletes by speeding up the temperature. On the other hand, on increasing the temperature, silica content increases. High thermal treatment increases the porosity and surface area of the adsorbent. It has been observed that as compared to untreated and chemically treated raw rice husk, thermally treated adsorbent have high porosity and large specific surface area.

The adsorption capacities of adsorbents studied for dichloromethane (DCM) were found to be high in the Rice husk heated at 300° C (RHH), followed by Rice husk modified with.0.5 M NaOH (RHN) and then Raw Rice husk (RRH). After a thermal and chemical treatment, the adsorption capacity of adsorbents increases. The adsorbents follow pseudo second-order kinetics. pH as a decisive role in the adsorption capacity of the adsorbents. Rice husk heated at 300°C (RHH) has shown more efficiency among the adsorbents under study. The adsorption capacity of untreated raw rice husk, rice husk heated at

300°C, and rice husk treated with 0.5 M NaOH for DCM was observed to be 45.25 mg/g 52.25, mg/g, and 53.85 mg/g respectively.

Besides the adsorption method, the bioremediation method was also used in the removal of dichloromethane. In bioremediation, microbial remediation was adopted in the present study. The utilization of microorganisms for the biodegradation of VOCs is in practice for many years. The microbes, namely, *Micrococcus albus, Gordonia amicalis, Enterobacter oryzendophyticus, Mycobacterium austroafricanum, Arthrobacter sp, Acinetobacter calcoaceticus, Nocardioides sp, Pseudoxanthomonas sp, Sphingomonas sp, Actinomyces globisporus, Cephalosporium sp, Micromonospora albus, Hyphomicrobium sp, Mycobacterium vaccae, Xanthobacter autotrophicus, Nocardia sp.* act as potent bio degraders for the removal of chemicals discharged from industries. The bacterium used in the present study was *Pseudomonas aeruginosa* after a literature survey of its capability to degrade organic compounds. After proper experimentation and analysis, the present study demonstrates the biodegradative properties of *Pseudomonas aeruginosa* to biodegrade the toxic volatile organic compound dichloromethane. The final concentration of dichloromethane after being treated with *Pseudomonas aeruginosa* evaluated was 3.11 mg/L and 15.33 mg/L and removal percentage was 93.78% and 92.33 % with initial concentrations of 50 mg/L and 200 mg/L of dichloromethane respectively.

After the completion of the adsorption and bioremediation method, the comparison of efficiencies of four adsorbents and bacteria for the removal of dichloromethane was also studied. The result of the final concentration of dichloromethane of four adsorbents- Coconut shells activated carbon (CSAC) Raw rice husk (RRH), Rice husk modified with 0.5 M NaOH (RHN), Rice husk heated at 300° C (RHH) (adsorption-conventional method) and Bacteria (bioremediation) was 69.3 mg/L, 74 mg/L, 41.2 mg/L, 37.3 mg/L, 15.33 mg/L respectively. The removal percentage of four adsorbents - coconut shell activated carbon (CSAC), raw rice husk (RRH), rice husk modified with 0.5 M NaOH (RHN), rice husk heated at 300° C (RHH), and bacteria were 65.35 %, 63%,78.4%, 81.35%, and 92.3% respectively.

We also applied an innovative approach to prepare a hybrid material and check its efficiency to degrade dichloromethane. As we already experimented and checked the efficiency of rice husk, coconut shell, and *Pseudomonas aeruginosa*, we prepared a hybrid material by combining the efficiency of all these three along with MOF (UiO66) and checked the efficiency of this hybrid material in the degradation of dichloromethane. It has been seen that *Pseudomonas aeruginosa* acts as a capable microbial degrader for volatile organic compounds such as DCM. When *Pseudomonas aeruginosa* with numbers of cells $8.2x10^9$ CFU/ml was used to biodegrade the 50 mg/L and 200 mg/L concentrations, out of which about 93 % of DCM was degraded**.** It shows the efficiency of *Pseudomonas aeruginosa* to biodegrade the DCM. The process demonstrates the transformation of a substrate into a product through microbial biochemical reactions. In this case, the pollutant acts as a substrate for energy generation for the specific microbe. Rice husk is an agricultural byproduct with cellulose and silica in its composition. The silica has the characteristics of porosity, light in weight with a larger surface area. Rice husk enhances the biodegradation efficacy along with adsorption of DCM when the *Pseudomonas aeruginosa* was used in combination with raw rice husk. Adsorbents prepared from the shell of coconut and Pseudomonas *aeruginosa* speeds up the adsorption of DCM up to 99%. It shows that the coconut shell and *Pseudomonas aeruginosa* acts as an appreciable hybrid material to biodegrade or adsorb organic compounds such as DCM. MOF is gaining momentum in the world of adsorbents due to its porosity and stability. The removal percentage *of Pseudomonas aeruginosa* with MOF (UiO66) is similar to the removal percentage of *Pseudomonas aeruginosa* with rice husk. Both have shown a removal percentage of 96%. The combination of *Pseudomonas aeruginosa*, rice husk, coconut shell activated carbon, and UiO66, speeds up the biodegradation ability by 98%.

In the present study, we hypothesized that the physicochemical parameters of the wastewater of the Kala Sanghian Drain will cross the limit set by WHO and the outcome of the study reveals the same as was assumed earlier. The values of physicochemical parameters mark the fact that industrial effluents are malodorous for both aquatic and terrestrial life and unguardedly confirm the toxicity of water bodies under current research work.

We assumed also that wastewater from the Kala Sanghian Drain will emit volatile organic compounds that cross the limit set by the WHO and these volatile organic compounds will be toxic to living organisms and the environment. Eleven compounds were identified in the wastewater of the Kala Sanghian drain namely, dichloromethane, cis-1,2-Dichloroethene, heptane, 1, 3, 5- Trimethylbenzene, 2-Chlorotoluene, bromobenzene, cumene, ethyl benzene and p-Isopropyltoluene, 1,1,1, Trichloroethane and tetrachloromethane. Among these eleven compounds dichloromethane, cis-1,2-Dichloroethene, 1,3,5- Trimethylbenzene, 2-Chlorotoluene, cumene, and tetrachloromethane acts as a carcinogen, teratogen, and mutagen.

The volatile organic compounds identified in the sites of the current study, although not crossed the limit set by WHO, as they were found in less concentration. But its duration of exposure has been set by environmental protection agencies such as ATSDR and EPA. Exposure to volatile organic compounds even in less concentration for a long time causes many severe physiological disorders and even acts as carcinogen, teratogen, and mutagen. Results from experimental studies of the effects of dichloromethane in humans indicate that acute neurobehavioral deficits may occur at less concentration with 4-8 hours of exposure. Even at less concentration, these chemicals affect psychomotor tasks, hand-eye coordination, visual response, and auditory vigilance. As the people are living in the vicinity and they are inhaling the toxic volatile organic compounds day and night continuously, in repercussion, they can suffer from skin, respiratory and neurological disorders as VOCs are very toxic even in small concentrations. In addition to this, these chemicals make the surrounding environment with bad smells.

The literature studies have clearly depicted the unfavorable effect of volatile organic compounds on the physiology of living organisms and environment. From the literature study, we analyzed that identified compounds are very toxic for living organisms and the environment. Acute exposure to heptane causes unconsciousness, feasible peripheral neuropathy, and neuroticism in the human nervous system. It has been reported to cause DNA damage in bacterial cells. Dichloromethane impairs visual, auditory, and motor functions. It is enlisted by the health organization as a human carcinogen. Due to its bioaccumulation potential, it is very toxic to the environment. cis-1, 2- Dichloroethene is toxic for human beings and responsible for the dysfunction of the central nervous system and respiratory system. This chlorinated hydrocarbon is considered a human carcinogen and harms the environment due to its non-degradable nature. A high concentration of cis-1, 2-Dichloroethene affects human females during pregnancy and causes birth defects. Trimethylbenzene has been reported in human cord blood and causes a reduction in fetal body weight. It is a known carcinogen and toxic for aquatic organisms too. 2-Chlorotoluene causes eye, skin, and respiratory problems in human beings. It acts as a teratogen and adds excessive toxicity to the aquatic ecosystem. Bromobenzene is reported as an environmental toxin that causes liver necrosis and massive hemorrhage in mice and acts as a hepatotoxic and neurotoxic agent. Cumene is reported as a mutagenic agent in some bacterial strains. Ethylbenzene is also a toxic compound. Its short-term exposure causes fatigue, headache, and drowsiness. While long-term exposure causes defects in the kidneys, liver, and nervous system. It is also responsible for pulmonary and cardiovascular disease. p-Isopropyltoluene can exert a genotoxic effect in drosophila, it also causes ozone depletion. 1, 1, 1-Trichloroethane impairs the physiology of the liver, nervous and circulatory systems. Tetrachloromethane on acute exposure manipulates the physiological functions in human beings and boosts the chances of liver cancer. This volatile organic compound damages the kidneys of animals too. Its exposure for a short duration leads to irritation of the eyes and throat and impairs the physiology of the nervous system in humans. It also acts as an ozone-depleting agent.

By elaborating on the work, one more hypothesis was assumed that adsorption and biodegradation will be very effective in the removal of VOCs. Dichloromethane has been chosen from the list of identified volatile organic compounds in the wastewater of the Kala Sanghian drain and removal of Dichloromethane was carried out by using four adsorbents prepared from the coconut shell and rice husks. This study reveals that satisfying the removal efficiency of dichloromethane can be achieved by using coconut shells and rice husks as adsorbents. The present study demonstrates the biodegradative properties of *Pseudomonas aeruginosa* to biodegrade the toxic volatile organic compound dichloromethane.

It has been hypothesized further that hybrid material with bacteria will show more efficiency in the adsorption and biodegradation of dichloromethane as compared to what they will do individually. The hybrid material prepared by using agricultural byproducts and MOF (UiO66) enhances the degradative ability of *Pseudomonas aeruginosa* as compared to their capability to degrade dichloromethane. Among various adsorbents, coconut shell-activated carbon helps *Pseudomonas aeruginosa* more in the process of biodegradation as compared to other adsorbents. Raw rice husk with *Pseudomonas aeruginosa* has almost given the same result in removal percentage which was achieved in the case of MOF with *Pseudomonas aeruginosa.* *Pueudomonas aeruginosa* is not alone sufficient enough to degrade dichloromethane as compared to when used with other adsorbents. Raw rice husk when used with *Pseudomonas aeruginosa* has shown less removal percentage for dichloromethane as compared to when used with coconut shell activated carbon. *Pseudomonas aeruginosa* with rice husk has shown almost the same removal percentage as *Pseudomonas aeruginosa* with rice husk, coconut shell activated carbon, and MOF has shown. The physicochemical values that crosses the limit of WHO and identification of volatile organic compounds that are responsible for carcinogenicity, teratogenicity, and mutagenicity in the wastewater of the Kala Sanghian Drain, demands seriously to bring forth the importance of treating the effluent properly before discharging them into the water bodies. It must be emphatically endorsed to make environmental protection rules and regulations sincerely and further implement them strictly with regular monitoring of various quality parameters. Quality regulation must be enforced and proper vigilance in industries should be set up with proper regulation of the effluent treatment plants. Honesty towards this direction assuredly armors the quality of water for successors. Industries should have a separate drainage system that pours its effluents into the non-residential areas away from humans and animals to safeguard them from toxicological impacts. To prevent salt load, eco-friendly methods should be utilized in chemical processes in tannery industries. It has been suggested that the textile effluents should be checked for toxicity potential microbiologically before discharging them in water bodies. As electroplating industries are responsible for the addition of acids in the water, suitable metallurgical options should be used for the recovery of these acids from their spent wash. The repeatedly and systematically monitoring and evaluation of the water quality index of industrial effluents is very essential before discharging them to make sure toxins-free water bodies.

More specifically, public awareness of the cause and sources of water contamination must be created so as to avoid further escalation of solid as well as liquid wastes in natural water bodies. To prevent the negative impact on water bodies and provide future generation a safe and secure life, the essential tools that can be used are proper control and monitoring of human activities. The current study indicates that there is an urgent need to take environmental issues seriously and create awareness in human society about the sources of pollution. The work should be carried out for monitoring wastewater treatment plants and building and implementing bioremediation strategies to reduce pollution in the concerned water body. Environmental laws should strictly imply to the industries for proper treatment, management, and disposal of wastewater and finding alternative methods for remediation of pollutants contaminating the wastewater.

Last, but not least, there is a vast scope for further studies dealing with the characterization of organic pollutants in the effluents from the tannery, leather, and electroplating industries. There is scope for more extensive study to remediate these volatile organic compounds that are very toxic and a big health hazard for the people living near these contaminated areas. In the future, there is high scope for using a variety of methods to reduce the pollutants responsible for bad odor as well as for the transmission of lethal diseases.

There is a bright prospect of using bio-materials in biodegradation and adsorption of malodorous compounds discharged into the environment. To understand the nature of chemicals released by the industries and possibility of method applied for their removal, there is need to make a transparent and strong bond between industries, researchers, and the scientific community. Further, there is a large scope for exploration of different microbial communities along with the agricultural byproducts to use, for study in depth their adsorptive and biodegradative ability for removal of organic compounds that can act as carcinogen, teratogen, and harm the environment directly or indirectly.

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9.PUBLICATIONS

List of Publications

1) Plight of Agro-irrigation Near Kala Sanghian Drain–A Systematic Case Study and Road Map for Sustainable Technologies- Annals of Biology 34 (2: 229-234, 2018-Scopus indexed)

2) Nano-Toxicity: A Bird's Eye View of Toxicological Aspects- Research and Development in Material Science (ISSN: 2576-8840, July 10, 2018)

3) Bioremediation of Industrial Effluents: A comprehensive Review and Perspectives- -Journal of the Gujarat Research Society (ISSN: 0374-8588, Volume 21 Issue 10s, November 2019)

4) Preparation, Characterization, and Assessment of Low Cost Green Adsorbent Prepared from Coconut Shell for Removal of Toxic Dichloromethane"- Annals of Biology (ISSN: 0970-0153, July 2022 (Accepted-Scopus indexed)

5) A comparative application of adsorbents prepared from rice husks in the removal of dichloromethane -5th International Conference on Advances in Agriculture Technology-(Abstract published-June-2022-ICAATAS, LPU, Agriculture department)

Annals of Biology 34 (2) : 229-234, 2018

Plight of Agro-irrigation Near Kala Sanghian Drain–A Systematic Case Study and Road Map for Sustainable Technologies

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ABSTRACT

Industries are milestones for the development and welfare of any nation. Despite the tremendous success industries bring about, there are several industries that are persistently discharging toxic effluents into the water bodies effecting flora, fauna and human populations. At a regional level, regardless of the efforts of Punjab Pollution Control Board (PPCB), the practice of discharging domestic wastes and untreated industrial effluents into drains, rivulets and water channels has been unprecedented. Kala Sanghian Drain is one among the various thoroughly polluted water bodies. It receives the noxious effluents from tannery industries of leather complex and electroplating industries situated at focal point. The pestilential effluents are deteriorating the environment and health of the human populations in Jalandhar and Kapurthala districts. The crops that are grown on the banks of Kala Sanghian Drain are undoubtedly affected by the polluted water of the drain. Upon entering the Sutlej river, this contaminated water has a bearing on agricultural farms of the entire Malwa belt of Punjab and some parts of Rajasthan.Till date, the scientific community has not plunged into analyzing the composition of waste water in this drain. Suggesting the officials or people at large without rational explanation would have futile results. Keeping things in perspective, analyzing the waste water of Kala Sanghian Drain and sharing the understanding would have considerable impact on various stake holders in the implementation of guidelines of the pollution control board. Additionally, developing cost-effective and ecofriendly technologies to treat waste water effluents right at the point of discharge would help protect the water bodies and environment at large. This article focuses on strategies to analyze the composition of waste water of Kala Sanghian Drain and various eco-friendly technologies with their plausible role in ecofriendly irrigation.

Key words : Toxic effluents, eco-friendly technologies, waste water, bioremediation

INTRODUCTION

The domestic and industrial effluents contain loads of pollutants with considerable threat to humans and environment (Chekroun *et al.*, 2014). Releasing effluent waste waters according to the guidelines given by international and national agencies would keep a check on the contamination of various water bodies. However, abiding by this in letter and spirit is still a farfetched dream for many countries (Gupta *et al.*, 2015). Afflicting tens of thousands of people all across the continents, water pollutants stand as one of the major threats to mankind and environment (Abbas *et al.*, 2014). The condition of Kala Sanghian Drain is one such sorry state of affairs in India. The severity of pollution of this drain is presented in Fig. 1. Several developed nations, for example, Australia, Universities have come forward to assist remediation of industrial effluents by establishing treatment plants right at the effluent outlets of the industries (June, 1999; Gonzalez *et al.*, 1997). Advocating technologies to clean up water bodies at a regional level will truly make a difference in the global perspective.

Many approaches have been applied so far to treat the contaminated waste water. Though effective to certain degree, the physico-chemical methods failed due to tremendous setbacks (Gonzalez *et al.*, 1982; Lorenz and Wackernagel, 1994; Brar *et al.*, 2006; Kurniawan *et al.*, 2006; Barakat, 2011; Bhatnagar and Kumari, 2013; Bilal *et al.*, 2013). These effects attracted the interest of researchers towards biologicals suitable for eco-friendly techniques. The various biological agents used to treat polluted water are bacteria, fungi, algae and higher plants. But all the biological methods also have certain drawbacks. Hence, there is not a single technology without setbacks. Restoring measures and finding improved solutions for sustainable eco-friendly technologies help resolving critical environmental issues. To this end, this article aims at bringing out plausible applications of conventinal and biological methods of waste water remediation.

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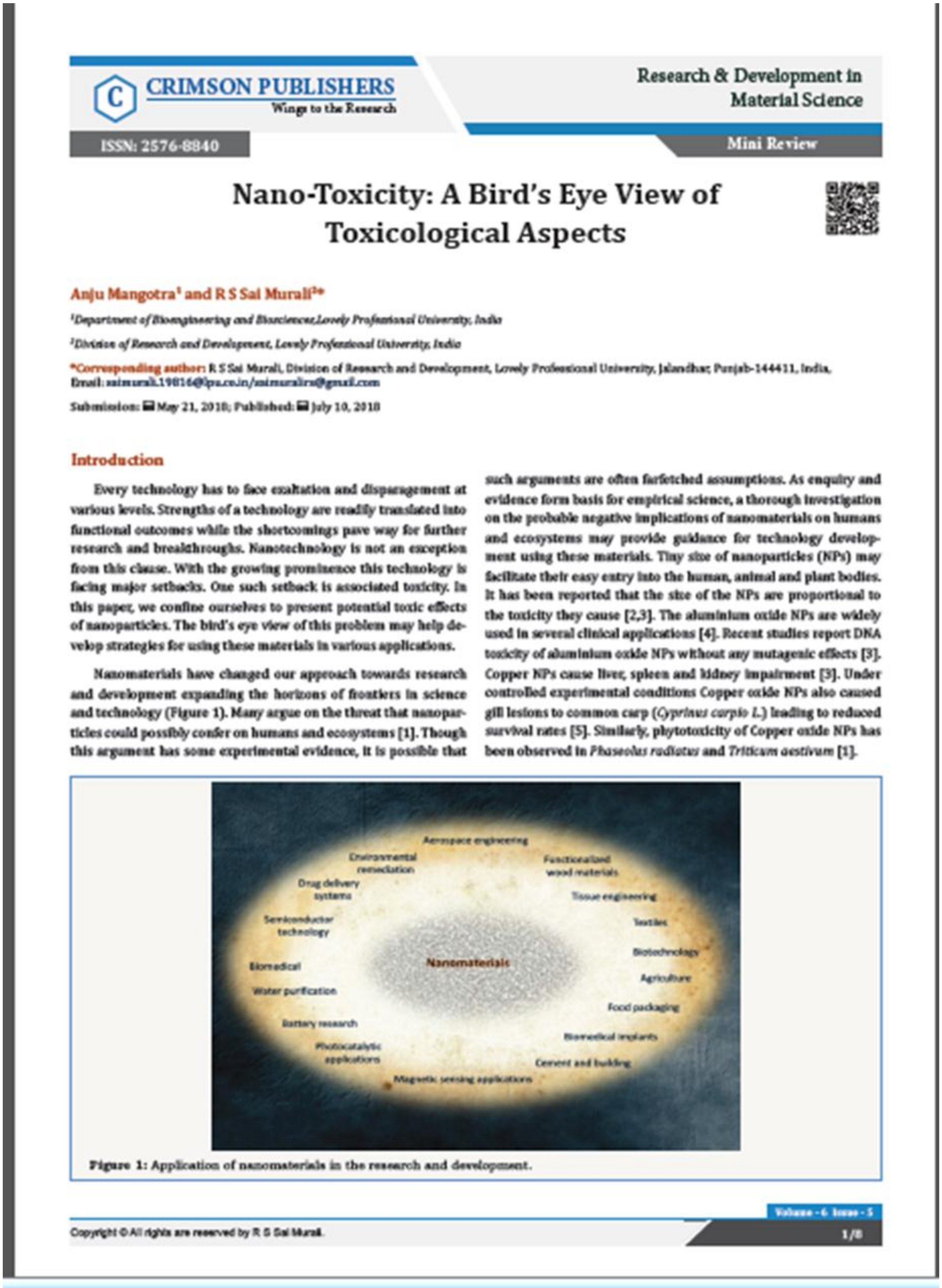

CRIMSON PUBLISHERS
Wings to the Research

Research & Development in Material Science

ISSN: 2576-8840

Mini Review

Nano-Toxicity: A Bird's Eye View of Toxicological Aspects

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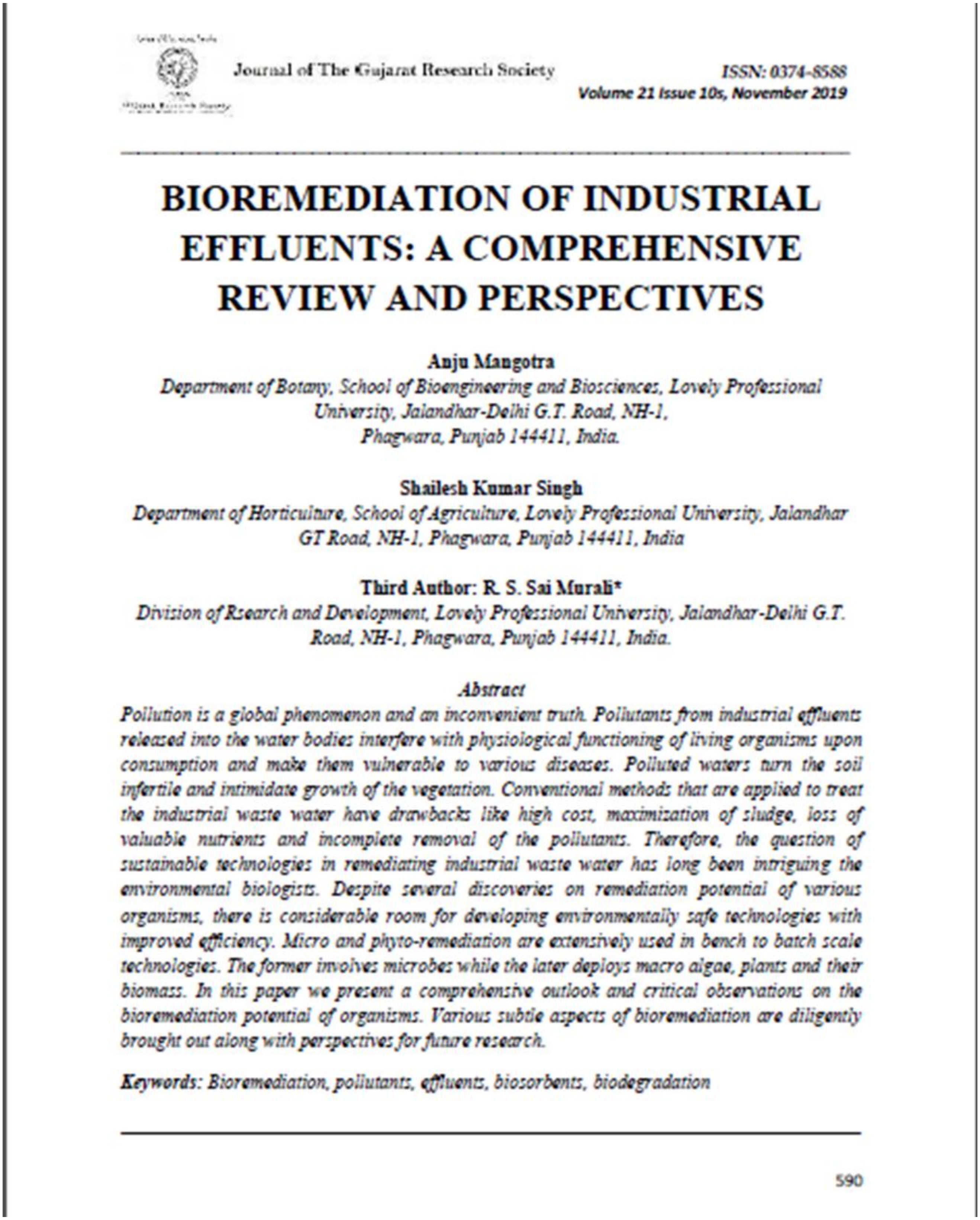
Submission: May 21, 2018; Published: July 10, 2018

Introduction

Every technology has to face exaltation and disparagement at various levels. Strengths of a technology are readily translated into functional outcomes while the shortcomings pave way for further research and breakthroughs. Nanotechnology is not an exception from this clause. With the growing prominence this technology is facing major setbacks. One such setback is associated toxicity. In this paper, we confine ourselves to present potential toxic effects of nanoparticles. The bird's eye view of this problem may help develop strategies for using these materials in various applications.

Nanomaterials have changed our approach towards research and development expanding the horizons of frontiers in science and technology (Figure 1). Many argue on the threat that nanoparticles could possibly confer on humans and ecosystems [1]. Though this argument has some experimental evidence, it is possible that such arguments are often farfetched assumptions. As enquiry and evidence form basis for empirical science, a thorough investigation on the probable negative implications of nanomaterials on humans and ecosystems may provide guidance for technology development using these materials. Tiny size of nanoparticles (NPs) may facilitate their easy entry into the human, animal and plant bodies. It has been reported that the size of the NPs are proportional to the toxicity they cause [2,3]. The aluminium oxide NPs are widely used in several clinical applications [4]. Recent studies report DNA toxicity of aluminium oxide NPs without any mutagenic effects [3]. Copper NPs cause liver, spleen and kidney impairment [3]. Under controlled experimental conditions Copper oxide NPs also caused gill lesions to common carp (*Cyprinus carpio L.*) leading to reduced survival rates [5]. Similarly, phytotoxicity of Copper oxide NPs has been observed in *Phaseolus radiatus* and *Triticum aestivum* [1].

Figure 1: Application of nanomaterials in the research and development.



Journal of The Gujarat Research Society
ISSN: 0374-8588
Volume 21 Issue 10s, November 2019

BIOREMEDIATION OF INDUSTRIAL EFFLUENTS: A COMPREHENSIVE REVIEW AND PERSPECTIVES

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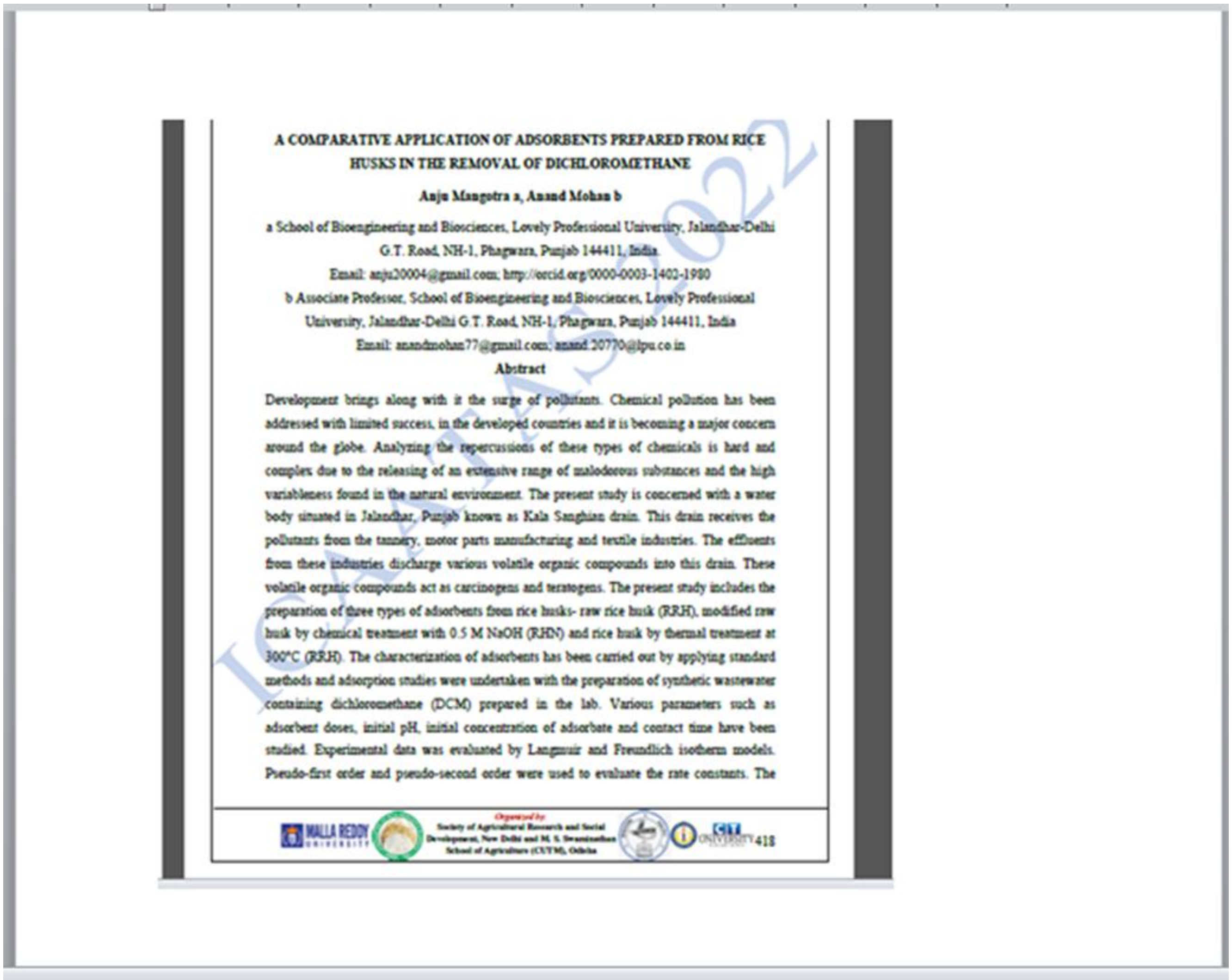
Abstract

Pollution is a global phenomenon and an inconvenient truth. Pollutants from industrial effluents released into the water bodies interfere with physiological functioning of living organisms upon consumption and make them vulnerable to various diseases. Polluted waters turn the soil infertile and intimidate growth of the vegetation. Conventional methods that are applied to treat the industrial waste water have drawbacks like high cost, maximization of sludge, loss of valuable nutrients and incomplete removal of the pollutants. Therefore, the question of sustainable technologies in remediating industrial waste water has long been intriguing the environmental biologists. Despite several discoveries on remediation potential of various organisms, there is considerable room for developing environmentally safe technologies with improved efficiency. Micro and phyto-remediation are extensively used in bench to batch scale technologies. The former involves microbes while the later deploys macro algae, plants and their biomass. In this paper we present a comprehensive outlook and critical observations on the bioremediation potential of organisms. Various subtle aspects of bioremediation are diligently brought out along with perspectives for future research.

***Keywords:** Bioremediation, pollutants, effluents, biosorbents, biodegradation*



A COMPARATIVE APPLICATION OF ADSORBENTS PREPARED FROM RICE HUSKS IN THE REMOVAL OF DICHLOROMETHANE

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Abstract

Development brings along with it the surge of pollutants. Chemical pollution has been addressed with limited success, in the developed countries and it is becoming a major concern around the globe. Analyzing the repercussions of these types of chemicals is hard and complex due to the releasing of an extensive range of malodorous substances and the high variableness found in the natural environment. The present study is concerned with a water body situated in Jalandhar, Punjab known as Kala Sanghian drain. This drain receives the pollutants from the tannery, motor parts manufacturing and textile industries. The effluents from these industries discharge various volatile organic compounds into this drain. These volatile organic compounds act as carcinogens and teratogens. The present study includes the preparation of three types of adsorbents from rice husks- raw rice husk (RRH), modified raw husk by chemical treatment with 0.5 M NaOH (RHN) and rice husk by thermal treatment at 300°C (RRH). The characterization of adsorbents has been carried out by applying standard methods and adsorption studies were undertaken with the preparation of synthetic wastewater containing dichloromethane (DCM) prepared in the lab. Various parameters such as adsorbent doses, initial pH, initial concentration of adsorbate and contact time have been studied. Experimental data was evaluated by Langmuir and Freundlich isotherm models. Pseudo-first order and pseudo-second order were used to evaluate the rate constants. The

Annals of Biology **39** (1) : 41-48, 2023

Impact Score : 0.32
(Scopus)

Preparation, Characterization and Assessment of Low Cost Green Adsorbent Prepared from Coconut Shell for Removal of Toxic Dichloromethane

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(Received: June 20, 2022; Accepted: July 21, 2022)

ABSTRACT

The seamless release of industrial effluents and deterioration of the physico-chemical properties of water are becoming a prolonged threat across the world. Industrial effluents including dichloromethane have deleterious effects on the water chemistry and make the zooplankton difficult to breathe and furthermore affect the metabolism of aerobic aquatic species. The aim of this study was to prepare low-cost porous coconut shell activated carbon (CSAC) and further analyze and investigate its capability to adsorb dichloromethane. The carbonization process was carried out at a temperature of 700°C. The activation of carbon was brought into forth by using phosphoric acid (H_3PO_4) as activating agent. Impregnation ratio was taken as 1 : 3 and the activation time was kept 1 h. The characterization of the resulting porous carbon was accomplished using sophisticated Fourier transform infrared spectroscopy and well-refined field emission electron microscopy. Adsorption capacities and removal percentages of samples were investigated by batch studies. Langmuir and Freundlich adsorption isotherms were used for the evaluation of adsorption process. The experimental data were best fitted in the Freundlich model. Various parameters such as thermodynamics, kinetics, pH and concentration of adsorbate, dosages of adsorbent and contact time on dichloromethane adsorption were investigated. The removal of dichloromethane by CSAC was evaluated to be 87.5%. The results of this study divulged that activated carbon produced from coconut shell by chemical activation was a justifiable adsorbent for treatment of volatile organic compounds in wastewaters.

Key words: Activated carbon, coconut shell, volatile compounds, chemical activation, isotherm models

10. CONFERENCES AND WORKSHOPS

List of conferences

1. Poster presentation in SAFNS, Conference, LPU (February, 2018).
2. Poster presentation in ICAATAS Conference, LPU (June,2022).
3. Oral presentation in National conference on Emerging trends in Engineering and technology, Chandigarh (May, 2022).

List of workshops

1. Workshop organized by Human Resource Development center, LPU (Nov.2018).
2. Workshop on Next Generation of Stem Scientists (NGSS), by Marwadi University,
   Rajkot, Gujarat (August, 2022).

11. Annexure I: Abbreviations

S.NO	Abbreviation	Full form
1	CSAC	Coconut shell activated carbon
2	RRH	Raw Rice Husk
3	RHN	Rice Husk modified with 0.5 M NaOH
4.	RHH	Rice husk heated at 300°C
5.	WHO	World Health Organization
6.	BIS	Bureau of Indian Standards
7.	DCM	Dichloromethane
8.	KSD	Kala Sanghian Drain
9.	EPA	Environmental Protection Agency
10.	ATSDR	Agency for Toxic substances and Disease
11.	BOD	Biological oxygen demand
12.	DO	Dissolved oxygen
13.	TDS	Total dissolved solids
14.	EC	Electrical conductivity
15.	VOCs	Volatile organic compounds

Annexure II: Chemical List

S.NO	Name of chemical	Company name
1	Manganese sulphate	Merck
2	Alkali-Iodide-azide reagent	Merck
3	Sulphuric acid (Conc.)	Merck
4	Starch indicator	Merck
5	Stock sodium thiosulphate 0.1 N	Merck
6	Standard sodium thiosulphate 0.025 N	Merck
7	Phosphate buffer	Merck
8	Calcium chloride	Merck
9	Ferric chloride	Merck
10	Sodium sulphate solution	Merck
11	1 N H_2SO_4 (Acid solution)	Merck
12	1 N NaOH (Alkali solution)	Merck
13	2-chloro-6-pyridine (Nitrification inhibitor)	Merck
14	Glucose-glutamic acid solution	Merck
15	Methanol	Merck
16	Fluorobenzene	Merck

17	Bromofluorobenzene	Merck
18	1,4 dichlorobenzene	Merck
19	Dichloromethane	Merck
20	Dil. H_2SO_4	Merck
21	Dil.HNO_3	Merk
22	0.5 M NaOH	Merck
23	Cetrimide agar	Merck
24	0.5% aqueous crystal violet	Merck
25	95% ethanol	Merck
26	Safranin	Merck
27	1% glucose	Merck
28	Tetramethyl-p-phenylene diamine dihydrochloride	Merck
29	a-naphthol	Merck
30	p-amino-dimethylaniline oxalate	Mecrk
31	Zirconium chloride	Merck
32	N,N-Dimethylformamide (DMF)	Merck
33	Terephthalic acid	Merck
34	Hydrogen acetate	Merck

35.	Cetrimide agar	Merck

RESULT FINDINGS

OF

16s rRNA TEST

RESULT FINDINGS

OF

16s rRNA TEST

BLAST ® » blastn suite » RID-22GC7XYA016

BLAST Results

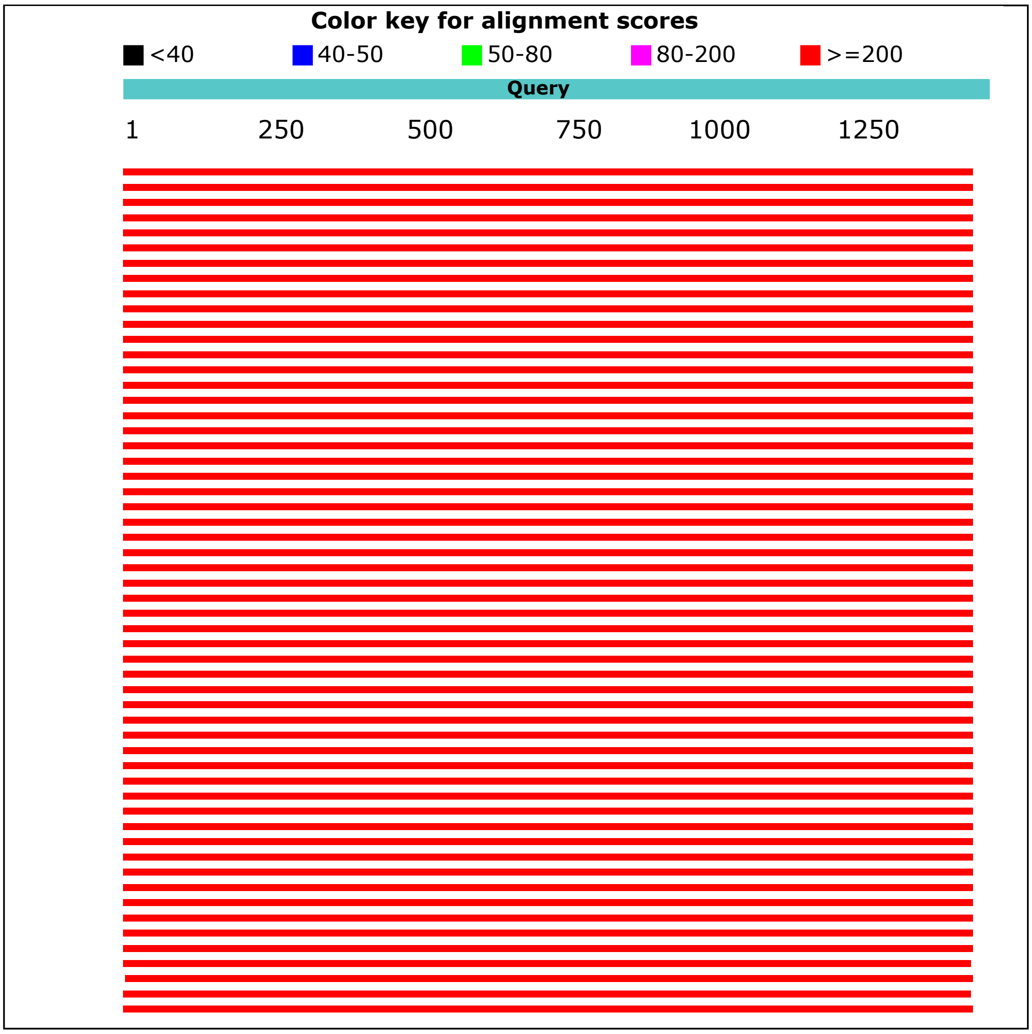
Questions/comments

Job title: Contig - SCDA1

RID	22GC7XYA016 (Expires on 03-28 15:11 pm)		
Query ID	lcl|Query_11803	**Database Name**	nt
Description	Contig - SCDA1	**Description**	Nucleotide collection (nt)
Molecule type	dna	**Program**	BLASTN 2.14.0+
Query Length	1410		

Graphic Summary

Distribution of the top 200 Blast Hits on 100 subject sequences



Descriptions

Sequences producing significant alignments:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas aeruginosa strain LGMT12 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MT337423.1
Pseudomonas sp. strain ZYD-2 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MT197312.1
Pseudomonas sp. strain InAD-148 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MF401349.1
Pseudomonas aeruginosa strain PA2 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MN636758.1
Pseudomonas aeruginosa strain B6 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MN551786.1
Pseudomonas sp. strain 206013 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MK949378.1
Pseudomonas sp. strain 206012 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MK949377.1
Pseudomonas sp. strain F30 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MK571443.1
Pseudomonas aeruginosa strain 610D5 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MH378333.1
Pseudomonas aeruginosa strain JBT18N 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MH628093.1
Pseudomonas aeruginosa strain GHJ12 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MG396955.1
Pseudomonas aeruginosa strain FQ5 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MF144458.1
Pseudomonas aeruginosa strain P-7 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KY885176.1
Pseudomonas aeruginosa strain D-1 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KY885165.1
Pseudomonas aeruginosa strain R4 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KU321274.1
Pseudomonas aeruginosa strain I2_6 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KT799669.1
Pseudomonas sp. strain EFB11 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	OP893815.1
Pseudomonas aeruginosa strain XSF-65 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	OP566521.1
Pseudomonas aeruginosa strain XSF-39 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	OP566520.1

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas aeruginosa strain LY.1 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	OM865894.1
Pseudomonas aeruginosa strain PA5-2-1 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KF977859.1
Pseudomonas aeruginosa strain P60 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KF670598.1
Pseudomonas aeruginosa strain MDM12 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MZ618961.1
Pseudomonas aeruginosa strain B19 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MZ425417.1
Pseudomonas aeruginosa strain D1 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KF113578.1
Pseudomonas aeruginosa strain SUB 10 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	KC920813.1
Pseudomonas aeruginosa strain OPGM3 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MZ150742.1
Pseudomonas aeruginosa strain NSJ003 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MT373468.1
Pseudomonas aeruginosa strain NSJ020 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MT373467.1
Bacterium P2A 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JX149546.1
Bacterium H1C 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JX149543.1
Pseudomonas aeruginosa strain R7-734 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JQ659920.1
Pseudomonas aeruginosa strain DQ2 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JQ669958.1
Pseudomonas aeruginosa strain IFS 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JQ041638.1
Pseudomonas aeruginosa strain FG10 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MW012614.1
Pseudomonas sp. strain ZM18 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	MT757946.1
Pseudomonas aeruginosa strain F1 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JN412064.1
Pseudomonas sp. G6 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	GQ221872.1
Pseudomonas sp. CIFRI D-TSB-6 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	JF784021.1
Pseudomonas sp. AGP-01 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	HM587311.1
Pseudomonas sp. Pseudo-EJB5 16S ribosomal RNA gene, partial	2604	2604	100%	0.0	100.00%	GU966670.1

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
sequence						
Pseudomonas aeruginosa strain HS9 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	GU323371.1
Pseudomonas aeruginosa strain BSF-g 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	GU121439.1
Pseudomonas aeruginosa NO6 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	FJ972534.1
Pseudomonas aeruginosa NO5 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	FJ972533.1
Pseudomonas aeruginosa isolate 9 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	FJ907192.1
Pseudomonas sp. Id-11 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	FJ598142.1
Pseudomonas aeruginosa strain RE7 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	FJ472580.1
Pseudomonas sp. HF8-8 16S ribosomal RNA gene, partial sequence	2604	2604	100%	0.0	100.00%	DQ288112.1
Pseudomonas aeruginosa strain NG4 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	100.00%	MN238694.1
Pseudomonas aeruginosa strain JYR-Pk-2011 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	100.00%	JQ792038.1
Pseudomonas aeruginosa strain P-5 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	100.00%	JN969044.1
Pseudomonas aeruginosa strain AJ-37 16S ribosomal RNA gene, partial sequence	2601	2601	99%	0.0	100.00%	KY465434.1
Pseudomonas aeruginosa strain D1402 16S ribosomal RNA gene, partial sequence	2601	2601	99%	0.0	100.00%	MW990114.1
Endophytic bacterium 202P-2 16S ribosomal RNA gene, partial sequence	2601	2601	99%	0.0	100.00%	JF901362.1
Pseudomonas aeruginosa strain SNDPR-01 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP119298.1
Pseudomonas aeruginosa strain M02 16S ribosomal RNA gene, partial sequence	2599	2599	100%	0.0	99.93%	OQ568312.1
Pseudomonas aeruginosa strain UASR_4 16S ribosomal RNA gene, partial sequence	2599	2599	100%	0.0	99.93%	OQ519934.1
Pseudomonas aeruginosa strain 0201761-1 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP117300.1
Pseudomonas aeruginosa strain HS18-89 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP084321.1
Pseudomonas aeruginosa strain B-3509 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP117974.1
Pseudomonas aeruginosa strain 2022CK-00828 chromosome,	2599	10397	100%	0.0	99.93%	CP117749.1

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
complete genome						
Pseudomonas aeruginosa strain MF1 chromosome, complete genome	2599	10391	100%	0.0	99.93%	CP117527.1
Pseudomonas aeruginosa strain PaLo1 chromosome, complete genome	2599	10378	100%	0.0	99.93%	CP075849.1
Pseudomonas aeruginosa strain PaLo2 chromosome, complete genome	2599	10391	100%	0.0	99.93%	CP075848.1
Pseudomonas aeruginosa strain PaLo3 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075847.1
Pseudomonas aeruginosa strain PaLo4 chromosome	2599	10397	100%	0.0	99.93%	CP075846.1
Pseudomonas aeruginosa strain PaLo6 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075844.1
Pseudomonas aeruginosa strain PaLo7 chromosome	2599	10391	100%	0.0	99.93%	CP075843.1
Pseudomonas aeruginosa strain PaLo9 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075841.1
Pseudomonas aeruginosa strain PaLo10 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075840.1
Pseudomonas aeruginosa strain PaLo11 chromosome, complete genome	2599	10391	100%	0.0	99.93%	CP075838.1
Pseudomonas aeruginosa strain PaLo12 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075836.1
Pseudomonas aeruginosa strain PaLo14 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075835.1
Pseudomonas aeruginosa strain PaLo17 chromosome, complete genome	2599	10303	100%	0.0	99.93%	CP075833.1
Pseudomonas aeruginosa strain PaLo20 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075832.1
Pseudomonas aeruginosa strain PaLo21 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075831.1
Pseudomonas aeruginosa strain PaLo22 chromosome, complete genome	2599	10384	100%	0.0	99.93%	CP075830.1
Pseudomonas aeruginosa strain PaLo26 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075828.1
Pseudomonas aeruginosa strain PaLo29 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075826.1
Pseudomonas aeruginosa strain PaLo30 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075825.1
Pseudomonas aeruginosa strain PaLo34 chromosome, complete genome	2599	10391	100%	0.0	99.93%	CP075822.1
Pseudomonas aeruginosa strain PaLo35 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075821.1

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas aeruginosa strain PaLo36 chromosome, complete genome	2599	10386	100%	0.0	99.93%	CP075820.1
Pseudomonas aeruginosa strain PaLo37 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075819.1
Pseudomonas aeruginosa strain PaLo38 chromosome, complete genome	2599	10373	100%	0.0	99.93%	CP075818.1
Pseudomonas aeruginosa strain PaLo39 chromosome, complete genome	2599	10386	100%	0.0	99.93%	CP075817.1
Pseudomonas aeruginosa strain PaLo40 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075816.1
Pseudomonas aeruginosa strain PaLo43 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075815.1
Pseudomonas aeruginosa strain PaLo44 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075814.1
Pseudomonas aeruginosa strain PaLo45 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075813.1
Pseudomonas aeruginosa strain PaLo46 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075812.1
Pseudomonas aeruginosa strain PaLo152 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075811.1
Pseudomonas aeruginosa strain PaLo166 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075810.1
Pseudomonas aeruginosa strain PaLo170 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075809.1
Pseudomonas aeruginosa strain PaLo185 chromosome	2599	10397	100%	0.0	99.93%	CP075808.1
Pseudomonas aeruginosa strain PaLo191 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075807.1
Pseudomonas aeruginosa strain PaLo226 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075806.1
Pseudomonas aeruginosa strain PaLo227 chromosome, complete genome	2599	10397	100%	0.0	99.93%	CP075805.1
Pseudomonas aeruginosa strain MDM18 16S ribosomal RNA gene, partial sequence	2597	2597	100%	0.0	99.86%	MZ618964.1

Alignments

Pseudomonas aeruginosa strain LGMT12 16S ribosomal RNA gene, partial sequence

Sequence ID: **MT337423.1** Length: 1418 Number of Matches: 1
Range 1: 7 to 1416

Score	Expect	Identities	Gaps	Strand	Frame
2604 bits(1410)	0.0()	1410/1410(100%)	0/1410(0%)	Plus/Plus	

Features:

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Sbjct  7    CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  66
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Query  61    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  120
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Sbjct  67    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  126

Query  121   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  186

Query  181   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  240
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Sbjct  187   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  246

Query  241   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  306

Query  301   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  366

Query  361   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  420
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Sbjct  367   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  426

Query  421   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  486

Query  481   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  540
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Sbjct  487   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  546

Query  541   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  606

Query  601   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  607   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  666

Query  661   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  667   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  726

Query  721   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  727   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  786

Query  781   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  787   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  846

Query  841   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  900
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Sbjct  847   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  906

Query  901   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  907   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  966

Query  961   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1020
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Sbjct  967   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1026

Query  1021  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1027  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1086

Query  1081  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1140
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Sbjct  1087  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1146

Query  1141  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1147  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1206

Query  1201  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1260
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Sbjct  1207  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1266

Query  1261  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1320
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Sbjct  1267  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1326

Query  1321  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1380
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Sbjct  1327  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1386

Query  1381  TAGTCTAACCGCAAGGGGGACGGTACCACG  1410
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Sbjct  1387  TAGTCTAACCGCAAGGGGGACGGTACCACG  1416
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Pseudomonas sp. strain ZYD-2 16S ribosomal RNA gene, partial sequence

Sequence ID: **MT197312.1** Length: 1439 Number of Matches: 1
Range 1: 18 to 1427

Score	Expect	Identities	Gaps	Strand	Frame
2604 bits(1410)	0.0()	1410/1410(100%)	0/1410(0%)	Plus/Plus	

Features:

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Sbjct  18    CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  77

Query  61    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  120
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Sbjct  78    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  137

Query  121   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  180
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Sbjct  138   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  197

Query  181   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  240
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Sbjct  198   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  257

Query  241   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  300
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Sbjct  258   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  317

Query  301   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  360
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Sbjct  318   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  377

Query  361   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  420
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Sbjct  378   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  437

Query  421   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  497

Query  481   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  540
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Sbjct  498   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  557

Query  541   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  600
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Sbjct  558   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  617

Query  601   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  660
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Sbjct  618   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  677

Query  661   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  720
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Sbjct  678   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  737

Query  721   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  780
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Sbjct  738   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  797

Query  781   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  840
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Sbjct  798   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  857

Query  841   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  900
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Sbjct  858   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  917

Query  901   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  960
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Sbjct  918   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  977

Query  961   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1020
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Sbjct  978   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1037

Query  1021  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1080
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Sbjct  1038  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1097

Query  1081  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1140
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Sbjct  1098  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1157

Query  1141  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1158  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1217

Query  1201  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1260
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Sbjct  1218  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1277

Query  1261  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1320
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Sbjct  1278  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1337

Query  1321  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1380
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Sbjct  1338  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1397

Query  1381  TAGTCTAACCGCAAGGGGGACGGTACCACG  1410
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Sbjct  1398  TAGTCTAACCGCAAGGGGGACGGTACCACG  1427
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Pseudomonas sp. strain InAD-148 16S ribosomal RNA gene, partial sequence
Sequence ID: **MF401349.1** Length: 1438 Number of Matches: 1
Range 1: 18 to 1427

Score	Expect	Identities	Gaps	Strand	Frame
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Features:

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Query  1     CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  60
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Sbjct  18    CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  77

Query  61    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  78    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  137

Query  121   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  180
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Sbjct  138   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  197

Query  181   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  240
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Sbjct  198   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  257

Query  241   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  300
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Sbjct  258   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  317

Query  301   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  360
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Sbjct  318   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  377

Query  361   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  378   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  437

Query  421   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  438   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  497

Query  481   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  540
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Sbjct  498   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  557

Query  541   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  600
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Sbjct  558   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  617

Query  601   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  618   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  677

Query  661   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  678   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  737

Query  721   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  780
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Sbjct  738   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  797

Query  781   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  840
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Sbjct  798   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  857

Query  841   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  900
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Sbjct  858   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  917

Query  901   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  960
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Sbjct  918   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  977

Query  961   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1020
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Sbjct  978   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1037

Query  1021  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1080
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Sbjct  1038  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1097

Query  1081  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1140
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Sbjct  1098  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1157

Query  1141  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1200
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Sbjct  1158  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1217

Query  1201  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1260
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Sbjct  1218  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1277

Query  1261  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1320
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Sbjct  1278  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1337

Query  1321  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1380
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1338  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1397

Query  1381  TAGTCTAACCGCAAGGGGGACGGTACCACG  1410
             ||||||||||||||||||||||||||||||
Sbjct  1398  TAGTCTAACCGCAAGGGGGACGGTACCACG  1427
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Pseudomonas aeruginosa strain PA2 16S ribosomal RNA gene, partial sequence

Sequence ID: **MN636758.1** Length: 1443 Number of Matches: 1

Range 1: 21 to 1430

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Sbjct  21    CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  80

Query  61    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  120
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Sbjct  81    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  140

Query  121   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  200

Query  181   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  260

Query  241   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  320

Query  301   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  380

Query  361   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  440

Query  421   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  500

Query  481   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  560

Query  541   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  561   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  620

Query  601   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  621   GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACAC  680

Query  661   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  681   CAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC  740

Query  721   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  780
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Sbjct  741   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  800

Query  781   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  801   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  860

Query  841   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  900
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Sbjct  861   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  920

Query  901   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  960
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Sbjct  921   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  980

Query  961   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  981   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1040

Query  1021  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1041  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1100

Query  1081  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1101  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1160

Query  1141  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1200
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Sbjct  1161  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1220

Query  1201  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1260
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Sbjct  1221  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1280

Query  1261  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1320
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Sbjct  1281  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1340

Query  1321  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1380
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Sbjct  1341  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1400

Query  1381  TAGTCTAACCGCAAGGGGGACGGTACCACG  1410
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Sbjct  1401  TAGTCTAACCGCAAGGGGGACGGTACCACG  1430
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Pseudomonas aeruginosa strain B6 16S ribosomal RNA gene, partial sequence

Sequence ID: **MN551786.1** Length: 1456 Number of Matches: 1

Range 1: 36 to 1445

Score	Expect	Identities	Gaps	Strand	Frame
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Features:

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Sbjct  36    CATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTA  95

Query  61    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  96    ATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCA  155

Query  121   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  180
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Sbjct  156   TACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT  215

Query  181   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  240
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Sbjct  216   CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAG  275

Query  241   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  300
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Sbjct  276   AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG  335

Query  301   GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC  360
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Query  361   TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT  420
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Query  421   TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAA  480
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Query  481   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  540
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Sbjct  516   GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT  575

Query  541   GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC  600
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Query  721   AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGAT  780
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Query  781   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  840
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Sbjct  816   CCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGC  875

Query  841   AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA  900
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Sbjct  936   TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG  995

Query  961   ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG  1020
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Query  1021  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1080
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Sbjct  1056  AGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGG  1115

Query  1081  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1140
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Sbjct  1116  GTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT  1175

Query  1141  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1200
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Sbjct  1176  CATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGC  1235

Query  1201  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1260
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Sbjct  1236  CAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAA  1295

Query  1261  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1320
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Sbjct  1296  CTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACG  1355

Query  1321  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1380
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Sbjct  1356  TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGC  1415

Query  1381  TAGTCTAACCGCAAGGGGGACGGTACCACG  1410
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Sbjct  1416  TAGTCTAACCGCAAGGGGGACGGTACCACG  1445
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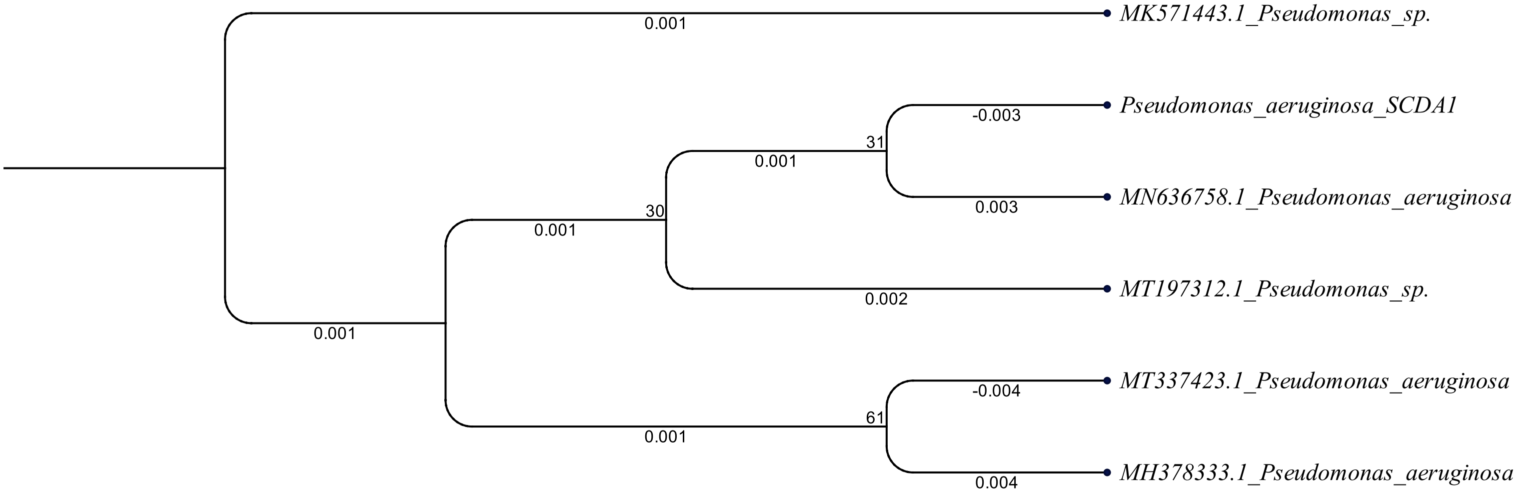
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