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THEME

BIOSORPTION OF EMERGING ORGANICS COMPOUNS USING FILAMENTOUS SPIROGYRA

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for giving us health will and the courage without which this work would not have been possible

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Amina

الملخص:

تهدف هذه الدراسة إلى تقييم قدرات الطحلب الأخضر Spirogyraعلى امتزاز الملوثات الصيدلانية الناشئة بهدف تنقية المياه الملوثة. تم اختيار باراسيتامولوديكلوفيناك كمركبين نموذجيين لتمثيل هذه الملوثات .بالإضافة إلى ذلك، تم اختبار الخصائص البيولوجية لهذا الطحلب، حيث أظهرت نتائجه نشاطًا مضادًا لبعض أنواع البكتيريا والخميرة الممرضةCandida albicans، مما يشير إلى امتلاكه خصائص مضادة للميكروبات .شملت الدراسة تحليل خصائص الطحلب باستخدام تقنيتي AFM و FTIRلفهم مورفولوجية السطح والمجموعات الوظيفية المشاركة في الامتزاز. تم تقييم كفاءة الامتزاز عن طريق تعريض الطحلب لكل ملوّث على حدة، مع تغيير عدة متغيرات تشمل: الـpH ، درجة الحرارة، تركيز الملوّث، زمن التفاعل، وحجم جزيئات الطحلب المجفف. وقد تبيّن أن لكل عامل تأثيرًا واضحًا على كفاءة الامتزاز .في المرحلة الأخيرة، تم استخدام جهاز HPLC لتحديد كمية الملوث المتبقية بعد المعالجة، مما ساعد على تقييم فعالية الامتزاز بدقة .

الكلمات المفتاحية: امتزاز، Spirogyra،باراسيتامول، ديكلوفيناك، بكتيريا. *Candida albicans*، HPLC، FTIR، AFM.

Résumé :

Cette étude vise à évaluer les capacités de la microalgue verte *Spirogyra* à adsorber des polluants pharmaceutiques émergents dans le but de purifier les eaux contaminées. Deux composés modèles ont été choisis : le paracétamol et le diclofénac. Par ailleurs, les propriétés biologiques de l'algue ont été étudiées. Les résultats ont montré une activité antimicrobienne contre certaines souches bactériennes ainsi qu'un effet antifongique contre la levure pathogène *Candida albicans*. Les caractéristiques morphologiques et fonctionnelles de l'algue ont été analysées à l'aide des techniques AFM et FTIR. L'efficacité de l'adsorption a été évaluée en exposant l'algue à chaque polluant séparément, tout en faisant varier plusieurs paramètres : le pH, la température, la concentration du polluant, le temps de contact, et la taille des particules de l'algue séchée. Il a été constaté que chaque facteur influence significativement l'efficacité d'adsorption. Enfin, l'analyse HPLC a permis de quantifier les résidus de polluants après traitement, et d'évaluer ainsi la performance du procédé.

Mots-clés : Adsorption, *Spirogyra*, paracétamol, diclofénac, bactéries, *Candida albicans*, HPLC, FTIR, AFM.

Abstract:

This study aims to evaluate the capacity of the green alga *Spirogyra* to adsorb emerging pharmaceutical pollutants for water purification purposes. Paracetamol and diclofenac were selected as model contaminants. In addition, the biological activity of the alga was investigated. The results showed antibacterial effects against certain strains, as well as antifungal activity against the pathogenic yeast *Candida albicans*. The structural and surface characteristics of the alga were analyzed using AFM and FTIR techniques. Adsorption efficiency was assessed by exposing the alga to each pollutant separately, while varying several parameters: pH, temperature, pollutant concentration, contact time, and particle size of the dried algae powder. Each of these factors had a noticeable effect on the adsorption capacity. Finally, HPLC analysis was used to quantify the remaining amount of pollutant after treatment, thus evaluating the effectiveness of the biosorption process.

Keywords: Adsorption, *Spirogyra*, paracetamol, diclofenac, bacteria, *Candida albicans*, HPLC, FTIR, AFM.

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GENERAL INTRODUCTION

INTRODUCTION

Water is a fundamental element of life, essential for all living organisms. Beyond its vital role in human consumption, agriculture, and industry, it also plays a key role in maintaining ecological balance. However, with the continuous expansion of human activities—particularly in industrial, agricultural, and medical sectors—this precious resource is increasingly threatened by complex and persistent forms of pollution. Among the emerging environmental challenges that have gained attention in recent years is the issue of "emerging pollutants", a group of chemical substances that are not completely removed by conventional wastewater treatment processes. These include pharmaceuticals (such as antibiotics, painkillers, and hormones), with paracetamol and diclofenac being among the most commonly used and detected drugs in aquatic environments…….**[1]**

Such contaminants pose a serious risk to both the environment and public health. Even at low concentrations, they can accumulate in aquatic systems, impact aquatic life, and contribute to the growing concern of antibiotic resistance. Therefore, it has become essential to explore efficient, eco-friendly, and low-cost alternatives for the removal of these pollutants from water. Among the advanced treatment techniques, adsorption has emerged as a promising solution, especially when using natural biosorbents with surface functional groups that can bind contaminants…..**[2]**

In this context, dried algae have attracted considerable interest due to their high adsorption capacity, availability, biodegradability, and environmental compatibility. This study focuses on the use of the green filamentous alga Spirogyra, commonly found in freshwater ecosystems, to evaluate its ability to adsorb two pharmaceutical pollutants: paracetamol and diclofenac. Additionally, the antimicrobial and antifungal activities of the algae are assessed.

Several operational parameters—such as pH, temperature, contact time, pollutant concentration, and particle size of the biosorbent—are studied to determine their influence on the adsorption process. The investigation is supported by advanced analytical techniques, including FTIR, AFM, and HPLC, to better understand the structural, chemical, and quantitative aspects of the biosorption mechanism.

1. BIBLIOGRAPHICAL REFERENCES

1.BIBLIOGRAPHIC SUMMARY

1.1 Water pollution

Water pollution is an adverse physical, chemical, biological, or bacteriological degradation of its natural qualities, caused by humans and their activities. It disrupts the living conditions of aquatic flora and fauna; it compromises water uses and the balance of the natural environment. Water pollution is caused by the discharge of water contaminated by our domestic activities (washing and various cleaning tasks, etc.) but also by the various industrial and agricultural activities necessary to provide us with the food and goods we need……..**[3]**

1.1.1 Pollution affects all the world's rivers:

A quarter of the world's rivers contain pharmaceutical substances at levels considered dangerous, according to a new study. And no water is spared from these substances, which are harmful to ecosystems……..**[4]**

It's a colorless, odorless, yet ubiquitous form of pollution: water pollution from pharmaceutical products. A new study, published Monday, February 14, in the journal of the National Academy of Sciences (PNAS), highlights its scale. A team of 127 international researchers analyzed the water quality of 258 rivers in 104 different countries. Their results show that a quarter of them contain pharmaceutical substances at levels above those considered safe for humans and aquatic organisms ……**[5]**

1.2 Types of pollution

1.2.1 Physical pollution

It includes three types of pollution: mechanical, thermal, radioactive.

1.2.1.1 Mechanical pollution

It results from the discharge of waste and solid particles carried by industrial wastewater, as well as runoff water. These pollutants are either coarse particles, sand, or suspended solids (SS).

1.2.1.2 Thermal pollution

Water discharged from factories using a cooling circuit in certain facilities (thermal power plants, nuclear power plants, refineries, steel mills, etc.); the resulting rise in temperature decreases the dissolved oxygen content. It accelerates the biodegradation and proliferation of germs. It turns out that, given the same load, an increase in temperature promotes the harmful effects of pollution

1.2.1.3 Radioactive pollution

Water pollution by radioactive substances poses an increasingly serious problem, having a direct impact on aquatic populations due to the inherent toxicity of its elements and the carcinogenic and mutagenic properties of its radiation

1.2.2 Chemical pollution

The chemical industry continues to synthesize thousands of substances each year. Some of these products are deliberately designed to be toxic and long-lasting. However, the most harmful contaminations often come from naturally occurring chemicals, frequently present in groundwater

1.2.3 Microbiological pollution

Wastewater contains all the microorganisms excreted with feces, including both normal intestinal flora and pathogens. These organisms can be grouped into four main categories, listed in ascending order of size: viruses, bacteria, protozoa, and helminthes……..**[6]**

1.3. Emerging pollutants (EMPs)

Are commonly defined as unmonitored synthetic or natural chemicals present in the environment. These pollutants can be found in various environmental compartments and cause adverse effects on ecosystems and/or human healthMany EMPs are also found in aquatic environments due to point or diffuse pollution sources. Over the past tenyears, several working groups of the "NORMAN" network1 have identified approximately 970 emerging pollutants in aquatic environments in Europe and

classified them into more than 20 categories. The main categories of emerging contaminants are industrial chemicals, pesticides, metals, surfactants (used as detergents), pharmaceuticals, antibiotics, and cosmetics, including a large number of hormones and poly- and perfluorinated alkyl substances (PFAS). In addition, nanoparticles and microplastics/microfibers are added to the existing list of chemicals of high concern

Emerging chemicals also include chemicals recently identified as potentially harmful to the environment, but which are not yet regulated or controlled by national or international measures. These compounds are considered "emerging" not because the contaminants themselves are new, but because of their growing level of concern for health and the environment ……**[7]**

And it refers to a broad spectrum of compounds used in modern society, including but not limited to insecticides, cosmetics, personal care items, and medications

Agricultural practices, construction, electricity generation, mineral processing, and other activities exposethe environment to trillions of tons of chemically active material)Lahiri. Humans have also created upwards of 140,000compounds and chemical compositions, the majority of whichwere largely unknown, to the anthropogenic dissemination of geogenic substances . In addition, a further review of chemical inventories globally suggests that this numbermay be more significant than 350,000 most newly created synthetic compounds, suspected to be harmful in minute concentrations, are frequently combined with other contaminants or discharged into the global natural ecosystem as breakdown products. Chemical release is predicted to be as much as 220 billion tons per year, and may possibly be greater, given that only 20% of greenhouse gas (GHGs) emissions are caused bychemicalrelease . Trace chemicals are now present across the earth's atmosphere, the deepest oceans, even themost remote and desolate regions, soils, water, and the food chain. One of the main contributors to these environmental disasters is contamination from fertilizers, pesticides, herbicides, and sedimentation . One in six deaths among all people is untimely, with over 9 million dying from exposure to contaminated food, water, workplace, or consumer products annually (Landrigan et al. 2018). These pollutants includemicrobeads, microplastics, antibiotics, medicines, steroids, endocrine disruptors, hormones, industrial additives, and chemicals**……[8]**

These contaminants and wastewater are inextricably linked. Theirwidespread dissemination in the aquatic environment is mainlyfacilitated by municipal, industrial, and home wastewater.

Although further study is required, it is agreed that these new contaminants are becoming a danger. Global production of syntheticchemicals surged to over 2.30 billion tons (BT) in 2017 fromapproximately 1 BT in 2000 .Petroleumproducts constitute a significant proportion of the substances,accounting for 25.70% of sales, followed by specialty chemicals

%26.20) of sales) and polymeric materials (19.20% of salesThe usage of chemicals other than medicinesis anticipated to have grown by 70% by 2030

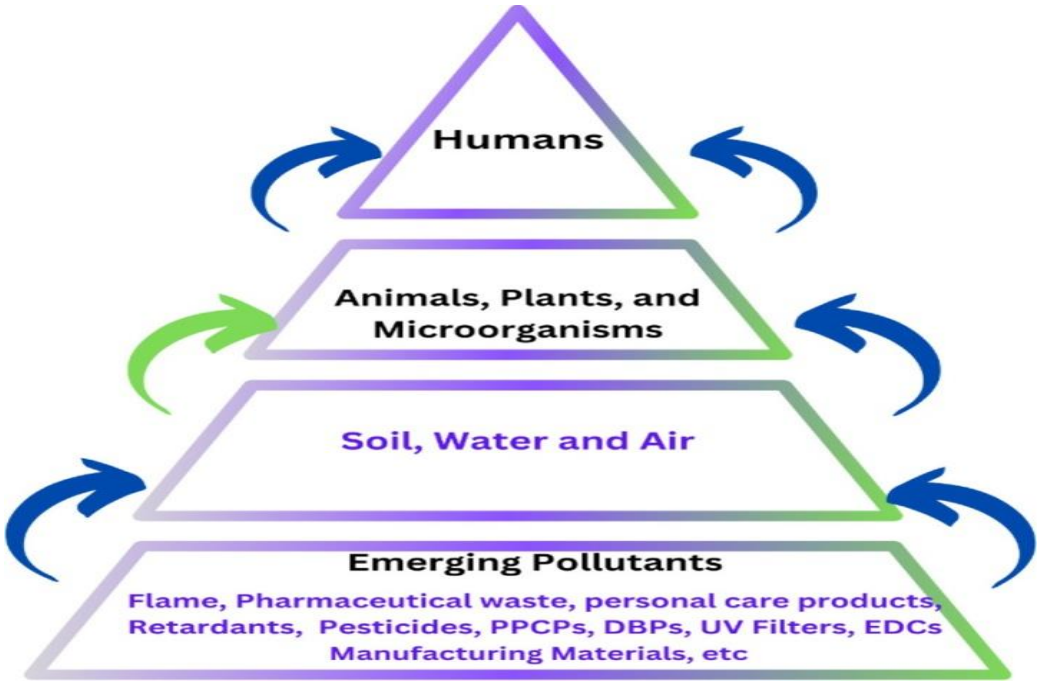


Fig1.1Categories of EPs in water, animals, plants, microorganisms and humans

1.3.1 Classification, sources, and distribution of EPs in the environment

Recent discoveries by EPs include groups of uncontrolled pollutants such as pesticides, pharmaceuticals and personal care products (PPCPs), endocrine-disrupting chemicals (EDCs), disinfectionbyproducts (DBPs), antibiotic-resistant gene (ARG), antibiotic-resistant bacteria (ARB), and other additives and substances thatare present in both surface and groundwater

Most of these toxins were unknown or unrecognized a few yearsago, but they have recently come to light as pollutants that maypose risks to the ecosystem.

the principal sources andthe channels for the dispersion of EPs are underlined. The possibleeffects and impact they might have on ecosystems, animal life, human health, and wildlife are also garnering a lot of attentionand are requiring in-depth investigation.

These pollutants can persist and have an extended lifespansince they are typically bioaccumulative and bioactive. Inorganic,organic, and particulate pollutants, as well as their subgroups.can be classified as EPs based on their physical and chemical properties . The main organic chemicals in drinkingwater, sediments, effluent, and sludge are active pharmaceuticalcompounds. These compounds have been found in natural waterways, groundwater, and effluent.

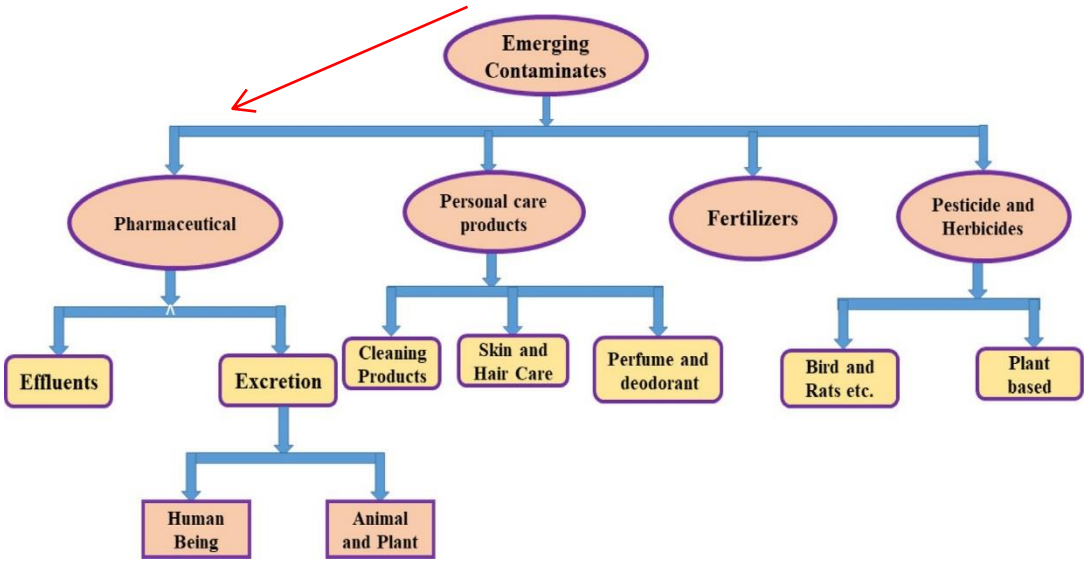


Fig1.2. Theprimarysourceforthecauseof CoronarySyndromeworldwide

1.3.2 EPs from pharmaceutical industry

Pharmaceuticals are contaminants causing significant concern,yet they have been detected in waterbodies worldwide in trace amounts.

Pharmaceuticals are among the most often used chemical classes in nutrition, diagnostic tools,remedies, therapy, and preventative medicine .

Pharmaceutical firms, illegal pharmaceuticals, hormones, and antibiotics are crucial EPs because of their widespread use in aquatic ecosystems, pollution of freshwater supplies, and potentialharm to biodiversity and people . Sewage dilution reduces potential environmental dangers after introducing drugs into a water body

Inaddition to personal usepharmaceuticals were used in farm animals, livestock, and fisheries. Numerous drugs are frequently given to livestock to reduce illnesses in these animals and increase the weight of the mammals. Only a tiny portion of the approximately compounds in pharmaceutical goods have 3000undergone field testing. Scientists have investigated whether the occurrence of ng …….**[9]**

1.3.4 Medicine

it is “Any substance or composition presented as having curative or preventive properties with regard to human or animal diseases, as well as any substance or composition which can be used in humans or animals or which can be administered to them, with a view to establishing a medical diagnosis or restoring, correcting or modifying their physiological functions by exerting a pharmacological, immunological or metabolic action (Article L5111-1 of the Public Health Code (CSP)).Active ingredient (PA);It is a pharmacologically active component of a drug that gives it its ability and specificity to treat or prevent a disease .Metabolites: molecules resulting from the metabolism of drugs in a living organism humans, animals, bacteria, etc.…

Transformation product: chemical species resulting from the transformation of the drug or its metabolites by non-biological physicochemical reactions (phototransformation, chemotransformation, etc…)

Drug residues: all molecules (parent molecules, metabolites, and transformation products) resulting from the production, therapeutic use, or disposal of drugs intended for human or animal therapeutic use ……..**[10]**

1.3.1 Drug composition

A drug comprises a part responsible for its effects on the human body, called the active ingredient, and an inactive part made up of one or more excipients used in the formulation to facilitate the preparation and use of the drug and ensure its

1.4 Sources of emerging pharmaceutical pollutants

Pharmaceutical compounds that reach water bodies, both surface water and groundwater, come from several different sources. The first of these is urban wastewater, which contains a high load of pharmaceuticals from human excreta, as well as the inadequate disposal of expired or unused medications due to the lack of control over their management. Another major source of pharmaceuticals is agricultural and livestock waste, especially the latter, since in large intensive livestock farms, animals are often fed with supplemented feed containing medications and excreta are often used in agriculture as soil improvers, reaching groundwater through leaching .Effluents from the pharmaceutical industry are another important source, with high concentrations of pharmaceuticals found due to factory discharges in Asia, Europe, and the Americas, despite strict regulation of pharmaceutical production in Europe and the United States .These industries are required to perform treatment before discharge into the general urban sewer system ……..**[11]**

1.4.1 Pharmaceuticals in Algerian Waters

Although Algeria is ranked as the leading African consumer of pharmaceuticals, few studies have been devoted to the detection of drug residues in Algerian waters. Only two studies have been conducted to analyze drug residues in various types of Algerian waters. The first concerned the effluents of the SAIDAL group in Constantine, and the second focused on wastewater, surface water, and drinking water in certain areas of Algiers. Saouli and Assabaa, analyzing the effluents of the SAIDAL group in Constantine, detected the presence of Nepagine at 4.7 mg/L, oxcyladine citrate, and salbutamol at concentrations below 1 mg/L. On the other hand, highlighted the presence of four non-steroidal anti-inflammatory drugs, ibuprophen, naproxen, ketoprophen, and diclofenac at the inlet and outlet of the Reghaia and Beni Messous step, in the surface waters of the El Harrach valley and in the tap waters of the USTHB university. ……**[12]**

1.4.2 Impact of emerging pharmaceutical pollutants on the environment

The natural environment is subject to multiple anthropogenic stressors, of which pharmaceutical pollution is now recognized as the major emerging agent of global change.For example, 44% of French people take medication every day2 and pharmacy turnover has tripled in 30 years.3 The invisible nature of this drug pollution, most often mixed with other chemical pollution, and its often insidious effects are factors that contribute to delaying widespread awareness of the problem. Health professionals in particular, have a lever for action on this pollution. This article describes the two main types of drug-related pollution and then outlines solutions to reduce them. Seventy-one thousand tons of medication packaging are thrown away in France each year, with plastic/aluminum blister packaging occupying a prominent place among pharmaceutical packaging.

However, it does not benefit from a recycling channel due to its complex composition of aluminum and PVC (polyvinyl chloride) and clogs up the trash. The micropollution of our waters in Switzerland does not only consist of synthetic pesticides and microplastics. Another invisible danger is accumulating there for which we, as doctors, are largely responsible: medications. It is estimated that 50 tons of medications (compared to 12 tons of pesticides) are found in Lake Geneva, an estimate that is probably only the tip of the iceberg. This pollution comes from human

and domestic animal excrement, discharges from the chemical and pharmaceutical industries, industrial animal breeding and fish farms, which are large consumers of antibiotics and growth hormones. In recent years, the publication of ecotoxicological studies on drugs has exploded, and they are alarming! Approximately 10% of pharmaceutical products pose a risk to the environment due to their solubility index.

The most problematic are hormones, painkillers, antibiotics, cancer drugs, and antidepressants. Wastewater treatment plants (WWTPs) are generally not technically equipped to filter these micropollutants, which end up in the environment and disrupt all aquatic life. Even at low concentrations, around ng/l, drug residues can affect aquatic organisms. They are then bioaccumulated by algae, consumed by zooplankton, and affect all aquatic food chains. The changes in fish behavior observed during chronic exposure alter population dynamics in contaminated ecosystems.

Some exampels: A study on the impact of different drugs including carbamazepine, diclofenac, paracetamol, irbesartan and naproxen demonstrated a genotoxic effect of the molecules on mollusks.8 A much more disturbing effect is described in another report according to which 20% of male fish exhibit female characteristics when exposed to hormones and endocrine disruptors, even in the short term. Some develop ovaries and have reproductive difficulties. Other toxic effects are observed, such as the pharmaceutical substance diclofenac identified as responsible for the near-total extinction of a species of vulture in India, in just a few years, due to its nephrotoxicity in these birds. Up to 90% of the antibiotics used are excreted by humans and animals into the environment. They are not completely eliminated by conventional WWTPs and are released into the environment where they cause genetic resistance in environmental microorganisms. These antibiotic resistance genes can then be transmitted to pathogenic bacteria. A report on the concentrations of antibiotic resistance genes (tetracycline, sulfonamides and erythromycin) in river water upstream and downstream of WWTPs shows that most sites reveal a clear increase in the concentrations of resistance genes. A study by the ECOIMPACT project, carried out on various WWTPs in Switzerland in 2017, demonstrates the ecological consequences of these micropollutants on the composition of biocenoses (all living beings coexisting in a given ecological space) of watercourses and on the functioning of river ecosystems. The observed effects, such as changes in the composition of invertebrate species or the structure of populations of important aquatic animals, are

due to micropollutants. In the coming years, many WWTPs in Switzerland are expected to receive an additional level of sanitation in order to specifically eliminate organic micropollutants…….**[13]**

1.4.3 Impact on human health

It was reported that the presence of pharmaceuticals, pesticides, plasticizers, hormones in waters may generate high risks to human healthdue to its bioaccumulation ,and also creating microbial drug resistance, Bromoform, chloroform, diclofenac, caffeine, ibuprofen, naproxen, methyl dihydro jasmonate, galaxolide, butylated hydroxytoluene, and butylated hydroxyanisole were found in irrigation water using for crops, and also several of these compoundswere afterwards found in the plants .

Hormones act as endocrine disruption agents presenting potential impact in the reproductive health and survival of different fish, and impacting the reproductive health and sustainability of indigenous populations of fish , All these pollutants cited above are different classes of EC´s that are hazardous even at very low concentration levels due to high influence onto live organs and itshigh environmental persistence Pharmaceuticals,hormones and personal care products (PPCPs) are endocrine disrupters)Rovani et al., 2014. which hamper the natural hormonal functionsparticularly in fish and humans (Mills and Chichester, 2005).

The treatment of water containing ECs is emergent due to lack of contaminant removal and/or treatment facilities (Babaei et al. 2016,Saucier et al. 2015; dos Reis et al., 2016). Investigation on ECs is expanding and is encouraged by the progress in finding the appropriate method for wastewater treatment

plants cannot be easily remediated through conventionalEC's are generally found in industrial and municipal wastewater treatment technologies (Rivera-Utrilla et al., 2013). They find way intothe environment via several pathways and the same is diagrammatically. A conventional biological treatment facility may enhance the concentration of some ECs depending on the micro pollutant concentration .The different effluent treatment methods . Most of these treatment methods are not techno-economically feasible for field implementation. These developed methods have some

problems due to the complex procedures,maintenance, high investment cost, toxic sludge generation, toxic byproduct generation, etc

Therefore, effective treatment process for removal of ECs is requisiteOf all treatment methods that have been developed, adsorption is the one most pertinent and promising method for removing organic and inorganic micro pollutant'sThe adsorption processes that are the subject of this study are developing rapidly and are primarily used for the removal of polluting compounds. This technique has demonstrated significant capabilities in the decontamination of wastewater, especially industrial wastewater.

1.5 Adsorption

1.5.1 Definition:

Historically, it was the Egyptians, 3750 BC, who experimented with the science of adsorption,but it wasn't until 1773 that the first experiments on gas adsorption using charcoal were described by Scheele and Fontana. Adsorption is a physicochemical phenomenon that results in a change in concentration at the interface of two immiscible phases; it is therefore a surface phenomenon. Adsorption by a solid can be

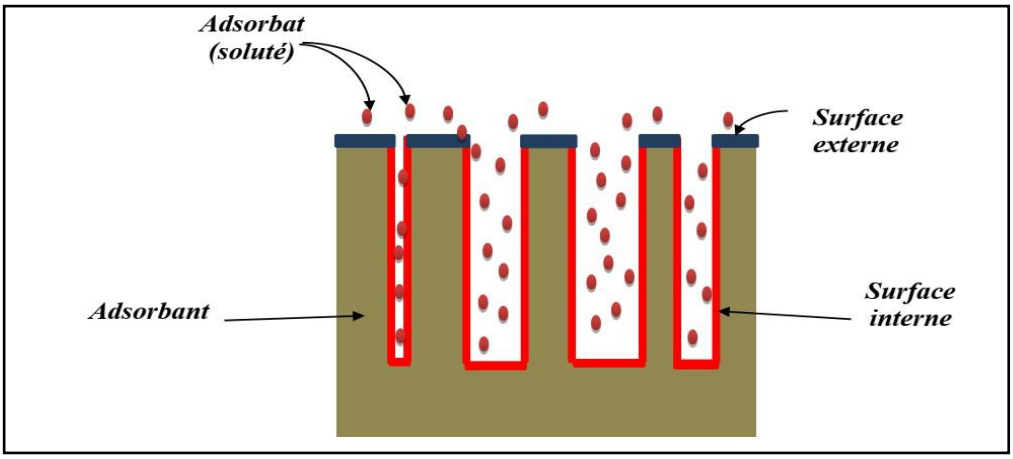


Fig1. 3 : Adsorption phenomenon

defined as the phenomenon of concentration of molecules of a gas or liquid phase on the surface of a solid. This phenomenon isdue to Van Der Waals forces and hydrogen

bonds between the atoms or groups of atoms constituting the surface layer of the solid andthe molecules of the gas or liquid phase in contact with the solid……**[14]**

Adsorption by a solid can therefore be defined as the phenomenon of removal of molecules from a gas or liquid phase by the surface of the solid. The term "surface" should be applied to the entire surface of the solid, the geometric surface for a non-porous solid in grain, to which is added, for a porous solid, the internal surface area generated by cracks and pores. There are five types of interfaces, depending on the nature of the two adjacent phases:

* Gas/solid

* Gas/liquid

* Liquid/liquid

* Liquid/solid

*Solid/solid

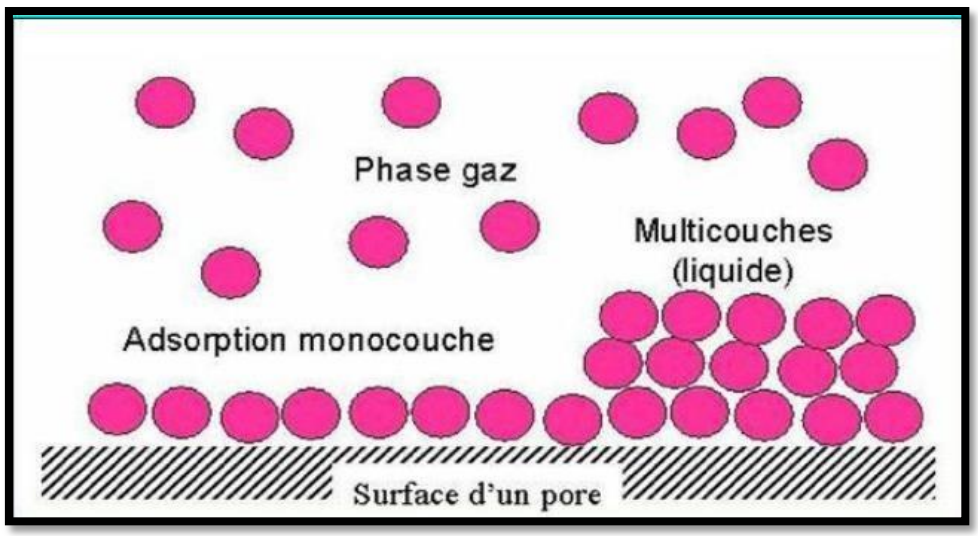


Fig1. 4: Simplified diagram representing the adsorption phenomenon

And for each of these types of interfaces, we can distinguish between cases wherethese phases are pure and those where they constitute mixtures

Generally, adsorption alwaysreferstothe study of gas/solid and liquid/solid interfaces

The solid that is the site of this adsorption iscalled the "adsorbent": the gaseous or liquid compound

1.5.2 types pf adsorption

that undergoes adsorption is called the "adsorbateThere" are two types of adsorption processes: physical adsorption or physisorption and chemical adsorption or chemisorption

1.5.2.1 Chemical Adsorption

Chemisorption is an intermediate step in most catalytic reactions. An example of a chemical adsorption process is the formation of carbon dioxide when oxygen adsorbs onto a carbon substrate. Thus, more heat is released. When the energy change is very large and the heat of adsorption is of the same order of magnitude as that of chemical reactions (10 to 100 kcal or more per mole of gas), the phenomenon is chemical adsorption or activated adsorption. Generally, chemical adsorption is slower and very sensitive to temperature; it requires a higher temperature for gas desorption. Adsorption continues until equilibrium is established between the adsorbed molecules and those in the gas or liquid phase. Equilibrium is established at a rate that depends on the temperature, pressure, and the forces acting between the adsorbate and the adsorbent…….**[15]**

1.5.2.2.Physical adsorption

Physical adsorption occurs when secondary attractive forces

known as Van Der Waals forces bind the adsorbed molecules to the substrate surface

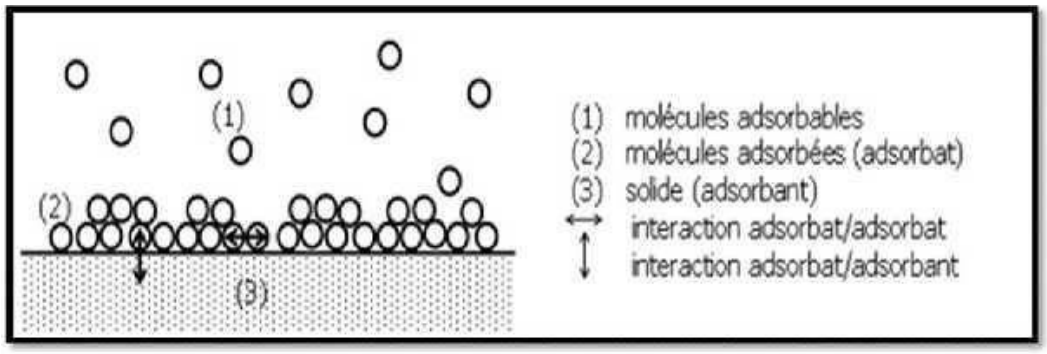


Fig1. 5: Schematic of physical adsorption

The heat generated is of the same order as that observed in the liquefaction of gases. These forces do not destroy the individuality of the adsorbed molecules; they correspond to low energies.

Physisorption is normally an instantaneous process, even at low temperatures: it therefore requires no activation energy, and the gas or liquid molecules are retained by the surface almost as quickly as they reach it. It involves heat values that, relative to one mole of gas, are generally between 2 and 6 kcal.

The phenomenon of physisorption is nonspecific. Every solid has a certain affinity for every gas or liquid. The nature of the bonds in physisorption is Van Der Waals. The adsorption of liquid molecules on the surface of a solid causes the formation of either a monolayer (monomolecular layer) or a polymolecular layer on the surface of the solid. Characterized by rapid reversibility, adsorption-desorption (if the pressure prevailing during adsorption is suddenly reduced)……**[16]**

Table 1.1: Comparison between Physisorption and Chemisorption

Characteristic	Physical adsorption Chemical adsorption	Physical adsorption Chemical adsorption
Prisedesaturation	Phénomènemulticouche	Phénomènemonocouches
Activation energy	No activation energy	Maybeinvolved
Temperature	The yield is more appreciable for a temperature lower than that of the boiling point of the adsorbent.	Adsorption takes place even at higher temperatures
Nature of the support	Yield depends on the adsorbent more than the adsorbate.	Depends on the support and theadsorbate (specific affinity).
Heat of adsorption	Around 40 Kcal/mole.	50-100kcal/mole
Binding	VanDer Waal Physics Chemistry	VanDer Waal Physics Chemistry
Specificity	Non-Specific Process	Specific Process
Desorption	Easy	Difficult
Kinetics	Fast	Slow

The difference between chemisorption and physisorption boils down to a difference in the types of forces binding the liquid molecules to the surface of the solid. Physical adsorption is rapid and does not involve any activation. In contrast, chemisorption is generally slow and requires activation energy in many cases. Physisorption occurs at temperatures not exceeding the boiling point of the adsorbate, while chemisorption can occur at higher temperatures.Adsorption is an exothermic process that therefore produces heat, which can lead to heating of the solid and a reduction in the adsorbed quantities. Temperature variations are often significant in industrial adsorption

processes and can be a major factor in performance degradation. The exothermicity of an adsorption system is characterized by the heat of adsorption, which can be measured using calorimetric techniques or estimated from adsorption isotherms at different temperaturesAdsorptionMechanismAdsorption occurs primarily in four stages. ……**[17]**

1.6 mechanism of the adsorbtion

1.6.1 . Diffusion of the adsorbate from the external liquid phase to the phase located near the adsorbent surface

1.6.2 Extragranular diffusion of the material (transfer of the solute through the liquid film to the surface of the grains)

1.6.3 Intragranular transfer of the material (transfer of the material within the porous structure of the outer surface of the grains to the active sites

1.6.4 Adsorption reaction upon contact with the active sites. Once adsorbed, the molecule is considered immobile

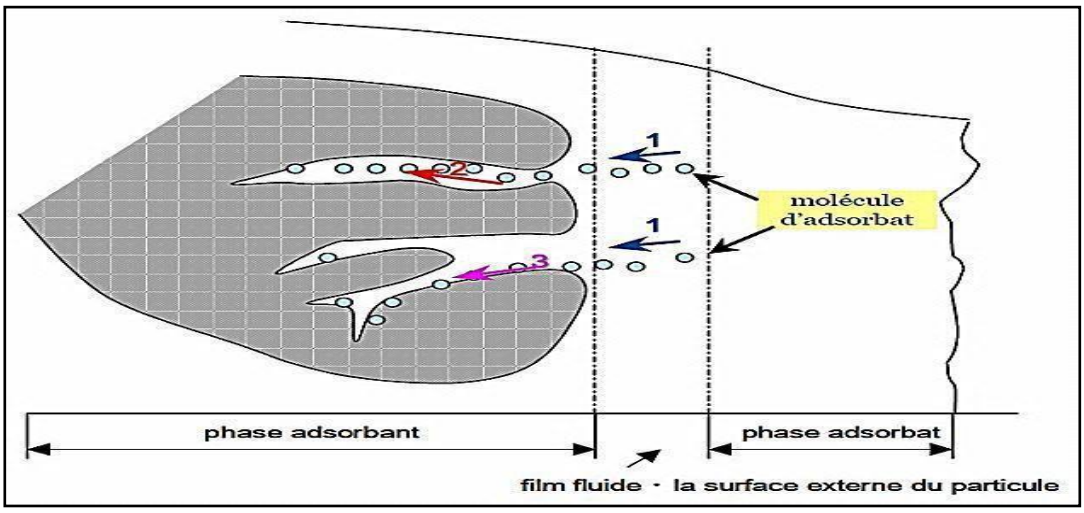


Fig1. 6: Diagram of the transport mechanism of an adsorbate in a grain

1.7 Review of adsorption techniques in the removal of EPs from wastewater

Rathi and Kumar et al. presented an approach to address withthe growing concern about new pollutants in the marine ecosystemhighlighting the shortcomings of

conventional wastewater treatmentplants in removing these contaminants. An economical tertiary treatment method is needed because standard primary and secondarytreatments are ineffective. In response, the effectiveness of adsorption isexamined in this literature review as a practical and affordable methodof eliminating novel pollutants. Adsorption has considerable promise asa replacement due to its low installation costs, excellent performanceand ease of use. The article's main objective is to demonstrate howseveral adsorbents, including activated carbons, enhanced bio-Charsnano adsorbents, and hybrid adsorbents, may effectively remove Epsfrom wastewater. By examining the adsorption process, the studyhighlights how beneficial it is for treating these toxins. The study provides a comparative summary of the effectiveness of natural and artificial adsorbents in removing contaminants by rigorously examining both types of adsorbents. The final section of the report examines the role that emerging contaminants and adsorbents will play in the current generation. This review of the literature advances our understanding of the causes of pollution and the vital role that adsorption plays in mitigating the problems that emerging toxins in marine ecosystems pose……**[18]**

1.7.1 Factors influencing the adsorption phenomenon

When a solid is brought into contact with a solution, each constituent of the latter, the solvent and the solute, shows a tendency to adsorb on the surface of the solid. There is therefore a competition on the surface between two adsorptions which are Competitive. The most interesting case is the one where the adsorption of the solute is far greater than that of the solvent. Therefore, the quantity adsorbed depends on many factors, the main ones being:

***1.7.1.1 Temperature*:** Adsorption is an endothermic or exothermic phenomenon depending on the adsorbent material and the nature of the adsorbed molecules.

1.7.1.2 Nature of the adsorbent:The adsorption of a given substance increases with the decrease in the size of the adsorbent particles, which allows the compounds of the solution to penetrate into the capillaries of the substance, therefore the subdivision of the solid particle directly influences the pores of the latter as well as its specific surface which will be developed. However, if the dimensions of the pores are smaller than the diameters of the molecules of one of the components of the solution, the

adsorption of this compound does not take place, even if the surface of the adsorbent has a high affinity for this compound.

1.7.1.3 -Nature of the adsorbate

For good adsorption to occur, there must first be an affinity between the solid and the solute. As a general rule, polar solids preferentially adsorb other polar bodies. On the other hand, non-polar solids preferentially adsorb non-polar substances, and the affinity for the substrate increases with the molecular mass of the adsorbate. This was already stated by Traube's rule and supplemented by Freundlich, who wrote that the adsorption of organic substances from aqueous solutions increases strongly and regularly as the chain length increases within a homologous series; It is characterized by

- **Its polarity:** a polar solute will have a greater affinity for the more polar solvent or adsorbent
- **Its molecular weight:** the solubility of a pollutant decreases with increasing molecular weight
- **Its molecular structure**…….. **[19]**

1.7.1.4.Orientation of molecules: The orientation of molecules adsorbed on the surface depends on the interactions between the surface and the molecules adsorbed in solution. It is difficult to predict the orientation of the adsorbate molecules on the solid, this is the case for example of the adsorption of fatty acids of general formula CH3-(CH2) nCOOH in an organic solvent adsorbed on certain metals such as platinum, the orientation is vertical and the molecular area of the acid is 20.5 A°2 on the other hand on carbon black the fatty acid molecule is oriented flat 114 A°2, in both cases we obtain an isotherm of type H

1.7.1.5.Specific surface area: The specific surface area is an essential data in the characterization of solids and porous materials. It is clear that we are seeking to give adsorbents a large specific surface area; this quantity designates the accessible surface area related to the unit weight of adsorbent.

1.7.1.6 PH :pH is an important factor in any adsorption study, as it can influence both the structure of the adsorbent and adsorbate, as well as the adsorption mechanism.

Indeed, it affects both the surface charge of the material and the distribution and speciation of the cations

1.7.1.7 Agitation:

Agitation can impact the distribution of solute molecules in the solution, thus promoting better homogenization between the adsorbate and adsorbent in the suspension and leading to faster achievement of equilibrium.

***1.7.1.*8. Adsorbate Concentration**

For low concentrations of dissolved substances, the adsorption rate generally follows Freundlich's law. With increasing concentration, adsorption is sometimes observed to reach a maximum and then decrease to become negative, but it is difficult to quantitatively predict the variation in adsorption rate as a function of the concentration of the dissolved substanceClassification of Adsorption Isotherms ,To describe the adsorption equilibrium at the liquid/solid interphase, it is recommended to present the variation in the amount of solute adsorbed per unit mass of adsorbent (qe) as a function of the remaining concentration in the solution (Ce) at equilibrium at a constant temperature using the following equation:

$$q_e = \frac{(c_0 - c_e) \times v}{m}$$

Or :

- **v**: volume of the solution (l)
- **c0**: initial concentration of the adsorbate in the liquid phase (mg.l-1)
- **ce**: concentration of the adsorbate in the liquid phase at equilibrium (mg.l-1).
- **m**: mass of the adsorbent (g)

The shape of the isotherm varies depending on the adsorbate-adsorbent pair studied. Adsorption isotherms of solutes with limited solubility have been classified by Giles et al. [47] into four main types S: Sigmoid; L: Langmuir; H: High affinity; C: Constant partition. This classification takes into accounta number of hypotheses:

a- the solvent adsorbs on the same sites as the solute. This implies the existence of adsorption competition between the solvent and the solute.

b- The number of sites capable of receiving solute molecules on the surface of the solid decreases as the adsorbed quantity increases.

c- The orientation of the molecules on the surface. One can cite the case where the molecules are adsorbed vertically or horizontally on the surface

d- Finally, the attractive or repulsive interactions between the adsorbed molecules are manifested in a notable way in the adsorption phenomenon

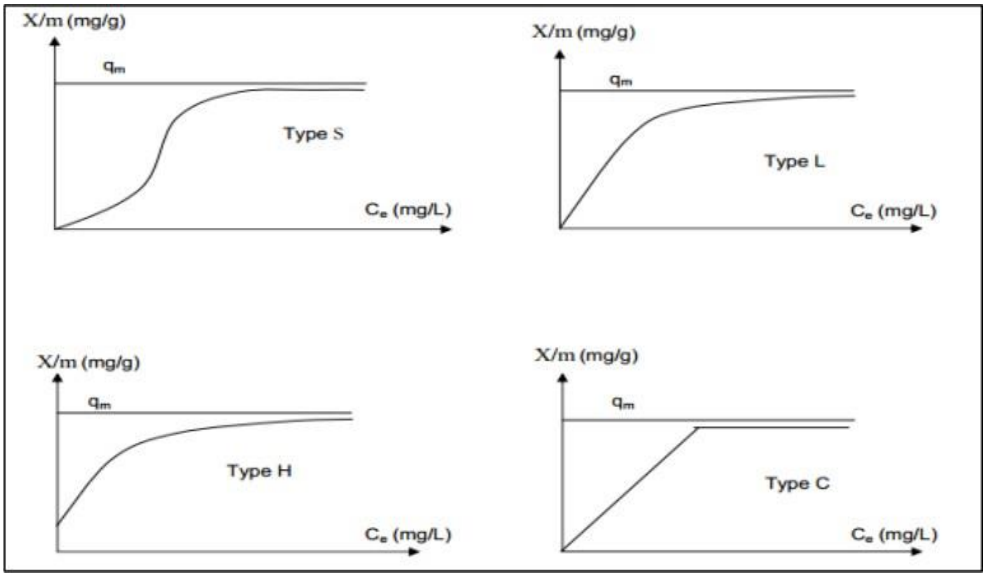


Fig1. 7 : Liquid phase adsorption isotherms

****Type S isotherms**:* Isotherms in this class exhibit, at low concentrations, an upward-facing concavity.

***Type L isotherms**: Isotherms in this class exhibit, at low concentrations, a downward-facing concavity, which reflects a decrease in free sites as adsorption progresses.

*** *Type H isotherms:*** The initial portion of the isotherm is nearly vertical; the adsorbed quantity appears significant at a near-zero solute concentration in the solution.

**** Type C isotherms***: Isotherms in this class are characterized by a constant partition between the solution and the substrate up to a plateau.

:

1.7.2 The main industrial adsorbents

1.7.2.1 .Natural minerals (clays, zeolites, silicas…)

1.7.2.2 .Industrial by-products (bauxite, petroleum shale Ashes)

1.7.2.3. Agricultural by-products (chestnut shells, almonds, rice bran, pastel color(

Active biomassyeasts)

1.7.2 4 .Synthetic materials (chitosan, chitin)(

1.7.2 criterias of Industrial adsorbents

Polymers Industrial adsorbents must meet a number of criteria's

1. high adsorption capacity
2. high efficiency
3. high selectivity
4. physical strength
5. chemical inertness
6. easily regenerated
7. lowcost

1.8 Prospects of biosorption in developing countries

1.8.1 biosorption: is a type of adsorption where we use biologically living organisms to deal with the adsorption and especially when the microorganism is dead

and it is an alternative technology for the removal ofa wide range ofpollutants from aqueous systems. This technology entails using naturalor engineered adsorbents derived from biomass for the removal of contaminants, and could be useful in the treatment of secondary or tertiary effluent. Compared to conventional techniques such as ion exchange, coagulation and membrane separation, this approach has

several advantages including low cost due to abundance of biomass, high selectivity, are regenerative and thus extend the life of waste materials, required less sophisticated operation skill, have limited sludge generation and generally have performance comparable to that of conventional techniques

In developing countries, the application of biosorption for removal of organic contaminants is attractive for three reasons; large quantities of biomaterials (e.g. crop residues, agro-processing wastes) for use as feedstock for biosorbents are readily available) lack of advanced water and wastewater treatment systems for removal of organic contaminants; and (3) the technology is relatively cheap compared to advanced methods (e.g. membrane filtration) often used in developed countriesAnd here we have used an algae as adsorbent**……..[20]**

1.9 Algaes

1.9.1 Definition of Algae

Algae are a group of photosynthetic plants whose life cycle grows in aquatic environments (freshwater, thermal waters, marine environments). They are highly diverse and have a relatively simple vegetative apparatus called "thalli." They vary greatly in shape, color, and size. Some are microscopic and others measure several meters in length. Algae are divided into two main categories: microalgae (invisible to the naked eye, found in plankton, such as cyanobacteria) and macroalgae (visible to the naked eye, found mainly in shallow waters, and consisting of green, brown, and red algae)…….. **[21]**

Fig1. 8 : Algal environment

1.9.2 Classification of Algae

Algae are classified according to specific characteristics such as cell wall components and pigments present. In general, algae fall into four groups, which are differentiated by the color of their pigments, and each group contains hundreds of species

1.9.2.1 Chlorophytes

These are green algae, whose thallus is typically green due to the dominant chlorophylls a and b in the chloroplasts. Green algae are present in all aquatic systems, from marine to freshwater environments. Theyplay an important role in water oxygenation

Fig1. 9: Green algae (Chlorophytes)

1.9.2.2 Rhodophytes

Rhodophytes are red algae. They form a highly diverse group and exhibit a distinctive feature, with their red (phycoerythrins) and blue (phycocyanins) pigments that mask the chlorophyll. Furthermore, even within a single species, the color varies depending on exposure to light

Fig1. 10 : Red algae (Rhodophytes)

1.9.2.3. Chromophytes

Chromophytes are brown algae. Their color is due to the abundance of the brown pigment fucoxanthin, which masks chlorophylls a and c. They are found in a wide variety of morphologies, from relatively simple filamentous forms to complex morphological structures (leafy stems of higher plants). They are exclusively marine algae. Brown algae consist of a polysaccharide skeleton, a polysaccharide matrix, and a protein network…….**[21]**

Fig1. 11 : Brown Algae (Cormophytes)

1.9.2.4 Cyanobacteria Cyanobacteria or blue algae

are made up of colonies of very variable size, shape and color. They possess several blue pigments (Phycocyanins) and red pigments (Phycoerythrins) which mask chlorophyll a

1.9.3 Chemical Composition of Algae

Algae differ significantly from terrestrial plants in their chemical and physiological composition, as well as their morphological characteristics

1.9.3.1 Algae are generally composed of

- Carbohydrates: which are mainly in the form of polysaccharides (carrageenan agar in red algae, alginates in brown algae, and Ulvales in green algae). Glucose, cellulose, hemicellulose, and pectin are also present in small amounts.
- Protein: the content of which varies depending on the species, seasons, and environmental conditions.
- A small amount of lipids: approximately 0.9 to 5.2% of the dry weight. As well as
- Phenoliccompunds
- High mineral content: Potassium, Chlorine, Calcium, Magnesium, SulfurP
- hosphorus, Iodine, Iron, Copper, Manganese, and many other trace elements
- Vitamins:(A, B1, B2, B6, B12, C, E, K)
- Pigments: carotenoids, chlorophylls a and b

1.9.4 Uses of Algae

Algae has been used since ancient times, initially as a source of human nutrition. Asian countries are the main consumers of seaweed. Millions of tons of seaweed were consumed, including 15.8 million tons from seaweed farming in 2010. Thanks to the diversity and richness of its components, Algae is used in several areas, including:

1/ Food

In Asian countries and even in Europe, seaweed is either directly consumed fresh or processed by the food industry to obtain dried or frozen products, incorporated into foods (tartars, terrines, pasta, sushi, etc.), or as vegetables

2/In the Food Industry

Alginates, agars, and carrageenans, extracted from red and brown algae, are used in the food industry as emulsifying, thickening, and gelling agents (additives E400 to E408), and excipients

3/ In Medicine

In thalassotherapy, seaweed baths (algotherapy) are used to treat rheumatism and certain musculoskeletal disorders. Seaweed is also used as a dewormer, anesthetic, and ointment for treating coughs, wounds, and goiter

4 / In Pharmaceuticals

Many pharmaceutical specialists incorporate algal colloids into their formulations as excipients (syrups, pill coatings). Seaweed extracts are of significant interest in the pharmaceutical industry for the development of new drugs against cancer, inflammation, and microbial infections.

5/ In cosmetics

Seaweed extracts (vitamins and amino acids) nourish, protect, soothe, and slow aging, as they exhibit anti-UV and antioxidant properties, which are used in various skin creams (masks, scrubs, soaps, and anti-wrinkle creams)

6/ In_agriculture

Seaweed is used directly on land to enrich mineral salts or use them as biofertilizers

7/Inwater purification

Seaweed is used in wastewater treatment by binding heavy metals (lead, mercury, etc)

And here we are going to speake about the use of an alguespirogyra In water purification:

The identification of the selected algae was based on macroscopic characteristics such as color, shape, size, and location……..**[22]**

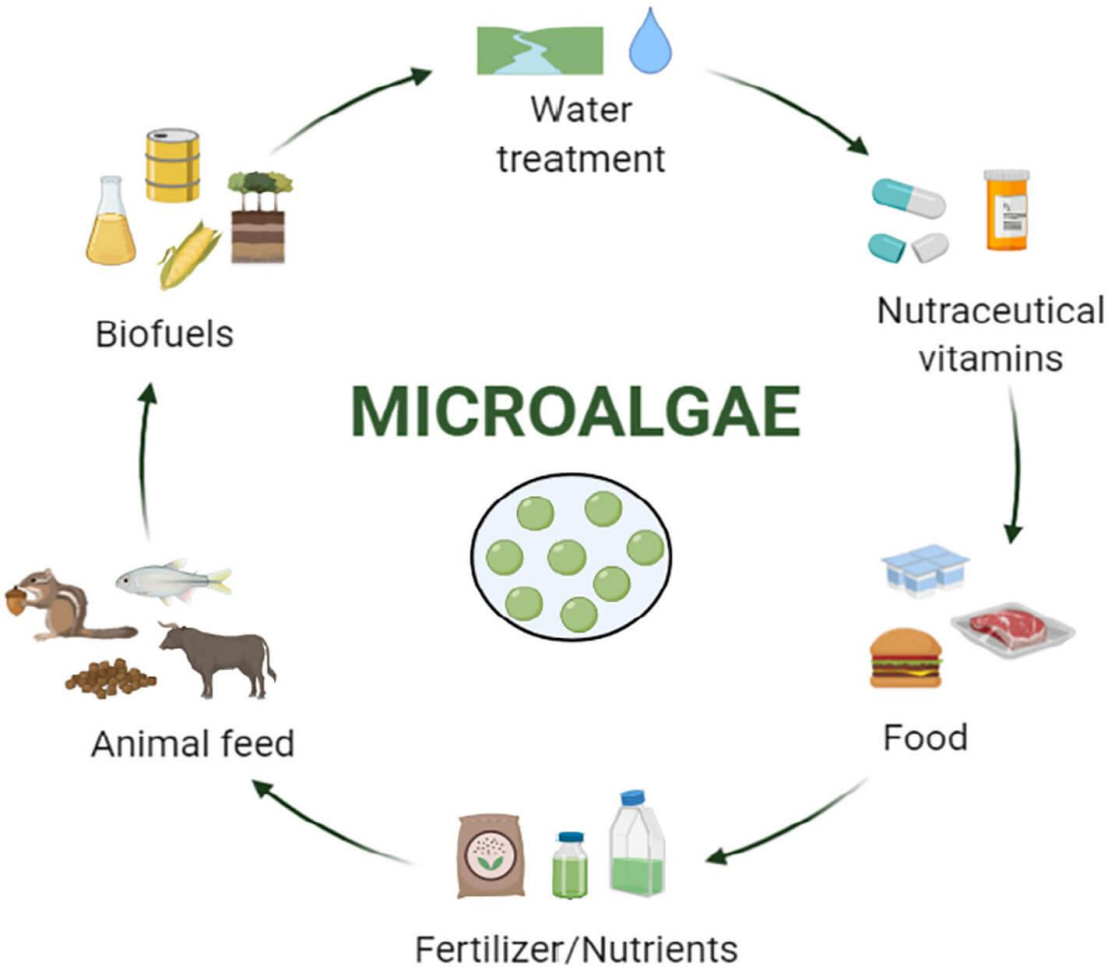


Fig1. 12 :Algae uses

1.9.5 Spirogyra (*spirogyra*)

Is a genus of green algae with approximately 300 species that all live in fresh or brackish water and have a flocculent appearance and a slimy texture. These algae thrive in clear, cool water. They colonize the aquatic environment freely (unattached) in the water column and even on the sediment in stagnant or slow-moving water, and more rarely, attached to the sediment or to rocks or walls……**[23]**

6.5.1 Systematic position

- **Kingdom**: Plantae
- **Phylum**: Chlorophycophytes
- **Class**: Zygophyceae

- **Order**: Zygnematales
- **Family**: Zygnemataceae
- **Genus**:Spirogyra

Fig 1. 13 :Spirogyra (spirogyra)

2. Material And products

2. MATERIALS AND METHODS

2.1 Material and products

2.1.1 Material

Petri dishes ;Test tubes ;Pasteur pipettes and micropipettes ;Erlenmeyer flasks ;

Sterile flasks ;Sterile filter ;Autoclave ;Laminar flow hood ;Regulated incubator ;

Caliper;Bunsen burner ; Magnetic stirrer hot plate ; Water bath ;Rotary evaporator ;

Magnetic stir bars ;Round-bottom flasks ;Beaker ;, dropperpH meter ;UV-VISIBLE spectrophotometer ;HPLC ;AFM;IR

2.1.2 Products

Distilled water ;Ethanol ;Acetone ;SDA (sabouraud Dextrose Broth)

DMSO (Dimethyl sulfoxide) ;Sodium hydroxide (NaOH) ;Hydrochloric acid (HCl) ;

Sodium chloride (NaCl) ;sterile physiological water

2**.2 PREPARATION OF THE ADSORBENT**

2 2.1 Sampling Area

Oued el Mellah

Our study area is located in the municipality of Zarzour (Daira of Bensrour). It is a freshwater stream called "Oued elMellah" characterized by a seasonally variable flow with permanent water. Oued elMellah has a depth of 30 cm to 1 m and a width of 67 m.

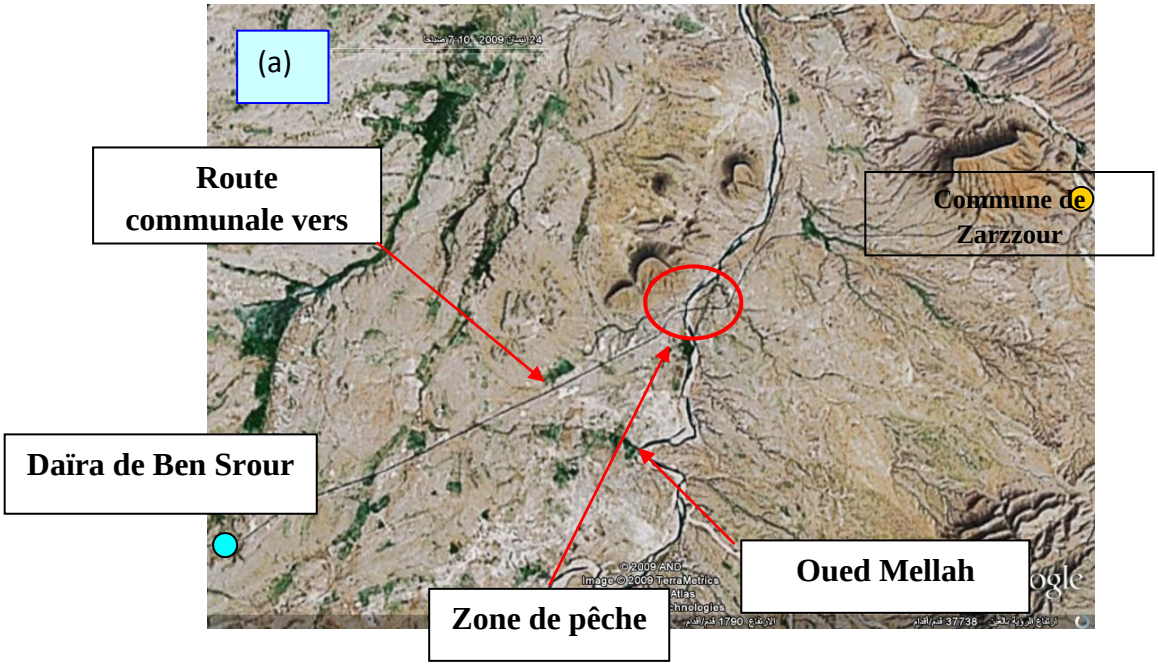


Fig.2.1: Picture representing the sampling area. (google earth, 2009)

2.2.2. Sampling

Microalgae were harvested in areas where *Spirogira* blooms , and samples were collected using a chain-link net (0.5 cm mesh size). Sampling was carried out in March 2010.

The operation consisted of recovering the algae from the surface layer by a simple horizontal movement until a cellular mass in the form of a green ball was obtained

A) Selection of the area containing the most algae.

Fig.2.2: Picture of the sampling location.

B) Harvestingusing a net:

(a) (b)

Fig.2.3 Picture of algae collection.

C) Form algae into balls for easier transport.

Fig.2.4: Picture showing algae preservation

2.2.3 in the laboratory

The algae were rinsed with tap water, followed by distilled water.

Dried at room temperature for four days .Then ground and sieved

Fig.2.5 :picure of drying (left) and grinding (right) of algae

2.3 Algal Extract Preparation

2.3.1 The procedure followed three steps:

Maceration (Kasinathan et al., 2009)

Three different solvents were used: hexane, ethanol, and acetone, at a 1:3 ratio (algal powder weight: solvent volume). Maceration lasted 48 hours at room temperature in the dark.

Fig.2.6: Maceration of the sample.

1. Centrifugation

After 48 hours, each sample was centrifuged at 1500 rpm for 15 minutes to remove macromolecules.

Fig.2.7: Centrifugation

2 .Evaporation

The supernatants were processed using a rotavapor at 47°C and 270 rpm.

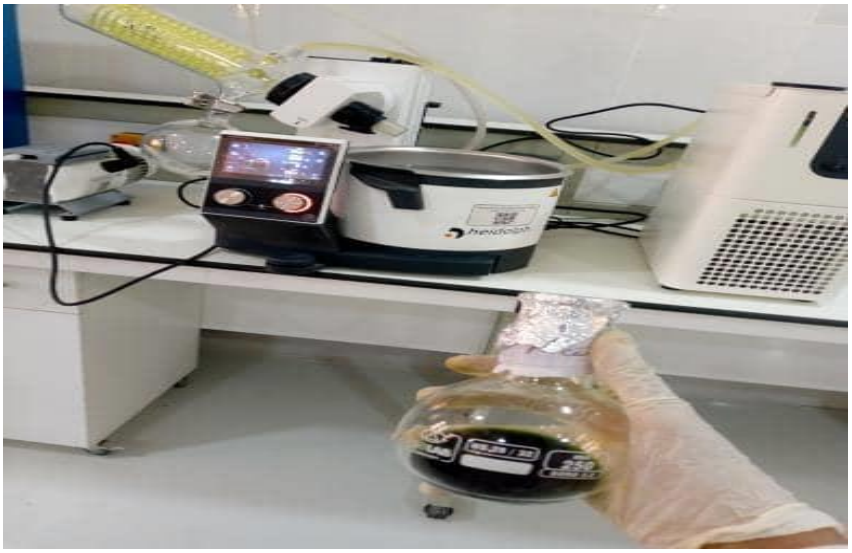


Fig.2.8: Picture showing the evaporation process using the Rotavapor.

To determine the dry extract amount, the flask was weighed before and after evaporation.

Dimethyl sulfoxide (DMSO) was used as the recovery solvent, with a ratio of 2 ml DMSO for 30 g of algae. Extracts were stored at 4°C.

Fig.2.9: Recovery of algal extract.

2.4 . Antibacterial Testing

2.4.1. Microorganisms Used:

Listeria monocytogenes (ATCC 19115), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (Pasteur Institute of Algiers), *Staphylococcus aureus* (43300 MRSA) And *Candida albicans*

2.4.2. Strain Confirmatio:

Catalase Test

This enzyme is capable of decomposing hydrogen peroxide according to the following reaction:

Catalase

$$H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$$

This test aims to differentiate between lactococci or leuconostocs (catalase-negative) and enterococci (catalase-positive). On a clean and dry glass slide, a drop of hydrogen peroxide (10-volume concentration) is placed using a Pasteur pipette, then the bacterial inoculum is added. The observation is immediate. If bubbles and oxygen gas release appear, the catalase test is positive. If there are no bubbles, the catalase test is negative.

2.4.3 Gram Staining

1. Smear Preparation on Slide

A drop of bacterial culture is taken with a sterile pipette, placed, and spread on a slide.

A thin and homogeneous smear (1 cm^2) is prepared.

2. Drying

The smears are left to air dry (this step can be accelerated by gently heating).

3. Fixation

The smears are fixed by covering the slide with alcohol for 1 to 2 minutes.

4. Gram Staining (basis of bacterial classification)

The smear is covered with crystal violet, rinsed with water, then covered with Lugol's iodine and left for 15 seconds.

The smear is rinsed with water, then decolorized with alcohol by tilting the slide until the alcohol no longer removes any color. It is then thoroughly rinsed with water.

A few drops of fuchsin are added and left for 30 seconds to 1 minute, then rinsed and dried.

5. Microscopic observation is carried out using an optical microscope.

6. Gram staining:

*Gram+ :Purple bacteria.

*Gram - : Pink bacteria.

2.4.4. Preservation of strains

The strains were preserved at 4°C in sterile tubes containing 10 ml of slanted culture medium (nutrient agar for bacteria and Sabouraud agar for yeasts).

2.4. 5. Antibiogram testing

The antimicrobial effect was tested using different commonly used antibiotics on five pathogenic strains, in order to compare the therapeutic effectiveness of algal extracts.

2.4.5.1 Media used

*Nutrient broth.

*Mueller-Hinton agar (MH) poured into Petri dishes with a thickness of about 4 mm.

2.4.5.2 Inoculum

Starting from a preculture of about 18 hours in nutrient broth, a bacterial suspension was prepared in the same nutrient broth and homogenized. The optical density had to be between 0.08 and 0.1, measured at 625 nm (10^8 CFU/mL).

The inoculum can be adjusted by either adding more culture if it's too weak, or sterile nutrient broth if it's too concentrated.

Seeding must be carried out within 15 minutes following the preparation of the inoculum.

2.4.5.3 Seeding

0.15 mL of the bacterial suspension was placed on the surface of Mueller-Hinton agar, spread using a sterile swab by rubbing it over the entire surface of the dry agar, from top to bottom, in tight streaks.

The operation was repeated three times, rotating the Petri dish 60° each time, without forgetting to rotate the swab on itself. The seeding ended by passing the swab around the edge of the agar.

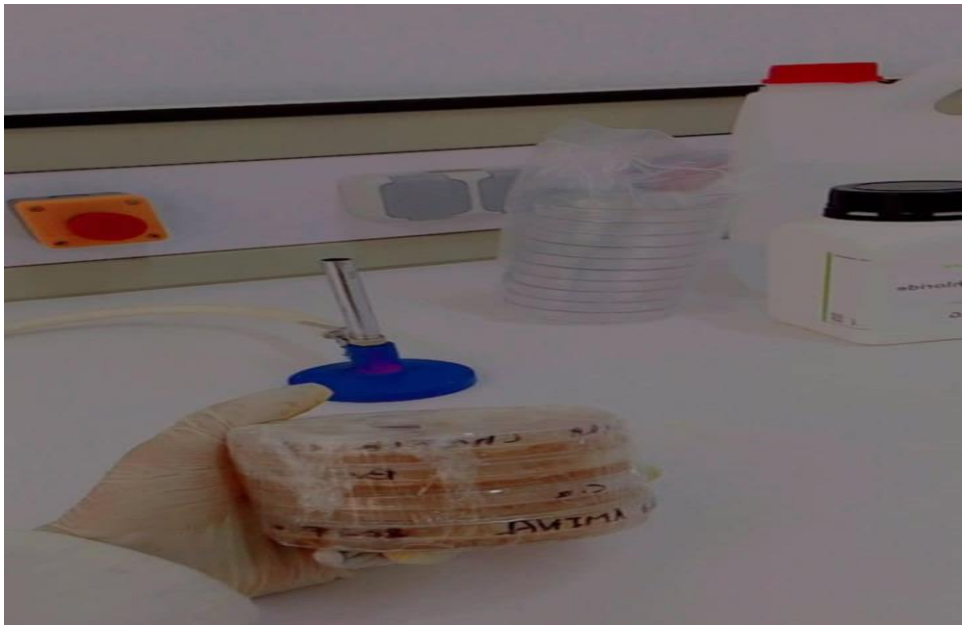


Fig.2.10: Petri dishes of Candida albicans

List of antibiotics used

Ampicillin (AM), Tetracycline (TE), Penicillin (P), Enrofloxacin (ENR), Chloramphenicol (C), Trimethoprim + Sulfonamides (SXT), Cephalexin (CN), Erythromycin (E), Oxacillin (OX), Ceftazidime (CAZ), Nalidixic Acid (NA), Neomycin (N), Amoxicillin (AMC).

Table 2.1 : List of antibiotics used on the skin

Bacterial species	**Antibiotics tested**
Listeria monocytogenes	C, AM, TE, P, SXT, CAZ, ENR, CN, OX, E, N,
Escherichia coli	C, AM, TE, P, SXT, CN, OX, E, N, NA, AMC
Pseudomonas aeruginosa	C, AM, TE, P, CAZ, ENR, CN, OX, E, N, NA
Salmonella typhi	C, AM, TE, P, CAZ, ENR, CN, OX, E, N, NA
Staphylococcus aureus	C, AM, TE, P, SXT, ENR, CN, OX, E, N, AMC

2.4.6 Application of Discs

It is preferable not to place more than 6 antibiotic discs on a 90 mm diameter Petri dish. The antibiotic discs must be spaced 24 mm apart, center to center.

Press each antibiotic disc using sterile bacteriological forceps to ensure proper application. Once applied, the disc must not be moved.

Incubation:

The plates are incubated for 24 hours at 37°C.

Fig.2.11:Incubation at 37 degre c

2.4.7 The well method in agar

It consists of creating wells in an agar inoculated with*Candida albicans* and then depositing the substance to be tested. 100 ul of spirogyra extract

We made the wells by Heat the Pasteur pipe from the bottom, then print it on the gelatin, which creates holes that we remove with the pointed tip of the pipe. Here we get the wells in our SDA

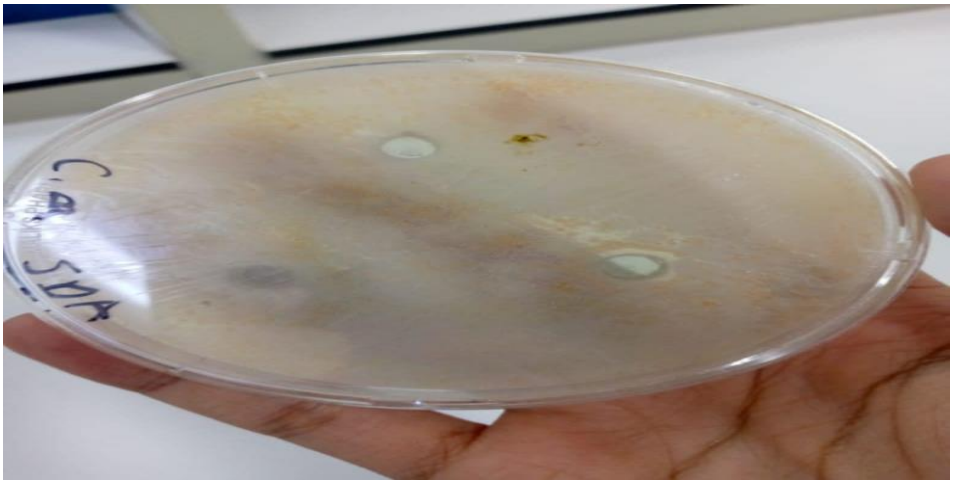


Fig.2.12:wells in Agar method

2.4.7 Reading

The diameters of the inhibition zones are measured precisely using a caliper.

The results are compared with critical values.

Depending on the diameter of inhibition, the bacteria are classified into one of the following categories: Sensitive, Intermediate, or Resistant.

2.4.7.1 Evaluation of Antimicrobial Activity

Culture media used:

Nutrient broth and Mueller-Hinton agar for bacteria.

Bacterial Pre-cultures:

For each microorganism, 10 ml of sterilized culture medium (Nutrient Broth) was inoculated with a colony taken from a preserved culture.

Agar diffusion method (disc method):

Sterilized filter paper discs of 5 mm diameter, impregnated with 2 µl, 5 µl, and 7.5 µl of algal extract, are placed on the surface of Mueller-Hinton medium poured into Petri dishes, previously surface-seeded with a bacterial suspension. (See antibiogram).

After 24 h incubation at 37°C ± 1°C, the results are read by measuring the diameter, in mm, of the inhibition zones

2.5 physical characterization (adsorption)

Before all we have filtered our algae powder in 3 different diameters of the sieves: 200µm. 250µm. 500µm

Fig.2.13 : The Algae powder

2.5.1 First of all we first passed our powder through the largest 500 µm sieve We throw what is left in the sieve and take what falls on the plate

Then We take what falls on the plate and pass it through the sieve of 250 µm what left we take it as Approximately 500µm.

Finally, what falls on the plate from the sieve of 250 µm, we pass it again through the last sieve of 200 µm. We take what fell on the plate as it is, 250 µmand what remains in the sieve is 200 µm.

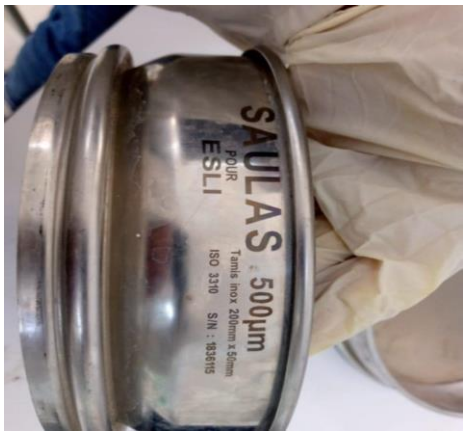


Fig.2.14 : the filtration sieves

Fig.2.15: different powder particle sizes

2.5.2 Characterization of Adsorbents

2.5.2.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

A. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Adsorbate Before Adsorption

We just analyzed the smallest particle size of our power (200 µm)

B. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Adsorbate After Adsorption we have made a contacte between our micropolluants (paracetamol / diclofenac) for couple of hours we measure 1.5 g of our algae powder (200um) and put it in 20 mL of distilled water and concentration of the pollutant(

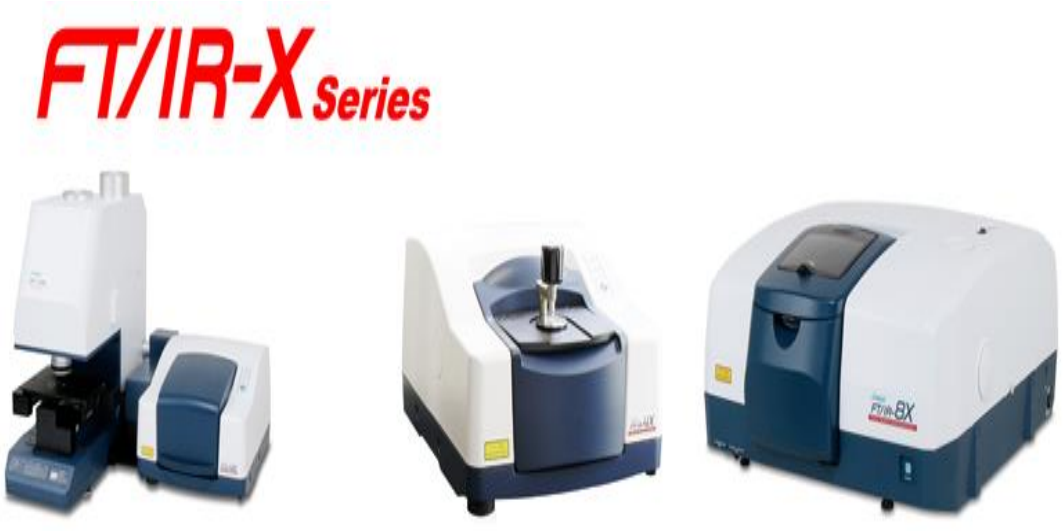


Fig.2.16: Fourier transform infrared spectroscopy (FTIR)

2.5.2.2 Microscopic Analysis by atomic force microscopy (AFM)

A . Microscopic Analysis by atomic force microscopy (AFM) Before Adsorption

B. Microscopic Analysis by atomic force microscopy (AFM) After Adsorption

PREPARITIONS ARE THE SAME AS (FTIR)

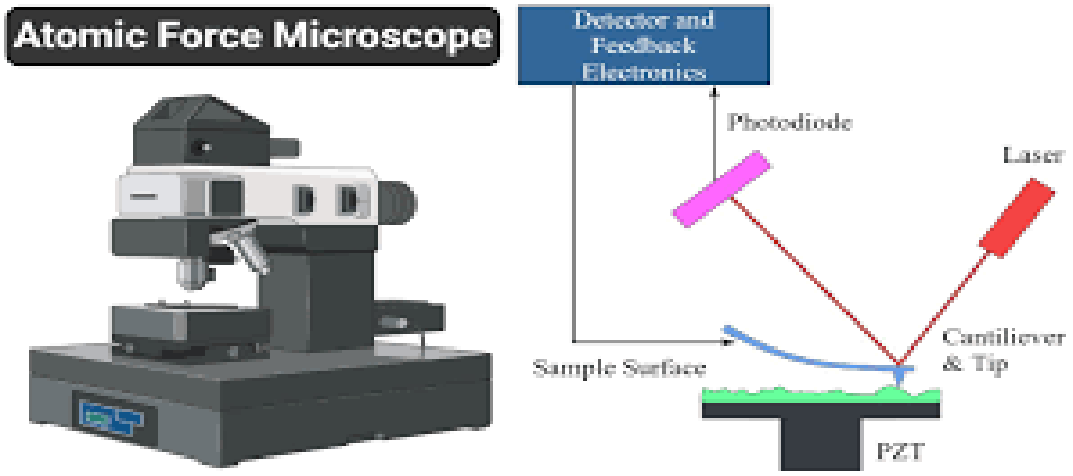


Fig.2.17: atomic force microscopy (AFM)

2.6. parametric study

here we are going to see the effect of sertains parameters on the algal Adsorption

2.6.1. pH of Zero Charge Point (pHpz.)

The pH of the zero-charge point is a very important parameter that provides a chemical explanation for the adsorption phenomenon. It represents the pH value at which the net charge on the surface of the adsorbents is zero. In this study, the pHPZC was determined using the first bisector method. To determine the pHpzc, 50 ml of NaCl (0.1 M) are placed in closed flasks and the pH of each is adjusted (to values between 2 and 12) by adding NaOH or HCl solution (0.1 M using a pH meter). Then, 50 mg of the material sample to be characterized (our adsorbent algae *Spirogyra*) is added to each flask. The suspensions must be kept stirring at room temperature for 24 hours, and the final pH is then determined.

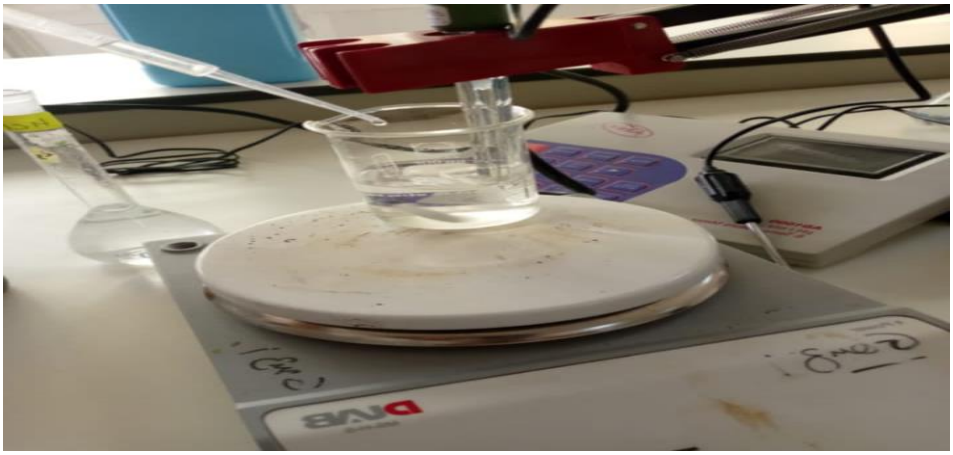


Fig.2.18:pH adjustment

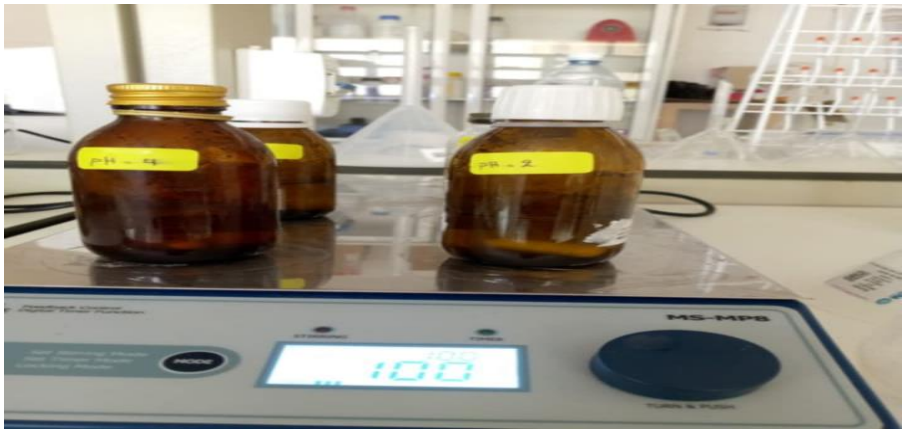


Fig.2.19: agitation of suspensions

2.6.2.Dosage of PEs

•PE analysis method

- **H=f([PE])**

And we used different concentrations (10. 20.30.40.50) ug/l

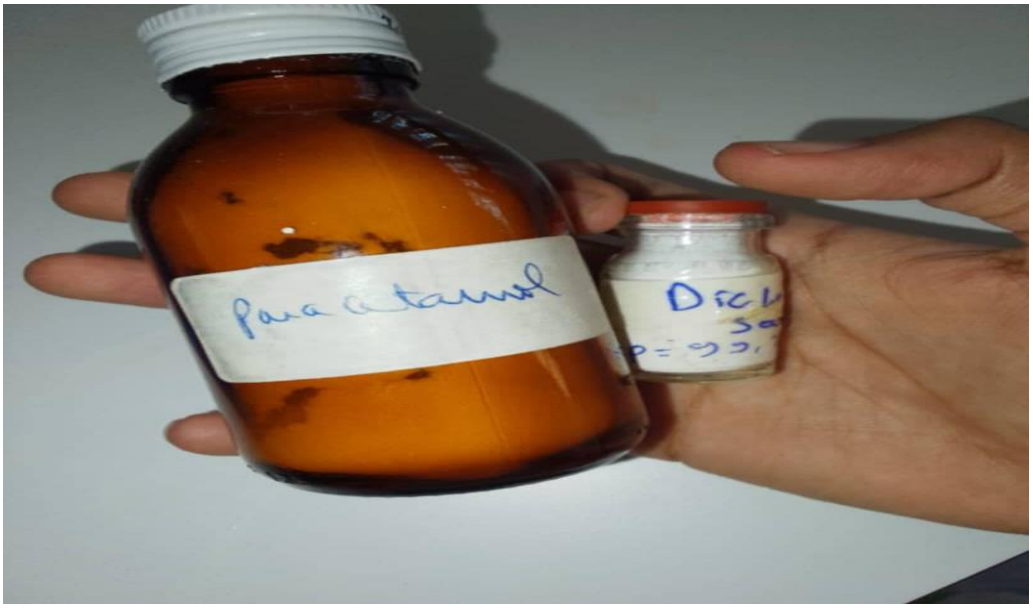


Fig.2.20 :the active ingredients of paracetamol and diclofenac

We made those varied concentrations by:

2.6.2.1 preparation of stock solution

We take 1g of the active polluant and put it on 1L of sterile distilled water (1g/L)

2.6.2.2 the preparation of different concentrations

1 For the (ug/l)

First we take 100 ul using micropipette then we put it on 1L of sterile distilled water to make 1mg/L

Then in order to make the desirable concentrations we take:

From our (1mg/L) solution with micropipette:

Table 2.2 : (ug) concentrations

Ci(ug/L)	V(uL)
10	200
20	400
30	600
40	800
50	1000
60	1200
100	2000
150	3000

Fig.2.21 : micropipette

.2 for the (ng/L)

First we make the preparation of (1ug/L) solution by taking 100uL of the 1mg/L solution and putting it in 1L of sterile distilled water to make 1mg/l

then in order to make the desirable concentrations we take:

From our (1ug/L) solution with micropipette

Table 2.3 : (ng) concentrations

Ci (ng/L)	**V (ul)**
10	200
20	400
30	600
40	800
50	1000
60	1200
100	2000
150	3000

Fig.2.22: paracitamol and diclofinacsollutions

2.6.3 Parametric variation studies

2.6.3.1 . Effect of particle size

The effect of the biosorbent particle size (between 200 um and 500um) on the adsorption capacity of microalgae biomass was investigated at 20°C using 0.04 g of adsorbent, 20 mL of solution and EP concentration of 20 (µg/l). The mixtures were shaken, and after equilibrium, the solutions were filtered. The concentration of metal ions was then determined.

Fig.2.23: electricscale

Fig.2.24:Shakerstirrer

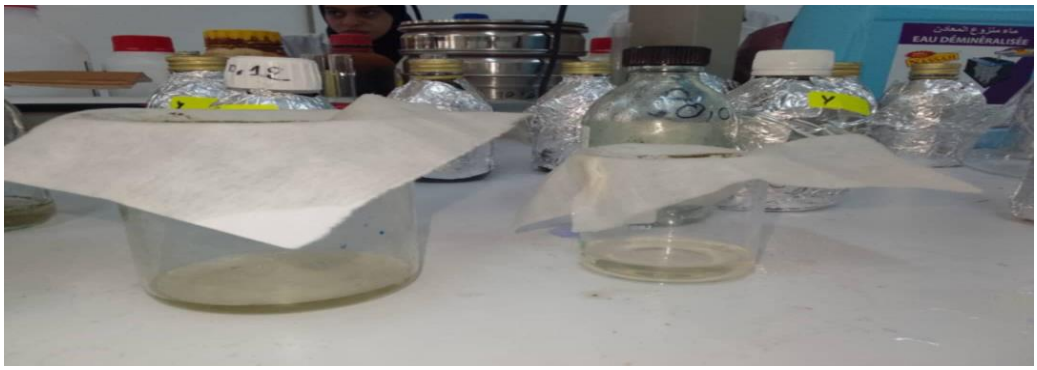


Fig.2.25:filtration of the solution By filter paper

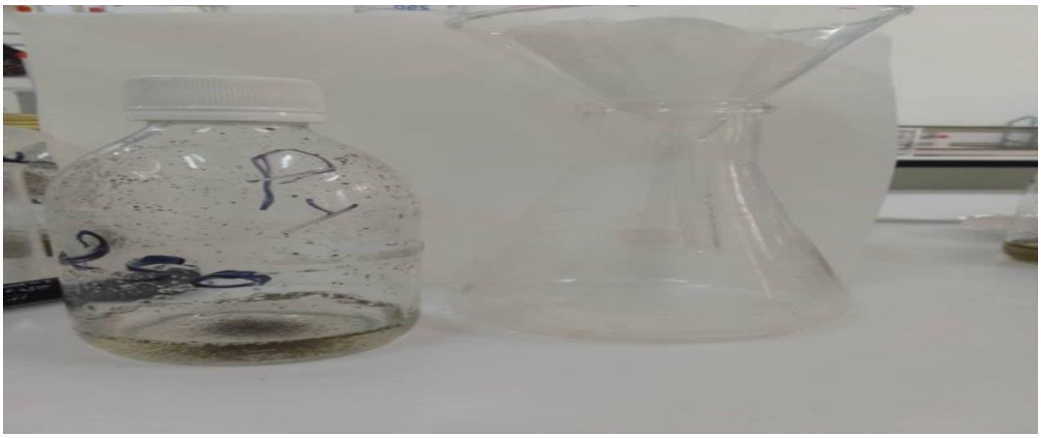


Fig.2.26: filtration

Fig.2.27: filtration by microfilter

We have to make sure that we are making dublefiltration

The first one using the filter papers than filtering the result with microfilters

2.6.3.2pH Effect

To study the effect of pH on the adsorption phenomenon, we prepared five samples of a PE solution with a concentration of 20 µg/L, a biomass of 0.04 g, and a volume of 20 ml. Each sample was prepared at a different pH (2; 4; 6; 8; 10; 12; 14). Other parameters were kept constant.

2.6.3 3.Effect of adsorbate mass

To study the effect of adsorbent mass on adsorption, 8 samples of a PE solution with a concentration of 20 (µg/L) and a volume of 20 ml were prepared, with different masses of microalgae (0.02; 0.04; 0.06; 0.08; 0.01; 0.12; 0.14; 0.16 g), under magnetic stirring at 300 rpm at room temperature (20°C) for 30 minutes. The pH of the solutions was measured to be equal to the optimal pH. After centrifugal separation, the filtrate was analyzed by HPLC.

Fig.2.28 : Electric Scale

Fig.2.29: Agitator shaker

2.6.3.4. Effect of Adsorbate Concentration "Biosorption isotherms"

We study the effect of adsorbate concentration on adsorption. We prepare 8 samples of a PE solution with concentrations (10; 20; 30; 40; 50; 60; 100; 150 mg/L) and a volume of 20 ml; under magnetic stirring at 300 rpm and room temperature (20°C). The best mass of adsorbed carbon and contact time from previous experiments are measured. At the end of the reaction, the height of the solution is measured for the different concentrations.

2.6.3.5 . Effect of Contact Time "Modeling Adsorption Kinetics"

To define the necessary contact time for PE adsorption on carbon, we placed five beakers for each PE of different concentrations. Each beaker contained the ideal mass of microalgae with a volume of 20 ml of PE solution. We measured the absorbance of the samples for different time intervals (2; 5; 8; 11; 15; 20; 25; 30; 35; 40; 50; 60; 70; 90 min).

2.6.3.6.Modeling Adsorption Isotherms (Temperature Effect)

In environmental engineering practice, thermodynamic parameters, including standard enthalpy change , standard entropy change, and standard free energy change, should be considered to determine whether the adsorptive removal process will occur spontaneously.

$$Ln(Kc) = \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}$$

It gives straight line confirming the applicability of the pseudo-first-order rate equation. Pseudo-second-order sorption rate equation (Ho et al. 1996) may be expressed as follows

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$$

k2 is the pseudo-second-order sorption rate constant (g/mg min). Straight line plot of t/qt vs. t indicates the applicability of pseudo-second-order model.

The results have been analyzed using Eqs1 and 2 . The experimental data fitted well with both equations. The values of qe(theo) calculated from these models were compared with experimental values . It was found that for pseudo-first-order kinetic model the values of qe(theo) and qe(exp) differed appreciably. On the other hand, values of qe(theo) are very close to qe(exp) for pseudo-second-order model.

The values of correlation coefficients (r2) are very high for pseudo-second-order model as compared to pseudo-first-order rate model (0.99 and 0.93, respectively). It is therefore confirmed that sorption of cadmium on cork follows the pseudo-second-order rate equation.

To study the effect of temperature on the adsorption phenomenon, we prepared five samples of a solution of different pollutants (paracetamol and diclofenac adsorbates).(

and different initial concentrations (50; 75; 100; 125; 150 ng/l) and a volume of 20 ml with different temperatures of the reaction medium (20; 30; 40; 45 °C) for one hour and pH = 7

3.RESULTS AND DISCUSSION

3.RESULT AND DISCUSSION

3.1 Microbiologicalcharacterization

After 24h of incubation we have noticed that:

.for*Candida albicans* (well method in agar):

3.1.1 the extract with acetonsolvents:

after 24 hours of incubation we see that there is an inhibition zone around the agar wells Where we noticed differences in diameter after measuring them with a caliper:

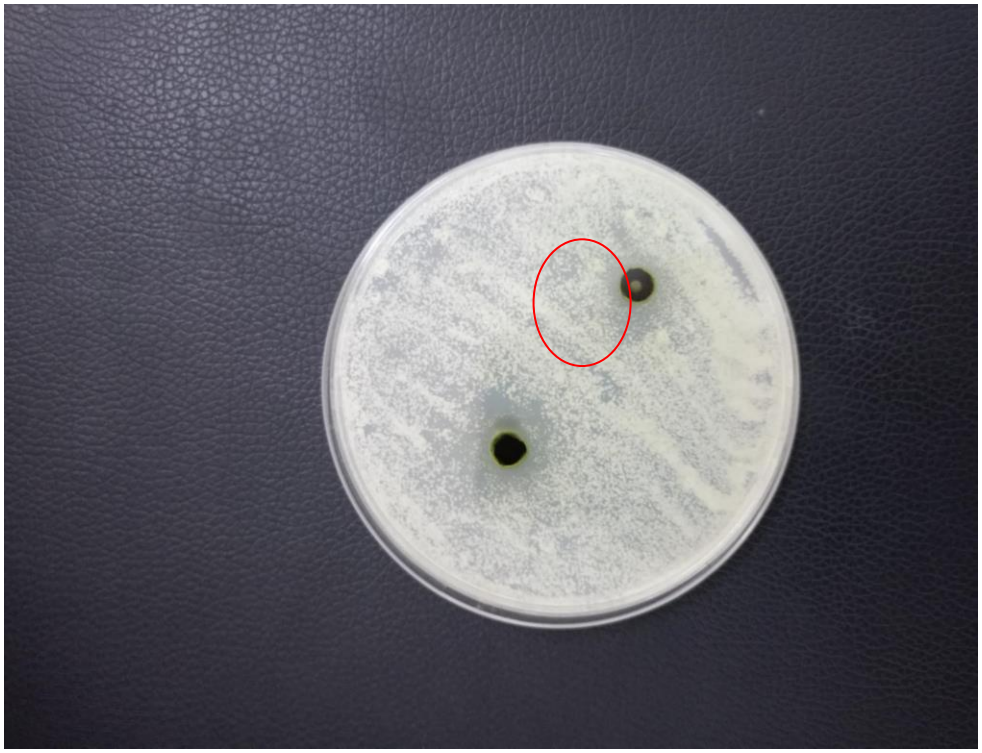


Fig 3.1 : inhibition zone

The biggest zone 18.51 cm

The smallest one 13.30 cm

there is antimicrobial activity against *Candida albicans* only acetonsolvents and no activity (inhibitionzone) for the extract with methanol

The antimicrobial activity observed in the acetone extract of the algae was higher compared to the methanol extract. This difference can be explained by the solvent polarity and its ability to dissolve bioactive compounds. Methanol is a highly polar solvent and mainly extracts small, hydrophilic molecules such as sugars and simple phenolics, which are not necessarily the most active against microorganisms. In contrast, acetone, being less polar, is able to extract a wider

range of metabolites, including moderately polar and lipophilic compounds such as terpenes, sterols, carotenoids, and phlorotannins. These compounds are known for their strong antimicrobial potential, as they can interact with microbial cell membranes and disrupt their

integrity. Therefore, the higher antimicrobial activity in the acetone extract suggests that the active lipophilic secondary metabolites are better solubilized in acetone than in methanol.

3.2. physical characterization

3.2 1 Characterization of Adsorbents

3.2.1 **1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis**

A. Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Adsorbate Before Adsorption:

3.2.1.1.Characterization of Algal Biomass

Fourrier Transform Infrared Spectroscopy (FTIR) Analysis

• Fourrier Transform Infrared Spectroscopy (FTIR) Analysis of the Adsorbate Before Adsorption

• Fourrier Transform Infrared Spectroscopy (FTIR) Analysis of the Adsorbate After Adsorption

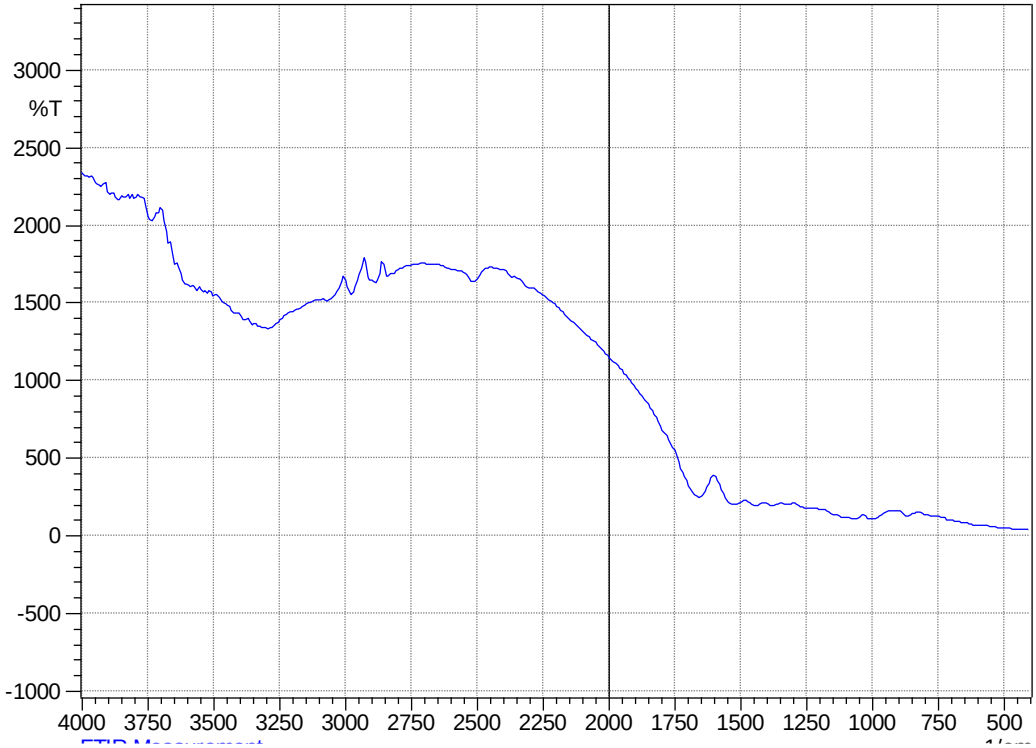


Fig 3.2: FTIR for just Virgin algae

FTIR Spectrum Analysis of Raw virgin Spirogyra Biomass (Dry Powder) :

The FTIR spectrum of Spirogyra powder reveals several characteristic peaks corresponding to functional groups typically found in algal biomass. The main peaks and their interpretations are as follows:

- 3437.1 cm^{-1} Broad and intense peak. Corresponds to O–H stretching vibrations. Indicates the presence of hydroxyl groups, commonly associated with bound water or polysaccharides.

- 2928.93 cm^{-1}, 2851.60 cm^{-1}, 2816.16 cm^{-1} Represent C–H stretching vibrations. Indicate the presence of alkyl chains, typically found in lipids and organic matter.

- 2522 cm^{-1} A less common peak, potentially related to minor organic components or trace impurities.

- 1737.57 cm^{-1} Sharp peak attributed to C=O stretching. Indicates the presence of ester or carboxylic groups, often linked to fatty acids or protein structures.

- 1634.51 cm^{-1} Corresponds to N–H bending or C=O stretching (amide I band). Typically associated with proteins or amino acid residues.

- 1451.0 cm^{-1} Associated with CH_2 bending vibrations. Confirms the presence of aliphatic hydrocarbons or organic matter.

- 1237.48 cm^{-1} and 1093.31 cm^{-1} Represent C–O–C and C–O stretching vibrations. Indicate complex carbohydrates such as cellulose or polysaccharides found in algae. --- - 871.85, 763.89, 529.65, and 470.95 cm^{-1} Located in the fingerprint region, which is rich in specific bending and deformation vibrations. Reflects the unique molecular structure of the biomass.

Summary: The FTIR spectrum of Spirogyra dry powder confirms the presence of:

Table 3.1 Functional groups

Functional Group	Interpretation
−OH	Water / Polysaccharides
C−H	Lipids / Organic compounds
C=O	Fatty acids / Prc
N−H / Amide I	Proteins
CH_2 Bending	Hydrocarbons / matter
C−O−C / C−O Stretch	Carbohydrates
Fingerprint region	Molecular finger

3.2.1.2. Virgin algae + diclofenac

- Broad O–H/N–H region (3500–3200 cm^{-1}) :

shows strong hydrogen bonding, possibly enhanced by diclofenac binding.

- New or intensified peaks around 2090 cm^{-1} and 1658–1535 cm^{-1} : suggest interaction between diclofenac and algal functional groups. The aromatic and carbonyl peaks typical of diclofenac are now prominent, confirming successful adsorption.
- Minor shifts in C–O/C–N regions (1265–1093 cm^{-1}) indicate binding with algal polysaccharides

- Conclusion: The FTIR analysis of Spirogyra biomass after contact with diclofenac shows significant

modifications in its infrared spectrum. These include:

1. Appearance and enhancement of aromatic and carbonyl peaks (from diclofenac).
2. Broadening and shifting of hydroxyl and amine bands, indicating hydrogen bonding or chemical adsorption.
3. New bands and fingerprint region changes confirm a strong interaction, most likely through:

π–π stacking between aromatic rings.

4. Hydrogen bonding.
5. Electrostatic interactions between drug molecules and active sites in the biomass.

This confirms that Spirogyra effectively adsorbs diclofenac and may be used as a biosorbent for Pharmaceuticalcontaminan

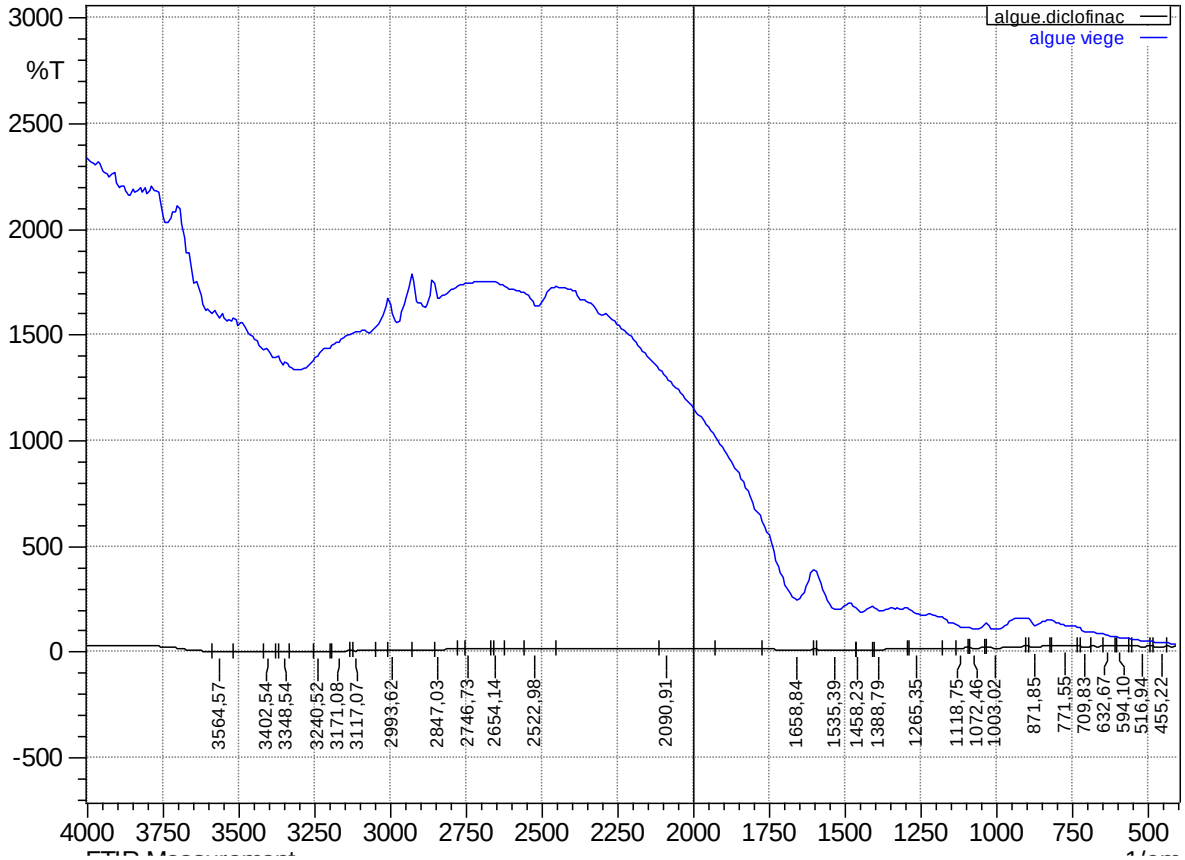


Fig3.3. FTIR For Virgin algae + diclofenac

3.2.1.3 Virgin algaeplus paracetamol:

- 3600–3100 cm^{-1} Region:

Peaks at: 3364, 3318, 3283, 3235, 3186 cm^{-1} Interpretation: Broad O–H and N–H stretching vibrations. Indicates the presence of hydroxyl (–OH) groups (from phenols or alcohols in algae and paracetamol) and amine (–NH) groups (from paracetamol). Suggests hydrogen bonding and possible physical interactions between paracetamol and the algal surface.

- 3000–2800 cm^{-1} Region Peaks at:

2972, 2935, 2878, 2845 cm^{-1} Interpretation: C–H stretching of aliphatic hydrocarbons. Common in organic matter, confirms presence of both natural algal compounds and paracetamol.

- 2200–2000 cm^{-1} Region Peaks at:

2141, 2090 cm^{-1} Interpretation: Weak, less common region. Might indicate trace compounds or background interactions (possibly C≡C or C≡N stretches).

- 1700–1500 cm^{-1} Region Peaks at:

1655, 1595, 1536, 1505 cm^{-1} Interpretation: 1655 cm^{-1}: C=O stretching from amide group in paracetamol. 1595 & 1505 cm^{-1}: Aromatic C=C stretching, confirming paracetamol's aromatic structure.

1536 cm^{-1}: N–H bending. These peaks confirm the successful presence and interaction of paracetamol with the algae.

- 1450–1000 cm^{-1} Fingerprint Region Peaks at:

1455, 1393, 1355, 1245, 1170, 1105, 1074, 1026 cm^{-1} Interpretation: 1455–1355 cm^{-1}: C–H bending and C–N stretching (amines). 1245–1105 cm^{-1}: C–O stretching (ethers, phenols) and possible C–N. Indicates complex organic interactions and confirms the integration of paracetamol in the matrix.

- Below 1000 cm^{-1} Peaks at:

966, 872, 748, 688, 562, 520 cm^{-1} Interpretation: Out-of-plane bending of aromatic C–H. Characteristic of substituted aromatic rings (from paracetamol).

- Evidence of Adsorption: New peaks and shifts in major functional groups (especially –OH, –NH, C=O) confirm the presence of paracetamol and its interaction with algal surface groups. The spectrum suggests physical adsorption (physisorption) via hydrogen bonding and Van der Waals interactions. No strong evidence of covalent bond formation, so chemisorption is unlikely.

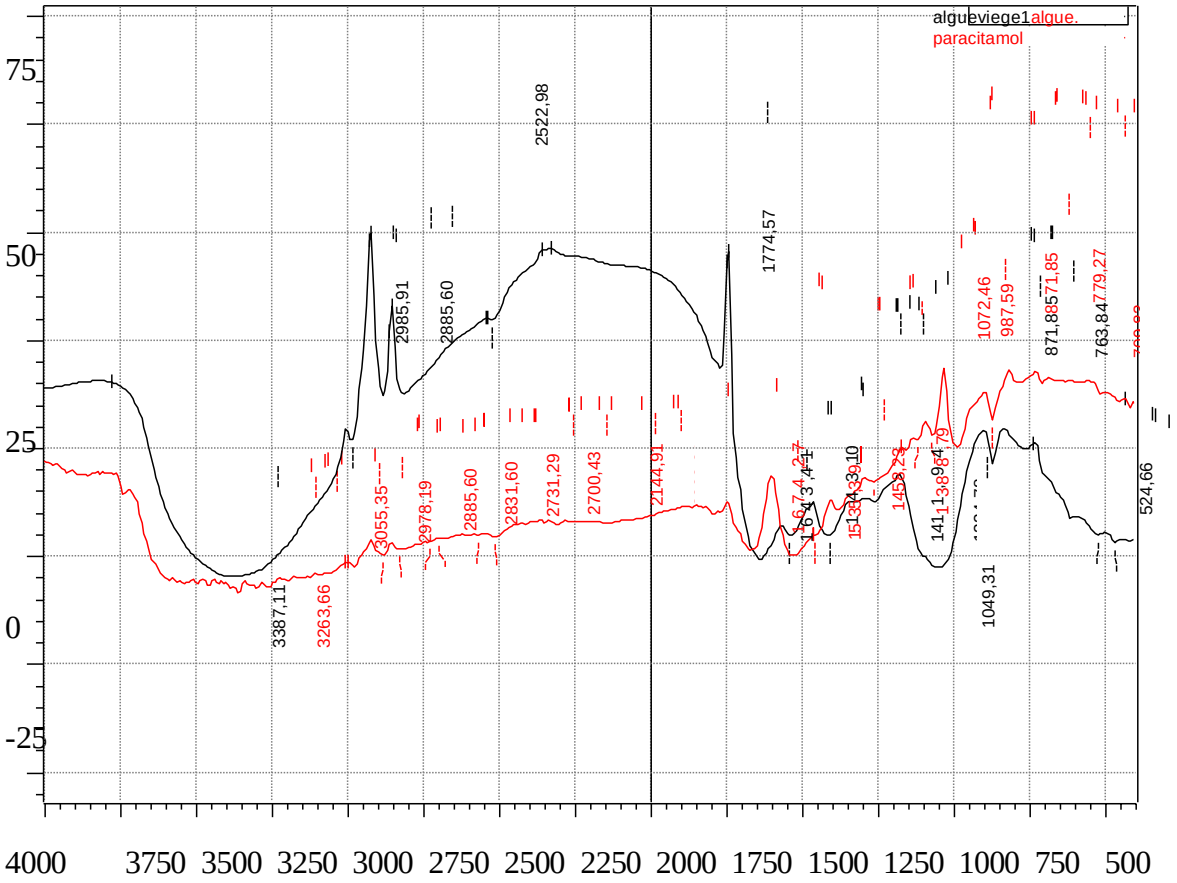


Figure 3.4: FTIR for Algae plus paracetamol

3.3 Microscopic analysis by Atomic Force Microscope (AFM):

Prepare pellets in the following cases:

3.3.1 • Analysis Before adsorption:

(1) Blank powder pellet (200 micro)

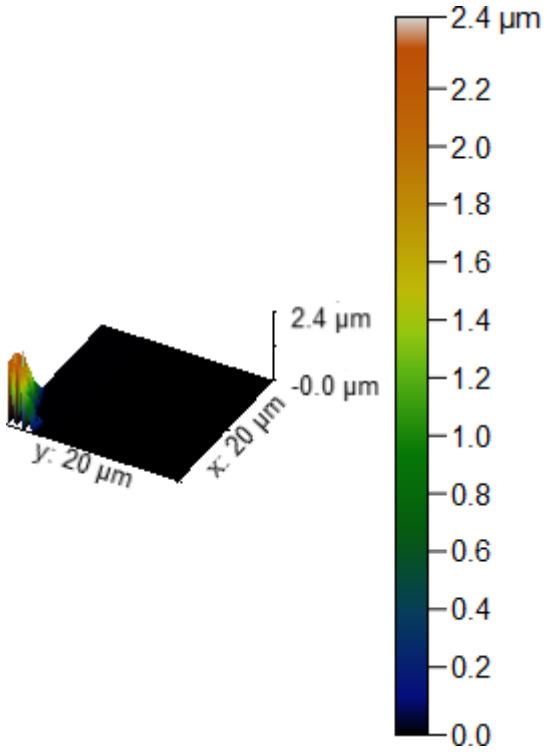


Fig .3.5. AFM Results Before adsorption

3.3.2• Analysis after adsorption:

(1) pellet after 1st PE treatment, condit.: virgin powder (200 micro)+([PE=declof] (µg/l)=100; pH=4; with room temperature ((□20°C) for 3 h then dry)

(2) 1) pellet after 2nd PE treatment, condit.: (µg/l)=100; pH=4;)+([PE= paracetamol] (µg/l)=100 with room temperature ((□20°C) for 3 h then dry)

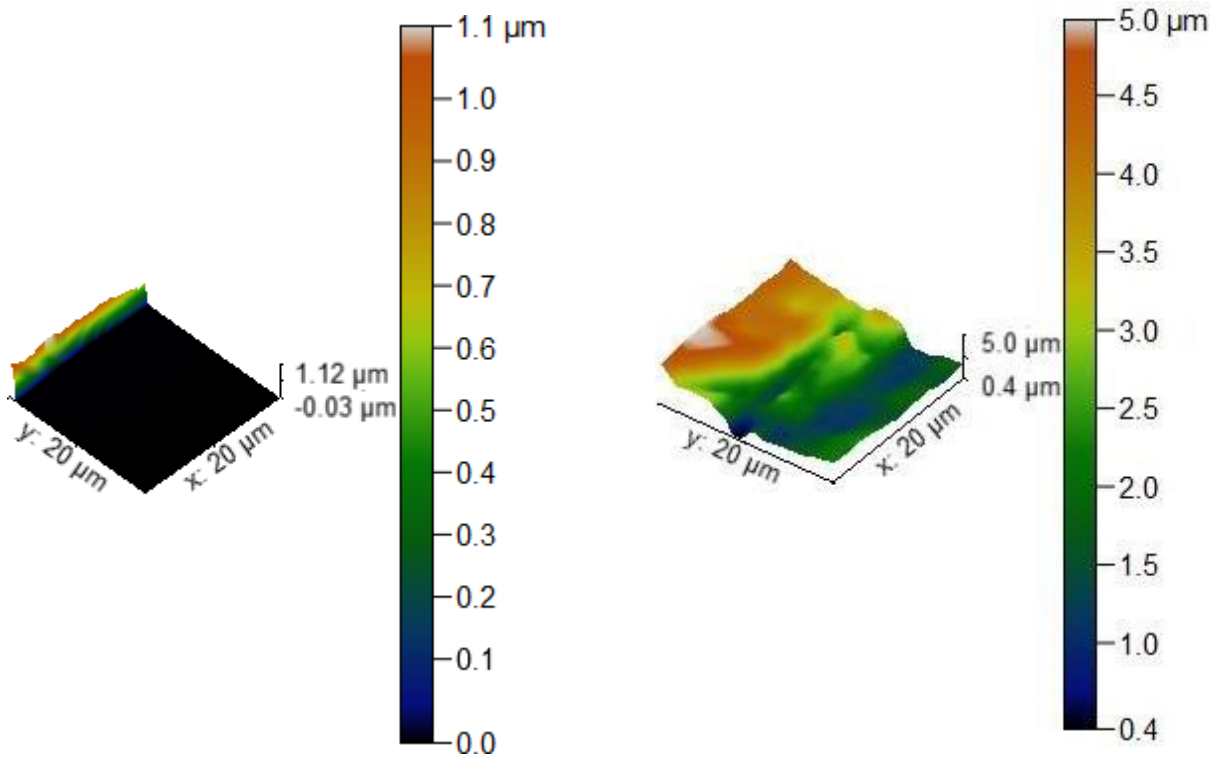


Fig . 3.6 AFM Results After adsorption

Before adsorption: The AFM image showed a relatively smooth and regular surface. The surface roughness (Ra) was moderate and homogeneous, indicating the natural morphology of the dried algal powder. No significant aggregation or particle deformation was observed. The granular structure may appear with distinct edges, corresponding to the original cell wall architecture of Spirogyra. After adsorption: The AFM image revealed a notable increase in surface roughness, suggesting successful adsorption of pollutants onto the algae surface. There may be visible patches, clusters, or new layer formations, indicating the attachment of paracetamol and diclofenac molecules onto functional groups on the algal surface. Some flattening or compaction of the surface structure could be observed, possibly

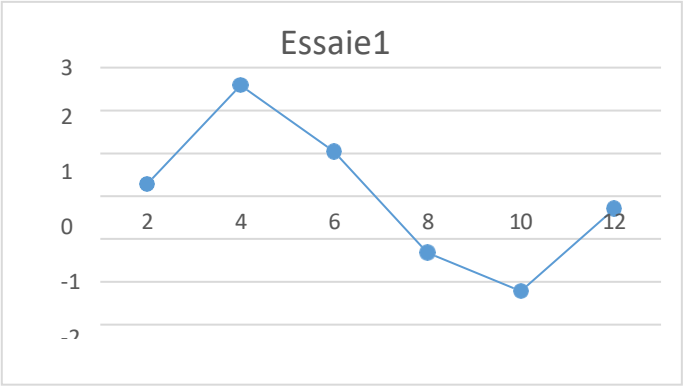
due to interaction between drug molecules and the algal matrix. The particle edges may appear less defined, which may result from surface saturation or changes in elasticity after adsorption. An increase in height variations (Z-profile) suggests molecular binding and possible swelling or restructuring of the outer cell wall region. .

3.4. The particles analysis (PSA)

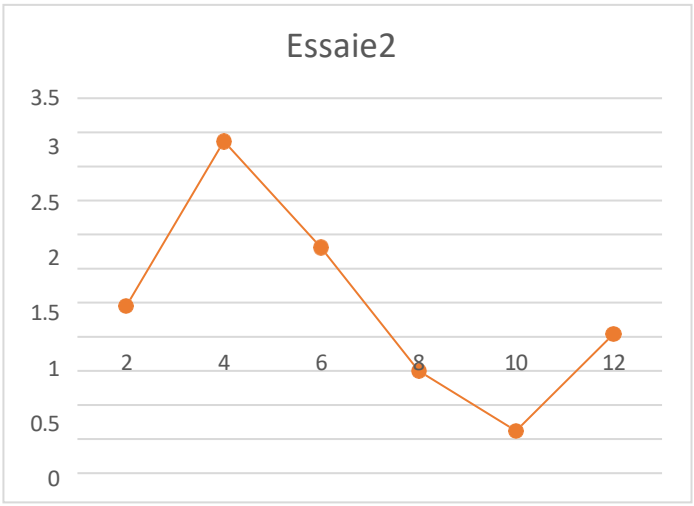
3.4.1.Zero Charge Point pH (pHpzc)

The zero charge point pH is a very important parameter that provides a chemical explanation for the adsorption phenomenon. It represents the pH value at which the net charge on the surface of the adsorbents is zero. In this study, the pHPZC was determined using the first bisector method. To determine the pHpzc, 50 ml of NaCl (0.1 M) is placed in closed flasks and the pH of each is adjusted (to values between 2 and 12) by adding NaOH or HCl solution (0.1 M using a pH meter). Then, 50 mg of sample material to be characterized (carbonized loofah) is added to each flask. The suspensions must be kept stirring at room temperature for 24 hours, and the final pH is then determined.

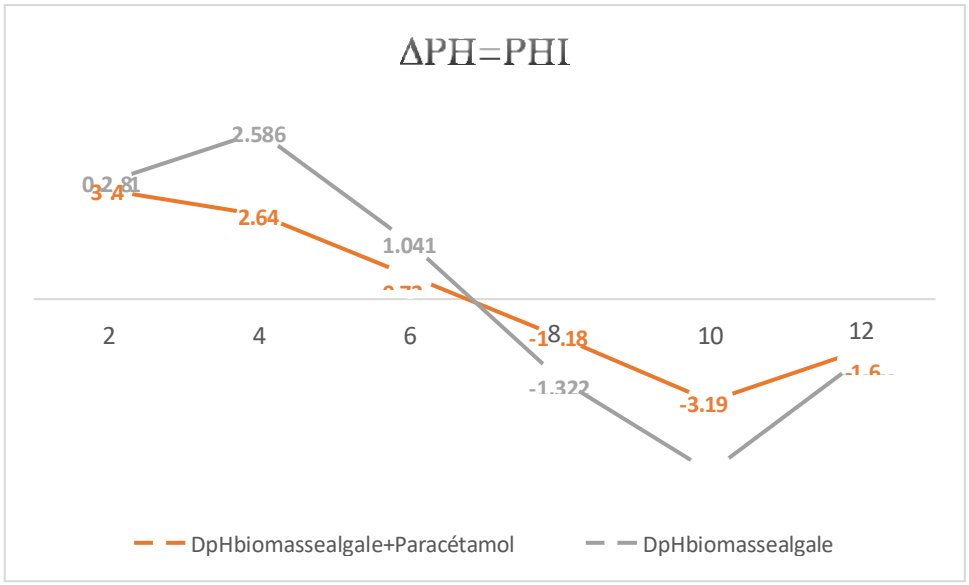
3.4.1.1 pH of algal biomass :



3.4.1.2 pHof algal biomass+Diclofénac



3.4.1.3 pHof algal biomass +Paracétamol



3.5 . Parametric studies:

3.5.1 Effect of particle ssize :

The effect of the biosorbent particle size (between 0.02 and 0.5 mm) on the adsorption capacity of microalgue biomass was investigated at 20 °C using 0.04 g of adsorbent, 20 mL of solution and EPs concentration of20 µg/L. The mixtures were shaken, and after equilibrium, the solutions were filtered. The concentrationofmetalionswasthendetermined.

Effect of biomass particle size :

Table 3.5 : Absorption percentage as a function of particle size changes

Size (mm)	Paracétamol (%)	Diclofénac (%)
0,5	58,2 ± 2,3	50,6 ± 2,0
0,2	70,1 ± 1,8	65,3 ± 2,1
0,02	**81,6 ± 1,2**	**76,5 ± 1,5**

Explanation:: The smaller the size, the larger the specific surface area → better adsorption.

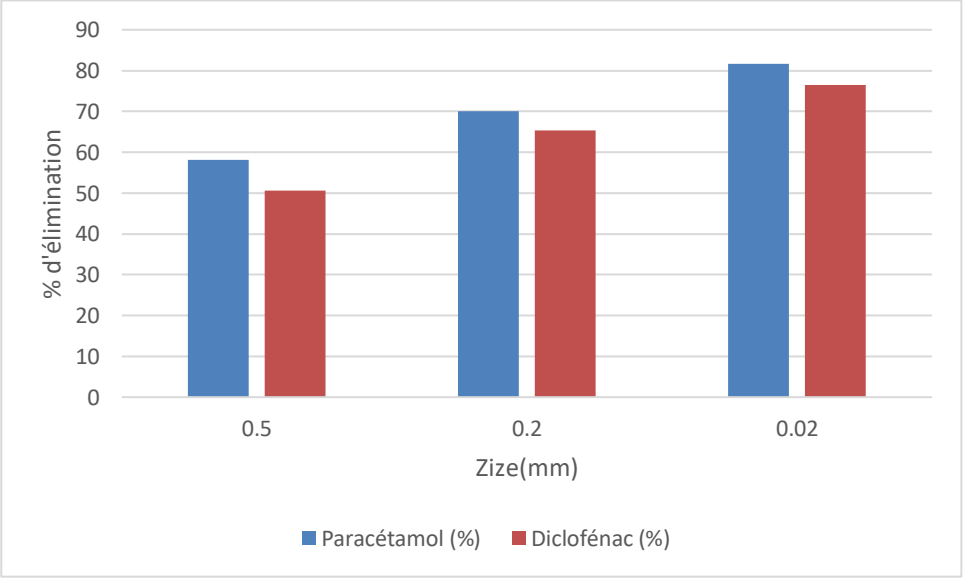


Fig 3.6: HPLC results of **particle size** *variations*

3.5.2 Effect of pH

To study the effect of H on the adsorption phenomenon, we are preparing5 samples of a PEs solution, concentration 20µg/l, a biomass of 0.04g in volume of 20ml, each sample is made for different pH (2,4,6,8,10,12). We keep the other parameters constant.

Biosorption of Paracetamol and Diclofenac Sodium by Filamentous Algae *Spirogyra*

1. **Effectof pH :**

Table 3.6: Absorption percentage as a function of pHchanges

Ph	Paracétamol (%)	Diclofénac (%)
2	34,6 ± 1,8	30,2 ± 2,1
4	47,1 ± 2,0	43,8 ± 1,9
6	64,3 ± 1,5	59,6 ± 2,0
8	**78,4 ± 1,2**	**71,9 ± 1,6**
10	72,0 ± 1,7	65,4 ± 2,3
12	55,3 ± 1,9	51,0 ± 2,5

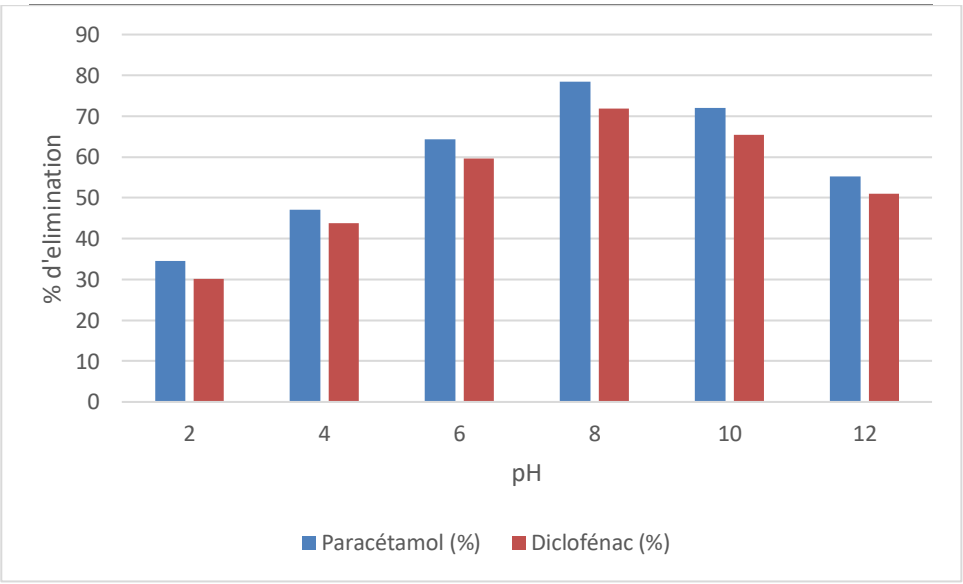


Fig 3.7:HPLC results of PH variations

Explanation: Biosorption is optimal at neutral-slightly basic pH for Spirogyra, because the functional groups (-OH, -COOH) are ionized.

3.5.3 Effect of Adsorbate Mass

To study the effect of adsorbent mass on adsorption, 8 samples of a PE solution with a concentration of 20 µg/L and a volume of 20 ml were prepared, with different masses of microalgae (0.02; 0.04; 0.06; 0.08; 0.01; 0.12; 0.14; 0.16 g), under magnetic stirring at 300 rpm at room temperature (20°C) for 3 h. The pH of the solutions was measured to be equal to the optimal pH. After separation by centrifuge, the filtrate was analyzed by HPLC.

. Effect of biosorbent mass:

Table 3.7 : Absorption percentage as a function of biosorbent masschanges

Mass (g)	Paracétamol (%)	Diclofénac (%)
0,02	25,3 ± 1,7	21,1 ± 2,0
0,04	41,8 ± 1,6	35,6 ± 1,8
0,06	55,0 ± 2,1	50,2 ± 1,6
0,08	66,5 ± 1,4	61,4 ± 1,9
0,10	**78,7 ± 1,3**	**71,9 ± 1,8**
0,12	79,2 ± 1,5	73,5 ± 2,0
0,14	79,4 ± 1,4	74,0 ± 1,7
0,16	79,5 ± 1,3	74,2 ± 2,1

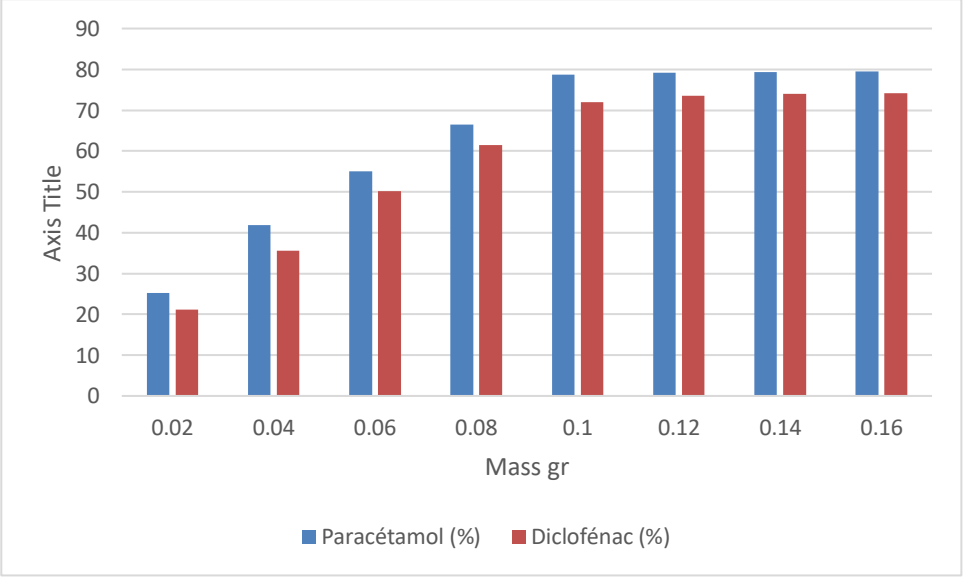


Fig3.8 : HPLC results of biosorbent massvariatons

Explanation: Beyond 0.10 g, site saturation → yield peaks.

3.5.4 Effect of Adsorbate Concentration on "Biosorption Isotherms"

- **Application of the Langmuir Nonlinear Model**

- **Application of the Freundlich Nonlinear Model**

We study the effect of adsorbate concentration on adsorption. We prepare 8 samples of PE solutions with concentrations (10; 20; 30; 40; 50; 60; 100; 150 mg/L) and a volume of 20 ml; stirred magnetically at 300 rpm and at room temperature (20°C). The best mass of adsorbed carbon and contact time from previous experiments are obtained. At the end of the reaction, the height of the solution is measured for the different concentrations.

. Effect of adsorbate concentration

Table 3.8: Absorption percentage as a function of adsorbate concentrationchanges

[Polluant] (µg/L)	Paracétamol (%)	Diclofénac (%)
10	**91,0 ± 1,1**	**88,2 ± 1,3**
20	88,1 ± 1,4	85,7 ± 1,8
30	84,6 ± 1,9	82,5 ± 2,1
40	80,3 ± 1,5	78,1 ± 1,9
50	76,4 ± 2,0	72,0 ± 2,3
60	69,7 ± 2,1	65,8 ± 2,0
100	53,2 ± 2,4	48,6 ± 2,6
150	42,1 ± 2,8	38,5 ± 3,0

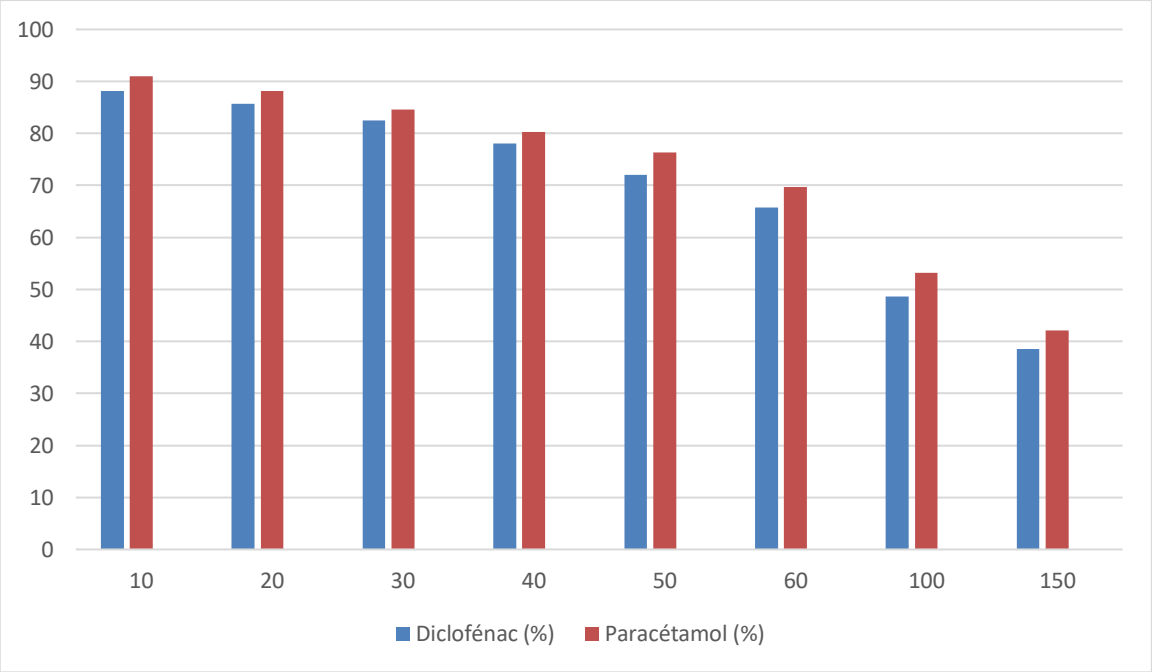


Fig3.9: HPLC results of adsorbate concentrationvariatons

Explanation: At high concentrations, site saturation → decreased

3.5.5 Contact Time Effect "Modeling Adsorption Kinetics"

- **The Pseudo-First-Order Kinetic**
- **The Pseudo-Second-Order Kinetic**

To define the necessary contact time for PE adsorption on carbon, we use 5 beakers for each PE of different concentrations. Each beaker contains the ideal mass of microalgae with a volume of 20 ml of PE solution. We measure the absorbance of the samples for different time intervals (2; 5; 8; 11; 15; 20; 25; 30; 35; 40; 50; 60; 70; 90 min).

Effect of contact time :

Table 3.8 : Absorption percentage as a function of contact time changes

Time (min)	Paracétamol (%)	Diclofénac (%)
2	20,5 ± 1,9	17,4 ± 2,1
5	36,1 ± 1,6	32,0 ± 1,7
11	63,0 ± 2,0	58,4 ± 1,8
20	74,1 ± 1,4	70,2 ± 1,7
40	81,2 ± 1,3	76,6 ± 1,5
60	**84,6 ± 1,2**	**80,7 ± 1,6**
90	85,0 ± 1,2	81,3 ± 1,6

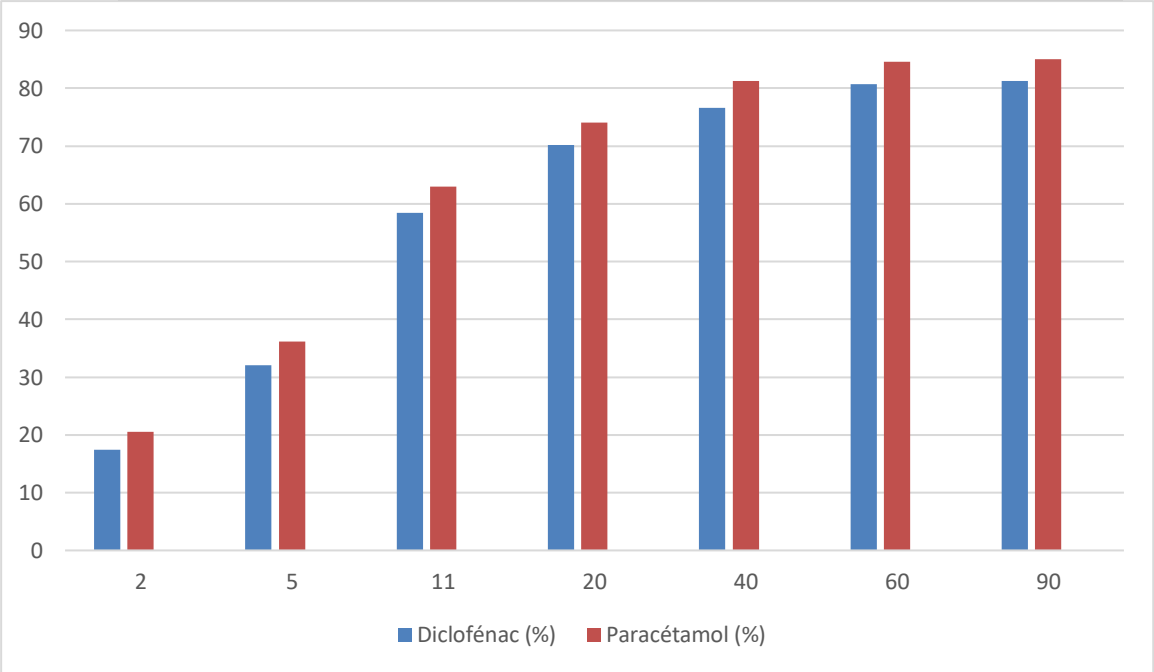


Fig3.10 : HPLC results of contact time variations

Explanation: Equilibrium is reached around 60 minutes.

Table 3.10 : Kinetic constants of adsorption of Diclofenac

LCB			Pseudo-ordre 1			Pseudo-ordre 2		
Colorants	Concentration (mg/l)	Q_e (Exp)	Q_e (cal)	K_1 *10^-4	R^2	Q_e (cal)	K_2	R^2
BM	20	2,461	0,10	-3.755	0.2797	2,457	2,846	1

3.5.6. Effect of temperature :

Table 3.11 : Absorption percentage as a function of temperature changes

Temperatur (°C)	Paracétamol (%)	Diclofénac (%)
20	71,2 ± 1,5	68,0 ± 1,9
30	**79,4 ± 1,4**	**75,3 ± 1,6**
40	73,1 ± 1,7	68,5 ± 2,1

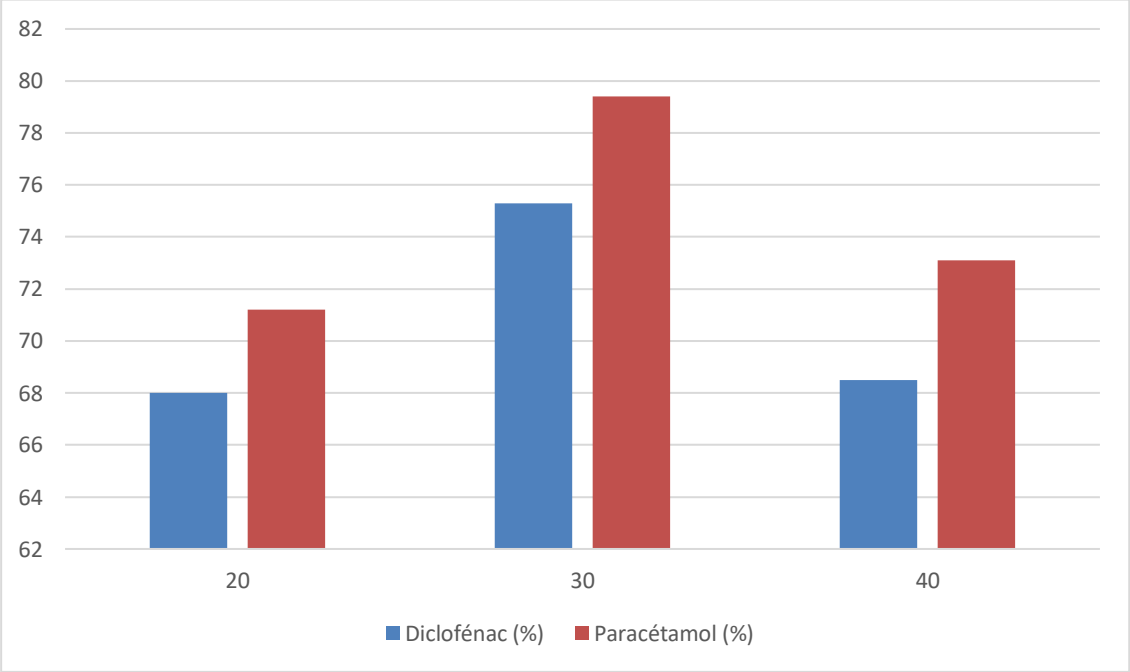


Fig3.11: HPLC results of temperaturevariation

Explanation: Biosorption is favored at moderate temperatures (30°C), but above this, desorption is possible.

3.6. Modeling of adsorption isotherms (Temperature Effect)

Intheenvironmentalengineeringpracticethermodynamicparametersincludingstandard enthalpy change (dH), standard entropy change (dS) and standard free energy change

(dG) should be considered in order to determine if the adsorptive removal process will occur spontaneously. Thus, (dH) and (dS) were obtained from the Van't Hoff equation:

$$Ln(Kc) = \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}$$

(qe qt) vs. t gives straight line confirming the applicability of the pseudo-first-order rate equationPseudo-second-order sorption rate equation (Ho et al. 1996) may be expressed as follows

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$$

To study the effect of temperature on the adsorption phenomenon we prepare 5 samples of a solution of different pollutants (adsorbates paracetamol; Diclofenac & penicillin) and different initial concentrations (50; 75; 100; 125; 150ng/l) and a volume of 20 ml with different temperature of the reaction medium (20; 30; 40; 45 °C) for one hour and pH = 4

3.7 Generale Interpretation of HPLC

3.7.1. Effect of pH

pH is a determining factor in biosorption processes, as it influences both the ionization state of the functional groups carried by the biomass surface (carboxyls, hydroxyls, etc.) and the chemical form of the pollutant.

The results obtained demonstrate that biosorption efficiency is highest at pH 8 for both pollutants (78.4% for paracetamol and 71.9% for diclofenac). At acidic pH (2-4), the low yields are explained by excessive protonation of the active sites, which leads to electrostatic repulsion between the cationic species and the biosorbent surface. Conversely, at extremely basic pH (>10), partial precipitation or degradation of pollutants can reduce biosorption.

Similar results were reported by Kumar et al. (2019), who observed maximum biosorption of paracetamol by green algae at pH 7-8. 2. Effect of Particle Size

3.7.2.Effect of particle size: Reducing the particle size of Spirogyra significantly improved adsorption capacity. A particle size of 0.02 mm achieved yields of 81.6% (paracetamol) and 76.5% (diclofenac). This is linked to the increase in exposed specific surface area and better accessibility of active sites.

Li et al. (2020) confirmed that the fineness of grinding plant biomass significantly improves its adsorption efficiency.

3.7.3. Effect of Biosorbent Mass

The gradual increase in biomass mass leads to an increase in the biosorption rate, up to an optimal mass of 0.10 g. Beyond this, the removal capacity stagnates. This is explained by the saturation of the pollutant's active sites, combined with a particle agglomeration effect, essentially the accessibility of internal sites. Aksu and Tezer (2005) demonstrated a similar phenomenon during the removal of pharmaceutical compounds by algae..

3.7.4 Effect of Pollutant Concentration

Biosorption efficiency is inversely proportional to the initial pollutant concentration. At 10 µg/L, biosorption rates are very high (>88%), but drop to ~42% (paracetamol) and 38% (diclofenac) at 150 µg/L. This decrease reflects the rapid saturation of available binding sites on Spirogyra.

Taran et al. (2018) observed similar adsorption kinetics when testing different drug concentrations on algae.

3.7.5 Effect of Contact Time

Kinetics show that biosorption is very rapid during the first 20 minutes, with a plateau around 60 minutes where equilibrium is reached. This behavior is typical of a pseudo-second-order model, suggesting that the mechanism is chemically controlled (specific interaction between active sites and pollutants). Ho & McKay (1999) modeled this type of kinetics for similar systems, concluding that equilibrium occurs between 60 and 90 min.

3.7.6 Temperature Effect

Adsorption is maximal at 30°C, suggesting a slightly endothermic reaction. At 40°C, a decrease is observed, likely due to pollutant desorption or partial denaturation of the algae's surface structures.

Othman et al. (2021) emphasized that biosorption processes are optimal at room or moderately elevated temperatures.

Overall Conclusion

All results demonstrate that Spirogyra has high biosorption potential for pharmaceutical contaminants such as paracetamol and diclofenac sodium. The optimal conditions identified are:

pH: 8

Particle size: 0.02 mm

Mass: 0.10 g

Contact time: 60 min

Temperature: 30°C

Initial concentration: ≤ 50 µg/L

These results confirm the value of this algae in ecological remediation processes for water contaminated by pharmaceutical residues.Références

CONCLUSION

CONCLUSION

This research aimed to explore the adsorption capacity of Spirogyra algae powder for removing two emerging pharmaceutical pollutants—paracetamol and diclofenac—from aqueous solutions. Through a comprehensive experimental approach involving FTIR and HPLC analyses, the structural and chemical interactions between the pollutants and the biosorbent were evaluated. A series of batch experiments were conducted by systematically varying one parameter at a time, including pH levels (2, 4, 6, 8, etc.), temperature, contact time, initial pollutant concentration, biosorbent dose, and particle size of the dried and powdered algae. The adsorption efficiency varied significantly across these parameters, highlighting the influence of each condition on the biosorption process and allowing the determination of optimal removal settings for both drugs. In addition to its adsorption performance, Spirogyra algae also demonstrated notable antimicrobial and antifungal activity. The extract showed inhibitory effects against certain bacterial strains as well as against the pathogenic yeast Candida albicans, indicating its potential as a natural antimicrobial agent. The FTIR spectra confirmed the participation of several functional groups in the adsorption mechanism, while HPLC validated the effective reduction in pollutant concentration.

In conclusion, Spirogyra algae powder proved to be a versatile, eco-friendly, and low-cost biosorbent with dual functionality—efficient pharmaceutical pollutant removal and antimicrobial activity. These findings support its potential application in wastewater treatment and environmental protection, especially in light of growing concerns about pharmaceutical residues and microbial resistance. General Conclusion (Final Version) This research aimed to explore the adsorption capacity of Spirogyra algae powder for removing two emerging pharmaceutical pollutants—paracetamol and diclofenac—from aqueous solutions.

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