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Tudor POPESCU

INTERACTION DE CERTAINES ESPECES D'ALGUES ET CONTAMINANTS METALLIQUES ET ORGANIQUES DANS LE MILIEU AQUATIQUE

Soutenue le 12 décembre 2016 devant le Jury composé de :

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A ma petite fille …

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Glossary

AAS: Atomic Absorption Spectroscopy

ADB: Biomass Absolute Dried

ADN: Deoxyribonucleic acid

ASE: Accelerated Solvent Extraction

ASV: Anodic Stripping Voltametry

ATP: Adenosine Triphosphate

BPA: Bisphenol A

CCD: Charge Coupled Device

COD: Dissolved Organic Carbon

CTC: Collision Cell Technology method

$DCIPH_2$: Dichlorophenolindophenol

DL - Detection Limit

EDTA: Ethylenediaminetetraacetic acid

ETM: Metal Trace Element

FS: Full Scan mode

GC: Gas Chromatography

GC-MS: Gas chromatography - mass spectrometry

HAB: Harmful Algal Blooms

HPLC: High Performance Liquid Chromatography

ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

LLE: Liquid-Liquid extraction

MRM: Multiple Residual Monitoring

MS: Mass Spectrometry

NAD^+: Nicotinamide adenine dinucleotide

$NADP^+$: Nicotinamide adenine dinucleotide phosphate

NTA: Aminopolycarboxylic acid

PAH: Polycyclic Aromatic Hydrocarbon

PHA: Polyhydroxyalkanoates

POP: Persistants Organic Polluants

RPS: Exocellular polysaccharides

SIS: Selected Ion Storage

SSM: Suspended Solid Matter

TDS: Fixed residue

UV: Ultra-Violet

WWTP: Wastewater Treatment Plant

Résumé

La révolution industrielle et technologique avec l'urbanisation intensive, la croissance démographique a attiré l'attention de l'humanité pour la nécessité d'adopter le développement durable pour préserver l'environnement.

Chimisation intense de l'agriculture et des processus industriels, l'augmentation de l'exploitation minière, le traitement des ressources minérales incomplètes ensemble avec le développement des transports, l'infrastructure routière, les services et l'augmentation de la consommation médicaments conduit à la pollution émergente des écosystèmes avec diverses micropolluants minéraux, organique et organométallique extrêmement toxique.

Les polluants organiques (persistants, produits pharmaceutiques, hormones et bisphénols) ainsi que le polluants minéraux (métaux lourds et sel minéraux) sont susceptibles de développer une toxicité non négligeable pour la santé humaine et le bon fonctionnement de l'écosystème global, y compris l'atmosphère, les ressources en eaux, les sols, les sédiments et les biotes.

Une de plus mobile phase, celle aquatique est et exposé le plus a la pollution conduisant à de graves problèmes environnementaux. C'est un danger très important pour l'humanité, menaçant la destruction des bassins d'eau de la biosphère avec des conséquences importantes dans l'approvisionnement en eau et influence directe dans la sante de la population.

Les volumes des eaux usées restent croissants et continuent d'augmenter de façon permanente. Afin de remédier la situation actuelle de l'eau de surface, il est nécessaire d'isoler ces substances toxiques de l'eau et en soumettant ces dernières au processus de traitement, le retourné plus tard être au circuit.

Dans le présent travail, on s'intéresse à la bioremédiation des polluants organique et minéraux dessus mentionnées par les algues bleu vertes.

La première partie de la thèse: ,,**Interaction de certaines espèces d'algues et contaminants métalliques et organiques dans le milieu aquatique,,** est consacrée à la caractérisation des souches algales utilisées suivi de méthodes de l'élevage algale en laboratoire pour la production de la biomasse au moindre cout prête pour les processus de bioremédiation des micropolluants. L'élevage des espèces algale sur milieux culturelles provenant des stations de traitement des eaux non-diluées va nous offrir un double avantage : traitement tertiaire des effluents ainsi que milieux gratuits pour l'élevage algale.

Une seconde partie consacre à la méthode de bioaccumulation des polluants organiques et minéraux sur la phase solide algale libre tant qu'en forme immobilisée avec l'évaluation de consommation et dégradation des micropolluants polluants

Mots clés : *Eau, contamination, micropolluants, bioremédiation, algues bleu-vertes.*

Abstract (Română)

Revoluţia industrială şi tehnologică împreună cu creşterea demografică şi urbanizarea intensivă au atras atenţia omenirii asupra necesităţii conservării si dezvoltării durabile a mediului ambiant.

Chimizarea intensă a agriculturii şi a proceselor industriale, creşterea extracţiei miniere si procesarea incompletă a resurselor minerale împreună cu dezvoltarea transporturilor, infrastructurii drumurilor, serviciilor şi consumului medicamentos a dus la poluarea emergentă a ecosistemelor cu micropoluanţi minerali, organici şi organo-metalici extrem de toxici.

Poluanţii organici persistenţi, produsele farmaceutice, bisfenolii şi hormonii precum şi micropoluanţii minerali (metale grele şi săruri minerale) sunt susceptibile de a dezvolta o toxicitate semnificativă pentru sănătatea umană şi pentru buna funcţionare a ecosistemului global, inclusiv atmosfera, resursele de apă, soluri, sedimente şi biota.

Cea mai expusă si mobilă fază care este expusă poluării este cea acvatică ceea ce duce la grave probleme de mediu, distrugînd biocenozele bazinelor acvatice cu importante consecinţe în aprovizionarea cu apă potabilă de calitate, influenţînd direct sănatatea populaţiei.

Volumul apelor reziduale se menţine permanent la o cotă ridicată şi în continuă creştere. Pentru redresarea situaţiei actuale a apelor de suprafaţă este necesar de a izola aceste substanţe toxice din ape şi de a le supune proceselor de epurare, ca mai apoi să fie reîntoarse în circuit.

În lucrarea de faţă, suntem interesaţi de bioremedierea poluanţilor organici şi minerali menţionaţi cu ajutorul alge verzi albastre.

Prima parte a tezei: **„Interactiunea unor specii de alge cu contaminantii metalici si organici in mediul acvatic„** este consacrată caracterizării tulpinilor algale utilizate, metodelor de producere în laborator a biomasei algale cu un sinecost ieftin pentru utilizarea ulterioară în procesele de bioremediere a micropoluanţilor. Creşterea speciilor de alge pe mediile culturale ale apelor nediluate la ieşirea din staţiile de epurare ne va oferi un dublu avantaj: tratarea terţiară a apelor reziduale, precum şi mediu de cultură gratuit pentru cultivarea algelor.

Cea de a doua parte a tezei este consacrată metodelor bioremedierii poluanţilor organici şi minerali pe suport algal atît liber cît şi în formă imobilizată. Studiul fost efectuat cu evaluarea degradării poluanţilor organici cît şi evaluării consumului macro si microelementelor din mediu poluat.

Cuvinte cheie: *apă, contaminare, micropoluanţi, bioremediere, algele albastre-verzi.*

Abstract (English)

The industrial and technological revolutions, causing intensive urbanization and population growth, have drawn the attention of humanity onto the necessity for conservation and sustainable environment development.

Intense use of chemicals in agricultural and industrial processes, increased mining, incomplete processing of mineral resources together with the development of transport, road infrastructure, services and the increase in drug consumption led to an emerging pollution of the ecosystems, infecting them with various minerals, organic and organo-metallic micro-pollutants which are extremely toxic.

Organic pollutants (persistent, pharmaceuticals, hormones and bisphenols) and the mineral contaminants (heavy metals and mineral salts) are likely to cause significant damage to human health and the proper functioning of the global ecosystem, including the atmosphere, water resources, soils, sediments and biota.

Water, being the most mobile phase, is the most exposed to pollution leading to serious environmental problems. This is a very significant threat to humanity, risking the destruction of global water basins, having important consequences on the water supply and a direct influence on population health.

The volumes of wastewaters are increasing and continue to increase permanently. In order to remedy the present situation of the surface water, it is necessary to isolate these toxic substances from water and subject them to the treatment process so that, ultimately, to put them back in circuit.

In the present work, we are interested in the bioremediation of organic and mineral pollutants mentioned above by the use of blue green algae.

The first part of the PhD thesis: ,,**Interaction of certain algae and metallic and organic contaminants in the aquatic environment,,** is dedicated to the characterization of utilized algal strains, of the methods that were used for algal biomass production in the laboratory with a cheap prime cost biomass for future bioremediation processes of micro-pollutants.

Growth of algal species on undiluted waters from the treatment plants will offer us a double advantage: third party treatment as well as free environment for algae farming.

The second part of PhD thesis is dedicated to the characterization of bioaccumulation processes of organic and mineral pollutants on a free as well as an immobilized algal state, with the evaluation of consumer and degradation of micro-pollutants.

Keywords: *Water, contamination, micro, bioremediation, blue-green algae.*

INTRODUCTION

Water is an essential element of civilization. It is as the basis of large varieties of activities including water supply, irrigation, energy, industry, aquaculture, recreation, transportation. However, water resources can be polluted by various sources of pollution such as the industrial discharges, agricultural and urban runoff and municipal waste. For the protection of aquatic life, water pollution should be minimized or removed to satisfy the protection requirements of the European Water Framework Directive of 23 October 2000 adopted by the Council and European Parliament [3]. The contamination of aquatic ecosystems becomes more and more severe for some region. Among the most toxic contaminants, there are metal trace elements (ETM) or heavy metals. Human activities are largely responsible for the contamination of aquatic ecosystems by ETM. Indeed, these elements are presents in the runoff of agricultural soils containing plant protection products based on copper and arsenic, and effluent discharges. Three quarters of organic carbon in the waste water is present in the form of carbohydrates, fats, proteins, amino acids, and volatile acids [4]. The inorganic components contain high amount of sodium, calcium, potassium, magnesium, chloride, sulfate, phosphate, ammonium bicarbonate and heavy metals [5, 6]. This complex mixture of natural organic and inorganic materials and synthetic compounds can be treated with the blue-green algae. This fascinating idea was launched fifty-nine years ago in the United States by Oswald and Gotaas (1957) [7] and has since been extensively tested in many countries [8-10]. Bio-treatment with micro algae is particularly attractive thanks to the photosynthetic capacity responsible for the conversion of solar energy into useful energy from biomass via the consumption of nutrients in polluted waters. Blue-green algae are microorganisms that are part of the phytoplankton community of the aquatic environment. They are classified in the same group as bacteria, which are recognized as being more primitive algae. For this reason, the blue-green algae are also called "cyanobacteria". However, blue-green algae have common characteristics with algae, including pigments in their cells that allow photosynthesis. The term "blue-green" refers to their blue (phycocyanin) and green (chlorophyll) pigments, which dominate in most species.

Study Objectives

As a first step, growing of the three species of algae were studied on synthetic and culture media from the output of Villeneuve d'Ascq, the water treatment plant (Nord Pas de Calais Regions, France), following the dynamics of nutrient elements in the affected areas, macro and microelements, algae productivity and pH of analyzed medium.

A second step concerning the algal biomass obtained we've achieved in all new experiences for both mineral and organic micro-pollutants bioaccumulation in free form of algae suspension as well as in the immobilizer form.

The investigation of biodegradation of a lot of organic micro pollutants such as pharmaceutical products, bisphenols and HAPs was as a function of time in free and entrapped in Na-alginate beads form of blue algae.

The biosorption of cadmium (II) ions by the biomass of algae *Nostoc gelatinosum* and *Spirulina platensis* immobilized and empty alginate beads was investigated as a function of time and concentration of initial cadmium (II). Seven consecutive cycles of adsorption-regeneration in a natural water treatment system were studied. Experience was performed in a batch system with artificial agitation and natural illumination of algae samples by monitoring the change of pH values. A control set of Na-alginate empty beads in triplicate was performed.

A variety of studies concerning the dynamics of nutrients as well as macro and trace elements concentration in wastewater environments are analyzed and linked to productivity and environmental pH.

Chapter 1

Microalgae

Cyanophytes

I.1. General characteristics of blue-green algae

Cyanophytes

The microscopic algae present in marine or salty waters, are among the first living creatures that appeared on Earth 3.5 billion years ago. Their collection and use is old and not new, although few species are actually listed. Today, the most widely used microalgae (Spirulina, Chlorella, Dunaliella and Haematoccus) represent a combined market of over 500 million dollars. Contrary to popular belief, the production of high added-value molecules (health, food supplements and cosmetics) is the fastest growing segment in the short term, before biomaterials and animal food, and especially to the use of micro-algae for pollution control and energy production. While Asia, and particularly China, is the largest producer of microalgae in the world, in Europe, it is mainly Germany and Spain, which have historically invested in the sector. France is in a paradoxical position: pioneer in upstream research (1st row for publications and 4th in the world for patent filing in 2010), it represents 5% of investments on projects worldwide and provides insignificant industrial production.

Dominated by a relatively mature market of *Spirulina platensis*, microalgae production is sometimes presented as an original solution working for sustainable development of rural areas. Being an additional source of revenue and recovery of polluted sites through their breeding, economic field can save space and acquiring the food and high quality water. To this end, the production, processing and marketing of high-value co-products through the joint development of a value chain will only be meaningful if these activities are part of a relevant territorial pattern and if the structure of that value chain is relatively balanced between global players of algal production [11].

Among micro algae, cyanobacteria are one of the oldest and most successful life forms on Earth. Fossil remains of blue-green algae were found in the layers of the Earth's crust, the age of which exceeds 3.5 billion years. These were the first vegetable organisms, which as a result of photosynthesis, gave a boost to the development of life on the Earth. Despite their exaggerated age, this group of microscopic organisms evolved very little during the development life, both in terms of morphological and taxonomic diversity. In comparison with other algae taxonomic groups, for example with the bacilariophyts, chlorophytes or the cryzophyts, the blue-green algae are a group composed of a small number of species (1,700).

However, the cyanophyts algae are spread over the water conditions, and under the most extreme conditions, ground bearing life [12]. Long evolutionary history and photoautotrophic way of life allowed them to colonize different habitats and is currently the most important group of organisms, numerically speaking. Cyanophytes terrestrial algae prefer moist soil surface. But there are a significant number of species that inhabit tree shells, wet walls of various buildings, monuments of art, often damaging their appearance. The outcome of blue-green algal metabolism, more than anything else, includes removing from the environment a range of organic acids that break down mineral substances, the concrete, cause metal corrosion, thereby causing the outstanding damage to buildings [12]. Since algae are the simplest cyanophytes organisms, phototrophs plants, they have retained to this day the capacity to accommodate to the most extreme environmental conditions and to entertain both autotrophic and heterotrophic. Even if their biotechnology potential is highly appreciated globally with a biomass estimate to the thousand million tons, their use by humans is still insufficiently developed.

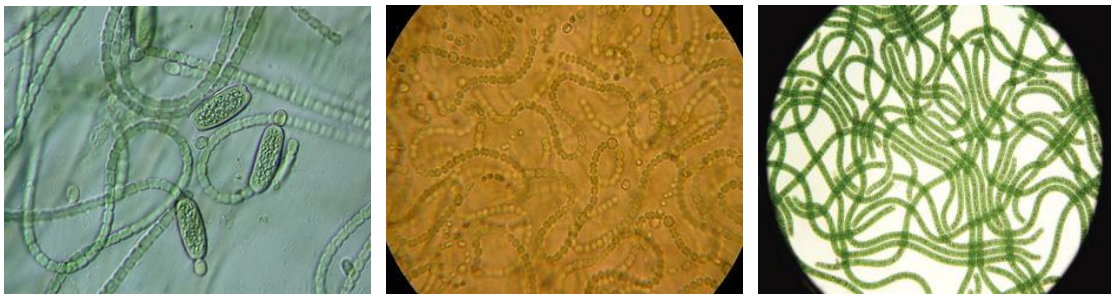
I.1.1. Cyanobacterial water flowers

In the presence of a large amount of phosphorus, cyanobacteria can reproduce rapidly then form a bloom. This represents an important density of cyanobacteria that the phenomenon is generally visible to the naked eye, can reach tens of thousands to several million cells per milliliter in an aquatic environment. When overflowing cyanobacteria is found only on the surface, it is called "scum".

Fig.2.1. Cyanobacterial water flowers

Because of the wind, the foam is often crowded near the shore. Indeed, cyanobacterial blooms are observed especially in aquatic environments enriched with phosphorus surplus. When a stream becomes green it means that it gets too much of this nutrient. Nutrient inputs to the aquatic environment can come from different sources: manure, compost or fertilizer applied to soils or lawns, septic systems, discharges of municipal and industrial waste water untreated or insufficiently treated, etc.

I.1.2. Characteristics of blue-green algae used



A B C

Fig.1.2 (A) *Cylindrospermum licheniforme (Bory) Kütz*, (B) *Nostoc gelatinosum (Schousb) Elenk* and (C) *Spirulina platensis (Nordst.) Geitl. CAUSM. 26*

I.1.2.1. Name of the strain of the species:

- *Cylindrospermum licheniforme (Bory) Kütz*
- *Nostoc gelatinosum (Schousb) Elenk.*
- *Spirulina platensis (Nordst.) Geitl. CAUSM. 26*

I.1.2.2. Entitled attributed to the strain of the applicant:

- *Cylindrospermum licheniforme (Bory) Kütz.*
- *Nostoc gelatinosum (Schousb) Elenk.*
- *Spirulina platensis (Nordst.) Geitl*

I.1.2.3. Origin of strains

- The blue-green algae *Cylindrospermum licheniforme (Bory) Kütz.* was collected in 2007 from soil samples collected from the wheat crop in the ground near the

town Cimislia, Republic of Moldova and simultaneously from the sunflower crop near the village Bozieni, Hincesti district. The strain was obtained by the method of inoculation on solid and liquid media.

- The *Nostoc gelatinosum (Schousb) Elenk.* strain was selected from soil samples taken on agricultural land Bogdanovca village, Cimislia district, Republic of Moldova by the same sowing methods.
- The *Spirulina platensis (Nordst.) Geitl. CALU-835* strain was selected in a pond in Ungheni district, near the village of Nicolaevca, Republic of Moldova in June 1979. It is noted that the sample revealed in the pond close to village Nicolaevca the *Spirulina platensis (Nordst.) Geitl. CALU-835* is almost in monoculture. Therefore, it was easy to select pure strain. During sampling, absolutely all filaments *Spirulina platensis (Nordst.) Geitl. CALU-835* was coiled. Being passed in laboratory conditions on nutrient medium Zarouk in approximately 40 days all trichomes was decoiled.

The strains were identified in the laboratory, Algologie, of Moldova State University by Professor Salaru Vasile and colleagues.

I.1.2.4. Morphological and cultural characteristics of stem

The strain *Cylindrospermum licheniforme (Bory) Kütz* presents asymmetric filaments together or free cells in the larger base bluish color - green cylindrical. Vegetative cells or square, the length and the width is 4-5 and 2.5- 4 μ respectively. The heterocysts apical cones are located only at the end of basal trichome. The spores of the algae have an average length of 21 μ and width of 12.2 μ and are linked to heterocysts. A specific feature of this species is the presence of a membrane stained brown - yellow in the spores, generally smooth and thin, that after two weeks of cultivation thickens, reaching 2.44 μ.

The strain *Nostoc gelatinosum (Schousb) Elenk* cells are uniformly distributed in colonies with brown fibers. The cells are rectangular-length dimensions 5.12 to 10.05 μ and a width of 2.44 to 7.32 μ. Sometimes there are conical heterocysts (10.0 to 5.12 μ-length and 3.66 to 6.1 μ-width). During aging, interlayers heterocysts culturing a diameter of 7.3 μ and elongated spores of a size in length between 7.32 and 14.64 μ and width between 4.88 and 8.54 μ.

The *Spirulina platensis (Nordst.) Geitl. CALU-835* stem consists of solitary linear filaments with 350-1000 microns in length and width up to 12-15 microns. Coloration of filaments is pronounced dark green, which differs from every other strain of *Spirulina platensis* described before. The cells have the form of drums with many grains across the walls partitions. Each cell is provided with a large number of gaseous vacuoles, compared with other strains of *Spirulina platensis*, of an indeterminate shape that refracts strong light during a microscopic analysis and therefore there appear some bright spots. The strain is multiplied by fragmentation of trichomes in hormogoniums.

I.1.2.5. The physiological and biochemical products synthesized by the strains

The main biologically active substances from the strain of blue-green algae *Cylindrospermum lichenoid (Bory) Kütz* contain: carbohydrates - 41.01%; lipids - 15.92%; proteins - 18.87% and pigments: allophycocyanine - 0.63%; phycocyanin - 0.83%.

The *Nostoc gelatinosum (Schousb) Elenk* strain grows well in a liquid medium Gusev at 23-27 ° C and forms around it a gelatinous mass. The main biologically active substances are proteins - 18.87%; lipids - 9.88%; sugars - 11.52%; of allophycocyanin pigments - 0.7%; phycocyanin - 0.14%.

The *Spirulina platensis (Nordst.) Geitl. CALU-835* strains have a productivity of 10-12 g / m2 dry weight matter per day. Biomass includes: protein - 65-70% carbohydrates - 15-16% fat - 5.5 to 6.8%, nucleic acids - 4.3% β-carotene - 0.42 to 0.44%, ascorbic acid - 80-100 mg / 100 g biomass dry tocopherol - 30-35 mg/100 g of dry biomass. The best grows of strain is at pH - 9.5-10 on Zarruc medium, a composition of which can be seen in the Annex1. It differs from other *Spirulina platensis* strains in that it supports the increase in nutrient medium of Na-bicarbonate up to 22-25 g/L.

I.1.2.6. The field of application of the strains

Algae are classified in a group of biologically active endogenous substances represented by proteins (proteids), vitamins, lipids, carbohydrates, enzymes and plant hormones [13]. *Cylindrospermum licheniforme (Bory) Kütz* is an alternative source of biologically active substances that can be used in crops, livestock and agriculture. *Nostoc gelatinosum (Schousb) Elenk* form colonial structures (air or water) highly resistant to UV,

radioactivity, the basic pH, desiccation and to various environmental stresses (including thermal shock). *Spirulina platensis (Nordst.) Geitl. CALU-835* is one of the biologically active supplements which are a source of easily digestible protein moreover being rich in vitamins, amino acids, trace elements, unsaturated fatty acids. Extensive use of this algae strain was limited by the exaggerated cost. It can already grow in wastewaters culture media thus solve the problem of biosorption of heavy metals from solutions [12].

I.1.2.7. The strains production parameters

Cylindrospermum licheniforme (Bory) Kütz: Absolute Dry Biomass (ADB) - 1, 28 g/L.
Nostoc gelatinosum (Schousb) Elenk- (ADB) - 1, 68 g/L
Spirulina platensis (Nordst.) Geitl. CALU-835 - 2, 3 g/L.

I.1.2.8. The method for determining the strains reproductive activity

For all three strains reproductive determination is the breeding capacity by the method of weighing the algal biomass filtered from a liter of algal suspension. The report is in terms of Absolute Dry Biomass (ADB) at a temperature of 105 ° C.

I.1.2.9. Cultivation media for breeding blue-green algae. Conditions and components of algae culture medium

The cultivation media of *Cylindrospermum licheniforme (Bory) Kütz* strains is made such that the average temperature conditions of 25 ° C and 1000 lx illumination within 24 hours to be ensured. The optimal environment for the development of the strain has the following composition: K_2HPO_4 - 0.2 g/L; $MgSO_4$.$7H_2O$ - 0.2 g /L; $CaCl_2$. $2H_2O$- trace; $FeCl_3$ - trace. The medium is prepared in double distilled water.

The cultivation media of *Nostoc gelatinosum (Schousb) Elenk* strains is made such that the temperature conditions of 23-27 ° C and 1000 lx illumination within 24 hours to be ensured. The optimal environment for the development of the strain is the *Gusev* medium that has the following composition: $MgSO_4.7H_2O$ - 0.2 g/L; KH_2PO_4 - 0.1 g/L; $CaCl_2$ - 0.1 g/L; $FeCl_3$ - 0.008 g /L. The medium is prepared in tap water. All recipes from the culture media of blue-green algae are exposed in Annex 1.

The cultivation media of *Spirulina platensis (Nordst.) Geitl. CALU-835* strains are made such that the temperature conditions of 23-27 ° C and 1000 lx illumination within 24 hours to be ensured. The optimal environment for the development of the strain is the *Gusev* medium that has the following composition: $NaHCO_3$ -16.8 g/L; $NaNO_3$ -2.5 g/L; $K_2HPO_4.3H_2O$ -0.5 g/L; K_2SO_4 .1 g/L; NaCl -1 g/L; $MgSO_4.7H_2O$ - 0,2 g/L; $CaCl_2.6H_2O$ - 0.04 g/L; $FeSO_4$ .0.01 g/L; EDTA -0.08 g/L; micronutrients solution - 4 ml at a pH- 9.5.

I.1.3. Algal metabolites

Cyanobacterial hydrogen has been considered as a very promising source of alternative energy, and has now been made commercially available. In addition to these applications, cyanobacteria are also used in aquaculture, wastewater treatment, food, fertilizers, production of secondary metabolites including exopolysaccharides, vitamins, toxins, enzymes and pharmaceuticals [14]. Products from algal biomass are used in wastewater treatment. Thus we intend to develop technologies of cultivation of algae in the laboratory that would combine treating wastewater from sewage treatment plants by raising the algal biomass and using it subsequently for treating heavy metals in very low concentrations present in surface waters.

Sulphonated exopolysaccharides were generally considered to be specific of EPS of eukaryotic algae, therefore should be considered as a characteristic of cyanobacteria. The poly-anionic characteristic of the exopolysaccharide of blue-green algae is responsible for its capacity to provide strong pseudo plastic solutions at concentrations that appear exceptionally low for biological polymers, although the minimum value of about 0.15 g/L would probably be reached, as for other polymer, at concentrations of 1 to 2 g/L [15]. The range of biological activity of secondary metabolites isolated from cyanobacteria includes antibacterial, antifungal, antialgal, antiprotozoal and antiviral activities [14]. Some preparations are used for prevention purposes or viral encephalitis due to the presence of sulphonated polysaccharides [16, 17].

With regard to the production and the releasing of *exocellular polysaccharides* (RPS) into the culture medium, more than one hundred cyanobacterial strains, belonging to twenty different genera, have been investigated in the last 60 years. The chemical properties show that such polysaccharides are complex anionic heteropolymers, in about 80% cases containing six to ten different monosaccharides. On the whole, the ten monosaccharides in cyanobacterial RPSs are: the hexoses glucose, galactose and mannose, the pentoses ribose,

arabinose and xylose, the deoxyhexosesfucose and rhamnose and the acidic hexoses glucuronic and galacturonic acid. In about 90% cases containing one or more uronic acids; almost all have non-saccharide components, such as peptidic moieties, acetyl, pyruvyl and/or sulphate groups. Based on such ingredients, and their anionic character, common to most cyanobacterial RPSs, these cyanobacterial RPSs show promise as cation-chelating compounds [18] and the residual capsulated cyanobacterial biomass, even following an RPS extraction, could be an effective cation-chelating material [19]. Various applications among others such as improvement of water holding capacity of soil, detoxification of heavy metals and radionuclide's contaminated water and removal of solid matter from water reservoirs have been proposed for cyanobacterial EPS [20].

The extracellular release of polysaccharides by phytoplankton may constitute the mucilaginous capsules and sheets typical of some microalgae species, or dissolve in the external environment [21]. Polysaccharide excretion takes place during all growth phases, whereas its rate of release per cell is enhanced in the exponential and stationary phases [22]. Polysaccharides characterized by high concentrations of charged components (like uronic acids, sulfate or phosphate groups, pyruvate ketals) usually form stable gels in the presence of metallic ion and are the most promising for the removal of toxic metals from polluted waters [18]. However, the mere determination of the quantity of charged groups is not enough for anticipating the actual metal binding capability of a polymer, because, depending on the conformational structure of the macromolecules, some of the charged groups could be hardly accessible for the ions.

Bioflocculants are extracellular macromolecules that are known for their ability to clarify turbid water [23]. Since the benthic photoautotrophic cyanobacteria occupy a low-light zone, water-clarifying bio-flocculants are important to the photosynthetic activity of these organisms. Because of their agglutinating properties, the bio-flocculants may also remove soluble nutrients from the water column, resulting in heterotrophic activity in the sediment region. In addition, if the bio-flocculants have numerous negatively charged binding sites, they may remove cations from the environment, thereby protecting cells from the toxic effects of heavy metal [23]. In their paper [23] report that microbial mats that were dominated by cyanobacteria produced negatively charged polysaccharide bio-flocculants. The relationship between metal removal and bio-flocculants production proves that once stimulated, bio-flocculants production continued at an elevated level even after the metal concentration decreased. In the period between days 6 and 8 there was rapid production of the bio-

flocculants and a corresponding decrease in the metal concentration. Multiple additions of metal resulted in insignificant increases in bio-flocculants production. Controls that contained no metal revealed that metal (Mn) was necessary for stimulation of bio-flocculants production [23].

Siderophores. Over the past six decades it has been shown that many micro-organisms produce high affinity ferric-iron transport compounds. There are two general classes of these microbial ferric-chelating compounds, secondary hydroxamates and catechol's, collectively termed siderophores[24, 25]. It can be argued that microalgae, particularly at the alkaline pH and aerobic conditions in the photic zone, should possess a siderophore-mediated iron uptake system in order to render the insoluble colloidal iron into an available form. Siderophore activity has been detected in various marine ecosystems [26]. Murphy et al. [27] suggested that hydroxamate chelators produced by blue-green algae may antagonize growth of other microalgae and hence be a factor in blue-green algal blooms. In 1976, Simpson & Neilands [28] isolated schizokinen, the di-hydroxamate siderophore produced by *Bacillus megaterium*, from the culture supernatant of iron-starved blue-green algae *Anabaena sp.*

I.1.4. Algal toxins

Secondary metabolites relate to compounds that are not used by the body to its primary metabolism, namely cell division or metabolism. Secondary metabolites include compounds which act as hormones, antibiotics, and toxins allele-chemicals. The toxins are secondary compounds that have a detrimental effect on other tissues, cells or organisms [13]. As yet we do not know why cyanobacteria produce toxins; we can assume that they can function as protective compounds as much as anti-herbivore compounds are in vascular plants. Cyanobacteria, or blue-green algae, owe their name to the presence of photosynthetic pigments. Cyanobacteria are found in freshwater all over the world. It was recently revised regarding the classification and forty toxigenic species were identified. Freshwater cyanobacteria can accumulate in surface water supplies as "blooms" and can focus on the surface in the form of blue "scum". Cyanobacteria produce an unparalleled variety of toxins. In the last decade, biosynthetic pathways have been assigned to the majority of the known toxin families. Every year in the summer months, recurrent mass developments of microscopic algae, so-called harmful algal blooms (HABs), become a matter of public concern. Many of the HAB-causing organisms are known to produce toxins that have a

variety of adverse effects, such as acute diarrhea, skin irritation, liver damage neurotoxicity in humans and domestic animals and death in wild animals. Cyanobacteria become particularly dominant as surface scum in freshwater lakes and salty water in the summer months, but may also form dense benthic mats in marine and freshwater habitats. Health risks from cyanobacteria are posed by swimming and other recreational activities, by drinking water or otherwise consuming tissues or dietary supplements that have accumulated the toxins. The mass development of blooms is also connected with significant economic costs on a global scale due to the necessity of bloom management and cyanobacteria removal as well as the negative effects on farming and recreation [29]. Copper sulfate at 1 ppm (1µg/ml) causes lysis of cyanobacteria within a few hours and the release into the water of any toxic cellular components [30].

I.1.4.1. Identity of cyanobacterial toxins

Cyanobacterial toxins include neurotoxins (ex., anatoxins), hepatotoxins (ex., microcystins), skin irritants and other toxins. Hepatotoxins and neurotoxins are produced by cyanobacteria commonly in surface water supplies and therefore seem the most relevant to water supplies [31, 32]. Neurotoxins, however, are relatively unstable and are therefore considered that they are not as widespread as hepatotoxins in water supplies. They do not appear to have the same level of chronic risk [32]. However, it should be noted that quantitative data on concentrations of neurotoxins in water supplies are limited. It may be that neurotoxins are more widespread than currently believed, particularly given that we have established a link between many neuro-toxigens algae and death of both livestock and pets. Most hepatotoxins are collectively called microcystins, from *Microcystis aeruginosa* hepatotoxin the first to have been isolated. There are isolated fifty different microcystins and the same flower water can produce more. Little is known about the factors leading to the production of toxins by cyanobacteria. Laboratory studies show that some of the environmental factors mentioned above such as temperature, light, nitrogen concentrations, carbon availability (as bicarbonate, carbonate and carbon dioxide), the concentrations of phosphate and pH, could be significant. This is the reason why the toxin production varies considerably between different strains of the same species. The differences in genetic and metabolic processes may also play an important role in the production of these secondary metabolites. Cyanobacterial toxins tend to be associated with cyanobacterial cells and may be membrane bound or be present in the Free State within the cells.

Toxin concentrations do not necessarily coincide with the maximum volume of the algal biomass. The concentration of toxins cyanobacterial biomass unit can vary considerably over time, regardless of fluctuations in the population of blue algae, performed studies showing that toxins are released mainly when cells age and die and release their contents passively [31]. Various physical, chemical and biological factors influence the development of cyanobacteria blooms.

I.1.5. Preventing of blue-green algae blooms in the freshwater bodies

Control techniques of blue-green algae populations in freshwater bodies represent some measures to prevent mass development of algae derived largely from the same cultivation techniques with which we meet below. One needs therefore to establish a deficiency in nutrients (especially N, P) through proper management of river basins which limits the intake of nutrients such as urban wastewater effluents, storm water from cultivated soil. One of the possibilities in the long term period is the addition of chemicals to the source of polluted water to reduce the availability of nutrients (e.g. $FeCl_3$ to precipitate P), but we realize that this variant is not very ecological and it needs a long period of time. An alternative way of uncontrolled population growth of blue-green algae is the application of physical methods such as the exclusion of light, or artificial reservoir delamination. Using chemicals such as alum and gypsum as algistats is a possible short-term path to control algal bloom for small sized communities. On the other hand, using algaecide's such as $CuSO_4$ or Cl_2 is not desired because they lead to the elimination from the cianophytes mature algae bloom sites of toxins in water body. Last but not least we can say that the use of algaecides destroy the all possible algae species other that cyanobacteria, reducing the competition by the seconds. Blue-green algae can thus gain more ground by uncontrolled development.

Chapter II

MATERIALS AND METHODS

II.1. Laboratory presentation

The Analytical Chemistry and Marine Team (ECAM) from the University of Lille 1, Science and Technology was founded in 1981 at the University of Lille 1, Science and Technology. In January 2006, the team joined the Laboratory of Sedimentary Processes and Results Areas (PBDS), which later became the Géosystèmes laboratory in 2008. This research group is associated with the CNRS as mixed research unit (UMR 8157). Since January 1, 2015, the ECAM Geosystems joined the Laboratory Spectrochimie Infrared and Raman (LASIR- UMR 8516) in team Physical Chemistry of the Environment. ECAM focuses its research on two axes: one axis is based on the origin and fate of metal pollutants; the second is the study of the origin, behavior and fate of organic contaminants, and organic matter in natural environments. The Algological Team from Republic of Moldova State University was founded at the same University by Professor Vasile Salaru and is until today one of the reference laboratories in the field of algae growth and preservation of algae cultures in an Eastern European Region.

II.2. Algae cultivation

II.2.1. The necessity to cultivate cyanobacteria

Most of the microalgae biomass uses for biotechnology is produced industrially or by cultivating extensive ensuring illumination and a sufficient exchange of gases (cultivation autotrophic) or through alternative cultivation to the use of organic substrates as carbon source and energy (or heterotrophic cultivation mixotroph). Applied phycology scored important achievements in microalgae cultivation and exploitation of the resources but the global production of algal biomass is still low (9.000 -10.000 t dry matter per year) and biomass prices are high, ranging from 10 to 300 EUR/kg [33]. Cyanobacteria have been identified as a rich source of biologically active compounds with antiviral, antibacterial, antifungal and anticancer activities [34, 35]. Several strains of cyanobacteria like *Spirulina platensis*, were found to accumulate polyhydroxyalkanoates (PHA), which can be used as a substitute for non-biodegradable petrochemical-based plastics. These are of particular interest as PHA producers because of their minimal nutrient requirements for growth and capability of accumulating PHA by oxygenic photosynthesis because cyanobacteria fix CO_2 from the atmosphere and turn it into PHA under nitrogen limiting conditions [14]. Other studies

reported the ability of cyanobacteria to oxidize oil components [36] and other complex organic compounds such as surfactants and herbicides [37].

Even if the cyanobacteria are not directly responsible for the degradation of these compounds, but the association of cyanobacteria alongside with the aerobic organotrophs facilitated the degradation process and both groups constituted ideal consortia for degradation of petroleum and other complex organic compounds [38]. Cyanobacteria within these consortia facilitated the degradation processes by providing the associated oil-degrading bacteria with the necessary oxygen, organics and fixed nitrogen.

II.2.2. Arguments of algae cultivation

For recovery and exploitation of re-generable resources it is necessary to increase the scale of algae obtained by major cultivation. Growing algae, algal biomass can be used in the broad practice. Seaweed cultivation may be performed under open field and closed - in the laboratory. The advantages of algae cultivation in the laboratory were found to be highest, characterized by obtaining organic products free from impurities, which were and are important environmental issues and health background. The first attempts of cultivating algae in culture laboratory began after more than 130 years, in the context of plant physiology research.

Growing algae under control of vital activities is one of the perspective directions of aquaculture, biotechnology and practical algology. At present, the interest in mass cultivation of algae has increased greatly. This growing has noticeable benefits and very good results were obtained from algal biomass application in vast areas. In recent years a highly successful industrialization is assigned by microscopic algae's photosynthesis-based, able to absorb carbon from the atmosphere and release oxygen. This presents a great argument for the use of algae in the global warming process improvement. Growing algae is substantiated by the fact that algae are the first food chain of aquatic and terrestrial ecosystems and constitute the primary organic matter from inorganic components of the environment. Thus, this increases interest in the practical application of algae to increase production and ensure the equilibrium of ecosystems. Compared with algae grown under controlled conditions in the laboratory, algae collected in nature are less secure because algal biomass jointly meets many organisms (such as bacteria and invertebrates) that are difficult to remove and sometimes as a result dangerous for human and animal consumption. Growing in laboratory conditions is more expensive but has increased security because the crops grown are first isolated and deprived

of other organisms.

Lately, at a global scale, red, brown, green and blue-green algae are widely cultivated in large amounts. According to a quantitative criterion, algal biomass is about 20% of global aquaculture. Among the most important algae grown for biomass use in food, one mentions 81 species of red algae, 54 species of brown algae, 25 species of green algae, and 8 species of blue-green algae. Another argument for industrial cultivation of algae biomass is their biochemical content. Algae are used to protect the environment, creating sources of bonds to various chemicals that are eliminated in the environment and at the same time produced a wide amount of biologically active substances. Algae participate in the formation of hydro-biocenoses, influencing the organoleptic properties of water, the formation of surface water quality. It should be listed here the great capacity of oxygen enriched water, and the purification of contaminated water with biogenic elements, heavy metals, radionuclides, contributing to the general treatment of all environmental components.

II.2.3. General Characteristics of algae cultivation

The algae cultivation in laboratory conditions presents a major scientific interest. To carry out laboratory experiments for obtaining algal biomass algal cultures are used. Growing algae in laboratory conditions consists of a set of complex operations which depend on the purpose and research tasks, specific biological characteristics of algae. Algae cultivation technique is a rather complicated process. Cultivation technique of each algal species is slightly different, but still largely the same basic steps are made. Algae cultivation stages are varied, but broadly six basic steps are achieved (Fig. 2.1.).

The first and most important step is collecting samples of algae from the wild in places where it develops massively (step 1), transporting them in the laboratory and keeping dense algal cultures on nutrient media where they were collected, or in the artificial nutrient media (stage 2), followed by a more difficult procedure for obtaining pure cultures of algae in nutrient media based on the use of artificial sometimes solid and liquid (step 3) base. The greatest difficulty is getting the cultivation of algae in a pure species in monoculture. Mono-algal culture species must be made free of bacteria and other microorganisms by applying specific techniques (step 4). After the successful implementation of the 4 stages, algae are cultivated under controlled conditions with the influence of different factors to determine the optimal conditions for algal biomass growth (step 5). One important thing is keeping algae in

collections, to ensure the viability of culture maintenance purposes, lengthy algae cultivation for future needs.

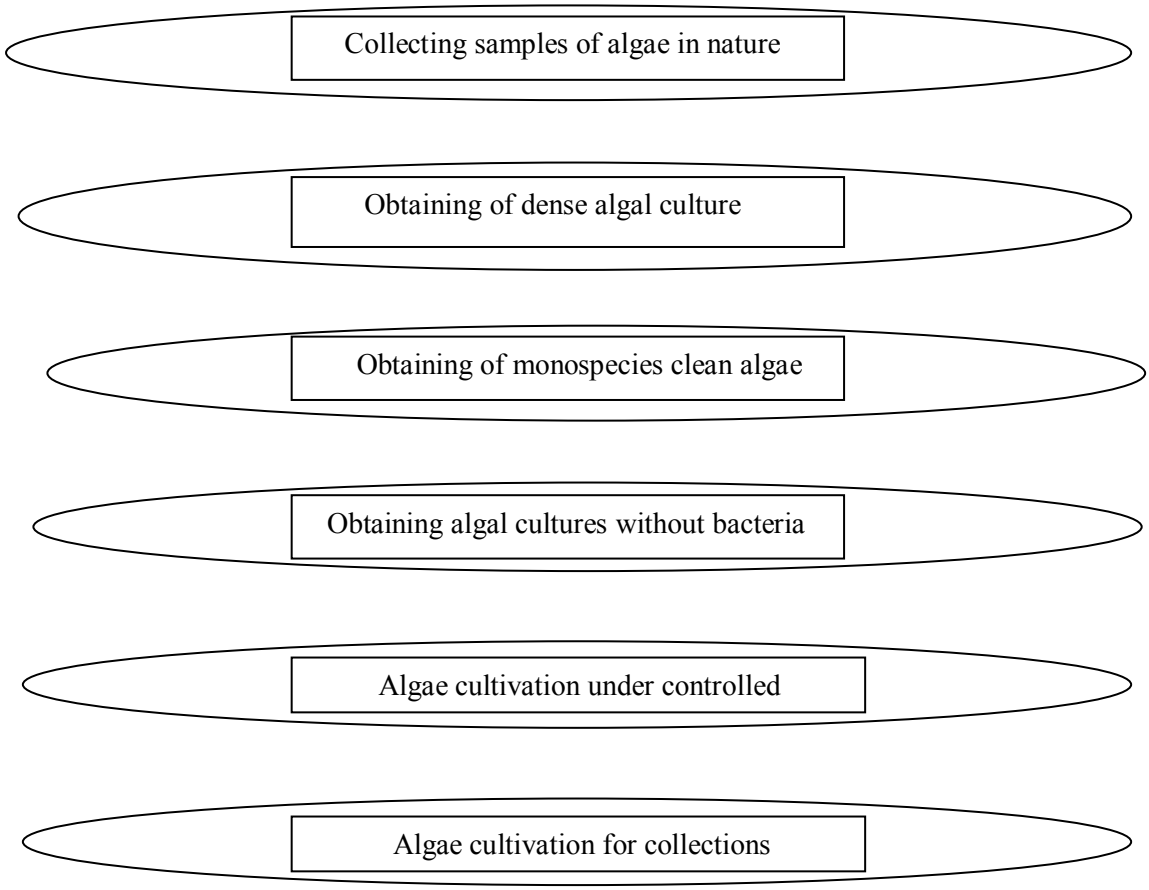


Fig.2.1. Generals stages of algae cultivation

Algae cultivation steps must be performed consecutively, one after another, to obtain pure algological cultures. Once obtained, these must be maintained and cultivated according to processes described by Dobrojan and al.[39] It is to be noted that each phase of cultivation of algae, shown in Figure 1, is conducted using specific methods and procedures and for obtaining algal biomass one must strictly observe the growing conditions. Thus, the effective cultivation of algae and algal biomass we described depends on the cultivation methods of algae.

II.2.4. Methods of algae cultivation. General characteristics of methods of algae cultivation

The most common methods used to cultivate algae, especially for industrial purposes are:
- Regular cultivation;
- Periodic deep cultivation;
- Multicyclic cultivation;
- Semi-continuous cultivation;
- Continuous cultivation.

II.2.4.1. Regular cultivation

Regular cultivation provides seeded inoculation material in nutrient medium (inoculation of the cells in the medium) at the beginning of the culture until reaching and maintaining suggested by population growth phase. The concentration of microorganisms in the cultivation process periodic increases, which stops the substrate or because of the limited inhibition of toxic metabolic products. Characteristic for this type of cultivation are incessant changes of physiological state of cells caused by changing conditions caused in turn by the activity of vital cells themselves. It thus appears that cultivation can maintain regular cell reproduction only for a limited time. After exponential phase of growth, the population begins to suffer due to insufficient nutrients and metabolic products are inhibited, leading to worsening physiological state of cells.

II.2.4.2. Periodic cultivation in depth

Periodic cultivation in depth is used in the cultivation of the liquid nutrient medium with a periodically stirring of crop to balance the growing conditions in different parts of the workload of plant cultivation. This led to the emergence of dynamic systems of cultivation in depth, endowed with special equipment. The cultivation of the fermentation regular ball, as compared with that at the surface, accelerate the growth and developed the so-called micro-algae at the expense of removing "hungry zone" around the cell and ensures a homogeneous culture "with ideal stirring"

II.2.4.3. Regular longterm cultivation

Regular longterm cultivation provides only one filling and emptying of the fermenters. The development cycle of microalgae cultivation process is longer regular account or insert (periodic or continuous), or due to keeping cells in the system (dialytic culture). Microalgae concentration and velocity periodic lengthy processes are higher compared to regular periodic processes. Thus, both the exponential phase is extended, and especially the linear growth phase. The addition of nutrients in the medium periodically turns the regular process time-consuming and is used generally in the manufacture of biosynthesis, which increases the performance of the process. At regular intervals cultures gradually accumulate metabolic products which inhibit the growth. To increase productivity or to accelerate the concentration of biomass dialysis is used.

The essence of this method is to develop in a limited space of an organic semipermeable membrane and the product is distributed outside of solution. The simplest method is the cultivation of its kind in polyethylene bags, but this is not widely spread. Regular long cultivation can be attributed to cultivate microalgae systems with added environmental dialytic as the addition increases the growth and development of algal populations, but this system is not continuous lack of biomass extraction procedure. In systems with added environmental dialytic total biomass increases until it reaches a state such that it becomes impossible stirring further.

Thus, the method allows to grow micro-organisms, including algae, to obtain a high biomass volume. Dialytic cultures are generally used in three cases: 1) to concentrate nedifundat; 2) to reduce product concentration difundat, inhibiting growth and increased production of biomass; 3) for acquisition and separation of the product difundat cells.

II.2.4.4. Multicyclic cultivation

Multicyclic cultivation is characterized in that crop cultivation is repeated many times, with multiple sterilization of environment. In multicyclic cultivation process the dependence between microalgae concentration and relative specific speed (in each cycle) are similar to those of regular cultivation. Growing multicyclic may be different. It can be carried out in a fermenter, repeating several times the complete cycle of development of the crop without interruption for sterilization. In a fermenter the cycle can be repeated or decreased, ending,

for example, with the exponential growth phase. Multicyclic growth provides the ability to implement multicyclic and multistadic process, which is based on the principle of repetition and follow regular cultivation, going through several fermenters in order to use culture on a long-term period. When the culture in a fermenter reaches an exponential growth phase condition it is transferred to another part of the fermentor. In the first reactor culture continue to grow until the next stage. When in the second fermenter the culture also reach the exponential state, some of it is transferred to the 3rd fermenter and so on. Since culture achieve only exponential phase, her aging or degeneration does not occur. Application of such methods ensures the reduction of the costs of obtaining a few times of the product, compared to regular cultivation method.

II.2.4.5. Semi-continuous cultivation

In semi-continuous systems the loading and unloading of fermenter is performed once, but in growing process of mass culture some of the content is discarded and the volume issued is replaced with freshly prepared nutrient medium. Thus removal-replacement system works. In fact, semi-continuous cultivation is characterized by the density and volume of removed culture and adding a fresh nutrient medium to the operational fermentor.

Established programme in the semi-continous systems is characterized by swinging of organisms concentration around one and the same relatively stable quantity with a specific rate of population growth, relatively constant. This type of cultivation can be carried out in omogenous open systems and can be applied to any fermentor used to regular cultivation, equipped with agitation and aeration systems.

II.2.4.6. Continued cultivation

Continued cultivation compared with regular cultivation, in continuous process nutrient medium is inserted permanently as permanent occurs and extracting biomass with vital products. The fixed regime in continuous cultivation of microalgae is characterized by stable concentration through the specific growth rate of the population. This type of dynamic cultivation is carried out in open systems, which can be both homogeneous and heterogeneous. These systems are capable of operating within a long time and in a steady state. Any periodic process can be transformed into continuous process flow.

Continuous cultivation creates the possibility of maintaining permanent growth conditions reaching an environment so nutrient content, which want only one of the factors may limit the growth. If in such a process the population density is determined by the chemical content of the environment (the concentration of limiting growth factor), it is called hemostat cultivation. Hemostat cultivation under strictly controlled conditions underpinning the study of physiological, biochemical and other general properties of microalgae cell cultures or otherwise. The phenomenon that occurs at long intervals under natural conditions, can be modeled in the laboratory, working with continuous culture, this could help us solve a large number of environmental problems in a very short time.

II.2.4.7. Synchronized cultivation

Cultures of blue-green algae even if they are taken at the same time a seed is a set of cells that are in different individual development stages. The tendency to get culture cells which are in a stage of development helped develop a synchronized method of division. The essence of the method is as follows: using various methods like mechanical extraction (Selective methods) influence through physical, chemical and biological, cells alga culture that divide a single physiological condition. Synchronized growing may be of two kinds: regular and continuous. The drawback of the first type of cultivation is in sync that crops lose their bodies relatively quickly (2-3 generations) capacity synchronized division. The second method relies on the fact that the content periodically extracted from the growing half at a time equals to a generation, at the same time it adds the same amount of fresh nutrient medium.

II.2.4.8. Intensive Algae Cultivation

Intensive growth of algae in laboratory research mainly requires obtaining relatively quickly, fairly large quantities of biomass. Intensive cultivation of algae can be effective if the following criteria:

1. Algal cultures must possess an increased intensity of photosynthesis in cultivation environments with high concentrations of mineral salts, which allows one to add less nutrients and achieve intensive growth of algae.

2. Algal cultures must possess a high energy growth with high intensity lighting

conditions. Optimum illumination of the suspension can be easily achieved by ensuring that all cells in suspension light use of high light intensity leads to illuminate large portions of cells, especially in turbulent suspension. Cultivation is more convenient for those crops having a higher coefficient of energy use.

3. Thermophilic algae cultures because high light intensity and temperature is a requirement of their growth. Thermophilic species of algae have a greater energy development, so they have a greater prospect in intensive cultivation.

4. Algal cultures must have the property to inhibit the growth of foreign microflora (bacteria and other species of algae).

5. The unicellular or colonial microscopic forms of algae are more affordable than intensive cultivation compared with macrophytes algae. It is argued that microalgae can easily adjust and the cultivation depth can be changed as well; they have greater contact with CO2 and light. Mucilage forming shapes bubbles etc. They can be equally accepted for intensive cultivation. In addition, it is required that the species of microalgae be well studied since they are in possession of a complicated development cycle. Most studied and applied in intensive cultivation are the green algae, unicellular protococoficees, blue-green algae and algal cultures diatoms.

6. Must be easily collected, separated from the nutrient medium or develop methods for the efficient implementation of these requirements. 7.
Algal cultures need to be studied in detail under laboratory conditions and cultural practices to be established.

II.3. Algae cultivation

II.3.1. Algae growth on different types of nutrient media

Research conducted during the 2011-2013 years in the Laboratory "Algologie" USM had a specific algologic investigational aspect. Thus, the selection of resistant and productive algae was possible, both on synthetic media as well as in the outcome of the cultivated culture media from municipal water treatment plants, loaded with excessive amounts of pollutants. Among these we can mention algae species *Tribonema viride, Nostoc gelatinosum, Cylindrospermum licheniforme* and *Spirulina platensis Nordst Geitl* [40] on Gromov-6 medium [41]as well as the studying of the physiological aspects of blue green algae on the

algae in the cultivation of various synthetic nutrient media [42].

This biological extract together with attraction to know the chemical processes and environmental balances that occur in the environment, has motivated us to seek to understand the reaction mechanisms of these particularly interesting to scientists worldwide cyanophyts and their use in bioremediation of heavy metal contaminated wastewaters. In a first context we tried to grow three species of blue-green algae *Spirulina platensis*, *Nostoc gelatinosum* and *Cylindrospermum licheniforme* in a wastewater medium from a treatment plant such as Villeneuve d'Ascq station, Nord-pas de Calais department. Each time a control grown on a specifically grown medium has been made to keep them also in pure culture. There were studied a large number of parameters such as biomass productivity, pH, dynamic of anions and cations during experience taking into account the processes of evaporation and concentration of environment. All these experiences were made taking into account the cultivation methods suggested above, as well as the specific requirements of physical and chemical factors of each species and the final purpose of their destination. How our purpose as the biomass increase production getting biomass useful for later experiences on the other hand studying the algal consumption of micro and macro nutrients necessary for growing algae from wastewater at the exit of the treatment plant, we chose working with regular method of cultivation. As achieving of our experiences occurred in small containers and the exposure to light and heat for prolonged periods of time requires a pretty intense evaporation process, we have chosen to fill up the volume of the working solution to the volume lost through evaporation. This was done once every 3 days by adding the weighing of bi-distilled water to reduce the loss due to evaporation and concentration of solutions respectively.

It has served, therefore, a twofold objective:

1. Tertiary treatment of wastewaters leaving the station to avoid the formation of further algal blooms and

2. Obtaining cheap algal biomass which will be used in bioaccumulation experiences of emerging pollution such as ETM and pollution by organics micro-pollutants.

Biomass obtained and recovered at the end of experiments will be characterized by productivity of biomass/total volume. The recovery made by algae filtration vacuum pump is not exposed to stress due to spinning cells and to recover the maximum amount of biomass. Biomass productivity was characterized and used in subsequent bioaccumulation experiences. In experiments we proposed following strains of blue-green algae *Nostoc gelatinosum* and

Cylindrospermum licheniforme growth on the culture media from exiting of wastewater treatment station Villeneuve d'Ascq, Nord-Pas de Calais, a French region in order to produce cheap biomass resources and to reduce pollution of surface water with biogenic elements (metallic elements and major anions as chlorides, nitrates, sulphates as well as the phosphates).

II.3.2. Controlled algae growth stages

A typical algal growth experiment shows three characteristics phases: (1) *a lag phase* in which algae grow but do not multiply, (2) *a logarithmic (exponential)* growth phase, and (3) *a limiting* growth *region*. The lag phase can vary in duration depending on the health and size of the initial inoculums. Inoculate taken from a culture in log phase and larger inoculate tend to have shorter lag periods. A number of reasons have been given for the lag period, including restoration of enzymes and substrate concentrations necessary for rapid growth and the algal modification of the nutrient to make nutrient components more available or to detoxify metals [43].

In the exponential phase the growth is characterized by a growth constant k or a mean doubling time (td = 0.693/k). In batch cultures the growth constant k is relatively insensitive to nutrient concentrations [43]. It is evident that two characteristics most amenable to a short term experiment are the effect of toxic metals on the length of the lag period and the effect of metal concentrations on growth rate [44]. One effect that will arise is the necessity of adding a nutrient supplement to support algal growth-the added nutrient will change the pH, the ionic strength, and add of a complexing agent (e.g. EDTA). These changes will definitely alter the metal speciation.

The presence of a *lag phase* in algae growth is evidence that the algae may change the chemistry of their immediate environment. Algae secrete much of their photosynthesized products into the surrounding medium. It has been estimated that approximately 10% of the organic carbon escapes as glycollic acid and 40% of the fixed nitrogen is liberated in extracellular form. The percentage of extracellular nitrogen is higher under suboptimum growth conditions and when algae are exposed to new environments [44].

Determining the phases of growth is quite complicated; sometimes it is necessary to establish the amount of biomass over a period of 2-24 hours, or 24-168 hours, depending on the stage of growth conditions: temperature, lighting, stirring the contents of the culture medium, etc. The essential feature of microalgae growth is growth rate, which determines

processes of photo-biosynthesis, depending on the speed of synthesis of biomass. Specific growth rate differs, however, from a phase to another, a fact noted by other researchers. In experiences of *Anabaenopsis sp* algae cultivation in Drew environment the average duration of growth phases was: lag - 1 day, log - 1 day, exponential phase - 7 days, stationary - 1 day, and decline growth phase - 3 days [45].

II.3.3. Preparation of blue-green algal innoculum

Inoculation of algae in their cultivation is done by special methods that vary depending on the growth characteristics of culture, adapting to the specific conditions of cultivation. Remember to mention the fact that algal inoculums may be obtained from crops grown on agar medium, because we can not get homogeneous inoculums. Thus, the culture grown on agar must first be transferred to liquid media increased after 15-20 days these environments can be taken in the quality algae inoculums. In case of intensively grow species, the homogenizetion are doing using the method of operation of the machine with mixing blades (device hundredth) exposing the culture unit 10 min. Culture obtained if 4-7µ has dimensions of the vessel sterile filtered through a blue filter slow.

Sometimes we get divided culture by centrifugation. In this case inoculation using separate cell fractions sedimented result of centrifugation.To prepare the inoculums used in the cultivation of algae we propose to perform the following steps:

- The extraction of algal culture inoculums which rose above the nutrient medium that is to be cultivated;
- The inoculums must be extracted from algae population from the middle exponential growth phase;
- The inoculums must be separated from the nutrient medium by filtration or centrifugation;
- The inoculums washing (with distilled water) and sterilizing;
- Administration of inoculums in optimal form and dose required;
- Stirring inoculated samples.

II.3.4. Amount of inoculums

One important thing is the amount of inoculums. Usually the amount of algae inoculums for regular cultivation is 0.4-0.5 g/L absolutely dry biomass calculation. To

continue cultivation the inoculums must be taken depending on the specific amount of exponential growth phase initiation (previously determined at regular cultivation).

II.3.5. Obtaining of algae pure cultures

To establish properties and functions of cells in algal research activities they should be in monoculture and be pure bacteriological. The idea of bacteriological pure algal culture has been submitted by Pasteur and Koch. The need to obtain pure cultures of algae cultivation derives from the need to obtain their pure biomass. This can then be used in scientific research, drug source or used in food or feed.

II.3.5.1. Obtaining of algae pure cultures using antibiotics

Treatment techniques in algal strains to antibiotics vary depending on the preparation, concentration and treatment time having a lethal effect on bacteria but not to algae. Algae antibiotic sensitivity is different and depends on the specific biological organism, concentration playing a very important role. Some concentrations contribute to the total destruction of algal strains and others just destroy bacteria and not affect algae. Thus, in his work, represented in Table1 (Annexe), Сиренко and al. [46] highlights doses of antibiotics required wich not affect the algal strains (but only bacteria).

The best method for combating bacteria cultures mono-algae is sequentially transfer the algal culture in a series of flasks with sterilized nutrient medium containing antibiotics which differ from one shot to another. They allow the algae to survive and possibly at low concentrations increase. The essence of the method is that the algal cultures at each crossing from one flask to another reduce the number of bacteria. This method allows the destruction of the bacteria step by step. This process is less toxic algal crop, than the ability to destroy all bacteria once. This method is advantageous because antibiotics can destroy some bacteria and other antibiotics can kill other bacteria, so finally we get a pure algal culture.

II.3.5.2. The use of filtration techniques for removal of bacteria from algal cultures

This method allows the removal of bacteria in algal cultures if they were smaller than algae cells. The method requires the use Nucleopore filter with a pore diameter of 8 μ because it allows the passage of microscopic algae cells [39].

II.3.5.3. The use of chemical methods for obtaining axenical algal cultures

Chemical methods consist of algae scrubbing with disinfectants of certain concentrations. The most common used antiseptic to sterilize algae is the follows:

- phenol (carbolic acid) in concentration of 0.01-1%;
- Rivanol, in concentration of 0.01-0.1%
- Ethanol, in concentration of 1-10%
- Detergents concentration of 1%.

For the blue green algae one of the most effective chemical sterilization methods is that of detergents using. After that the algae are exposed on agar nutrient media (2% glucose agar + 0.5%) for growth. The colonies grown on agar longer inoculated several times until it remains sterile bacteriological [39].

II.3.5.4. The use of agar media inoculation techniques for removal of bacteria from algal cultures

This method, one of the oldest methods of purification, can be applied only for algae that grow on the agar surface or inside it. It can be used both for purification of bacteria algae and to obtain mono-algal cultures and are often used in combination with ultrasound and centrifugation methods.

II.3.5.5. The use of UV light

The principle of this method consists in the removal of bacteria from algal cultures using ultraviolet light with an ultraviolet radiation type C. For this applies a germicidal lamp which generates a radiation of 254 nm is used. As a result, the ADN replication and transcription is preventing and their bacterial growth is inhibited leading to the cell death.

II.3.5.6. The use of ultrasounds

For samples with separate particles of algae one suggests the use of ultrasounds followed by centrifugation. This method was applied to remove bacteria cultures of algae cyanophytes [47]. Selecting species of algae in our experiences, most of all for the intensive rearing of their ability, or their adaptability to environmental conditions. To intensify photosynthesis and productivity, blue-green algae cultures must meet rules below, characterized by Dobrojan and al. [39] :

1. Algal cultures should have an increased intensity of photosynthesis cultivation on media with high concentrations of salts that would allow avoiding adding nutrient medium and achieving intensive growth of algae.

2. Algal cultures must be able to increase under high intensity illumination and must ensure sufficient illumination of portions of the cells, especially in turbulent suspension.

3. Be thermopile algae culture because high light intensity leads thermopile species growing demand - they have a large increase at high temperatures. Thermopiles algae species have a greater energy development and that they have a greater prospect in intensive cultivation.

4. Algal cultures must have the property to inhibit the growth of foreign micro flora (bacteria and other algae species);

5. The forms microscopic unicellular or colonial forms of algae are more affordable than intensive cultivation compared with algae macrophytes. This argues in that microalgae can easily adjust the cultivation depth can be shaken well and they have greater contact with CO_2 and light.

6. Shapes forming mucilage, bubbles, etc., can be equally accepted for intensive cultivation. Algal cultures should be easy to collect and separate from the nutrient medium or to develop the method for achieving these requirements.

7. Algal cultures must be studied in detail under laboratory conditions and cultural practices to be established.

II.3.6. Nutrient media for algae cultivation

Nutrient media presents a mixture of different chemicals in nature chemical complexity, provenance, consistency, which provides algae with substantial's nutrients and energy needed for growth and development. Currently known numerous nutrient media used to cultivate algae.

Nutrient media can be categorized three main criteria:

- Once consistency - liquid, semisolid, solid;
- Once chemical composition - natural, synthetic, semi-synthetic;
- Once frequency and purposes of use in the laboratory - special and usual [48].

The nutrient media recommended for artificial cultivation of algae are varied. Selecting nutrient media depends on the specific object and purpose of algae cultivation. Nutrient media can be liquid and solid. Liquid media are used for the production of algae biomass and for morphological, cytological, physiological, biochemical and other kind of researches. The solid media are used for keeping the algae in collections (algal museum).

One of the most essential aspects of algae cultivation technology is providing the necessary elements of mineral nutrition. For the synthesis of biologically active substances algal biomass need to be insured with macro- and micronutrients. These algal components of algal frame have a decisive role and determine the intensity of their development. The biogenic elements such as N and P are the components of living organisms being necessary for their development. Most algae need nitrogen for synthesis of proteins. For efficient growth of algae nutrient media must contain P, K, Mg, S. Among the key macro-elements that are necessary for the cultivation of blue-green algae includes sodium (Na) and calcium (Ca), which are required in reduced concentrations. Potassium is utilized in large quantities by algae and should be reflected in the nutrient media. Magnesium enters into the composition of chlorophyll, which is an essential pigment for photosynthesis, being present in multiple clades. The determining the optimal concentration of macro is approached and studied to date. Following data presented by Dobrojan [39] (Fig.2.2.) growing process of algae biomass is conditioned by consumption of biogenic elements that are manifested in three stages:

Stage A - biogenic substance consumption occurs in the cell without increasing the number of algal cells. In stage B the cells division is performed simultaneously with nutrient

intake, and whereas in stage C- dividing of cells takes place without consumption of nutritive substances from environment, due to the reserves of internal cells.

According to data of Гусева [49] the best concentration of nitrogen for algae growth is between 5and 10 mg/L in the form of NO_3^-. Ammoniated N (from NH_4^+), as compared to their nitric is consumed 10 times faster than blue-green algae. Nitrites (NO_2^-) can be also consumed by algae, but they amend the cell and that gets an unusual shape.

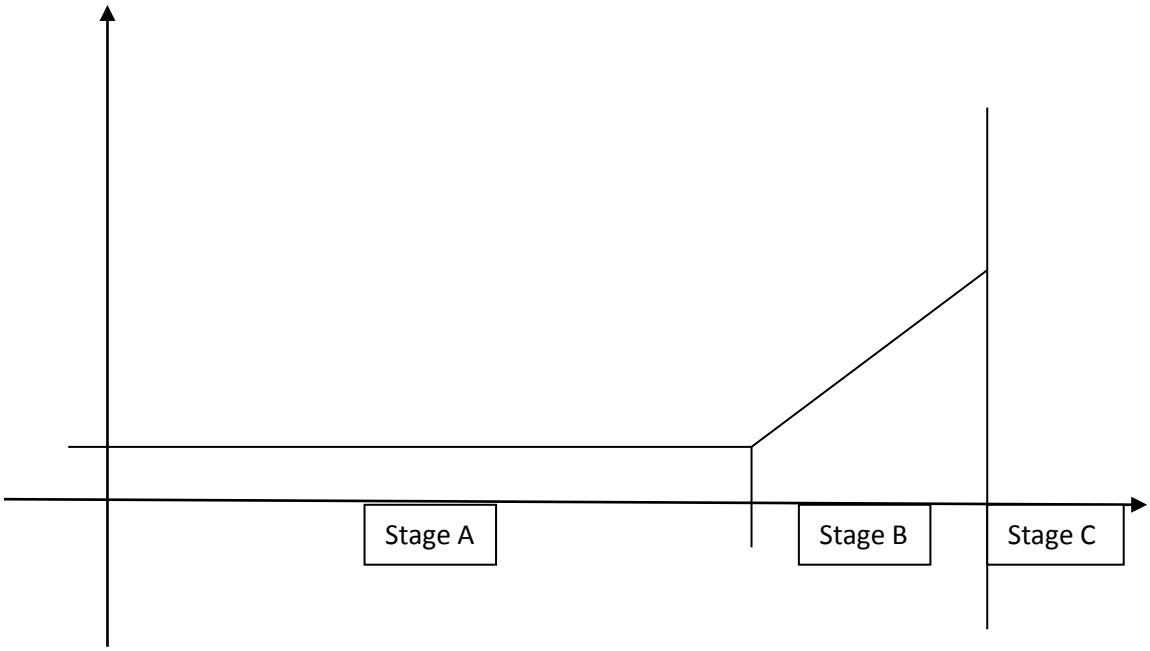


Fig.2.2. Stages of the nutrients consumption during growth of algae biomass[39].

II.4. The environemental conditions for algae cultivation

For ensuring an optimal growth and development of algal cells we need to follow several important environemental conditions among which temperature, lighting, shaking as well as nutrient and other macro and microelements in neededconcentrations. Often these conditions are factors that limit the growth of algae biomass in the cultivation and make the system ineffective.

II.4.1. Light

Lighting is one of the main conditions to be established and maintained for industrial algae cultivation. The algae are able to maintain optimal growth speed in a wide range of lighting, but this varies from one species to another. It is therefore important to determine the optimal intensity of illumination for cultivation of seaweed species which show much interest. For growing algae is an important thing establishment: light intensity; light regime, lighting and spectral composition of light. Light intensity, where growth routed algae is an environmental factor extremely important, not only by its significance in itself, the determining factor of photosynthesis, but also by the fact that, throughout the process of algal growth once with densification suspension in the nutrient medium, there is an effect of "self-shading" in the population of algae, which is in fact decreasing the intensity of light radiation to the culture in its entirety.

II.4.2. Turbidity

Increased turbidity cyanobacteria advantage compared to other algae. Cyanobacteria can use a wide spectrum of light for photosynthesis and can migrate to the surface in order to capture as much light. A very high turbidity may, however, reduce the availability of phosphate and limit their growth. Second, turbulence and high water flows do not promote the growth of cyanobacteria, as they interfere with their ability to maintain a certain position in the water column. Torrential rains can increase runoff volumes and concentration of nutrients in the water, which promotes blooms of training

II.4.3. pH (Hydrogen Potential)

pH measures the H^+ ion concentration of water. It reflects the balance between acid and base on a scale of 0 to 14. The value 7 is neutral. The range between 0 and 7 constitutes the acid medium, and between 7 and 14 the basic medium. The pH value can give information on the origin of water. For example, the surface water has a pH between 7 and 8. Groundwater has a pH between 5.5 and 8. A very basic pH shows a biological activity and intense evaporation. The pH of a lake or pond depends on age and discharged waste. During his

training, a lake has a basic pH (or alkaline) and gradually it becomes acidic (by fermentation of organic materials dissolved carbon dioxide with bicarbonate ion formation).

Blue-green algae usually occur more abundantly in hard-water than in soft-water lakes, but the importance of calcium and bicarbonate ions, which are largely responsible for hardness in fresh waters, may be related to pH buffering and the availability of free carbon dioxide. Most blue-green algae grow best in the range pH 7.5-9.0.

Shapiro (1973) [50] found that they became dominant over green-algae at high pH-values, indicating that the algae are efficient in deriving carbon from very low concentrations of free carbon dioxide. In culture media which are well buffered by high concentrations of bicarbonate ions the pH value (8.0-8.6) should be favorable to the growth of blue-green algae. In weakly buffered systems, fluctuations in pH may well be critical to the development of blue-green algae populations [51].

II.5. Macro and micro-elements in nutrient media for algae cultivation

The importance of trace elements contents for algae cultivation

Trace elements are necessary algae in small quantities, but of particular importance for their cultivation, as are part of many important enzymes. Trace elements can often be the limiting factors of algal cells increases. In their category includes 10 elements: Fe, Mn, Zn, Cu, B, Si, Mo, C, V, Co. From physiologically they can be divided into three groups: 1) necessary for photosynthesis-Mn, Fe, Cl, Zn and V; 2) required for the metabolism of nitrogen -Mo, B, Co, Fe; 3) necessary for other metabolic functions - Mn, B, Co, Cu, Si.

II.5.1. Calcium

Divalent cations alter light energy distribution between the photosystems and can increase light, saturated Photosystem II activity [52]. As others divalent cations are much less effective or are inhibitory the requirement for Ca^{2+} is specific, it can causes a twentyfold or greater increase in the rate of oxygen evolution by cell-free preparations of blue-green algae [52]. It acting close to the oxygen evolving reaction center of Photosystem II. As well as Ca^{2+}, Mg^{2+} have almost invariably been reported to be identical in their effects.

II.5.2. Magnesium

Magnesium plays an important role for algae, is a component of chlorophyll and many other organic links. It plays a major role in the phosphorylation reactions and helps regulate the metabolism of macroergic compounds and phosphoric acid. Intervenes in almost metabolic processes that require ATP [53]. The importance of Mg^{2+} for normal growth and cell division of micro-organisms was demonstrated by Webb M. [54], who showed that a deficiency or excess of Mg^{2+} inhibited growth and cell division, causing the formation of filaments. Enlargement of single-celled organisms therefore seems to be a general feature of Mg^{2+}-limitation [55]. Mg^{2+} might therefore control cell size through a universal mechanism for both prokaryotic and eukaryotic micro-organisms. Cell enlargement caused by Mg^{2+}-limitation could, on the other hand, be due to a different mechanism in each group of organisms.

The effects of magnesium on photosynthetic electron transport in membrane fragments of blue-green algaes, *Nostoc muscorum* (Strain 7119), noted a high stability and high rates of electron transport from water or reduced dichloro-phenolin-dophenol to $NADP^+$. In the blue-greens algae's the magnesium ions are required for multiple reasons including light-induced electron transport from water to $NADP^+$ but also for protection in the dark of the integrity of the water-photooxidizing system (Photosystem II). Membrane fragments suspended in the dark in a medium lacking Mg^{2+} lost the capacity to photo reduce $NADP^+$ with water on subsequent illumination [56].

II.5.3. Potassium

One of the irreplaceable nutrients needed for the algae cultivation is potassium which practically cannot be replaced with other elements, only some individual processes should be substituted with sodium and rubidium. Potassium is found in cells in substantially soluble and colloidal chemical structure determines the cell cytoplasm and active operation. Although not enter into the composition of proteins, lipids, carbohydrates, cells necessary for their synthesis. Potassium consumption is achieved through a process linked metabolically active cellular energy use and light conditions or when autotrophic heterotrophic nutrition using organic ties. K^+ accumulation is not irreversibly interlinked. Potassium ion transport into and out of cells of blue-greens algae occurs by an energy-dependent, active mechanism which is sensitive to the *trans* concentration of K^+ [57]. The gross fluxes frequently show dependence

on the concentration of the ion on the opposite or *trans* side of the membrane, for example sodium efflux from various cells [58].

II.5.4. Chloride

As well as magnesium cations, chloride anions could substitute, but less effectively, the two effects of magnesium ions cited above. By contrast, the photo-reduction of $NADP^+$ by $DCIPH_2$ was independent of Mg^{2+} (or CI^-) for the protection of the electron transport system in the dark or during the light reaction proper. Furthermore, high concentrations of $MgC1_2$ produced a strong inhibition of $NADP^+$ photo reduction with $DCIPH_2$ without significantly affecting the rate of $NADP^+$ photo reduction with water. The implications of these findings for the differential involvement of Photosystem I and Photosystem II in the photo reduction of $NADP^+$ with different electron donors are discussed [56].

II.5.5. Sodium

Sodium is a necessary element for blue-green algae cultivation, his lack from nutrient medium, leading to poor development of many cultures. Reducing of chlorophyll content, of organic nitrogen in the cells and eliminate the cells in the form of a large amount of organic carbon previously fixed are some effects of the lack of sodium nutrient medium for growing blue-green algae. Algal resistance to Na^+ in the form of NaCl is different, going from 30 g/L NaCl, in unicellular cyanophytes cells to 50-90 g/L at witch concentration the algal growth usually stops except for some species such as the genus *Microcystis, Synechocystis Aphanocapsa, Myxosarcina* which may increase concentrations of 90 g/L NaCl.

II.5.6. Sulfur

Sulfur is one of the necessary elements for growing algae. Enter into the composition of proteins, enzymes, peptides, amino acids containing sulfur, being a component of many other organic connections in algae cells. Some of the sulfur bonds participate in the oxidation-reduction reactions in the biosynthesis and metabolism of many substances. Sulfur is important in determining the properties and structure of the conversion of protein molecules. Most algae consume sulfur from sulfur-sulphates oxidative-bonds. According with Travieso and all data [59], blue-green algae need some quantities of sulfur for the conversion to

essential sulfured amino acids. In their study the efficient removal of H_2S is a special advantage of algal batch system. An initial level of 0.9 to 1% H_2S decrease to a final level of 0.3-0.4 % [59].

Russell and all suggests in their works [60] that algal sulfur metabolism includes two major processes not shared by either bacteria or fungi: the synthesis of the plant sulfolipid, 6-sulfoquinovosyl diglyceride [61], and the formation of sulfate esters of polysaccharides [62]. The plant sulpho-lipid is an essential component of the photosystem I electron transport system as well as sulfate esters of complex polysaccharides are synthesized in large amounts by various algae. The polysaccharides form mucilaginous slimes surrounding cells or colonies. The ester sulfate is hydrolyzed, enabling its detection as inorganic sulfate. Label incorporation into the fractions containing these compounds could help resolve what component of the microplankton is contributing most of the observed sulfate reduction [60].

II.5.7. Iron

Iron is an essential element in the life of any organization. It intervenes indeed in many metabolic and signaling functions. Adopting of two different ionic forms Fe^{2+} and Fe^{3+} makes iron an important player in the cell redox reactions, serving as catalytic center of enzymes. These enzymes are involved in central cellular processes such as transport of electrons, the activation of oxygen, the reduction of the peroxide, the synthesis of nucleotides or amino acids, the synthesis of ADN or photosynthesis. Synthesis by microorganism's siderophors is induced in iron deficiency in the medium.

Siderophores will bind ferric iron Fe^{3+} to form [Fe^{3+} siderophore] complex. This complex will reach the surface of the cell to bind to a receptor transmembrane protein. The complex then enters the cell through an ABC transporter type of carrier. Once in the cell, the complex will therefore dissociate, and ferric iron Fe^{3+} is reduced to ferrous iron Fe^{2+}. Thus, Fe^{2+} will be used by the cell in particular for enzyme functioning and the respiratory chain. The dissociated siderophore and iron ions will be then recycled to the extracellular medium by a pump efflux.

II.5.8. Nitrogen

Nitrogen is also an important nutrient for the production of microalga biomass. The nitrogen content of the biomass can range from 1% to more than 10% and is dependent upon the amount, the availability and the type of the nitrogen source [63]. It is an indispensable element for the biosynthesis of protein substances, molecules constituting the amino acids, nucleic acids, chlorophyll, alkaloids, amides, amines etc.

Common nitrogen removal methods such as bacterial nitrification/denitrification remove the majority of the nitrogen as N_2 gas, whereas algal treatment retains useful nitrogen compounds in the biomass [64]. The use of wastewater can offset the cost of commercial fertilizers otherwise needed for the production of algae, and wastewater treatment revenues can offset algae production costs. It is apparent that overcoming the current challenges to the production and harvesting of algae will be beneficial for both wastewater treatment and for the production of biofuels and bio-products [64]. Growing algae requires consideration of three primary nutrients: carbon, nitrogen, and phosphorus. Micronutrients required in trace amounts include silica, calcium, magnesium, potassium, iron, manganese, sulfur, zinc, copper, and cobalt, although the supply of these essential micronutrients rarely limits algal growth when wastewater is used [64]

II.5.9. Phosphorus

Phosphorous is also an essential macro-nutrient for microalgae growth [63]. Although cyanobacterial biomass do not need large amounts of phosphorus, as it contains less than 1% of it, phosphorus is an important growth limiting factor, especially in natural environments where phosphorus is limited [63]. But of quantitative increase is harmful to ecosystems. Phosphorus contributes to the formation of nucleic acids, co-enzymes of ($NADP^+$ to NAD^+, etc.), is composed of complex lipids, nucleotides, etc. Phosphorus has a favorable action on the biosynthesis of chlorophylls, participate in the processes of phosphorylation, biosynthesis and degradation of carbohydrates, lipids protein substances. Phosphorus is a nutrient that has a fundamental role in metabolic processes such as respiration and photosynthesis.

II.5.10. Oxygen

Challenge directly related to carbon dioxide supply is the removal of excess oxygen. Oxygen concentrations above air saturation begin to inhibit photosynthesis, and this byproduct must be removed in order to prevent photo-oxidative damage.

II.5.11. The Carbon content in the algae medium

The carbon content in the medium by culturing *Spirulina platensis* is one of the most important factors that determine the productivity of the culture. Growing *Spirulina platensis* on Zarrouk medium, the basis of which comprise hydrocarbons accompanied by decrease in the concentration of the latter, the accumulation of carbonates and increasing pH in the period of active growth of microalgae. From the available ionic form of CO_2 in the environment *Spirulina platensis* prefers HCO_3^-. After reaching stationary growth phase the reverse process is observed in algae: the concentration of carbonate ions is reduced, and the concentration of hydrocarbons ions begins grow [65]. This is due to the shift of the carbonate equilibrium toward hydrocarbons, which is caused by the withering away of the culture, the pH decrease and increases the role of assimilation of CO_2 from the air. At cultivation of the microalgae in a Zarrouk medium, which basis form the carbonates, the main source of inorganic carbon becomes a CO_2 air assimilated environment due to the high pH. As an additional carbon source in the first 8 - 9 days used hydrogen carbonates formed due carbonate equilibrium displacement towards HCO_3^-.

II.6. Conditions of medium in algae cultivation

II.6.1. Nutrient concentrations in algae cultivation

Vollenweider [66] concluded ever since 1968 that massive development of blue-green algae would be likely if nutrient concentrations exceeded 0.01 mg/L, P and 0.2-0.3 mg/L N at the end of the winter, or if the specific nutrient loading reached 0.2-0.5 g/ m^2/year P and 5-10 g/m^2/year of N, though he qualified this by saying that other factors were involved. Below these levels, however, it seems that growths of algal populations are likely, sooner or later, to become limited by phosphorus or nitrogen deficiencies. Nutrient concentration in the water is not a true measure of availability, since it ignores fluxes and the content in the algal cells.

Many algae, in fact, absorb and store far more than their immediate needs ('luxury consumption') when nutrients are freely available, and these may be sufficient to sustain two or three doublings despite apparent exhaustion in the water. This provides one explanation for the ability of blue-green algal populations to achieve bloom-forming proportions at times when extreme nutrient deficiency has set in. Some species of blue-green algae possess the ability to fix atmospheric nitrogen when combined sources of this element are present at low concentrations [67].

II.6.2. Nutrient ratio in the algae medium

One of the most important factors that are affects the algae community structure, in the conditions of unlimited access to biogenic substances are the size of N: P ratio. The classical ratio used in characterizing intake of biogenic substances in phytoplankton cells is called Redfield ratio which atomic ratio of C: N: P is 106: 16: 1 rule [68]. Analysis of the algae cells that were separated from debris, have shown that the ratio N: P for phytoplankton may vary within the limits from 5:1 to 15:1. Blue-green algae can develop even in amounts of nitrates and phosphates that are minimal and that may positively correlate with the content of organic substances in water. As a result of the investigations made by Zubcov and Ungureanu [68] it was established that not as much the phosphorus or nitrogen concentration are important as the N: P ratio. When the ratio N: P < 10 or the ratio of inorganic form of these elements is < 5, the development of phytoplankton is limited by nitrogen. When these ratios are 10-17 and 5-2, they limit the nitrogen and phosphorus, and when higher that 17 and 12 limit phosphorus.

II.6.3. Organic compounds in the algae medium

The rumors that the growth of blue-green algae is favored by dissolved organic compounds appears to be based solely on the work of Pearsall [69] who asserted that there was a strong positive relationship between dissolved organic matter and the proportion of blue-green algae in the phytoplankton. However, no specific requirement for an organic substrate among fresh-water forms is known [70].

Many planktonic species have been maintained for long periods in culture media to which the only organic compounds added were chelating agents, to maintain iron and other trace metals in solution [71]. Heterotrophy, the ability to grow on organic substrates in total

darkness, has been demonstrated in a number of blue-green algae, but is not universal [72, 73]. Apart from maintaining trace elements in solution, substantial quantities of oxidizable organic matter lower the concentration of dissolved oxygen, and may thus accelerate the onset of strongly reducing conditions. Many blue-green algae not only tolerate low oxygen tensions, but grow and fix nitrogen more actively under such conditions [67].

II.6.4. Temperature of the algae medium

Algal ambient temperature is one of the most important conditions of cultivation must be respected and maintained. Although blue-green algae are known as algae that grow in extreme conditions of temperature [74]. Some species is encountered in fountains with thermal waters and maintain their viability at up to 85°C, while others cause the phenomenon of "blooming snow" giving her red, black, green or bluish, depending on the correlation quantitatively protoplast composition of photosynthetic pigments in cells [75-77]. The differences between species at low and high temperature are potentially of significance for seasonal, altitudinal and latitudinal distribution.

Planktonic blue-green algae are widely supposed to have a preference for higher water temperatures. Temperature optima for some blue-green species are in the range 25-35°C. However some species such as *Anabaena flos-aquae* began to grow above 5°C, producing its most rapid rate of increase between 10 and 15°C, growth of Aphanizomenon flos-aquae sharply increased above 20°C, while *Microcystis aeruginosa*, had a wider temperature tolerance, its most prolific increase was above 17-18°C [51]. Some autors [78] showed that cyanobacterial dominance generally occurs at higher (>20°C) water temperatures because of temperature optima above this value, whereas temperature optima for other algal groups tend to be lower.

II.6.5. Floatability of algae in the medium

Certain cyanobacteria, such as *Spirulina platensis*, may further optimize their position in the water column as a function of the available light by actively modifying their buoyancy. This feature also allows cyanobacteria to migrate into the thermal gradients and use the nutrients confined in the deep cold water layer. Control is primarily by photosynthesis (by

producing carbohydrates) and deteriorates when the concentration of carbon dioxide is too low. The buoyancy cannot be controlled during the night.

II.6.6. Mixing of the algae medium

The role of mixing in affecting productivity of photoautotrophic microorganisms has been investigated since the early stages of developing the biotechnology for microalgal mass cultures.

II.6.7. Artificial chelating agents in the algae medium

The repression of toxicity by complexing agents has been documented quantitatively in phytoplankton cultures using well characterized artificial chelating agents and copper as the toxicant [79]. They confirm that certain phytoplankton can produce extracellular organic compounds capable of appreciably complexing copper but in culture media containing strong artificial chelating agents (EDTA, NTA, etc.), the influence of the exuded organic material would be negligible. In natural waters, where the algal densities are likely to be lower than the stationary phase, the extent of complexation would presumably decrease proportionally.

II.7. Algal productivity

Algae productivity or absolute dried biomass (ADB) was determined using an analytical balance wet method filters, the following expression:

$$\mathbf{ADB = (B\text{-}A \times X) \times Y\ (g / L)}, \qquad (2.1.)$$

Where:

A- Weight filter washed with 5 ml of MQ water,

B- Weight biomass filter and cooled to room temperature,

X - Recalculated coefficient for the algal suspension to 1 liter [11] and

Y- Coefficient recomputed for the suspension of dry algae to 105°C.

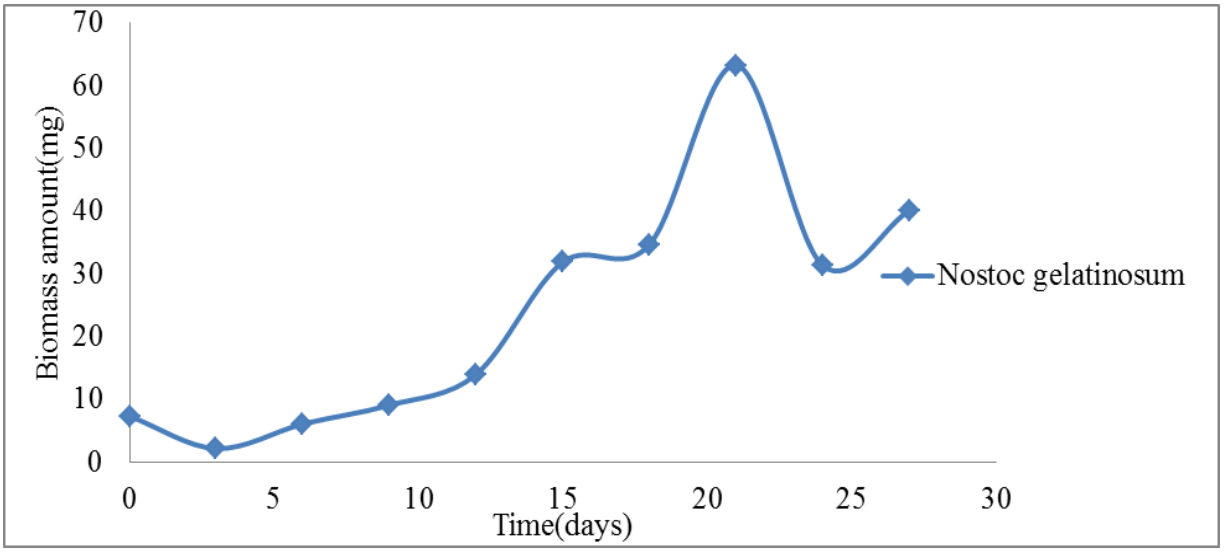


Fig.2.3. The productivity of *Nostoc gelatinosum* in a growth medium of un-diluted wastewater.

II.8. Forms of algae utilization in biotechnology applications, Algae immobilization

II.8.1. Definition and necessity of algae immobilization

Normally, the use of algal biomass freely suspended has drawbacks that include: small particle size, low strength and the difficulty of separating biomass and effluent [80]. Immobilization of algae in alginate beads is used to avoid these disadvantages. Immobilization of algae in biotechnology applications began there more than 49 years [81]. The first report on the study on the immobilized algae was published in 1966 [82] where used are chemically fixed Chlorella cells for the measurement of the reaction Hill [83]. An immobilized cell is defined as a living cell that, by natural or artificial means, is prevented from moving independently to its original location in all parts of the aqueous phase of the system.

II.8.2. Methods of blue-green algae immobilization

One of the major and practical limitations in algal treatment systems is harvesting or separation of algal biomass from the treated water discharge. An efficient removal of algal biomass is essential for recycling of the wastewater. A series of tests in order to obtain a simple and cost-effective technology for harvesting microalgae from the wastewater solutions

were made over the years. Among them processes ranging from simple sand filtration to energy-intensive centrifugation [84-86]. Different technology such as auto flocculation, self-aggregation by stopping aeration followed by decantation, especially for cyanobacteria havealso been practiced but without outstanding results. Therefore, de la Noüe & de Pauw have been proposed in 1988 immobilization of algal cells for wastewater treatment for circumventing the harvest problem as well as retaining the high-value algal biomass for further processing [87]. Among the numerous advantages of algae immobilization, we can include accelerated reaction rates due to increased cell density, increased cell walls permeability, no washout of cells, better operational stability, and of course greater flexibility in reactor design compared with conventional suspension systems. Following Mallick's assertions [83], six different types of immobilization methods can be distinguished i.e covalent coupling, affinity immobilization, adsorption, confinement in liquid-liquid emulsion, capture behind semi-permeable membranes and entrapment.

Of all these techniques last method is the most widely used both in laboratory experiments as well it is sometimes used in industrial processes and cell-based embedded. This method of entrapment suggested by the name itself, represent isolation of algal cells in a three-dimensional gel network. The structure of chosen material for algae immobilization allows to cells algal feel free in their compartments and through the formed pores the products of algal synthesis, substrates and other chemical products can easily broadcast to and from cells. The immobilization materials are synthetic (as acrylamide, polyurethane, polyvinyl, etc.) and natural polymer (collagen, agar, agarose, cellulose, alginate, carrageenan, etc.). Among them, alginates, carrageenan and agar is the most used (4). To be economically and attractive to the large-scale environment of potential users should follow the immobilization matrix of a series of conditions: 1. The effectiveness of the system to remove pollutants; 2. The cost of the polymer; 3. The cost of the immobilization process. Our immobilization method has several advantages: 1. Concentrates of high quantities of biomass that can be used as a byproduct; 2. Prevents filtration of treated wastewater, which can be used as is; 3. A high resistance to toxins treated wastewater; 4. Can immobilizes more of a microorganism (microalgae); 5. Easy to be applied by non-professionals; 6. In addition, the technique is environmentally friendly.

In our experiences, we chose to immobilize algal into natural gels obtained again from algae (brown algae) which are environmentally friendly, an organic product that is very

popular and economically profitable. From the most commonly used gels such as alginate and carrageenan we chose to work with Na-alginate.

II.8.3. The blue-green algae entrapment in Na-alginate matrix. Preparation of *Spirulina platensis* algae-alginate beads

Alginate beads with *Spirulina platensis*, last one extracted alive from the exponential phase of the Zarrouk synthetic medium of growth were synthesized. In this way, 3g wet weight of *Spirulina platensis* alive, removed from the upper layer during the centrifugation were placed in 250 ml MQ water in an Erlenmeyer flask and well agitated. In another flask in a same volume of MQ water was placed 2g of sodium alginate (AppliChem, PanReac), high viscosity and the last content will heat until the dissolution of the NA-alginate in the water. We allowed to the Erlenmeyer ball cooling to room temperature and we mix the both content of the alive algae and dissolved Na-alginate. The well homogenized mixture was going through a peristaltic pump in a one liter Nalgene beaker filled in a half with a solution of 0.5M $CaCl_2$. Upon contact of the contents passes through the peristaltic pump to the surface of the $CaCl_2$ 0.5 M solution a particulate solid of alginate beads algae measuring 1.5-2 mm are formed.

The algae-alginate beads are then shaken and allowed overnight in the solution 0.5 M $CaCl_2$. The same technology of immobilisation is applied and to others blue green algaes such as *Cylindrospermum licheniforme* and *Nostoc gelatinosum.*

Fig.2.4. Immobilization process of *Nostoc gelatinosum* blue green algae in Na-alginate beads

Once removed from the solidifying solution, the balls of algae-alginate compounds are rinsed 3 times in abundance with MQ water and are then rinsed with a solution of 0,5M of HCl diluted acid to remove ETM presented in the matrix content. After that it is again rinsed with MQ water to not allow the degradation of the blue-green algae cells.

II.9. Organic micro-pollutants detection

In organic micro-pollutants detection and analysis, it is important to pay close attention, before handling, and during all procedures (sampling, packaging, extraction and analysis) to avoid the risk of sample contamination. It is for these reasons that the glass is previously well washed with ultrapure water (Milli-Q) 18.2 MΩ cm resistivity. This water is produced by a millipore unit, and then the glass is cleaned with a specific detergent (Decon), acidified Milli-Q water, then initially rinsed with Milli-Q water and after with acetone. Finally, it was dried at 120 °C for a few hours.

II.9.1. Sample Processing

Detection of organic micro-pollutants in the different samples requires a methodology for rigorous analysis and careful attention to avoid the risk of contamination.

II.9.2. Filtration

Water samples from freely suspended algae are immediately filtered with a vacuum pump thought a glass microfibers Whatman 0,7 µm precalcined at 450°C overnight in order to separate the dissolved phase and suspended solids (SS) in our case the blue-green algae. Then dissolved phase were extracted by liquid-liquid extraction Extraction (LLE) in order to preserve the different organic micro-pollutants molecules of the contaminated solution. MES collected on filters, whose dry weight is determined by weighing guard are in the freezer for a later extraction by the extractor ASE (Accelerated Solvent Extraction) to determine phthalates, bisphenol and drug residues associated with algae.

II.9.3. Extraction of the dissolved phase. Liquid-Liquid extraction (LLE)

In order to extract the different pollutants studied, 750 ml of water are poured into a separatory funnel and then are spiked with internal standards present in the following Table 2.1.

Table 2.1. The different internal standards used.

Molecules of interest	**Internal standards, concentrations, doses**	
Drug residues	Caffeine $^{13}C3$ *100 ppm, 10 µL*	17-β-Estradiol-D2 *500 ppm, 10 µL*
Bisphenols	Bisphenol A- D_{16} 500 ppm, 10 µL	
Phtalates	Diisobutyl phthalate -3,4,5,5-d4 *250 ppm, 20 µL*	Diphenyl isophthalate 250 ppm, *20 µL*
PAHs	A mixture composed of: • Naphtalene d-8 (N-d8) • Acenaphthene d-10 (A-d10) • Phenanthrene d-10 (Phe d-10) • Pyrene d-10 (Pyr-d10) *100 ppm, 30 µl*	

The technique of liquid-liquid extraction (LLE) is a conventional technique which has proved its high efficiency extraction of wide varieties of organic micro-pollutants. In this study, the LLE was chosen because of its many benefits. LLE technique is used to purify and concentrate the samples prior to analysis by chromatographic techniques. Knowledge of certain properties of solvent (miscibility in water, density and toxicity) and solute (structure and lipophilicity) allows the choice of a suitable solvent and thus the optimization of the extraction. This is a manual technique, consuming large amounts of organic solvents and time. But despite these drawbacks, it is still used for the extraction of organic micro-pollutants because of its good efficiency in extracting large variety of organic contaminants.

II.9.4. The method of internal standards

Internal standards are used to correct errors or possible losses throughout the stages of handling. In fact, during calibration, it frequently happens that the detector response is not fully proportional to the concentration of the target analyte on the selected concentration range. This is particularly the case when the detection is by mass spectrometry (MS). In addition, losses during handling are possible as well as during extraction, chromatographic analysis or column purification. In this case, it is impossible to use the method of the external calibration for accurate quantification. The method of the internal standard is then used. It allows not only eliminating the injected volume and of the change in detector response over timing but also to correct any potential losses throughout the analytical protocol (extraction, purification, separation or pre-concentration). In addition, this method allows correcting the effects of matrix. To this, to each sample solution was added an internal standard of known concentration. Internal standards are compounds having a structure with very similar property of the compounds that we want to study. They were added with well-known concentrations in each range of standard and concentration in each sample. Then the extraction is done in a successive manner with dichloromethane and hexane. Firstly 60 mL of dichloromethane is added and stirred for 5 min chasing air. The mixture was then decanted to recover the organic fraction. This process is repeated twice. Second, we performed the same procedure but with twice 60 ml of hexane. Fig.2.5 illustrates an LLE extraction.

Fig.2.5 Separation funnels during the LLE extraction.

All organic fractions are combined in a tube tip. Any traces of water in the extract are removed by the addition of Sodium Sulfate (Na_2SO_4). This is important because the presence of water in the extracts can damage the chromatographic column and as the mass spectrometer (MS) detector. Then the extracts were pre-concentrated using a rotary evaporator and then under a nitrogen stream to obtain a final volume of 100 µL for analysis by GC-MS.

II.9.5. Accelerated solvent extraction (ASE)

As previously reported, some molecules have high water solubility and others have a capacity to bind to living organisms (phytoplankton). Therefore, it is important to analyze not only the dissolved part of the water column but also the suspended material (MES) representing the biomass of blue-green algae. Or the entire biomass filtration of 0.75 L of each sample was collected for extraction by ASE. Figure 2.6 shows a photograph and a schematic diagram of the ESA.

Fig.2.6. Simplified diagram of ESA, an actual photo and membership of an ASE cell

This device allows operate at temperatures and relatively high pressures, compared to other conventional mining techniques, which helps to improve the extraction efficiency for solid and semisolid matrices. It also helps save time, reduce the volume of solvent, maintain constant extraction conditions and give good repeatability by automation. It is an economical technique that respects the environment thanks to its effective extraction capacity with low solvent and in the short term. Filters containing the MES were dried, weighed to calculate the mass of MES. They are then inserted into the extraction cell and spiked with internal standards suitable to each family of molecules of interest, followed by filling the void volume of glass beads.

Two extraction procedures are employed in our experiments and are described below:

(i) Firstly, each sample is placed in the ASE extraction cell and then doped with appropriate internal standards. Then, with the ASE device, the cell containing the sample is loaded into the oven programmed at 110 ° C and under a pressure of 103 bars to maintain the solvent in the liquid state. A mixture of dichloromethane / acetone was used according to the method of Reid et al., 2009 [88]. The cell is preheated for 5 min and then kept static for 1 min with 3 static cycles. After each static time we purged for 2 minutes with nitrogen to recover the cell extract into a recovery vial

(ii) The second method employed with dichloromethane according Tronczynski et al. [89]. The extraction conditions were 5 min of pre-heating temperature of 100 °C, a static extraction time of 2 min with 5 static cycles. The pressure was 138 bar followed by a 3 min purging. The high purity nitrogen was used as purge gas.

II.9.6. Purification

During extraction, the interfering molecules may also be extracted together with the molecules of interest. Interference can then affect the detection and quantification of bisphenol and drug residues in the chromatographic analysis and can also damage the chromatographic columns and detectors. A purification step is necessary.

Firstly, activated copper is added to the extract to remove the elemental sulfur. The copper acting with sulfur form the CuS which is a black precipitate, then, removal of traces of water is done by addition of Na_2SO_4. The organic extracts are then transferred in tip tubes then pre-concentrate to 200 µL using a rotary evaporator followed by a stream of nitrogen.

II.9.7. Chromatographic analysis

The analysis methods based on gas chromatography (GC) and Liquid Chromatography High Perfomance (HPLC) are widely applied for the determination of organic micro-pollutants. One difference between the two techniques lies in the fact that the mobile phase of GPC is a gas whereas HPLC is liquid method. GC is known as a powerful technique for the separation of gaseous compounds or capable of being vaporized by heating without decomposition until the order of nanograms. The detector mass spectrometry (MS) coupled to the GC is considered a universal detector not only to quantify but to identify the majority of organic micro-pollutants contained in environmental matrices.

Fig.2.7. A photo of the GC-MS apparatus Varian 3900-coupled to a mass spectrometer (MS, Saturn 2000, Varian, USA) used for various analyzes.

The figure 2.7 shows the GC-MS apparatus used in our experiments. For natural samples, residues of pollutants are identified and quantified by gas chromatography (GC, Varian 3900) coupled to a mass spectrometer (MS, Saturn 2000, Varian, USA). The final extract is injected into the injector, then passed through a capillary column Phenomenex XLB (60 m length, 0.25 µm thick) with carrier gas (helium) maintained at 1.2 mL/min. The injector temperature was initially 280 °C and the oven of the GC is programmed as follows: the initial temperature was 70 °C held for 1 min before rising to 170 °C with a rate of 10 °C/min and up to 220 °C with a rate of 4 °C/min to finally reach a temperature of 280 °C with a speed of 2. °C/min which was maintained for 12.50 min. The molecules of interest are first identified by their retention time (RT) on the chromatogram and characteristic fragments (m/z) on the mass spectrum obtained during analysis in Full Scan mode (FS) of a standard solution. Then the quantification is done with the mixed method of analysis SIS/MRM (Selected Ion Storage (SIS)/Multiple Residual Monitoring (MRM).

II.9.8. Data Processing

For each family of organic micro-pollutants, calibration ranges are prepared at various concentrations ranging between 0.1 and 10 µg/mL and performed with standard commercial mixtures and doped with the appropriate internal standards. The areas of chromatographic peaks are proportional to the concentration of each molecule in the extracts and can be quantified using the calibration curve. The calibration allows to a linear regression equation as follows:

$$\frac{Ai}{Aei} = a\frac{Ci}{Cei} + b \qquad (2.2.)$$

Where:

Ai= Peak area of compound interest i,
Aei= Area of the internal standard peak,
Ci= Concentration of molecule of interest, i,
Cei = Concentration of the internal standard.

After determination of the regression line, it becomes possible to determine the concentration of the compound in the sample by the equation:

$$Ci = (\frac{Ai}{Aei} - b) * \frac{Cei}{a} \qquad (2.3.)$$

For each sample is then determined the concentration of each molecule in each phase (dissolved, particulate). Then, the concentration obtained relates to the water volume or to the mass of dry MY weighed to have a response in g/L or mg/g dry weight.

II.10. Mineral micro-pollutants detection

II.10.1. General methods of major and trace metals elements analysis in aquatic environment

Depending on the ionic strength, type, speciation (labile or not), concentration and pH in the solution of the metallic elements in a variety of methods of analysis in aqueous solutions exist (Tab.2.25). The dosage of the major elements and metals in water was performed by spectrometry. Depending on their concentration, samples are assayed by atomic emission spectrometer (ICP-AES for concentrations of mg/L the order), mass spectrometry (ICP-MS for concentrations of µg/L the order), or polarographically (ASV for concentrations of ng/L the order).

Table 2.2. General methods of chemical analysis of trace metal elements from polluted waters

Heavy Metal	**Discharge limit µg/L**	**Sampling**	**Delay of analyze**	**Conservator**	**Pre-treatment**	**Suggested methods**
Cd		recipient in polyethylene washed with hours and then rinsed in the double-distillated water	One month after addition of conservator. Filtration in situ if dosage of dissolved Cd	acidification HNO_3 a pH ≤ 2	acid digestion: -destruction of organic matter -dissolution of suspended Cd	colorimetry (sensitivity:0,02 mg/L) - polarographically (sensitivity: 0,001 mg/L) - atomic absorption spectrometry(AAS) (sensitivity:0,02 mg/L) or complexation and extraction (sensitivity:0,05 to 0,001 mg/L) - ICP AES or ICP MS - neutron-activation - fluorometrically
Pb		in the same way as Cadmium	in the same way as Cadmium	in the same way as Cadmium	in the same way as Cadmium	colorimetrically (sensitivity:0,02 mg/L) - polarographically (sensitivity: 0,001 mg/L) - atomic absorption spectrometry(AAS) (sensitivity:0,02 mg/L) or complexation and extraction (sensitivity:0,05 to 0,001 mg/L) -ICP AES or ICP MS

Cr III		-	-	-	-	- colorimetry (sensitivity:0,02 mg/L) - polarographically (sensitivity: 0,001 mg/L) - atomic absorption spectrometry(AAS) (sensitivity:0,02 mg/L) or complexation and extraction (sensitivity:0,05 to 0,001 mg/L) - ICP AES or ICP MS
Cr VI		-	-	-	-	- colorimetry (sensitivity:0,02 mg/L) - polarographically (sensitivity: 0,001 mg/L) - AAS after extraction (sensitivity: 0,001 mg/L) - ICP AES or ICP MS
Hg		-	-	-	-	- AAS - ICP
As		-	-	-	-	- colorimetry - ICP AES or ICP MS

.

II.10.2. The ICP-AES

Atomic emission spectrometry principle is based on the line emission specific light by atoms and excited ions at high temperatures (6000-10000 K) in an argon plasma. These lines are separated by a combination network – Prism then simultaneously detected by a CCD detector (charge coupled device). Standard solutions for calibration were prepared in similar dies to the samples to minimize the attenuation of the transmitted signals and thereby distort our results [4].

Fig. 2.8. Model ICP-AES (Varian Vista Pro) with its heating flame argon plasma at 6000K

II.10.3. The ICP-MS

Mass Spectrometry is based on the formation of ions in the argon plasma then on their separation in function of the ratio m/z (mass/charge) which takes place in a quadrupole mass analyzer. The ions are separated and finally counted by a multiplier type transducer electron. In some cases, (Cr, Co), spectroscopic polyatomic interferences forced us to use the CTC method (Collision Cell Technology), collision chamber with a mixture of helium-hydrogen gas to eliminate interference [90]. In our work the dosage of the major elements and metals in water was performed by spectrometry. Depending on their concentration, samples are assayed by atomic emission spectrometer (ICP-AES, type Vista Pro axial view, Varian) or mass spectrometry (ICP-MS, Thermo Elemental X7).

II.10.4. Major and trace metals elements analysis. Preparation of diluting solution 2% HNO_3

For preparation of dilution solution, we take 2ml of 100% HNO_3, we placed (using a micro pipette graduated in 1 ml) in a 100 ml flask and complete the flask up to volume with ultrapure water.

II.10.5. Calibration

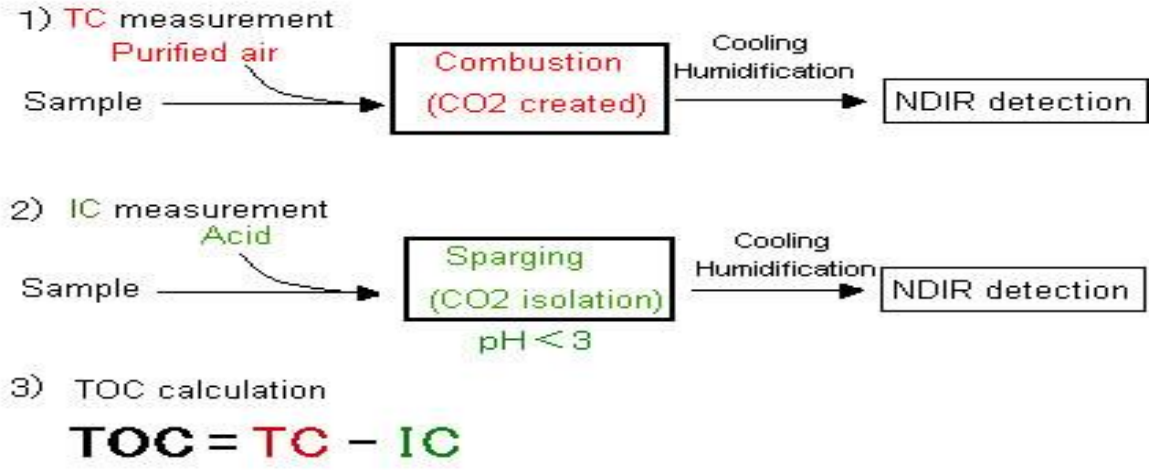
For each macro-element to be determined, a series of standards were prepared at different concentrations (depending on the type of metal), from a stock solution of 1000 ppm, in tubes 50, 50, 50, 25.25 and 10 ml respectively for the 6 standards followed by making up the volume with the dilution solution 2% nitric acid. Cadmium and other micro-elements standards are prepared from an intermediate solution with a concentration of 10 ppm. The intermediate solution is prepared also from a stock solution of 1000 ppm by taking a ml and diluting in a 100 ml flask with 2% nitric acid. To avoid possible interference due to the matrix, each standard is prepared by a mixture of concentration of different elements. For ICP-AES analysis, various standards have been used. For each metal element concentration corresponding to an absorbance and the computer traces the curve. From this curve, the computer gives a reading, after measuring the absorbance of each sample, studied the concentration of metals in the prepared solution (mg/L).

II.10.6. The Dissolved Organic Carbon

The assay was performed on Shimadzu TOC meter (TOC-V CSH model) as we see in Fig.2.9.

Fig.2.9. Shimadzu TOC meter (TOC-V CSH model)

The samples were analyzed according the method NPOC (Not Ventable Organic Carbon), which allows the determination of non-volatile organic compounds. The samples were first acidified to remove by means of an air flow inorganic compounds as CO2. Volatile organic compounds may also be removed during this step. The sample is then heated to 680°C; the mineralized organic matter releases CO2 which is supplied to an infrared detector.



It sends a response as a peak, the surface is used to calculate the concentration using a calibration curve with standard solutions of potassium hydrogeno-phtalates.

II.10.7. The analytical methods of anions analysis, Ionic chromatography

Dosage of anions such as chlorides, sulfates, nitrates and phosphates were assayed by ion chromatography provided with an eluent generator (Dionex EG 50). The separation is performed on an anionic column (Ion Pac AS18 4x250 mm) in particular according to the charge density ions, driven by a mobile phase (KOH). Cell conductivity can detect these anions versus retention time of each of them.

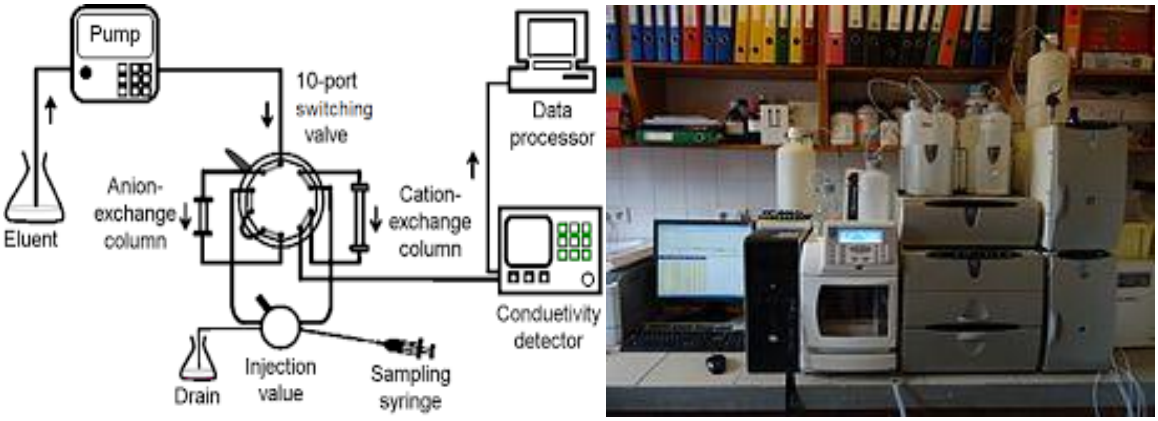


Fig.2.10. Ionic exchange chromatography Dionex EG 50 system and their operational principles

Chapter III

Pollutants in the aquatic environment

III. Main micro-pollutants in environment. Definition and classification of micro-pollutants

The micropolluants term encompasses tens of thousands of molecules. They are likely to have direct or indirect potential chronic effects on the environment or on human health even at low concentrations (µg/l or ng/l) [91]. This micropolluants can be classified as:

- Their origins
- Their sizes,
- The type of pollution that they cause,
- Their uses,
- Their effects on human health (proven, alleged or never observed)
- Their effects on the environment,
- Regulatory frameworks that affect them.

According to their nature, generally there are three main groups of micro-pollutants in the environment: ***minerals micro-pollutants*** (cadmium, lead, mercury, zinc, ...), the micro ***organo-metallic micro-pollutants*** such as methyl mercury and ***organic micro-pollutants*** such as pesticides, hydrocarbons, detergents, phthalates, bisphenol and drugs [91]. The first and last will be addressed in this report.

III.1. The mineral micro-pollutants, macro and trace elements, metals

A metal is a chemical element that has a density greater than 5 g/cm^3, a good conductor of heat and electricity, having characteristics of hardness and malleability, combines easily with other elements to form alloys used by the man since ancient times.

Macronutrients and micronutrients have a crucial role to blue-green algae and determine the intensity of development. These biogenic elements are always these substances in the composition of organisms and are necessary for development. Of these nitrogen molecules are part of all proteins, and phosphorus is a necessary component of nuclear substances, having an important role in redox reactions. Potassium, calcium, sulfur and magnesium are just as necessary as nitrogen and phosphorus. The optimal concentrations of macronutrients depend on the systematic position of algae. The doses required for cyanobacteria algae growth culture studied were determined by calculating these items from recipes nutritional environments (Annex 1). Environmental pollution by metals became extensive as mining and industrial

activities increased in the late 19th and early 20th century. The worldwide mine production of Cu, Cd, Pb, and Hg is considerable [92]. These pollutants, ultimately derived from a growing number of diverse anthropogenic sources (industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps, and mining operations), have progressively affected more and more different ecosystems [93].

In aquatic soluble fraction metals is in colloidal and especially in the form of metal cations. These metals are sometimes indispensable for the metabolism of living organisms in the aquatic environment but very often they can be toxic once their concentration in the aquatic environment exceeds a limit value. Some elements such as (Zn, Cu) have a vital character for life, for against, other (Cd, Pb) not currently have any biological known functions. Often in the aquatic environment seemingly innocuous chemicals depending on the valence and type of ligands with that are associated can become toxic. So it needs to study in particular any potentially toxic form of an element not only in dependent of concentration but also characterizing the toxic forms that may arise later in their environment.

In lately in environmental sciences, we hear more often improperly term heavy metals, referring to all heavy metals and metalloids present in trace concentrations, called, traces, regardless of which would be their molar mass. Yet it operates within the scientific term for metallically trace elements to avoid blurring, metals and metalloids whose content is inferior to 1mg/g in sediment and 5 mg/g in the water column [94].

III.1.1. Heavy metals in the aquatic environment, Trace Metallic Elements (ETM)

In the aquatic environment, a metal is defined as a chemical that can form metallic bonds and lose electrons to form cations [95]. These are present mostly in the environment in trace amounts: Mercury, Lead, Cadmium, Copper, Arsenic, Nickel, Zinc, Cobalt, and Manganese. The most toxic of these are Lead, Cadmium and Mercury. The origin of the metals in the aquatic environment is twofold. Naturally in the biosphere, they come on the one hand, mechanical and chemical weathering of rocks and soil leaching [95]. On the other hand, the anthropogenic contribution issue of industrial and domestic waste, mining and contaminated runoff from fertilizers and pesticides used in agriculture are all sources contributing to increased concentrations of heavy metals in the aquatic environment.

Heavy metals in water accumulated in the primary organisms such as algae and other aquatic plants [96] and spread throughout the food chain. They can reach concentrations threatening the survival of natural populations and present dangers to consumers of aquaculture products. Already in the 50s, their highly adverse effects have been highlighted following the fatal poisoning occurred in Minamata in Japan. The people had eaten fish contaminated with mercury discharges from a nearby factory. The disease then spread to the entire younger generation through breast milk [97].

Europe ruled by proposing in 2000 a definition that applies to European law and state-members, particularly in the field of waste "heavy metal" means "any compound of antimony, arsenic, cadmium, hexavalent chromium, copper, lead, mercury, nickel, selenium, tellurium, thallium and tin, as well as these materials in metallic form, if they are classified as dangerous substances "and more generally, a "dangerous substance" is "a substance that has been or will be classified as hazardous under Directive 67/548 / EEC or its subsequent amendments" [3].

ETM represent a dangerous group to the aquatic environment due to their persistence, toxicity and tendency to bio-accumulate in aquatic organisms and lead to unwanted biological effects [98]. In addition to the risk related to metals, their non-biodegradable nature allows their accumulation in different environmental compartments, hence the importance of studies to understand the behavior of these metals in the environment and deduce toxicity [99]. Fixed metal pollutants by suspended matter aquatic organisms and sediment but may in certain circumstances to re-solubilize in the water column and produce harmful effects on aquatic flora and fauna, but also on humans. It is thus necessary not only punctual quality assessment of the water but and of possible processes as a result of ETM interaction with organic or minerals forms present in the water column. Trace metals can control algal growth by acting either as toxicants (e.g. cadmium), or as limiting micronutrients (e.g. iron). Toxicity and nutrient limitation by trace metals depend on their chemical speciation in the medium [100, 101]. The speciation of metals in natural waters and in culture media is controlled by chemical processes such as precipitation, adsorption, and inorganic and organic complexation [102]. By releasing metabolites that complex metals, algae could, in principle, modify metal speciation in the medium and effectively control metal availability or toxicity in their external milieu. Blue-green algae produce weak organic acids and also strong metal-complexing agents ($^{e}K \geq 10^{7.5}$), during the later growth phases of batch culture. The metal-complexing agents produced by

blue-green algae may dominate the speciation of soluble metal in freshwater lakes during algal blooms [102].

III.1.2. Toxicity of heavy metals

The metals are generally separated into two categories according to their essential character or not living beings. Indeed, they may be necessary for the performance of biological processes (trace elements), is the case of iron (Fe), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), vanadium (V), Selenium (Se), Molybdenum (Mo), manganese (Mn), chromium (Cr), Arsenic (As) and titanium (Ti). In this case, their concentrations in organisms must meet the metabolic needs of the latter. Otherwise, a deficiency or excess of these essentials can lead to deleterious effects. Others are not necessary for life, and may even be harmful as mercury (Hg), Lead (Pb), cadmium (Cd) and Antimony (Sb). Cu, Fe, Zn and Mn are also discussed. Indeed, unlike previous, these four metals are considered trace elements are essential to the conduct of biological processes in metabolism and become toxic only above a certain threshold.

Table 3.1. Limits to not be exceeded for trace elements according to the (CODEX standards Codex Standard August 1, 1981 amended in June 1981, July 2001 and February 2008 Natural mineral waters.)

Element	Limits (mg/L)
Cr	0.05
Cu	1.0
As	0.01
Ba	0.7
B	5.0
Cd	0.003
Pb	0.01
Mn	0.04
Hg	0.001
Ni	0.02
Se	0.01

III.1.3. Toxicity of cadmium (Cd)

Cadmium has a high resistance to corrosion; its melting point is low; it has a good conductivity of electricity; its derivatives have good resistance to high temperatures; it has chemical characteristics similar to those of calcium, in particular the ionic radius, facilitating its penetration in organisms. Diffuse releases appear to be the first port of cadmium intake in aquatic environments and their importance relative to point sources is expected to increase in the future. Among these diffuse sources of cadmium, the use of phosphate fertilizers in agriculture, atmospheric deposition, diffuse sources of combustion (residential, waste fires ...) will be very difficult to reduce in the short term [103]. Cadmium is one of the most dangerous heavy metals. Even at low concentrations, it tends to accumulate in the renal cortex over very long periods it results in abnormal loss of protein in the urine (proteinuria) and causing urinary dysfunction in the elderly. The use of cadmium is in continuous decline. It was banned or its use has been restricted by EU regulation in several important applications (electrical and electronic equipment, coloration and stabilization of certain products, metal products surface treatment). Cadmium is being used in a wide variety of industrial processes, e.g., alloy preparation, metal plating, and electronics. It has been well recognized for its negative effect on the environment where it accumulates throughout the food chain, posing a serious threat to human health [104, 105]. This ion species is non-biodegradable and tend to accumulate in living organisms, causing various disorders for living organisms.

Table 3.2. Some of the most toxic heavy metals and their actions on the body

Particles	Toxicity
Pb	Nervous system disorder, liver disease and kidneys
Cd	Respiratory kidney problems
Hg	Nervous system disorders (memory, sensory coordination functions)
Ni	Respiratory diseases, asthma, birth defects, cancers
Cr	Anemia, dermatological disorders, cancer
As	Cardiovascular Disorders

III.1.4. Bioremediation of heavy metals (ETM)

Heavy metals have been released into the environment over long periods of time, throughout many activities of man. Once the metals have been released into the environment, they are difficult to be removed by physical or chemical means and most of them exhibit toxic effects on organisms. In addition, conventional physico-chemical means for removing heavy metals from wastes are generally very expensive [106]. *Azolla pinnata* and *Spirodela polyrhiza* showed profound ability to take up cadmium (Cd) from ambient medium. Cadmium (Cd) adsorption by test plants occurred rapidly during the initial stage of incubation, but the process slowed down and reached equilibrium in 120 min. This suggests the presence of two kinds of sites, reacting rapidly or slowly with cadmium (Cd). Dead plants accumulated two times more cadmium (Cd) than living plants, due perhaps to absence of a permeability barrier which resulted in cadmium accumulation at intracellular locations as well. Cadmium adsorbed by dead plants could be effectively displaced with chemicals like NaCl, $CaCl_2$ and EDTA. Cadmium sorption ability of dead plants did not diminish up to 10 successive cycles of sorption and desorption, as long as plants did not become fragmented [106].

According to the data provided by Wilde and Benemann [107] the most used solutions to treat metal-containing include the addition of chemicals (caustic, sulfide) for precipitation of metals and the use of ion exchange resins to bind the metals to a substrate. Other, less frequently used, processes include activated carbon adsorption, electro dialysis, and reverse osmosis. Bioremediation is better than precipitation in terms of ability to adjust to changes in pH and heavy metal concentrations, and better than ion exchange and reverse osmosis in terms of sensitivity to the presence of suspended solids, organics, and the presence of other heavy metals. Also, only ion exchange can compete with bio-removal in terms of residual heavy metal concentrations. Overall, according to the same authors [107] the limited data comparing bio-removal with conventional heavy metal removal methods indicate that several potential advantages are possible with bio-removal processes including:

1. Use of naturally abundant renewable biomaterials that can be cheaply produced;
2. Ability to treat large volumes of wastewater due to rapid kinetics;
3. High selectivity in terms of removal and recovery of specific heavy metals;
4. Ability to handle multiple heavy metals and mixed waste;
5. High affinity, reducing residual metals to below 1 ppb in many cases;

6. Less need for additional expensive process reagents which typically cause disposal and space problems;

7. Operation over a wide range of physicochemical conditions including temperature, pH, and presence of other ions (including Ca^{2+} and Mg^{2+});

8. Relatively low capital investment and low operational costs;

9. Greatly improved recovery of bound heavy metals from the biomass; and

10. Greatly reduced volume of hazardous waste produced.

Bioaccumulation is the process by which a living organism absorbs a substance at a greater speed than that with which it excretes where metabolizes. The cells will absorb the contaminants and the more hydrophobic compounds will be stored in tissues rich in lipids where they will tend to accumulate because of their persistent nature.

III.1.4.1. Phycoremediation of heavy metals (ETM)

This process characterizes the process of removing or biotransformation of pollutants by macro- or microalgae, using nutrients and xenobiotic substances including in wastewater and CO_2 from the air [108].
Some applications can remember phycoremediation:

- Sequestration of CO_2 emissions;
- Removing nutrients and xenobiotic compounds using algae-based bio-sorbents;
- Wastewater and metal treatment;
- Nutrient removal in waste and effluent rich in organic matter;
- The conversion and degradation of xenobiotics;
- The use of biosensors based on algae for the detection of toxic compounds.

If some applications are quite developed and are marketed intensive industrial others are just in the stage of initiation of research. The research in municipal wastewater treatment using algae was conducted over several decades by a number of researchers looking to overcome some limiting factors.

Heavy metal uptake by phytoremediation can be divided into a rapid passive bio-sorption on the cell surface followed by a slower intracellular bioaccumulation [109]. One of the most important mechanisms for survival of aquatic organisms, including algae to the

increased ETM concentrations is tolerance. Both tolerance itself as well as tolerance for ETM is characterized by the presence of complex systems neutralizing activity of the toxic metal cations. Organisms which have adapted their tolerance to high concentrations of metal ions inside the cell when in the exterior of cell concentrations are increased are called <<in accordance>>. Organisms that regulate and maintain cations concentration inside of the cell to a predefined, threshold level, are called, regulators. Basic mechanisms that participate in the regulation of metal content in the cell are generally branched into two: (1) Inputs are low because the cell has low membrane permeability or absorbent surface. (2) Outputs are increased due to the involvement of excretion systems own to living organisms. Both tolerance and regulation depends largely of the type of metal analyzed. In general, the concentrations of metals which have a physiological role in the body are adjusted when metals with no known physiological role are accumulated in the cell body. Depending on the donor-acceptor functions of the metal in contact with the cellular enzymatic system, metals are closely linked to a large group of substances such as metallothioneins and chelatins. These proteins are produced in an incredible amount with increasing of metalical cations amount (physiological or not). In this way induction of metalloproteins represents a protective mechanism against toxic effect of metal ions.

Additional function of metallothioneins include control of intracellular redox potential, cellular repair processes, growth and differentiation, where they are likely to serve as the source of Zn for newly synthesized enzymes, as well as regular molecules in gene expression [110]. From the data published from El-Enany and al [111], *Nostoc linckia* seems to be tolerant to heavy metals (Zn and Cd) and is able to accumulate this metal by adsorption on the pellets and/or through sequestration via metal-binding protein. Therefore it can be recommended it to be employed in the purification of waste contaminated with these heavy metals [111].

Metallothionein occupies a unique place in the catalog of metalloproteins as demonstrated by a primary sequence dominated by a 30% cysteine content (of the amino acid residues), with metal binding properties. This class of protein characterized by a high cysteine content, low molar mass (approx. 6000 for the mammalian protein), and lack of aromatic amino acid residues makes their remarkable for metal binding properties have been reported both in vivo and in vitro. Chemical and spectroscopic studies have shown that an unusually wide range of metals bind to metallothionein [112, 113]. Blue green algae affinities with ETM been demonstrated repeatedly in [114, 115]. The molecular mechanism by which green-blue

algae detoxifies the body were also studied intensively [116, 117]. While greater amounts of heavy metals can be accumulated by intracellular accumulation, toxicity problems are excluded by passive biosorption on the cell surface [109, 118]. Therefore, biosorption processes could be an alternative or a supplement to purification methods currently used.

III.1.5. Speciation

Speciation of an element is the determination of the individual physico-chemical forms of that element which together make up its total concentration in a sample [119]. Determining the total concentration of ETM will not provide enough information about its bioavailability in the cell or the possibility to interact with suspended material or sediment. For this is necessary such a measure in the element concentration which is bioavailable for the living organisms and that could study the aquatic toxicity of the metal to organism's cells separately and could allow understanding of ETM transport in rivers and estuaries generally. One of the most important differences between ETM and other toxic pollutants is that the firsts are not biodegradable and once they entered in the environment, their potential toxicity is controlled in large part by their physicochemical forms.

Some elements such as iron, iodine, copper, manganese, zinc, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon and nickel have been established as essential for organisms [119] while others such as arsenic, cadmium, lead and tungsten have no role for them toxic cell in the lowest possible dose. For all these essential ETM there is a threshold concentration between the essential and toxic that is essential to known for proper functioning of the organisms. If an essential element for the cells is in an exceeding concentration in relation with this threshold it is automatically toxic. Nowadays the people is subjected to much higher environmental concentration of metals that our ancestors. The modern human body is exposed to metal concentrations exceeding 500 times the concentrations to which were exposed a primitive man, being very close to the level of concentration of the clinical toxicity [120]. The smallest variation in the speciation of trace elements can dramatically change their bioavailability or toxicity. For example, change in the oxidation state of element can have a profound effect on bioavailability and toxicity. Chromium (III) is an essential element while chromium (VI) is highly toxic [121] as well as arsenic (III) is much more toxic than arsenic(V) [122].

More specifically speaking toxicity of a particular dissolved metal towards aquatic organisms related to its ability to react with a biological membrane and to transit it. The

possibility of transiting of membranes depends directly on the solubility of the metal-species in lipids (usually only uncharged organometallic species) or extents and rates of reaction of membrane transport protein studied with the studied metal ion. Metal-protein interaction, leading to carrier-mediated transport of the metal across the membrane, will, for bivalent ions, be thermodynamically favored when the metal is in the simplest chemical form, e.g., $Cu(H_2O)$, $CuCl^+$ or $Cu(OH)^+$.

In natural waters the concentration of many heavy metals and other elements is often below 1μg/L, and sometimes below 0.1 μg/L. It is for this reason that chemical analysis at these concentration levels must be carried out with specific and appropriate technics. There are severe problems of contamination from a multitude of sources and, at the same time, serious losses of metal can also occur by adsorption on the walls of sample bottles or analysis vessels. For this reason, a clean-room or at least a special room with a filtered air supply is necessary for work at the ultra-trace levels. Even in these conditions a large number of repetitions of the analysis should be made until the results could be validated putting a difficult test for the sensitivity of the analytical method and offering a challenging to chemical analyst skills.

The speciation measurements should not be acidified water sample storage up to prevent precipitation of humic materials that usually down carries some heavy metals. Freezing of freshwater is not allowed too, because ionic metals are continuously concentrated in the liquid.

III.2. Wastewaters and their discharge in water courses

Watercourses receive pollution from many different sources, which vary both in strength and volume. The utilized water in domestic, agricultural and industrial purposes spilled in natural open watercourses through sanitary sewer system is called wastewater.

The composition of wastewater is a reflection of the life styles and technologies practiced in the producing society [123]. It is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Urban waste waters are those that come from the mixture of water by faeces household with industrial waste and rainwater. When all the water is discharged together and led to the treatment plant, the sewage systems are mixed, and when rainwater am totally or partially driven directly into the river channel is separate [124]. Domestic wastewater often contains paper, urine, feces and synthetic detergents. Industrial effluents have various pollutants- both organic and inorganic-depending on the type

of industrial activity. Agricultural effluents have animal wastes, fertilizers and various pesticides [125]. Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals [5, 6]. In some countries around the world sometimes occurs wastewater discharges into watercourses without any treatment.

III.2.1. Wastewaters and their discharge in water courses of Republic of Moldova

According to the data presented by academician Gheorghe Duca [126] as well as Dontu in his PhD thesis [124], wastewater purification systems in Republic of Moldova are physically and morally outdated, it operates more than 25-30 years without being reconstructed and do not meet the treatment technologies. If in 1990, 304 stations were operating in the republic wastewater treatment, now it operates less than 50. Note that in towns on rivers Nistru and rivers Prut not operating as normal no treatment plant [127]. Over the past eight years the share of population connected sewage system in total water supply system does not exceed 39 percent [128]. Domestic sewage in our country is the main and most serious source pollution of small rivers, as most biological sewage treatment plants do not work. Of the 580 existing stations in the country, currently only 132 are equipped with necessary equipment. From this 78 functions and the other 54 is stationary. Such waters are discharged untreated directly into natural basins and causes, primarily their eutrophication, a phenomenon often detected in Bic rivers, Ikel, Cubolta, Cogalnic and other small rivers. The problem becomes more stringent water quality and aquatic resources that are excessive use in all sectors of the economy. A Republic of Moldovan citizen consumes on average about 594 m^3 of water per year, of which 293 m^3/year are surface resources.

Volume of wastewater discharged into natural receivers was 685-687 million m^3, including conventional pure - 550-552 million m^3, polluted - 10 to 14 million m^3, normative treated - 115- 119 million m^3 and 9.2 to 13.2 million m^3 insufficiently treated [128]. Analysis performed in 2011 by Moldavian State Hydrometeo Service shown that the content of heavy metals (total forms) in the analyzed sediments samples from diverse points of moldavian territories adjacent to transition of the most important hydrographical network (Fig 3.1) shown diverse concentration of analyzed elements:

- Copper - from 2.3 in 2010 (Lake Manta) up to 63.4 mg/kg in 2009 (river Bic, downstream of mun. Chisinau);
- Zinc - from 7.6 (Lake Manta) up to 189.8 mg/kg (river Bic, Chisinau downstream);
- Lead - from 2.9 (Lake Manta) up to 128.4 mg/kg (river Bic, Chisinau upstream);
- Nickel - from 5.3 (Lake Manta) up to 48.6 mg/kg (river Prut, Giurgiuleşti);
- Mangan - from 138.6 (Lake Manta) up to 1114.0 mg/kg (river Bic, Calarasi).

Fig.3.1. Physical map of rivers in Republic of Moldova

Data analysis made by Moldavian State Hydrometeo Service shows that the lowest content of heavy metals was recorded in Manta underwater lake sediments and the highest in Bic river sections Chisinau downstream and Calarasi upstream [128]. Compared with previous years, in 2010 the total copper content in most monitoring points was reduced. Indeed, there is a slight increase in Taraclia basin, reaching value of 19.7 mg/kg [128]. The pollution of natural waters can happen on purpose of numerous accidents. Domestic wastes constitute a source of pollution for various ecosystems because of sewerage leaks or

blowouts, oil are spilled mostly because of wreckage. Organic micro pollutants often contaminate natural basins as a result of washouts of surface soil layers or together with precipitation from the air where they were sprayed [125]. Suspended organic particles inhibit light penetration deep into the water hindering photosynthesis processes. Decomposition in water ecosystems of organic pollutants can create favorable environments for pathogenic organisms. Tension-active reagents like fats, oil and lubricants form films on water surface, which inhibit gas exchange between water and atmosphere and lower oxygen content in water. Detergents belong to a waste group of substances decrease water surface tension that comes in industrial effluents after use in flotation ore processing, depletion of the products in chemical technologies, obtaining of polymers, improvement of oil and natural gas drilling, prevention of corrosion [125]. This means a large number of organic and inorganic substances disturb the water quality, which are the main causes of eutrophication of the water body.

Wastewater is mainly treated by aerobic or anaerobic biological degradation. However, the treated water still contains inorganic compounds such as nitrate, ammonium and phosphate ions, which leads to eutrophication in lakes and cause harmful micro algal blooms [129]. They also proved to be powerful stimulants to algal growth and consequently formation of ‘‘algal blooms’’. Algal blooms can affect the water quality in several ways [5]. Oswald [86] estimates that the relative cost doubles with each additional stage of treatment. Furthermore, chemical-based treatments often lead to the contamination of the sludge by-product of conventional treatment plants. For example, a common tertiary treatment for phosphorus removal can cause increased levels of aluminum in the sludge and create problems of safe disposal. A complete tertiary process aimed at removing ammonium, nitrate and phosphate is estimated to be about four times more expensive than primary treatment [130].

Quaternary treatment intended for the removal of heavy metals, organic compounds (refractory and toxicants) and soluble minerals will be about eight to sixteen times more expensive than that of primary treatment, respectively [86]. Advanced treatments are generally based on technologically complex techniques, such as chemical precipitation, ozonation, reverse osmosis or carbon adsorption. These techniques include processes designed to remove particular nutrients, such as phosphorus or nitrogen, which can stimulate eutrophication in certain situations [5].

Bioremediation of wastewater may by accomplished by using algae and micro-organisms to eliminate or reduce polluting elements existing in them. The bio-treatment of

wastewater with algae to remove nutrients such as nitrogen and phosphorus and to provide oxygen for aerobic bacteria was proposed over 60 years ago by Oswald and Gotaas [131].

Algal treatment of wastewater, mediated through a combination of nutrient uptake, elevated pH, and high dissolved oxygen concentration, can offer an ecologically safe, less expensive, and more efficient means to remove nutrients and metals than conventional tertiary treatment. The cultivation of algae in wastewater offers the combined advantages of treating the wastewaters and simultaneously producing algal biomass, which can further be exploited as food additives (for aquaculture and animal feed), energy such as biogas and fuels, agriculture (fertilizers and soil conditioners), pharmaceuticals, cosmetics and other valuable chemicals as well as using in waste water polluted with organic or inorganic micro pollutants.

III.2.2.4. The nutrients in micro-algae cultivation on wastewaters

The nitrogen in sewage effluent arises primarily from metabolic inter-conversions of extra-derived compounds, whereas 50% or more of phosphorus arises from synthetic detergents. With the increasing use of inorganic nitrogenous fertilizers and the production of wastes from human and animal populations, there are signs of nitrogen (N) accumulation in the environment. In the case of N pollution, most concern stems from the possible health hazards that have been attributed to nitrite either directly as a causative factor of methemoglobinemia or indirectly as the source of nitrosamines [132]. Although the most widely utilized biological method for water treatment has focused mainly on removal by bacteria, during the last 20-25 years increasing attention has been paid to the possibility of using microalgae. The bacterial denitrification has the disadvantage of requiring the addition of an organic carbon source (usually methanol) as well as a strict anaerobic environment, which makes the system unstable in long-term operation.

Through the appropriate use of the nutrient uptake capabilities of photosynthetic cyanobacteria, various nutrients can be simultaneously removed from water. Biological N removal generally appears a valid option and offers some advantages over tertiary chemical.

The principal forms in which they occur in wastewater are NH_4^+ (ammonia), NO_2^- (nitrite), NO_3^- (nitrate) and PO_4^{3-} (orthophosphate).

Phosphate in wastewaters does not seem to present any problems for human health; phosphorus (P) removal from municipal and industrial wastewaters is required to protect water from eutrophication. At high pH, polyvalent cations, such as calcium and magnesium,

interact with phosphates to precipitate as an algal–mineral complex [133]. Therefore, this mechanism, which is referred to as auto-flocculation, contributes not only to the lowering of the phosphorus concentration, but also aids in the removal of the suspended algae from the effluent. However, wastewater often is deficient in calcium and magnesium. Nurdogan et al. [133] demonstrated that adding lime to the wastewater increased phosphorus removal efficiency to 90%.

Algae treatments' systems of offer a lot of benefits that can be achieved with little or no use of chemical additives and, furthermore, may offer the benefit of resource recovery and recycling. In the works of Hoffmann [134] we can see a comparison between algal-based treatment systems and conventional treatment for the removal of phosphorus. To achieve the desired level of treatment with algal systems, maximizing autotrophic production is of primary importance [135]. The basic principles of algal mass culture [134] must be applied, particularly the need of effectively harvest of the algal biomass in order to remove the sequestered nutrients. The largest body of information on how to accomplish acceptable levels of wastewater treatment with algae is available for suspended algal systems. This nutrient loading can be substantial since many conventional treatment plants discharge 10^6L of wastewater per day, and nutrients in wastewater can be three orders of magnitude more concentrated than in the receiving water [130, 134]

Together these two elements are known as nutrients and their removal is known as nutrient stripping [136].

III.2. Organic micro-pollutants, persistent organic pollutants or POPs

POPs are synthetic chemicals and are distinguished multitudes of chemicals by their persistence, toxicity, ability to be transported from long distance and bio-accumulate in living organisms [137]. They can also be transported in the food chain and are likely to be bio-concentrate or bio-accumulate from the first link of the food chain (For example: copepod, microalgae) to mammals including humans. This ability to bio-accumulate through the food chain causes adverse effects not only on the proper functioning of the environment but also on human health [138]. POPs are hydrophobic and lipophilic molecules hence their preferential tendency to adsorb on the solid matrix that enter in the aqueous phase of environment. Moreover, their persistent nature led to their resistance to physical, chemical or metabolic degradation.

Organic micro-pollutants include several types of compounds having one or more carbon atoms and hydrogen. They are mainly from human activities such as farming, urban and industrial. Each pollutant has its own physical and chemical properties which generally control their fate and behavior in the environment. Ultimately, these said properties can control their persistence, their degradability in the environment, or the phase in which they will preferentially focus (dissolved, gaseous or solid) well as their ability to bio accumulate in the food chain. The organic micro-pollutants come in the environment in trace and ultra-trace concentrations, from µg/L to ng/L in water and from µg/kg to ng/kg in the sediment.

Even present at trace, these molecules can have adverse effects on the functioning of the ecosystem and also on the entire biosphere. Hydrocarbons, polychlorinated biphenyls, pesticides, drug residues, bisphenol and phthalates are among the most dangerous and organic contaminants most commonly found in the environment, particularly aquatic. In our research, drug residues, phthalates and bisphenol were selected as study molecules of interest. Today, very little information is available at the local, regional, national or international on contamination levels, behavior and environmental fate of these three families of molecules. Indeed, for the environmental interest, they are subject to review only recently compared to hydrocarbons, polychlorinated biphenyls and pesticides that are relatively well documented.

III.2.1.1. Pharmaceuticals products

Pharmaceuticals products for human and veterinary use as well as personal care products are becoming a major source of environmental concern. The latest survey published in 2008 by the Organization for Economic Cooperation and Development (OECD) about 3 000 pharmaceutical substances are used annually in the European Union including 650 in France per year. The most used molecules are antibiotics, consumption of which has reached 12500 tons per year over the last decade [139]. Drug residues enter in the environment from various sources. Figure 1 shows the pathways for the introduction of drug residues in water. Indeed, organisms (humans and animals) reject from 50 to 80% of consumed active substances. Also, some people have the habit of throwing their unused drugs down the drain or toilet. For all these reasons, a significant quantity of drugs was found in sewage in rivers, streams and even in drinking water.

Indeed, the first objective of drugs being to ward off certain organizations or even to attack the immune system in order to better protect thereafter. Since these molecules are made for biologically active effects, you can logically speculate that drugs have an impact on aquatic and terrestrial species. For example, drugs may be able to feminize certain fish and limit annihilate see the reproduction of these fish.

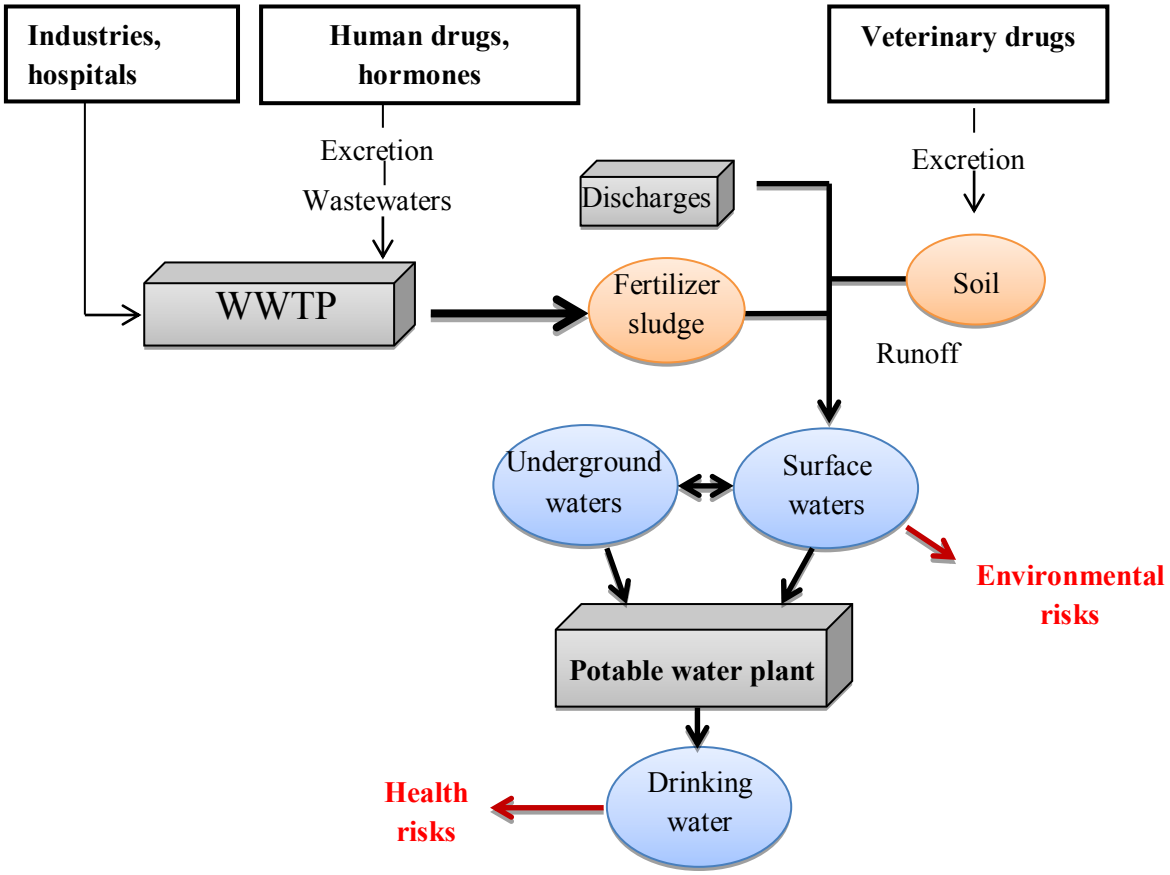


Fig.3.2. Different drug diffusion paths in the water cycle([1, 2])

III.7.1.2. The antibiotics

Antibiotics are used only by humans and the sources of contamination are numerous. In 2000 in France, 1391 tons of antibiotics were used for veterinary use, reflecting an increase of 5% between 1999 and 2000 [1, 2]. In 2006, the French Agency for Food Safety (AFSSA) estimated the overall antibiotic consumption to 2000 tons for France. Therefore, molecules are metabolized by the organisms; they are nevertheless not completely eliminated. Accordingly, it is found in soil and in water of many antibiotics waste. For example, are detected in feces of livestock and humans in hospital waste and urban waste, hospital and

farming. These antibiotic residues left behind in nature are introduced into the environment and mingle with the wildlife and the microorganisms of the soil. Thus, the bacteria in the soil impregnate antibiotics and begin to create new mechanisms of resistance [2]. As a result, when these bacteria affect us, the usual drugs will have no effect, since these organizations have a multi natural resistance acquired in nature.

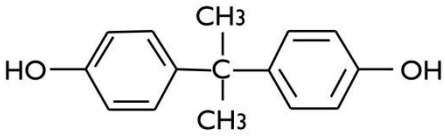
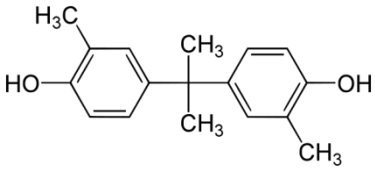
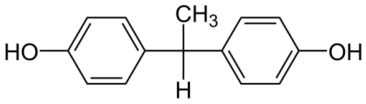
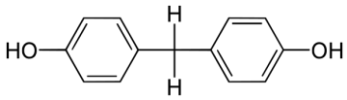
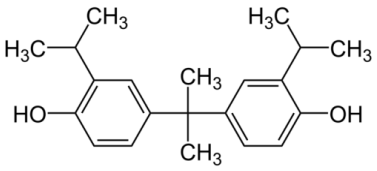
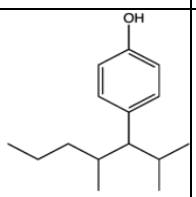
III.7.1.3. The hormones

Hormones are one of the most potent endocrine disrupting compounds as well as are considered also as emerging pollutants. Hormones can be differentiated in estrogens, androgens, and progestogens. Steroidal hormones and their synthetic analogs include estrogen and progestin contraceptives or hormone replacement therapy. The steroid hormones help controlling the metabolism, inflammations, immunological functions, water and salt balance, sexual development, and the capacity of withstanding illnesses [140, 141]. Their environmental impact is now clear and they act on non-target organisms at very low doses. These are powerful endocrine disrupters, accumulating in the tissues of some aquatic species; these compounds can have an impact on their morphology. For example, the feminization of fish due to releases including women urine containing synthetic hormones in birth control pills [142]. Fish are therefore more likely to be contaminated, being at the top of the food chain. These residues can accumulate in aquatic organisms from the first food chain to mammals-including human.

III.7.1.4. Bisphenols

Bisphenols are a family of organic compounds with two phenol functional groups. Most of them are related by a methyl bridge and the molecule is a diphenyl methane derivative. In addition, each bisphenol may be associated a number of derivative molecules which differ by the presence of alkyl radicals [142]. The molecules of interest in this study are presented in the below table. Bisphenol A (BPA) is a chemical compound most stable under normal environmental conditions but readily biodegradable. In addition, BPA has a low potential for bioaccumulation in fish. But after the oral and dermal exposure, BPA absorption is rapid and extensive.

Table 3.3. The physicochemical properties of Bisphenols and 4 nanophenol ([143], [141]).

Name and structure	Chemical formula	Mw (g/mol)	Solubility (mg/L)
Bisphenol A or 4,4'-(propane-2,2-diyl)diphenol 	$C_{15}H_{16}O_2$	228,29	120-300 (21,5 °C)
Bisphenol C or 4,4′-Ethylidenebisphenol 	$CH_3CH(C_6H_4OH)_2$	256,34	-
Bisphenol E or 4,4'-Ethylidenebisphenol 	$C_{14}H_{14}O_2$	214,26	-
Bisphenol F or 4,4′-Methylenediphenol 	$CH_2(C_6H_4OH)_2$	200,23	-
Bisphenol G or 2,2-Bis(4-hydroxy-3-isopropyl-phenyl)propane 	$C_{21}H_{28}O_2$	312,45	-
4-nanophénol 	$C_{15}H_{24}O$	220,35	5,76

The bisphenols, particularly bisphenol A (BPA), are known as endocrine disruptors. BPA is found in the linings of cans, soda cans in electric kettles or coffee machines or as a developer in the thermal printing papers like crates tickets [142]. The same author asserts that if the BPA was prohibited in baby bottles since January 2011 and the food containers were also prohibited from January 2016 [142], then it is still present in many products of daily life.

III.7.1.5. PAH's

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated from both natural and anthropogenic processes(Chemicals, steel, fire, combustion engines, municipal waste incinerators, transport and many other) [144], and have led to serious concern regarding the health of aquatic and human life through bioaccumulation [145, 146]. These compounds are characterized by complex chemical structures, constituted by assembling two or more benzene rings. They are said light or low molecular weight when the number of existing aromatic ring is ≤ 3 while those aromatic ring ≥ 4 are categorized among the heavy PAHs (or high Molecular weight). Bacteria and fungi are known to play important roles in the degradation of polycyclic aromatic hydrocarbons in the environment [147, 148]. However, the role of cyanobacteria (blue-green algae) has not been thoroughly investigated despite their wide distribution in aquatic ecosystems [149].

III.7.1.5.1. Fluorene

Fluorene is a PAH found in the smelting effluent from Norwegian fiords [150], in coal processing wastes [151], in crude oil and fuel oil [152], and in the tar coating of drinking water supply tanks [153]. Fluorene has also been found in water samples collected from contaminated areas in Puget Sound [154]. Fluorene is important as a precursor to a family of mutagenic compounds [155] and has been placed on the list of the U.S. Environmental Protection Agency's Priority Pollutants [156]. Laboratory studies indicate that fluorene is highly toxic to fish [157] and aquatic algae [158]. The persistence of a chemical in the ecosystem and its environmental fate are key factors in the assessment of contaminant hazard.

III.7.1.5.2. Phenanthrene

Phenanthrene, a tricyclic aromatic hydrocarbon, is widely distributed in the environment as a result of pyrolytic processes (4). Although phenanthrene is not mutagenic or carcinogenic, it has been shown to be toxic to aquatic organisms [159]. Interestingly, since it is the smallest aromatic hydrocarbon to have a "bay-region" and a "K-region," phenanthrene is often used as a model substrate for studies on the metabolism of carcinogenic polycyclic aromatic hydrocarbons [160-162]. Presently, bioremediation has been shown to be effective in remediating soils contaminated with low molecular weight PAHs [163-165]. On the other hand, the lack of microbial activity towards high molecular weight PAHs may be attributed to site specific environmental factors, such as bioavailability of the contaminant, nutrients, redox potential, etc.

III.7.1.5.3. Pyrene

One such high molecular weight PAH is benzo[a]-pyrene (BaP), a five-ring compound. BaP has been classified by the US Environmental Protection Agency (USEPA) as a priority pollutant: a compound selected on the basis of its known or suspected carcinogenicity, teratogenicity or acute toxicity [163]. BaP has been shown to cause genotoxic effects in a broad range of prokaryotic and mammalian cell assays, and as such, its occurrence in the environment is of great concern [163].

III.7.1.5.4. Fluoranthene

Fluoranthene is one of the 16 PAH priority pollutants classified by the US Environmental Protection Agency (USEPA) and its poses greater potential danger to the ecological environment because of its toxicity, mutagenicity, and carcinogencity (Hui 2003) and, in addition, it is generally present in greater amounts in marine environments. Thus, PAH pollution of marine environments is often characterized by fluoranthene. The solubility of fluoranthène in water is relatively low (0.20–0.26 mg/L) and its K_{OW} is 5.33. Fluoranthene is among the most ubiquitous and abundant pyrogenic PAH pollutants and therefore is frequently chosen as a model compound for ex situ biodegradation studies [146, 166, 167].

III.7.2. Sources and behavior of organic micro-pollutants in the environment

The environment can be subdivided into various compartments (air, surface water or groundwater, soil, sediment and biota) and which form systems interconnected. Chemical exchanges, physical or biological can take place between these compartments. Thus, waste/discharges of contaminants in one of the compartments can migrate in others. The proper functioning of the ecosystem can be so assigned and human health [168]. It is then important to know the mode of transfer and the form of distribution of a contaminant from a phase to another.

In the aquatic environment, one can distinguish the dissolved, particulate and sedimentary phase [169]. Sorption and partition of pollutants are as existing process between the water column, sediment interface and the nature of the organic materials located. In water, transport of organic pollutants is associated with the mobilization of suspended particles [170, 171].Contaminants bind to organic and inorganic particles to finally settle the bottom of lakes, rivers and seas. The origins of micro-pollutants in water are many and varied, there are still a few years, and few people cared about what was rejected in nature, imagining that it would address itself our releases. Organic micro-pollutants originate from a share of direct discharges of urban and industrial wastewater, and also to diffuse inputs associated with atmospheric deposition or contaminated runoff waters from agricultural soils [172]. These effluents charged residues are treated in the treatment plant before joining the aquatic environment (Fig.3.3).

However, veterinary residues meet the environment without purification stage. The flow of effluent from veterinary use is complex to follow. Indeed, the manure can reach groundwater percolation of animal excrement (in pastures) or by spraying. For example, in industrial livestock manure, concentrations can reach several milligrams of tetracycline per gram of manure from pigs or sheep [172, 173]. A certain number of pharmaceutical compounds are by their nature water soluble, biodegradable and have a short half-life. Compounds such as estrogen EE2 and E2 have high log K_{OW} (3.94 to 4.77) show a high affinity for soil and sediment [174]. Drug metabolites excreted in conjugated form, can be cleaved at the treatment in sewage treatment plants, releasing the active parent compound as has been shown for the E2 [175-177] and EE2 contraceptive pills [178].

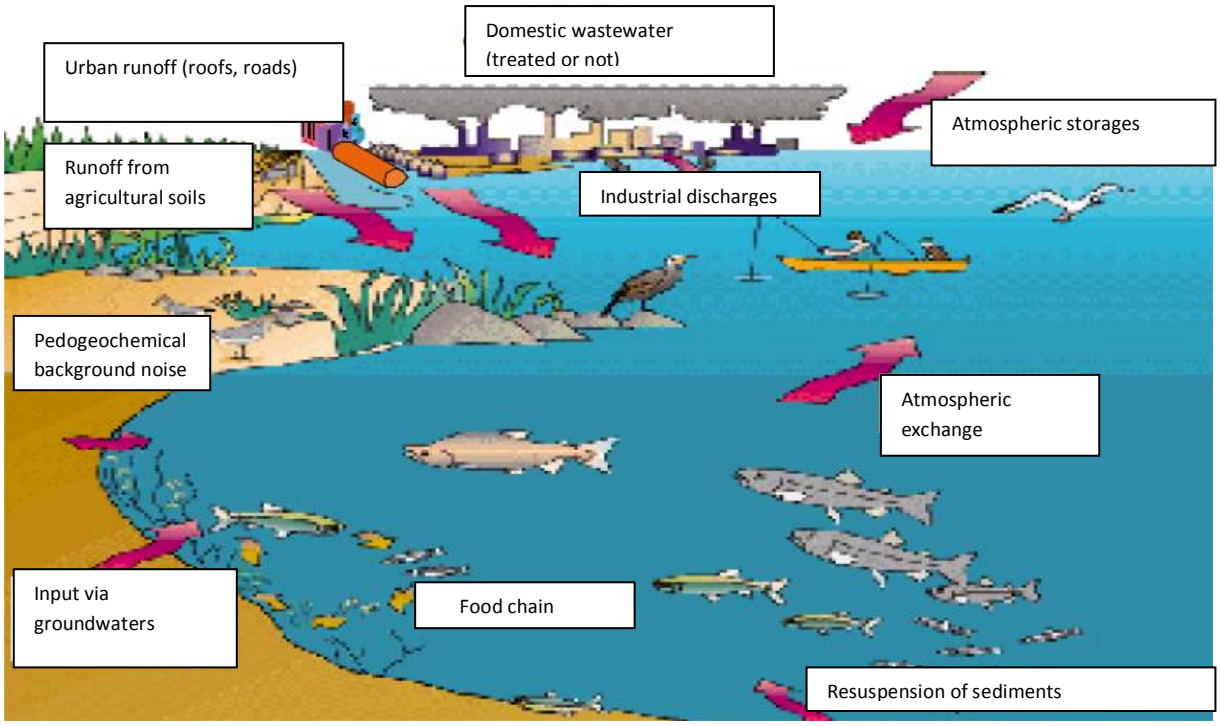


Fig.3.3. The different sources of contamination by organic micro-polluants ([173])

Chapter IV

Interaction of certain algae species with metallic and organic contaminants

IV.1. Bioremediation of organic micro-pollutants by blue-green algae

In our research concerning bioremediation of organic micro-pollutants by blue-green algae, 2 species of blue-green algae have been used: *Spirulina platensis* and *Nostoc gelatinosum.* Each of these species is recovered from their culture media in the exponential growth phase and is added in pre-established mass in their recently prepared culture media, an equal volume 1L. For the algae *Spirulina platensis* has used the Zarrouk media and the blue-green algae *Nostoc gelatinosum* in the Nr.6 middle whose recipes are presented in Annex no.1.

IV.1.1. Bioremediation of phthalates, bisphenol and residues of drugs by *Spirulina platensis* blue-green

In order to conduct a study on the feasibility of removing phthalates, bisphenol and residues of drugs and behavior by *Spirulina platensis* in contaminated water, three different series were prepared:

- ✓ Control ;
- ✓ Free *Spirulina platensis* (2 g/L)
- ✓ Immobilized in Na-alginate *Spirulina platensis* (2 g/L).

These different media were spiked with a concentration of 200 µL each of the following internal standards. Then they were distributed to 40 Erlenmeyer flasks.

Table 4.1. Internal standards selected for the feasibility study by *Spirulina platensis*.

Internal standards selected	• Caffeine, • Bisphenol A, • Progesterone, • α-Estradiol, • Acetylsalicylic Acid (Aspirin), • Naphtalene d-8 (N-d8) • Acenaphthene d-10 (A-d10) • Phenanthrene d-10 (Phe d-10) • Pyrene d10- (Pyr-d10)

During each day of extraction, three solutions were taken, filtered to be ready for extraction.

Fig.4.1. Preparation of various environments for bioremediation by *Spirulina platensis*.

IV.1.2. Bioremediation of HAP contaminated waters from urban wastewater treatment plant by *Nostoc gelatinosum* blue green-algae

IV.1.2.1. Chemicals and reagents

Crystals of phenanthrene, fluorene, fluoranthene and pyrene with 98% of purity were purchased from Acros organic (France). Acenaphthene-d10 (A-d10), phenanthrene-d10 (Phe-d10) and pyrene-d10 (Pyr-d10) were obtained from LGC-Promochem (Middlesex, UK) and used as internal standards. HPLC-grade solvents as hexane, dichloromethane (DCM) and acetone were purchased from Dislab (France). Glassware was systematically washed with detergent (Decon, East Sussex, UK), rinsed with acidified ultrapure water then with ultrapure water followed by acetone before drying at 120 °C prior to use. Nalgene flasks were also washed with the same way but by using double-distilled water instead of ultrapure water

IV.1.2.2. Growth conditions

For the PAH treatment, the experiment was conducted using two types of medium and a level of contamination equal to 0.1 mg/L. The first type of medium used 100% of filtered wastewater above described while the second one is a synthetic medium namely “Drew”. The Drew medium has the following composition in g/L: 0.2 K_2HPO_4, 0.2 $MgSO_4*7H_2O$; traces of $CaCl_2$ and $FeCl_3$. Before performing the bioremediation experimentation, cells were firstly grown in “Drew” and filtered non-diluted wastewater culture medium, without contamination, in order to accommodate this blue-green alga in theses chosen medium.

IV.1.2.3. Batch biosorption studies of *Nostoc gelatinosum* in PAH contaminated waters

As already above mentioned, the treated non-diluted wastewater solutions were collected from a wastewater treatment plant located in Villeneuve d’Ascq (59493), France then filtered by using a vacuum pump with cellulose acetate filters which pore diameter is 0.45 micrometers. The filtered non-diluted wastewater and the Drew medium were also physically sterilized, in clean flasks, by exposure to an ultraviolet light for 30min. Fresh algal strains were obtained from the previous accommodation culture of *Nostoc gelatinosum* by harvesting this culture in the logarithmic phase that corresponds to the sixth day of growth. Different amounts of algal biomass were tested in the two types of medium (non-diluted treated wastewater and Drew medium) and an amount of PAHs stock solution, obtained by

dissolving powders of phenanthrene, fluorene, pyrene and phenanthrene in acetone, were spiked to each type of the medium. The batch experiment details are described in Table 4.2. During the entire experience, each of the seventh samples contained 500 ml of the solution that always refilled with double-distilled water before each sampling in order to compensate the evaporation factor.

Table 4.2. Batch experiment details of *Nostoc gelatinosum* algae utilized in our experiments

N° Sample	Type of medium	Type of medium	PAH concentration (mg/L)
1	Wastewater	19.60	0.1
2	Wastewater	19.67	<LOQ
3	Wastewater	78.20	0.1
4	Wastewater	<LOQ	0.1
5	Drew	79.21	0.1
6	Drew	79.48	<LOQ
7	Drew	<LOQ	0.1

IV.2. Trace metal elements (ETM) in wastewaters, Seasonal campaigns of trace metal elements sampling and analysis in the waters of the Dniester River and Bic, the most strategic economic sites of Republic of Moldova

High pollution of the river Bic and the impact of this pollution on the river Dniester one of the most important rivers in Republic of Moldova [179] led us to the idea of making some sampling campaigns of surface waters in these two basins in several strategic points of high economic importance. They were targeted mainly points upstream and downstream flow of the river through the city of Chisinau, as well as in relation to treatment plant wastewater in the capital but also upstream and downstream of the overflow of the river Bic in river Dniester, the most important resource of drinking water of the country.

Seasonal analysis of heavy metals using ICP-MS of the most important metal pollutants (ETM) present below shows mineral heavy metal pollution of the both rivers during the winter-spring-summer and autumn of 2015.

Throughout the year 2015 significant concentrations of ETM in surface waters of the Republic of Moldova can be observed. However, some concentrations vary depending on the season and the sampling site.

The analysis results indicate that the maximum content of total chromium was recorded in waters downstream of the wastewater treatment station in Chisinau municipality, with a maximum of 3,5µg / L in February 2015. Cobalt is present in concentrations up to 0,6µg / L in May 2015 in the estuary of Gura Bicului. Nickel concentration value is not exceeding 5 µg / L in February both in downtown Chisinau and upstream from the treatment plant of the city. The concentrations of 2,25µg / L of lead that can be detected in Ghidighici lake waters also in February 2015 are highlighting possible sources of the pollution of the lake or the historic pollution of the sediment that was not removed for about 50 years.

The zinc concentrations quantity detected in surface waters of the Republic of Moldova in May 2015 in the estuary of Bic river at Gura Bicului sampling site are exceeding the CMA (10 µg / L) value, registering concentrations of (27µg / L), these values are decreasing in September up to 7µg / L concentration values in the same site. Significant concentrations of Zinc are determined in the surface waters of Bic river in the sampling site of Criuleni (about 17µg / L), in spring season. In downstream waters of the wastewaters treatment plant from Chisinau, in February 2015 the Zinc concentrations were 15µg / L, and 12µg / L in September 2015. In almost all analyzed sites was detected that the maximum admissible concentration of copper in the surface waters of the Republic of Moldova (1µg / L) is reaching the upper limit, except the sampling sites of Gura Bicului (6µg / L), Ghidighici (2,5µg / L) and the Chisinau municipality with about 2µg / L.

The concentration of aluminum decreases from 45µg / L in February 2015, to about 3µg / L in September 2015. There were registered concentrations of Titan up to 0,9µg / L in May, Vanadium up to 2,7µg / L in May, and Cadmium up to 2,7µg / L in the same period.

Data analysis shows that ETM content (total forms) in analyzed water samples from sites of the Republic of Moldova is insignificant and does not exceed CMA in the majority of analyzed water samples.

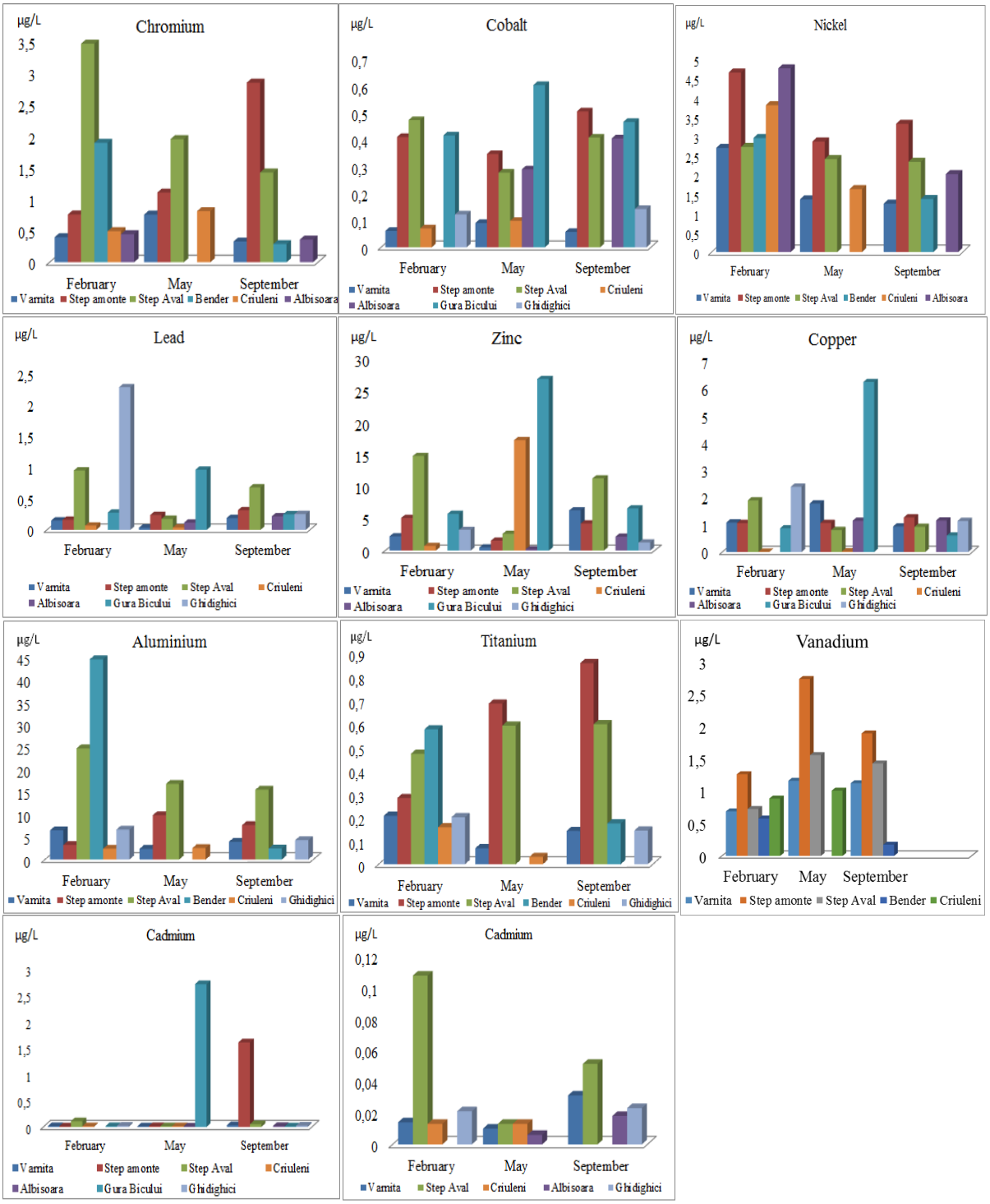


Fig.4.2. Heavy metals concentrations in surface wastes of Republic of Moldova

III.2.1. Seasonal campaigns of Dissolved Organic Carbon (DOC) analysis in the waters of the Dniester River and Bic

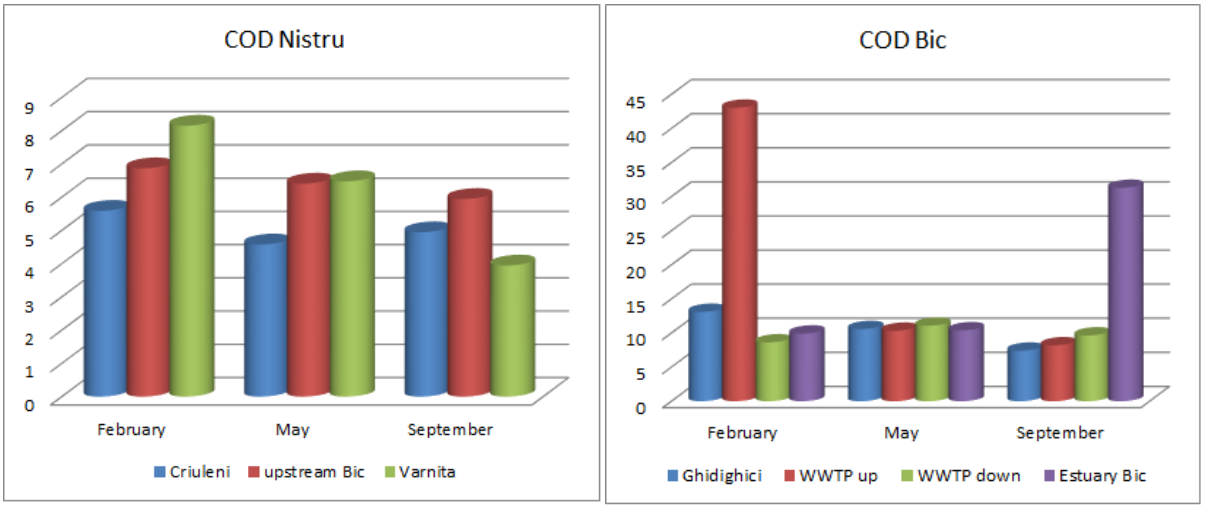


Fig.4.3. Dissolved Organic Carbon concentrations in water of rivers Bic and Dniester

Dissolved Organic Carbon (DOC) concentration in waters of the Dniester and Bic rivers describes a descending trend throughout the year 2015. Thus, the highest values in Dniester river could be observed in February 2015, the values that reach 8mg / L in Varnita sampling site with a minimum of 3,5mg / L reached in September in the same site. On the river Bic the most important DOC values were recorded in February 2015, upstream of the wastewater treatment plant Chisinau (42mg / L) and in September 2015 in the estuary of Bic river (30mg/L). The last concentration is due to the important amounts of MES (2,98mg / L) which was determined during this period.

IV.3. Blue-green algae growth

In our research concerning bioremediation of micro-pollutants by blue-green algae, several species of blue-green algae among which *Spirulina platensis*, *Nostoc gelatinosum* and *Cylindrospermum licheniforme* have been used. Each of these species is recovered from their culture media in the exponential growth phase and is added in pre-established mass in their recently prepared culture media. Cheeped in collection of all algae species was achieved in different environments specific to each particular species. All these culture mediums are exposed in Annex 1. *Spirulina platensis* was raised and experimented in the Zarrouk media. The blue-green algae *Nostoc gelatinosum* and *Cylindrospermum licheniforme* were cultivated

in the Nr.6 and Drew middle whose recipes are presented also in Annex 1. In order to obtain fresh and cheap algae biomass for our bioremediation experiences, growth of these species were performed in laboratory by apply the method of regular cultivation in wastewater nutrient media from the exit of treatment plant.

IV.3.1. Bioremediation of anionic micro-pollutants from WWTP in batch by blue-green *Nostoc gelatinosum* algae

The bioremediation of anionic macro-pollutants by blue-green *Nostoc gelatinosum* algae growing in regular growth conditions were performed in non-diluted wastewaters environments from output of WWTP Villeneuve d'Ascq (Fig.4.4.).

Fig.4.4. The regular cultivation method of *Nostoc gelatinosum* blue-green algae to undiluted wastewater from the exit of treatment plant in laboratory conditions.

IV.3.2. Prepare of the inoculums

In order to prepare the inoculums used in the cultivation of algae have met the following steps:

• Extraction of the algae inoculums is carried out from the same nutrient medium like the one that we want to work further.

• Extract the inoculums is obtained from the middle of the exponential growth phase culture
• The inoculums is separated from the nutrient medium by filtration or centrifugation;
• The inoculums is wash with distilled water followed by sterilization;
• Seeding with inoculums with a required optimal dose. Generally, the amount of inoculums algae is 0.4-0.5 g/L, calculated on absolutely dry biomass (ADB) - for the regular cultivation. The following cultivation conditions were kept during growth: Agitation - twice a day and temperature - 29.0±1.0°C and light intensity - 144W/m^2.

IV.3.3. Preparation of dishes

The experiments were carried out in 32 Erlenmeyer flasks carefully washed with detergent Decon Laboratories Limited (Conway Street, Hove Sussex BN3, 3LY) then rinsed with pure water and then immersed in a 0.5 M HNO_3 solution for 24 hours followed by rinsing with ultra-pure water. Drying using an oven at 105 °C for glass bottles intended for PAH analysis is to host a laminar flow for metals were realize. After drying the vials were sterilized by exposure to UV for 30 minutes.

IV.3.4. Organism, culture and growth conditions

In our experiments the blue-green algae *Nostoc gelatinosum (Schousb) Elenk* and *Cylindrospermum lichenoid (Bory) Kütz* were grown on a culture medium composed of wastewater collected at the exit of wastewater treatment plant Villeneuve d'Ascq, Department, Nord Pas de Calais (France). The medium used is composed from undiluted 100% wastewater. For its filtration was used the vacuum pump with cellulose acetate filters (for the major analysis and SSM) or glass filters (for PAH analysis) with a pore diameter of 0.45 micrometers. The secondary effluent previously undergoes sterilization by exposure to UV for 30 minutes.

After cooling, the sterile medium was weighed and inoculated with freshly harvested algae strains log phase corresponding to the sixth day of growth. Very important is the fact to avoid the stress of adapting to a new algal cell culture medium. This is the purpose for which our experience before the blue-green algae were grown in the same culture medium, the same concentrations and environmental conditions of the experimental medium. The experimental conditions during the 24-day growth cycle were: temperature - 21 °C ± 1.0 °C; periodic agitation - 3 times a day and 190W/m^2 continuous lighting natural pH. Experimental details of

the lots are described in Table 1. During the experiment, the samples contained 500 ml of the solution were partially covered with a sheet of perforated paper to reduce the evaporation factor. At predetermined time intervals (1, 3, 6, 9, 12, 15, 18 and 21 days) the samples taken for residual metal ions, anions and amounts of biomass in the solution was taken by filtering in a container using acetate cellulose filters with an electrically vacuum pump. The SSM of undiluted wastewater is 24.27 mg/L.

IV.3.5. Determination of Algal Productivity

Algal productivity or Biomass Absolute Dried (ADB) was determined using an analytical balance by wet filters method, following expression:

$$ADB = (B\text{-}A) \times X \times Y \text{ (g /L)}, \qquad (4.1.)$$

Where

A-weight filter washed with 5ml of bi-distilled water,

B- weight biomass filter and cooled to room temperature,

X-coefficient for the recalculation of algal suspension to 1 liter [11] and

Y-coefficients for the recalculation of dry algal suspension at 105C

In our experiment this relation may be expressed as follows:

$$ADB = (B\text{-}A \times 2) \times 0{,}036 \text{ (g /L)}, \qquad (4.2.)$$

Productivity evolution of *Nostoc gelatinosum* blue-green algae describes a curve as we can see in Fig.4.1

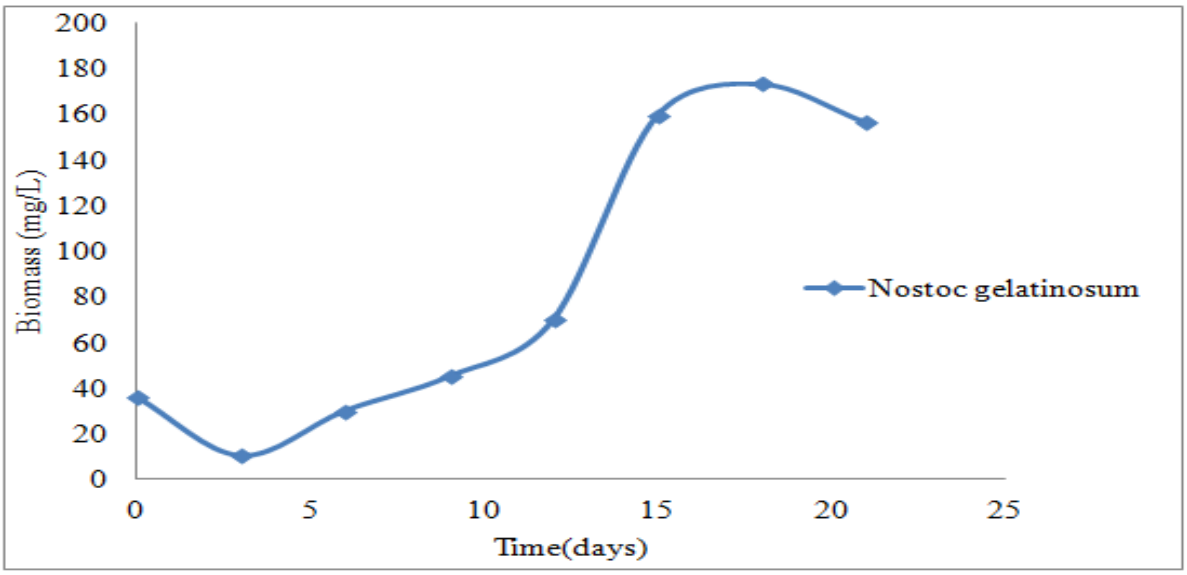


Fig.4.5. *Nostoc gelatinosum* blue-green algae productivity on the WWTP un-diluted medium

IV.3.6. Effects of pH on bio sorption of Macronutrients and ETM

pH is an important parameter affecting the bio sorption process. At low pH values the cell wall ligands were closely associated with the hydronium ions H_3O^+ and restricted the approach of metal cations as a result of the repulsive force. As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface [180]. Crist et al. in 1981 [181] also suggested that zero-point charge, or isoelectric point, would be found at pH 3.0 for the algal biomass. Above this algal cells would have a net negative charge. The ionic state of ligands will be such as to promote reaction with metal ions. Cadmium (II) uptake by immobilized *Spirulina platensis* is also a function of pH solution, as observed in the other similarly works [182].

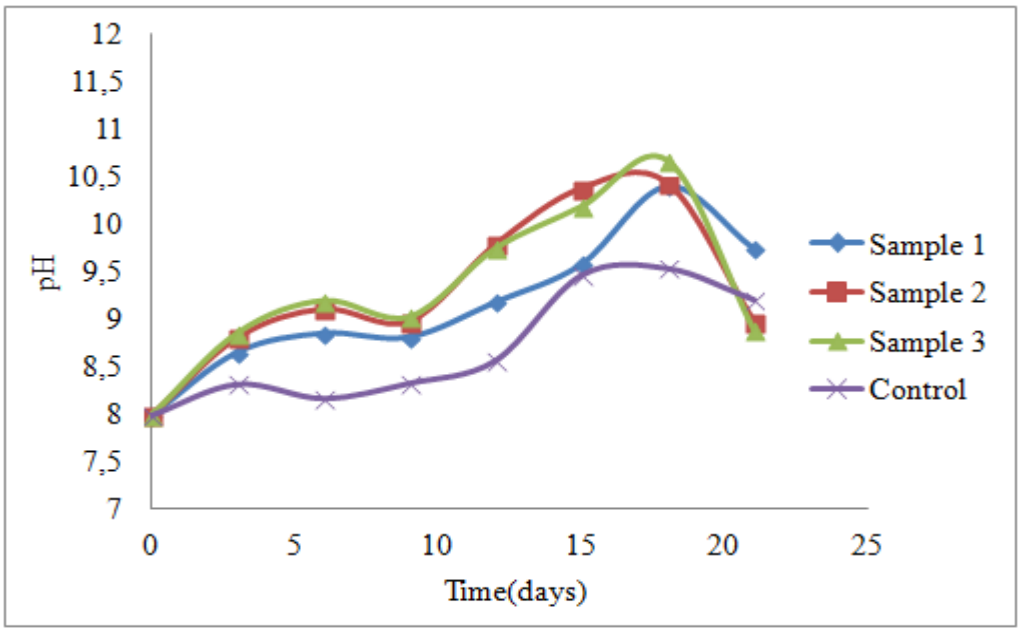


Fig.4.6. Evolution of pH in non-diluted wastewater growth media of *Nostoc gelatinosum* blue-green algae in batch

IV.3.7. Analytical methods of anions analysis

Dosage of anions such as chlorides, sulfates, nitrates and phosphates in analyzed medium were assayed by ionic chromatography. Statistical results were determined using Microsoft Office Excel 2010, by determination of the arithmetic mean (X) and standard error (x). In Fig.4.7, the correlation points of each anions concentration values are presented. The R^2 demonstrates a strong correlation of the four concentrations values of each analyzed nutrient.

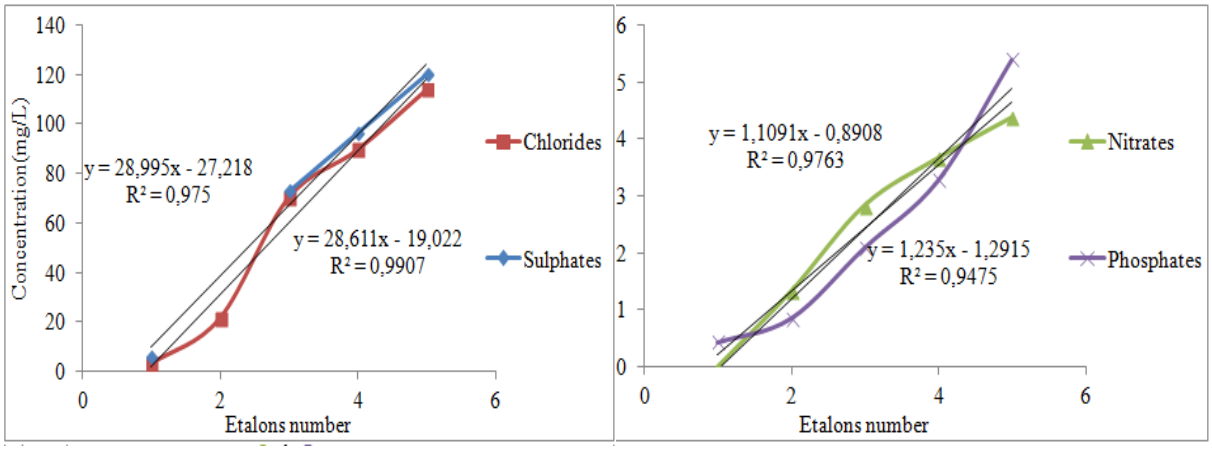


Fig.4.7. Linear regression for the experimental analyzed anions

IV.3.7.1. Sulfur

Sulfur is one of the elements necessary for growing algae. Entered in the composition of proteins, enzymes, peptides, amino acids containing sulfur, which is a component of many other organic compounds in algal cells. A portion of the sulfur bonds participating in redox reactions in the biosynthesis and metabolism of many substances. According Travieso et al.[59], blue-green algae need amounts of sulfur for conversion into sulfur amino acids essential. In nutrient medium, the sulfur is in the form of sulfate concentration which is not significantly reduced during the breeding of blue-green algae *Nostoc gelatinosum* compared to the control variant, decrease from 108.5 to 60, 56 mg/L for the experienced period (Fig.4.8.).

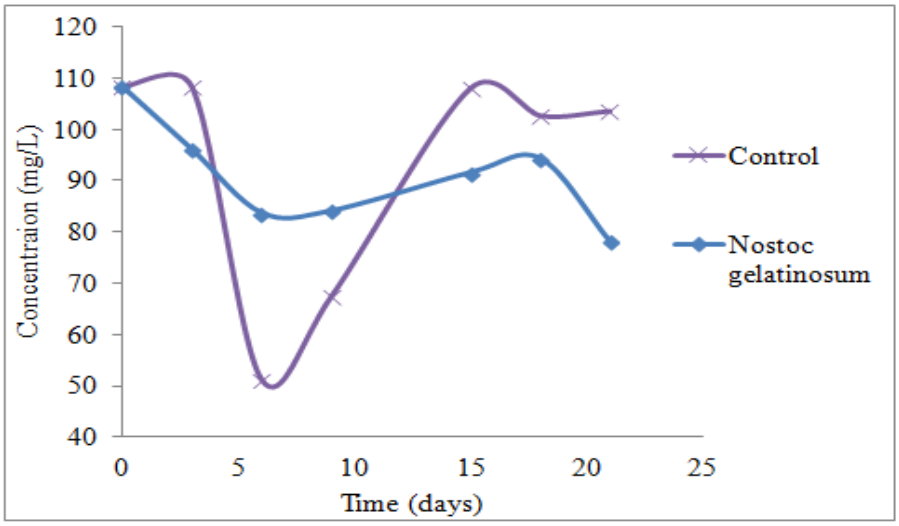


Fig.4.8. Evolution of sulfate consumption by the culture of blue-green algae *Nostoc gelatinosum* in non-diluted wastewater treatment plant Villeneuve d'Ascq, France

IV.3.7.2. Chlorides

As well as magnesium cations, chloride anions could replace, but less effectively, the magnesium ion effects, photo-reduction of $NADP^+$ in the dark or during the reaction of the appropriate light. The implications of the chloride anions in the photosystem I and II as electron donors are discussed by McSwain and all since 1976 [56]. The concentration of chlorine in the wastewater collected from treatment plant used as a nutrient environment varies with time and the presence of the algae in the solution. Thus, reducing of chloride concentrations from 120 mg/L at algal inoculums up to 65,5mg in the first 3 days can be observed in the samples with algae. Lower concentrations of chloride (31.6 mg/L in the 6th

day) can be detected in samples in the absence of algae. However, inflection point concentration curves indicate instability in these environments as well as in algal samples (Fig.4.9.). The concentrations of chlorides are buffered by algae in stable values.

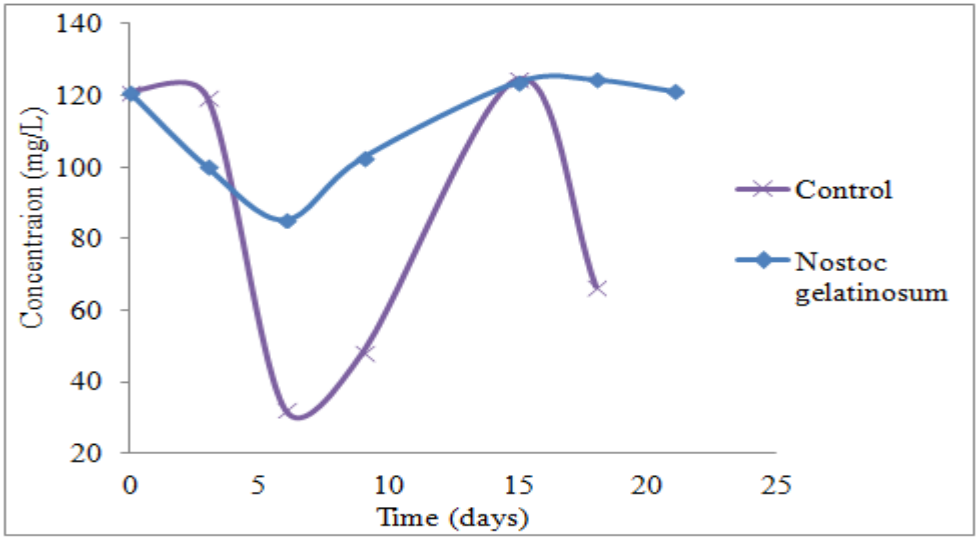


Fig.4.9. Evolution of consumption of chloride by the culture of blue-green algae *Nostoc gelatinosum* in non-diluted wastewater treatment plant Villeneuve d'Ascq.

IV.3.7.3. Nitrogen

Nitrogen is an element essential for the biosynthesis of protein substances, molecules constituting amino acids, nucleic acids, chlorophyll, alkaloids, amides, amines, etc. The maximum effect of nitrogen on the growth and development of algae is obtained if the optimal ratio of phosphorous and potassium is reached [183]. The use of wastewater can offset the cost of commercial fertilizers otherwise necessary for algae production, and wastewater treatment revenues can offset the cost of algae production. Nitrate concentrations in algal samples follow linear dynamics. The concentration was 3.7 mg/L on the day of inoculation, and totally reduces to the end of the experiment. In contrast, sample control keeps the same value (4.5 mg/L) during the last 2 weeks after a small increase in the 9th day of analysis from 3.7 mg/L to 4.5 mg/L (Fig.4.10.).

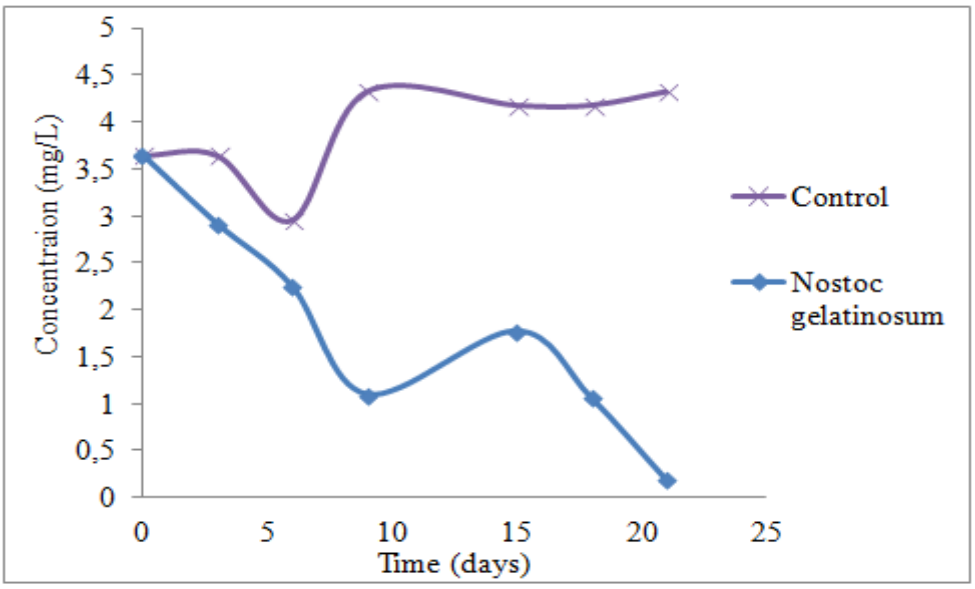


Fig.4.10. Evolution of nitrate consumption by the culture of blue-green algae *Nostoc gelatinosum* in non-diluted wastewater from Villeneuve d'Ascq, France treatment plant.

IV.3.7.4. Phosphorus

Phosphorus is also an essential macro nutrients for growth of microalgae [63]. Although cyanobacterial biomass does not require large amounts of phosphorus, because it contains less than 1% thereof. Phosphorus is an important factor limiting growth, especially in natural environments in which the phosphorus is limited [63] but the quantitative increase is harmful to ecosystems. This chemical element contributes to the formation of nucleic acids, co-enzymes (NAD^+ $NADP^+$, etc.) is composed of complex lipids, nucleotides, etc. Phosphorus has a favorable effect on the biosynthesis of chlorophylls, participate in phosphorylation process, biosynthesis and degradation of carbohydrates, protein substances of lipids. It is a nutrient that has a fundamental role in metabolic processes such as respiration and photosynthesis [183]. Nitrogen and phosphorus, therefore, are the two nutrients most concern in the analysis of a source of water for the growth of algae potential. To avoid the limitations of either, the molar ratio of water to feed ratio must match the stoichiometry of the algal biomass. This nitrogen/phosphorus ratio is often assumed to be the Redfield ratio of 16:1 [184].

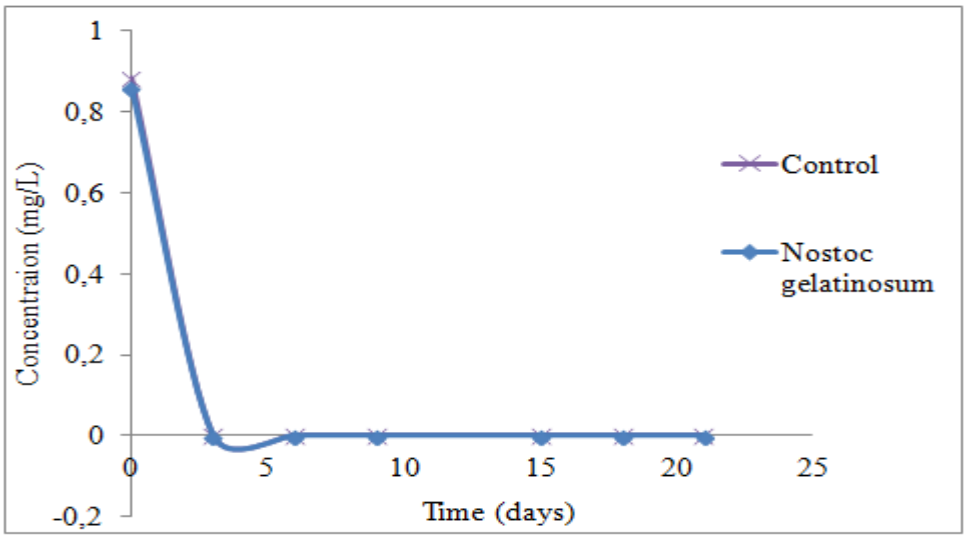


Fig.4.11. Evolution of consumption of phosphates by the culture of blue-green algae *Nostoc gelatinosum* in non-diluted wastewater treatment plant Villeneuve d'Ascq.

Initial concentration of orthophosphates in the culture media until the algae inoculation is within the limits 0.75 to 1.02 mg/L. But immediately they are consumed and one day after algae inoculation, there is no phosphate, the medium being a phosphorus deficiency (Fig.4.11.). However, this does not prevent increasing the productivity of algae. These results indicate that wastewater from the output of Villeneuve d'Ascq treatment plant can be used as a culture medium for blue-green algae. The nutrients that are present, leads to the growth of algal cells, this being well reduced.

IV.3.7.5. Studies of biosorption of cadmium (II) in batch

The experiments were conducted in Erlenmeyer flasks Nalgene 125 ml, each containing 100 ml of water solutions taken from the French basin of the Scheldt River (Fig.4.12.). The range of concentrations of cadmium solutions prepared (II) lies within the limits 25, 50 and 75 µg/L. Six samples were prepared for the experiment: three corresponding to 25, 50 and 75µg/L cadmium (II) in continental water samples containing immobilized biomass *Nostoc gelatinosum* in alginate beads. We noted Sample 1, Sample 2 and Sample 3 and the three blanks with the same concentrations range containing alginate beads without algae, denoted Control 1, Control 2 and Control 3 (Fig.4.12.).

Fig.4.12. Biosorption study of cadmium in a batch system

The initial pH of the solution was maintained to values naturally present in the environment. At the time of contamination with cadmium, bottles with aqueous solutions were stirred at 200 rpm, constant stirring speed for 2 hours to ensure that equilibrium is reached. After stirring, the first 5 ml sample (at t = 0 time) were taken, and a mixture of 0.5 g bio-sorbent costs is provided to already prepared solutions. At predetermined intervals of time (10min, 20min, 40min, 1h, 1h30, 2h, 3h, 6h, 9h and 24h) sampling to determine the residual concentration of metal ions in the solution are realized. pH measurements are made at time intervals of 0; 0.5 h; 1h; 1.5 h; 2 h; 3h; 6h; 9h and 24h.

IV.4. Bioremediation of Anionic macro-pollutants by algae in free and immobilized state in regular growth conditions

IV.4.1. Bioremediation of macro elements (anions) by the blue-green algae *Spirulina platensis* in regular grown in diluted wastewaters from livestock complex

IV.4.1.1. Preparation of media with the addition of waste water used in the cultivation of *Spirulina platensis*

For the preparation of media with the addition of wastewater, waste from livestock complex of sheep and poultry, collected directly from the stable channel or storage platforms was used. In the laboratory we have weigh 1kg of waste, and then we added 5 liters of tap water and leave stirring at 25 ° C for a period of 5 days under special air filtrations systems. Every 4-5 hours a manually shaking with a total 15 minutes per day is carried out. After 5 days, the supernatant sneak through 2-3 layers of cheesecloth to remove solid particles maximal possible. Prepared in such a manner, the liquid waste obtained from the sheep's, were used in experiments in different concentrations. The concentrations tested were obtained by diluting the liquid with distilled water at a rate of 5%; 10%; 15% and 20%.

IV.4.1.2. Regular grown of *Spirulina platensis* on media with the addition of livestock (from sheep's) wastewaters

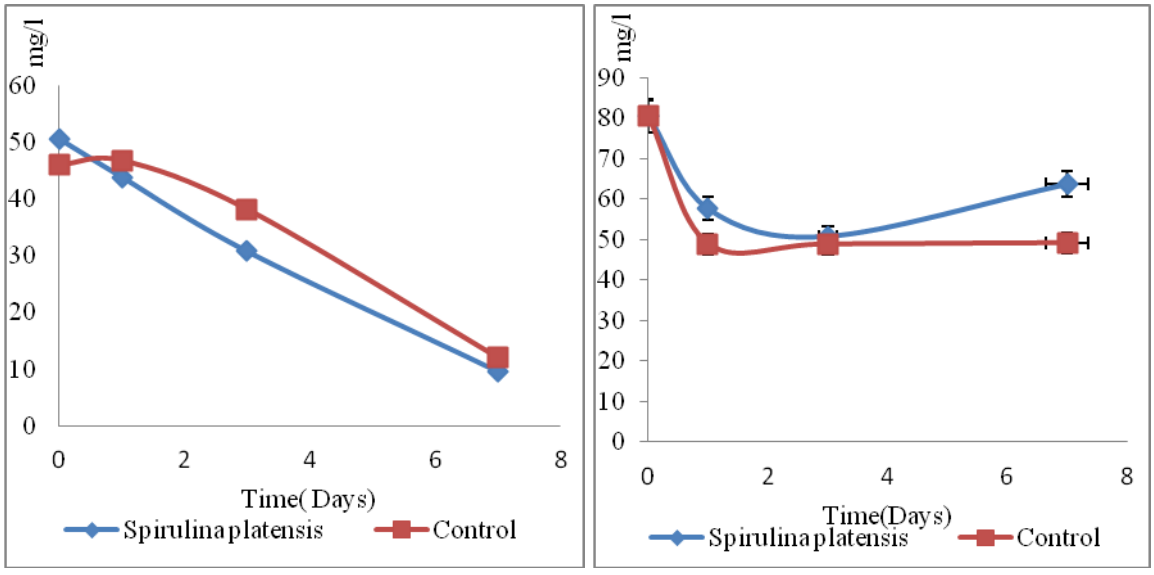
Regular grown of *Spirulina platensis* was performed on media prepared the way above mentioned, based on the content of nitrogen and phosphorus as elements of basic feed algae. Initial experiments were held in glass and plastic volume of 5 liters, the air temperature was 30 ± 1 ºC and continuous illumination-18 to 24 thousands erg/cm^2. The results of biomass cultivation of *Spirulina platensis* as well as the its use as a food additive in the form of extract, to increase the quality of physiological indices livestock on the example of domestic rabbits *Oryctolagus cuniculus* was being exposed [185].

IV.4.1.3. Regular grown of *Spirulina platensis* on media with the addition of livestock (pony) wastewaters

Regular grown of *Spirulina platensis* on media with the addition of livestock (from pony) wastewaters was performed on media prepared the way above mentioned, based on the

content of nitrogen and phosphorus as elements of basic feed algae. Initial experiments were held in glass and plastic volume of 5 L, the air temperature was 30 ± 1 ºC and continuous illumination-18 to 24 thousands erg/cm^2. The concentrations tested were obtained by diluting the liquid with double-distilled water at a rate of 2% and 5% and lasted for one week at environmental concentration of pH = 8.00-8.42 and 0.04 g/L of absolutely dry mass of fresh *Spirulina platensis* strain. As our goal was to track the dynamics of concentrations of anions especially N and P we will present you below our results. In order to establish the role of *Spirulina platensis* species in wastewaters treatment of waters with the addition of livestock (from pony) we analyzed some physico-chemical parameters of water from the cultivation of this species.

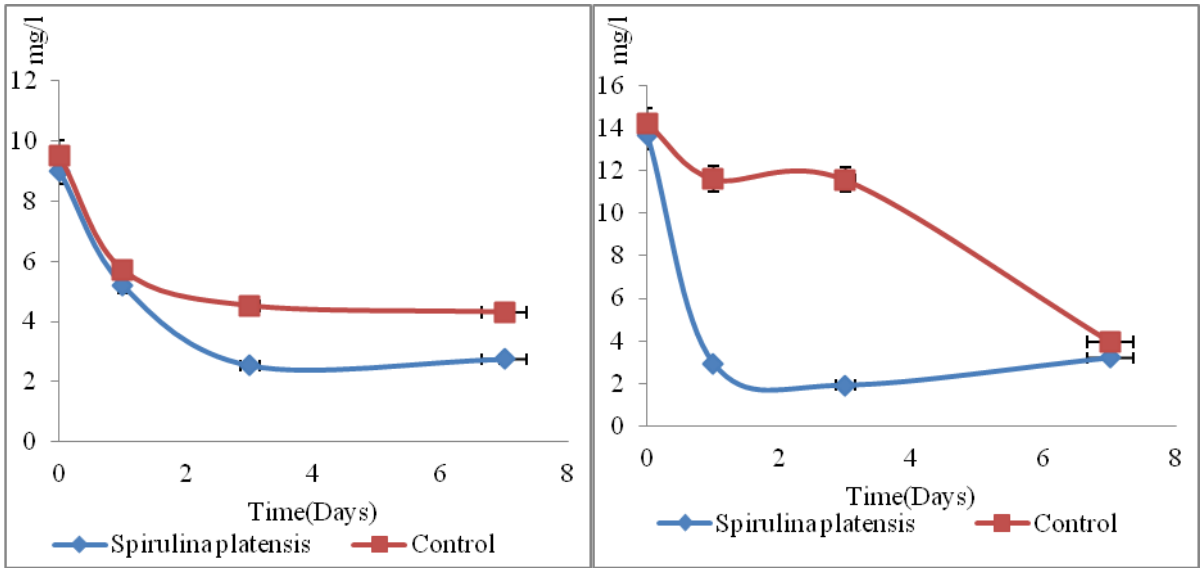
Evolution of chlorine ions consumption depends on the initial concentration of these ions and chlorine in wastewater and intensity of algal biomass growth. Thus, this anion concentration did not differ essentially in the algae sample compared to the control sample. The Fig.4.13 shows that in the lots of algae exposed to 2% of wastewater from livestock, the amount of chloride ion decreased almost similar in both sample inoculated, and in the control, which represents a decrease of 80.87 and 73.70% respectively compared to the initial sample. In version 5% of livestock wastewater this reduction is much smaller and it represented only 36.87% in the sample with algae and 39.98% in the control sample, at the 3-day of experience. A slight increase in the chlorine anion concentration values up to 63.82 mg/L noted towards the end of our experience, evidence that algae is not in the best physiological state due to high concentrations of biogenic substances present in the wastewater solution.



A B

Fig. 4.13. Evolution of Cl^- ion concentration in media with the addition of livestock (pony) wastewaters under the influence of algae *Spirulina platensis* (A - 2%, B - 5% diluted wastewater)

Evolution of nitrate ion consumptions (Fig.4.14.) shows a trend almost identical for the 2 concentrations of wastewater with high nitrate consumption during the 7 days of experience. The intensity of algal biomass growth lowers the concentration of biogenic elements in the algae sample with 69.5% and with 54.8% in control sample during the 7 days in the 2% wastewater sample analysis. The Fig.4.14 shows that in lots of algae exposed to 5% of wastewater from livestock, the nitrate concentrations decreased drastically from first day of experience with 78.56% and 86% in the next day, touching on the seventh day the values similar to those of the control sample located around 3.20 to 3.94 mg/L.

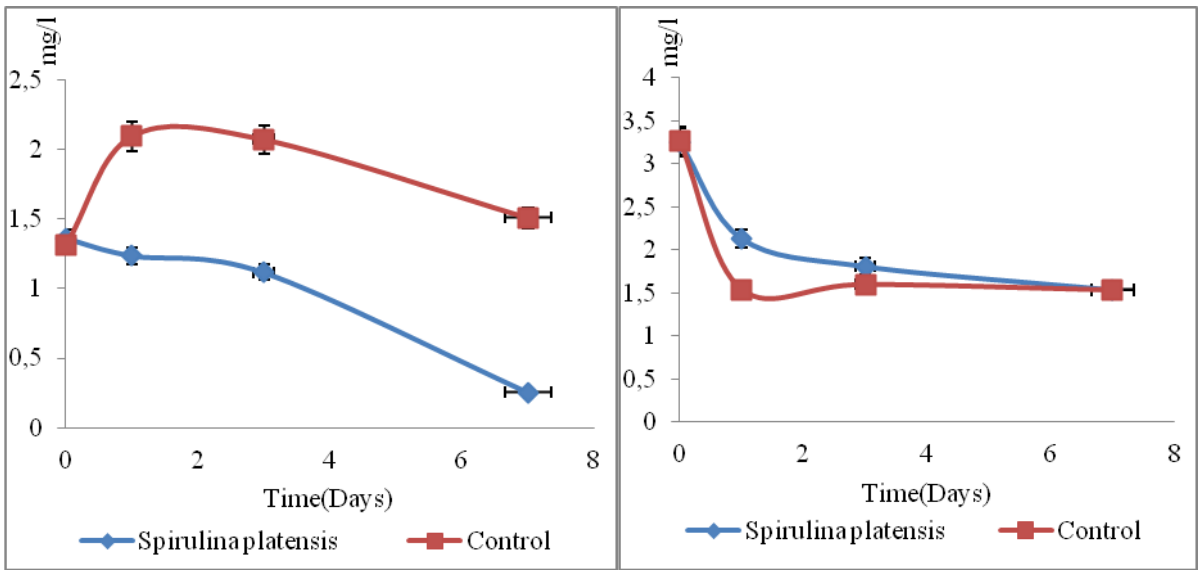


A B

Fig.4.14. Evolution of $NO3^-$ ion concentration in media with the addition of livestock (pony) wastewaters under the influence of algae *Spirulina platensis* (A - 2%, B - 5% diluted wastewater)

The same approximate values of 2.74 and 4.29 mg/L of nitrate in sample 2% wastewater we have in the 7th day of analysis, which suggests that these concentrations are limited consumption of algae in our wastewater system media with the addition of livestock. The best nitrate reduction is observed in the 3rd day of analysis for both 2% and 5% sample wastewater concentration treated with blue-green *Spirulina platensis* algae.

Phosphate ions consumption trends describe a different dynamic in the 2 different concentrations of wastewater whose initial concentrations are 1.30 mg/L in sample 2% and 3.25mg/L in 5% wastewater sample. High concentrations of this nutrient relative to the amount of algal inoculums in wastewater samples during the seventh days' experiment did not result in the entire reducing of phosphate contents as we had expected. That fact is logical in heavily polluted environments. Yet even in such conditions phosphate ion concentration was significantly reduced by about 81.2% in the algae sample in 7th day experience for sample of 2% wastewater compared to the control sample where these values, as we can see from in Fig. 4, on the contrary, grow. The fig.4.15 (B) shows the dynamics of phosphate consumption during the 7th days experience in the 5% wastewater sample with algae, follows a similarly trend to that of the Control sample, reaching the same values of 1.53 mg/L at the end of the examined period.



A B

Fig.4.15. Evolution of PO_4^{3-} ion concentration from media with the addition of livestock (pony) wastewaters treated by blue-green *Spirulina platensis algae* (A - 2%, B - 5% diluted wastewater)

In this connection we can conclude that the most effective consumption of phosphate anion is performed in 2% of wastewater of livestock wastewater sample inoculated with 0.04g (ADB) of *Spirulina platensis* in fresh.

IV.4.2. Bioremediation of macro elements (anions) by the xanthophylls algae *Tribonema viride in* regular grown in the filtered non-dilute wastewater from entering of Chisinau, Republic of Moldova treatment plant

Investigations of such researchers [124] in algae growth on culture media from wastewater plant exit diluted with distilled water in proportions starting from 1 up to with 50% clearly demonstrates that algae and most specifically macrophytes, such *Tribonema viride* grow well in environments with high concentrations of wastewater increasing the productivity and consuming large amounts of biogenic substances from the proposed environment. *Tribonema viride Pasch* is a species that belongs of Xanthophyta phylum, Xanthophyceae class, Tribonematales order, Tribonemataceae family and Tribonema genus. It represented by harsh filaments, assembled as cotton fibers, usually united, only some periods they come off and they are solitary. The cells are cylindrical with a length of 2-8 times higher that width or as form of keg, and width of 10-15 μm (Fig.4.16.).

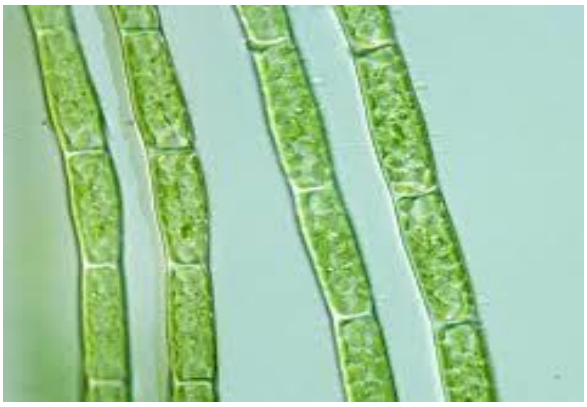


Fig.4.16. *Tribonema viride Pasch*

The cell membrane is well defined and has shape H form; it has 2 to 4 chromophores to a larger, discoid or uneven. The cell can contain oil droplets, leicosyne and crystals. Multiplication takes place by means of zoospors, aplanospors and achinetes and by direct division. It is the most widespread species of the genus Tribonema and is found in rivers, lakes and wastewaters. This species is involved in the experiences of wastewater treatment from the fish nursery, developing intensively in these purification plants installations [186]. We have decided to move forward and grow this strain of Xantophita algae on higher raw polluted water concentrations even from the entering in the treatment plant. For mechanical removal of waste, polluted water was passed through sieves with the size of 6mm followed by filtration through 3 layers of sand collected from quarry sand of Pitusca village in Calarasi

district. Unfortunately the lack of size analysis equipment and adsorbent surface chemical characteristics of sand that has not been made, many issues in this regard being able to be found in the work of Photo [187]. The fact is that after filtering through the filter sand, *Tribonema viride* algae has not only adapted to the non-dilute wastewater concentrations from treatment plant but has maintained its normal physiological form, continuing to develop at a rapid pace.

Regular grown of a xanthophylls algae *Tribonema viride* made both on wastewater from sewage treatment station as well as the synthetic culture media was made by methodology exposed in Dobrojan [12] and Dontu [124] in their PhD thesis. At inoculation of 2g/L of *Tribonema viride* algae on a wastewater alga from entering on the wastewater treatment plant Chisinau, we obtained the following dynamic productivity exposed in terms of Absolutely Dried Biomass (ADB). The wastewater has filtered through triple sand filter and treated with some tablets of activated carbon to obtain a transparent solution.

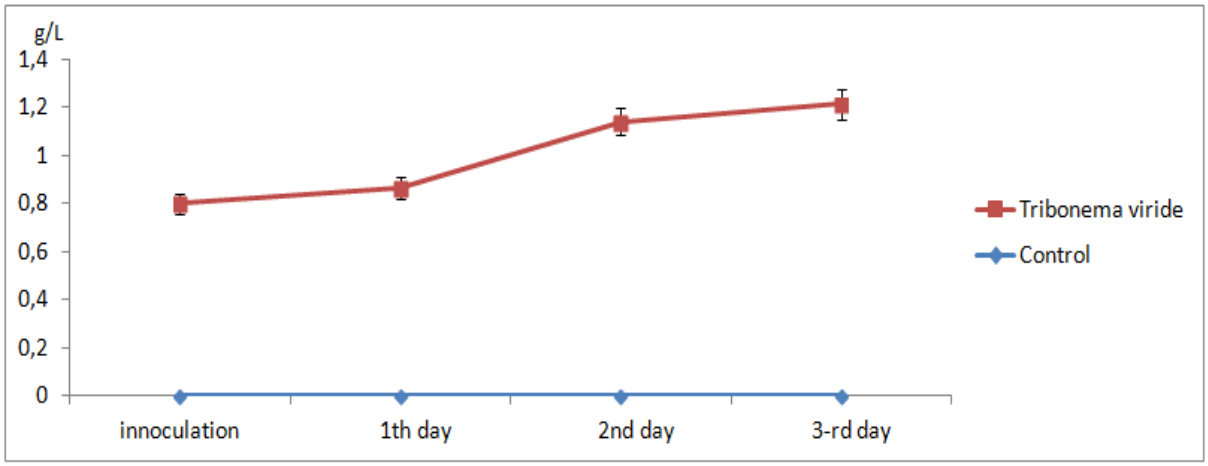


Fig.4.17. Evolution of ADB of *Tribonema viride* over 3 days of cultivation in wastewater culture medium from Chisinau (Republic of Moldova) wastewater treatment plant or poultry complexes

Thus we can see in Fig.4.17, that despite the low temperature recorded during this period of the year (mid-January with 15°C even inside the laboratory room), and high concentrations of nutrients (because the water has not been diluted, for economic reasons), *Tribonema viride* algae continue to develop intensely, touching in 3 days' worth 1.5 times the baseline algae inoculated. The main necessary ions for the growth and development of algae are ammonium ions, nitrate, nitrite, phosphate and carbonate. Because nitrification and denitrification changes due by bacteria in wastewater, between the ammonium and nitrate ions

amounts an inverse relationship is established. When NH_4^+ ion concentration increases, the nitrate ions decreases and vice versa.

The nitrate ion was analyzed because it is a necessary nutrient for algae growth but also a major pollutant of surface waters. Initial amount of nitrate (0.998 ± 0.087 mg/L) describes a constant decrease in algae sample *Tribonema viride*. Its concentration values reduce with 77.6% after the first day of experience up to 0.34 mg/L in the 2nd day of experience. As can be seen from the data of the Fig 4.18. valoriel substantially increase of the control sample, that practically doubled concentration in 2nd day of experience, or an increase of 92.2% in the day, and is maintaining its elevated in the 3-day experience in the control sample. In all experimental samples content of nitrate ion was reduced to almost zero in 2nd day of culture (values 0.220 ± 0.044 mg/L) maintains the values of 0.242 ± 0.044 mg/L and 3rd day of cultivation, unlike witness variants where high ion concentrations are maintained practically doubled from baseline by 46.2 and 92.5% after 2 days of algae cultivation. It is not excluded that for long periods of time nitrate ion concentration values begin to rise, because the algal biomass due to decomposition, but our goal was to expurgate in as short time even in highs nitrates concentrations from the imputs of municipal treatment plant.

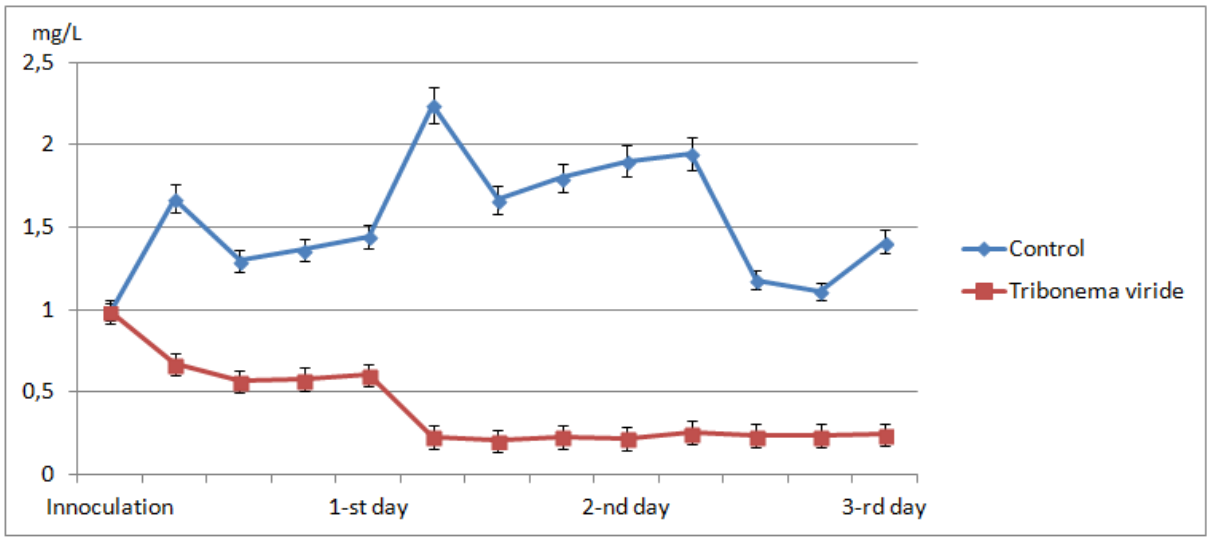


Fig.4.18. Evolution of NO_3^- in non-diluted wastewater medium from wastewater treatment plant treated by *Tribonema viride.*

The evolution of ammonium ion concentration was observed continuing decline of its values in all experimental variations and fluctuations evident in the control sample (Fig.4.19.).

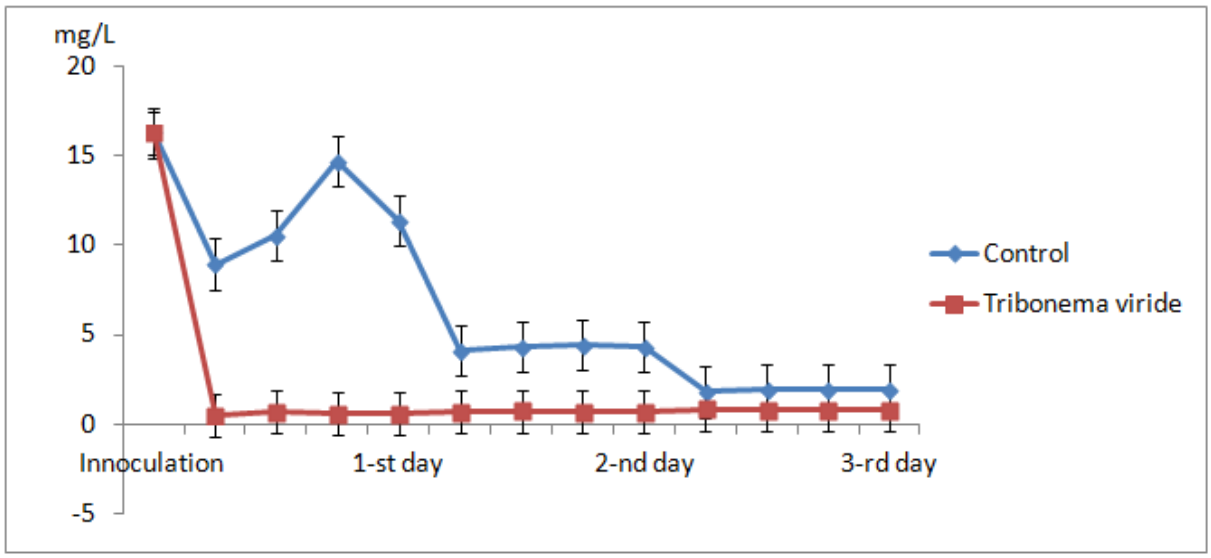


Fig.4.19. Evolution of $NH4^+$ ion concentration in non-diluted wastewater medium from wastewater treatment plant treated by *Tribonema viride*

Even after the first day of experience the amount of ammonium ion reduces completely in variants with algae inoculated, while in the blank reduction is observed only in the 3rd day experience. Decrease of the amount of ammonium ions depends directly on increasing of the amount of algal biomass represented in Fig.4.5. Initial concentration of phosphate ions in samples of wastewater from Chisinau wastewater treatment plant filtered through sand filters was 0.630 ± 0.049 mg/L. Following inoculation of 2 g/L biomass alive of *Tribonema viride* or 0.8 g/L absolutely dry biomass (ADB) of same algae species, the concentrations of phosphate ions are reduced up to 0.301±0.137 mg/L on the first day of exposure and to 0.043±0.056 mg/L in the second day of experience which represents a decrease of 91% (Fig.4.20).

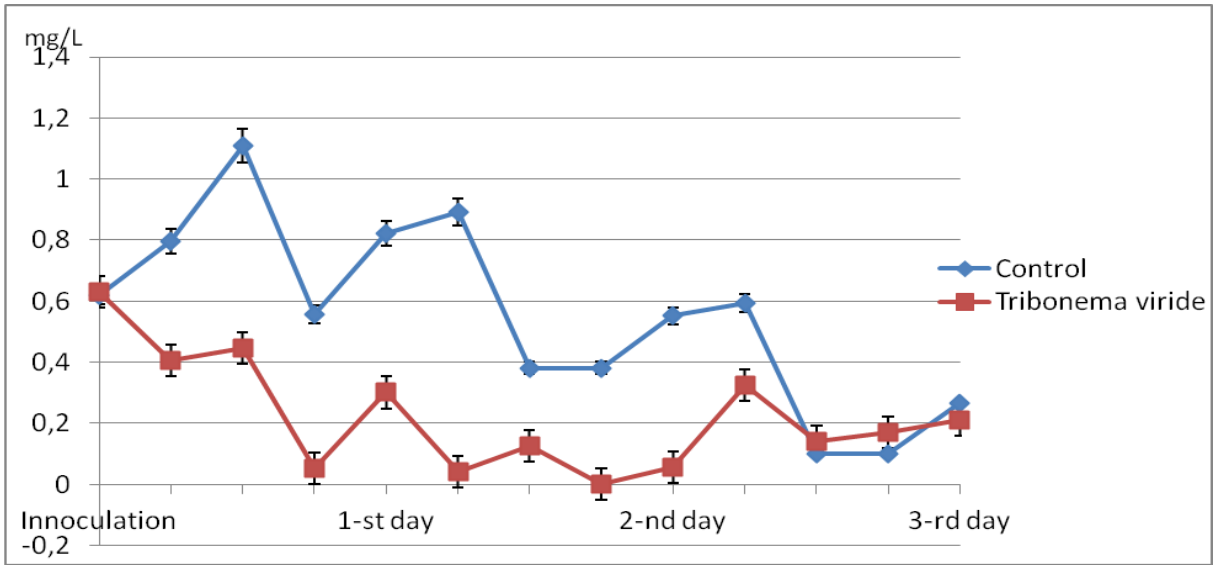


Fig.4.20. Evolution of PO_4^{3-} ion concentration in non-diluted wastewater medium from Chisinau wastewater treatment plant treated by *Tribonema viride*.

In the 3rd experimental day an equilibration of phosphorus concentrations in algae samples (0.212 ± 0.008 mg/L) and the control (0.266 ± 0.008 mg/L), takes places, which is absolutely normal taking into account the fact that phosphates are rapidly consumed in the water samples, being a limiting factor for growth. However, in algae test the consumption went very quickly reduce phosphates in 2-day of experience which is a good result for our experience (Fig.4.20.).

Fixed residue (TDS) represents all of dissolved substances in water (mg/L) and their characterization is an important indicator of the state of water mineralization. When treating wastewater from wastewater treatment plant with macrophyts algae such as *Tribonema viride* (Fig.4.21), the significant differences in the exchange of mineral water sample compared to the control sample, because the algae is that witch consume and includes the mineral salts in their biomass. Thus the highest consumption of mineral water was done in 2nd day, or 20% of the residue corresponding perfectly with the biggest increase in algal biomass in this period (Fig.4.21), demonstrating that algae consume large amounts of minerals. Since algal inoculums was recovered during their exponential growth phase from the same type of liquid medium the lag phase was only one day.

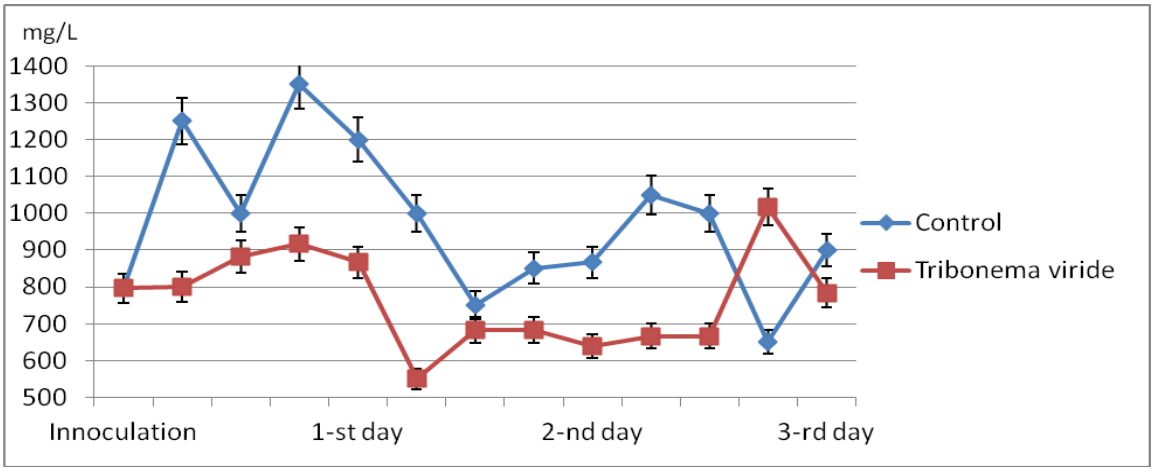


Fig.4.21. Evolution of TDS in non-diluted wastewater medium from wastewater treatment plant treated by *Tribonema viride*

Starting with the 2nd day, the exponential growth phase of our algae is put in place. Contrary, the control sample, recorded significant fluctuations with increases up to 50% of mineralization, tending to normalize around the initials values.

The amount of ions (HCO_3^-) in examined wastewaters during the 3rd days of experience was around 561±78 mg/L at inoculation. A decreasing plateau tendency we have in the algae sample, reaching 260±78 mg/L on the first day (Fig.4.22) and continuing to 105 ± 14 mg/L in the 3rd day. On the contrary in the control sample, hydrogen carbonate's concentrations describe decrease oscillations of only 13.04 % compared to the initial sample. This demonstrates that algae *Tribonema viride* consume HCO_3^- ions, produced in the result of decomposition of organic compounds in water, by reintegrate them into their biomass content.

We can conclude that in a very short time of only three days we have very good results of cleansing nutrients from wastewater of inputs of wastewater treatment plant Chisinau, following the initial chemical composition: NH_4^+ -16.29±0.10 mg/L; NO_3^- -0.998±0.087 mg/L; PO_4^3 -0.63±0.15 mg/L and HCO_3^- -561±78 mg/L. The period of 3 days is optimal for treatment of the medium with the algae xanthophylls. Role of species *Tribonema viride* in the process of wastewater treatment is estimated to reduce total quantity of ions analyzed within 2 days of exposure in the environment, increase the amount of biomass in this period leads us to conclude that these ions were being assimilated by algae.

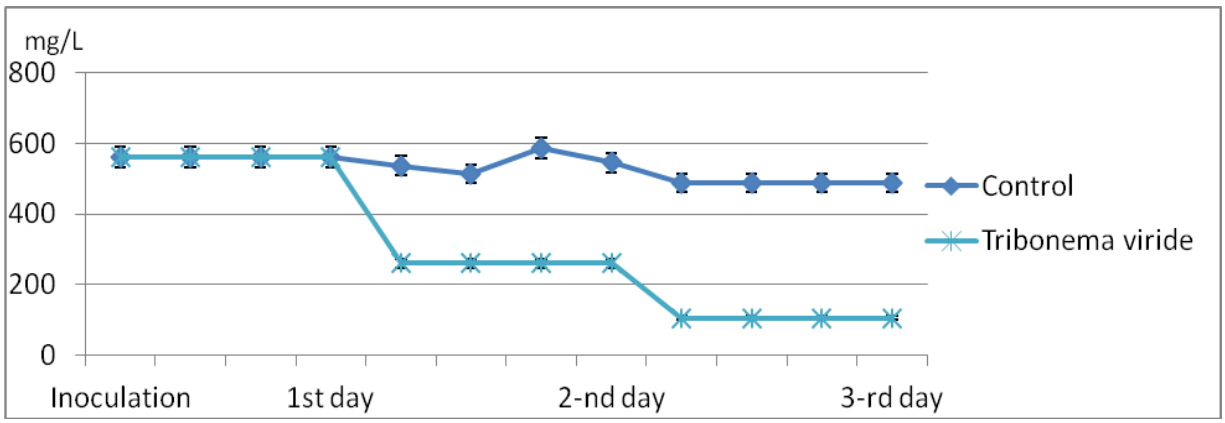


Fig.4.22. Evolution of HCO_3^- ions in non-diluted wastewater medium from wastewater treatment plant treated by *Tribonema viride*

IV.5. Bioremediation of Organic micro-pollutants by blue-green algae in free and immobilized state

IV.5.1. Bioremediation of pharmaceutical products (medicaments and hormones) by blue-green algae *Spirulina platensis*

For bioremediation feasibility study by *Spirulina platensis*, a range of calibration at various concentrations is carried out using mixtures of commercial standards and spiked with internal standards. The calibration allows having the linear regression equation as follows:

$$\frac{Ax}{Ai} = a * \frac{Cx}{Ci} + b \qquad (4.3.)$$

Where:

Ax = The peak area of the compound x,

Ai = Area of the internal standard peak,

Cx = known concentration,

Ci = internal standard concentration.

An example of the calibration curve is shown in the following Fig 4.23.

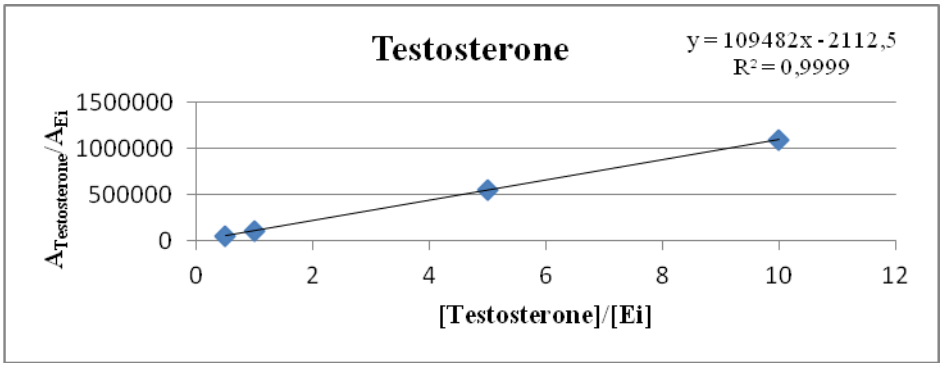


Fig 4.23. Testosterone calibration curve

The molecules of interest regarding disposal feasibility study by algae: (i) **Bisphenols**: Nonylphenol, bisphenol F, bisphenol A, bisphenol A, bisphenol C and bisphenol G. (ii) **Drug residues**: Estriol, α-Estradiol, Testosterone, Estrone and Progesterone. The calibration curve is performed for each molecule and the equations are presented in the following table with their correlation coefficients R^2.

Table 4.3. Equations of the calibration line for a few molecules of interest with their studied R^2 correlation coefficients.

Molecules of interest	Equations	R^2
4-Nonylphenol	647110x-10335	0,998
Bisphenol F	457460x-21738	0.997
Bisphenol A	362600x+13187	0.999
Bisphenol G	189758x+55581	0.985
Estriol	15996x-1286.4	0.999
α-Estradiol	326318x+40135	0.998
Estrone	70022x+15637	0.995
Testosterone	109482x-2112.5	0.999
Progesterone	422195x-364860	0.775

For each sample, based on the calibration lines presented above, then determines the concentration of each molecule of interest.

This study was planned for a 24 days' period, but it was suspended at the 21st day having regard to the complete degradation of micropollutants considered in all samples. Since the third day the elimination of different molecules of interest whether by free or immobilized

Spirulina platensis that was remarkable mostly for Estrone, Bisphenol G and Progesterone.

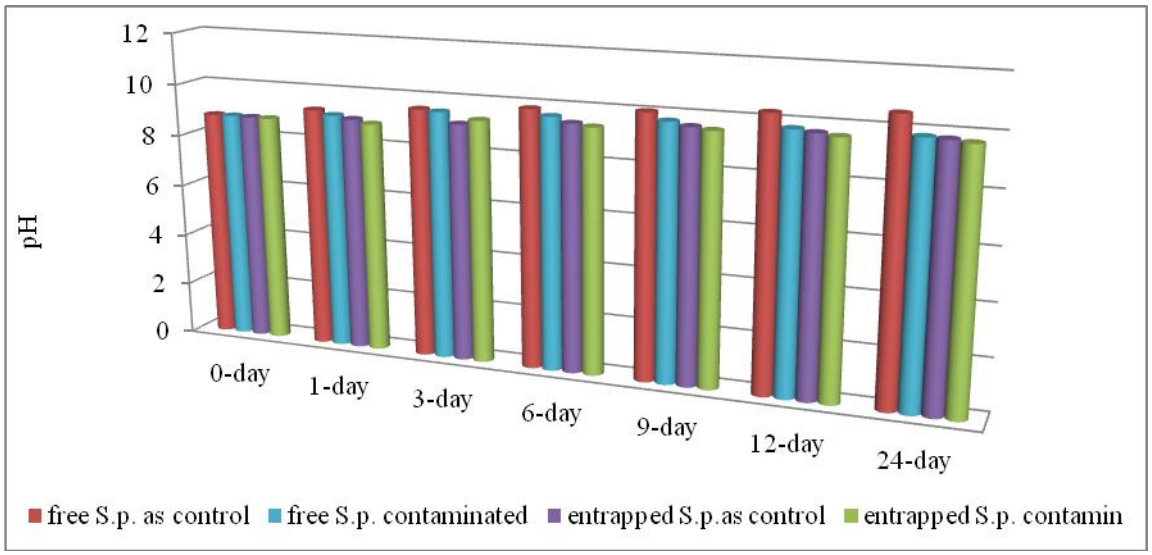


Fig.4.24. pH of solutions in bioremediation studies of pharmaceutical products (medicaments and hormones) by blue-green algae *Spirulina platensis*

According to the different results obtained for removing feasibility study of micro-pollutants by *Spirulina platensis* in water, it was noted that the performance of the immobilized Spirulina platensis in Na-alginate is similar to the free blue-green algae *Spirulina platensis* strains. However, from the 8th day, the immobilized in Na-alginat *Spirulina platensis* beads begins to degrade due to contaminated environments which lead us to believe that the free *Spirulina platensis* is more potential.

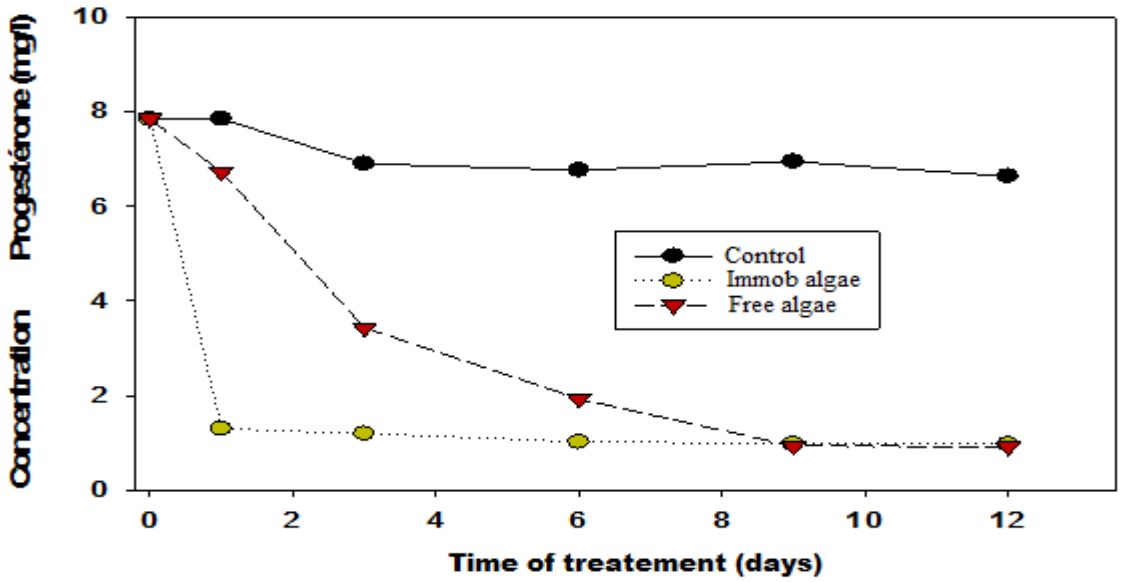


Fig.4.25. Progesterone elimination by free and immobilized in Na-alginate blue-green algae *Spirulina platensis* strain in Zarrouk medium

Figure 4.25 shows the decay level trends Progesterone by free and immobilized *Spirulina platensis* strain in Zarrouk medium. The degradation of progesterone is very fast with immobilized Spirulina; more than 85% of Progesterone in the water is removed in a single day (Fig.4.25.). In the presence of free *Spirulina platensis*, Progesterone in water is eliminated more gradually. More than 50% removal was obtained after 3 days and 80% removal was obtained after 7 days of exposure. The results reveal the great potential of *Spirulina platensis* to eliminate hormones surface water. Furthermore, and in addition to their bioremediation potential of water contaminated with drug residues, *Spirulina platensis* strain reveals its potential power bioremediation of polluted environments by bisphenols.

The Fig. 4.26 (D) illustrates the removal of Bisphenol G by *Spirulina platensis*, it is found that the degradation is very fast, in one day more than 80% of bisphenol G being eliminated. Additionally, and on the third day, all of the contaminant is degraded.

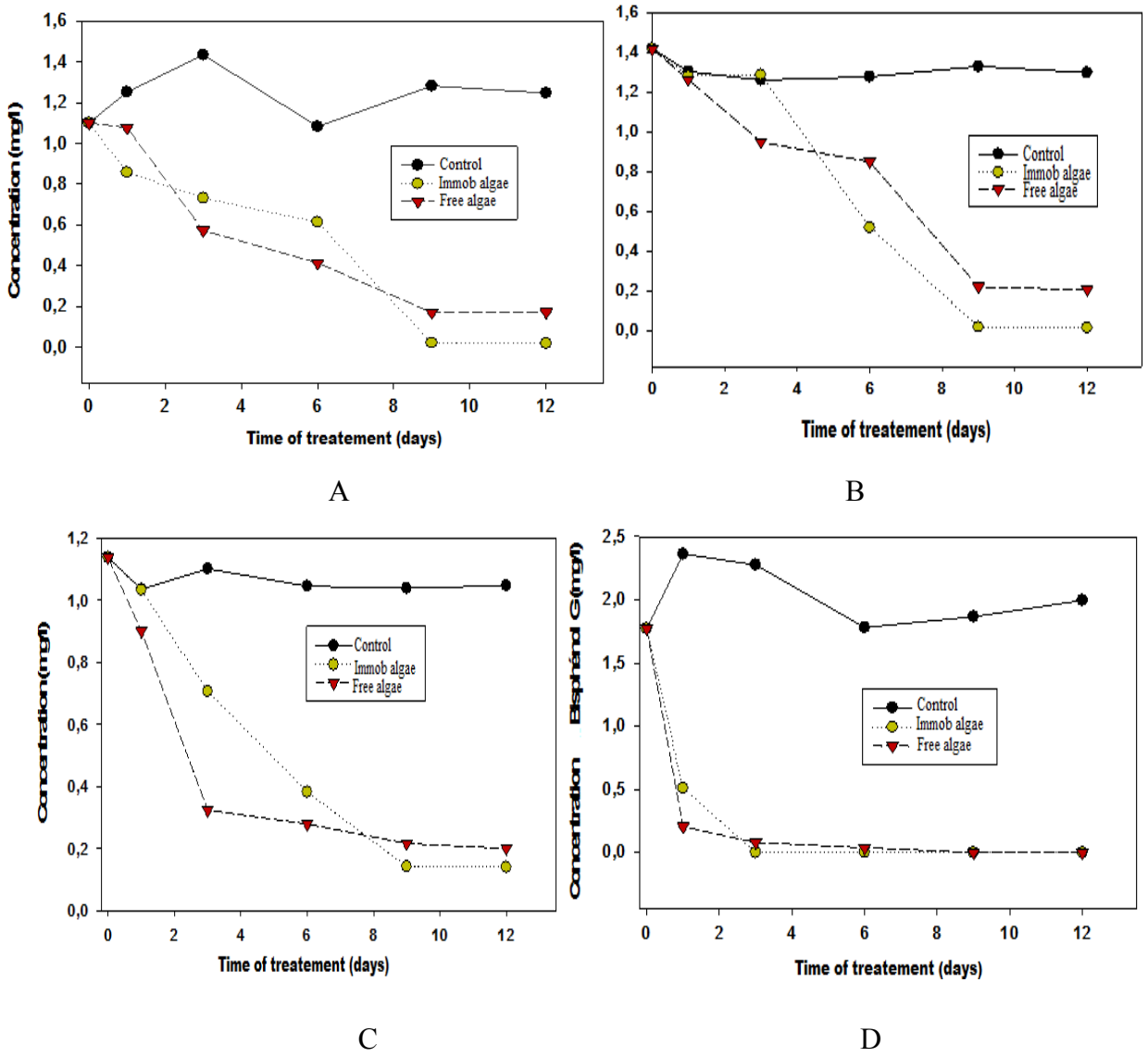


Fig.4.26. Removal of Bisphenol A (A), Bisphenol E (B), Bisphenol F(C) and Bisphenol G (D) by free and immobilized in Na-alginate blue-green algae *Spirulina platensis* strain in Zarrouk medium

The same Fig. 4.26 (A, B and C) shows a linear degradation of bisphenol A, bisphenol E and bisphenol F to the 9th day in Zarrouk medium with free and immobilized *Spirulina platensis* in of the samples values descending up to 80% in all 3 samples.

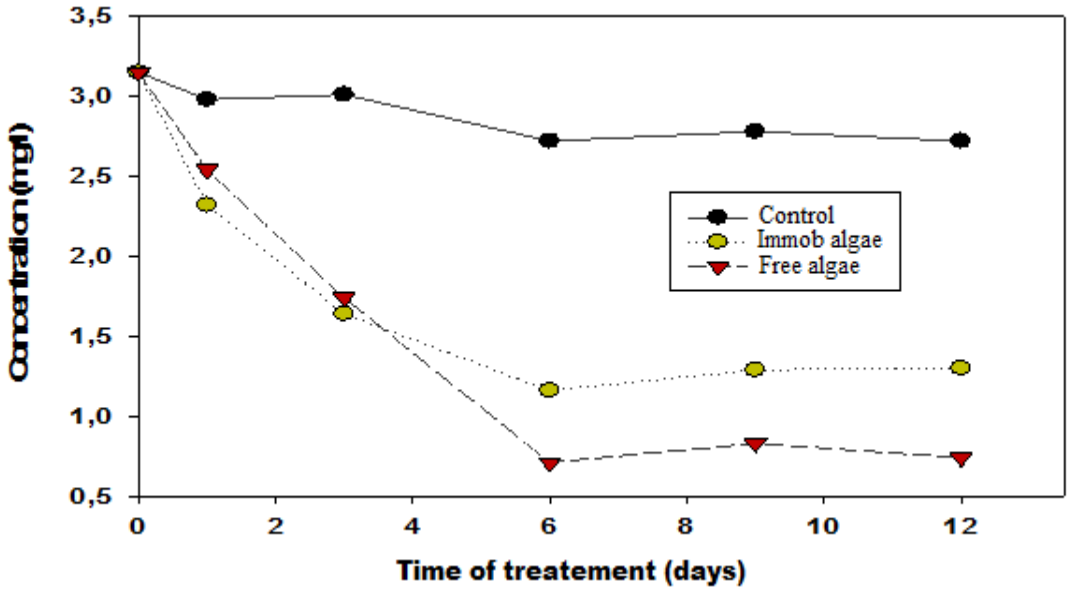


Fig.4.27. Removal of Estriol by free and immobilized in Na-alginate blue-green algae *Spirulina platensis* strain in Zarrouk medium

The Fig.4.27 shows the removal of Estriol by free blue-green algae *Spirulina platensis* in Zarrouk medium can be described by a 90% reducing in the sample with algae in a free form and 60% reducing in immobilized algae in Na-alginate already on the 6th day of analysis. Decreasing of Estriol stops here in the coming days remains to approximately constant values.

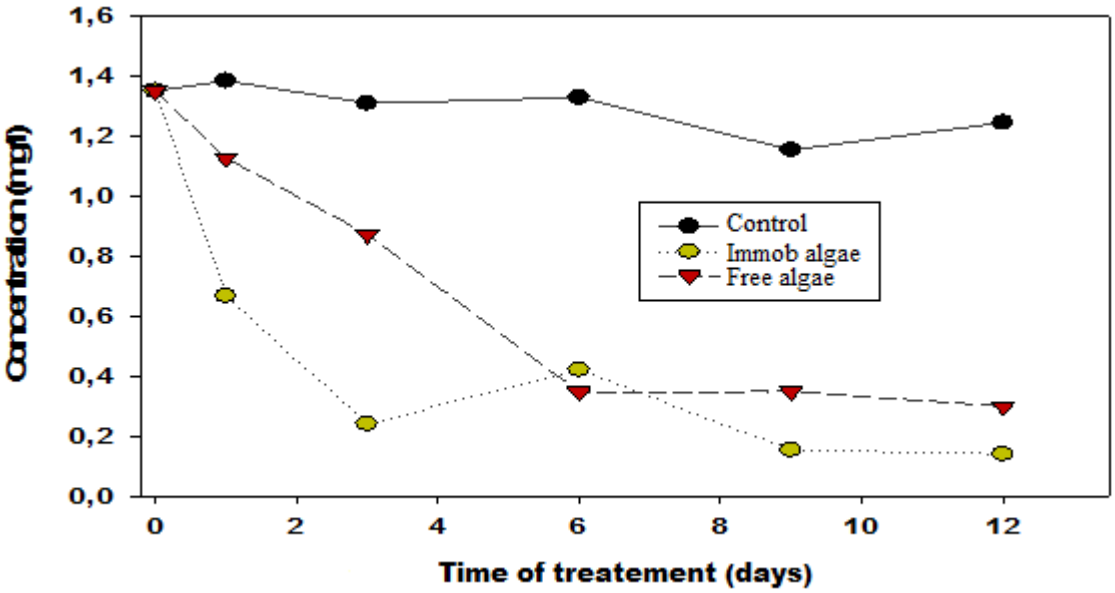


Fig.4.28. Removal of Testosterone by free and immobilized in Na-alginate blue-green algae *Spirulina platensis* strain in Zarrouk medium.

The Fig.4.28 illustrates the removal of testosterone by immobilizer *Spirulina platensis* algae requires only three days to reduce to 85% of initial concentration while in the 12th day this reduction reaching 88% of the concentration initial testosterone which was 1.37ng/L.

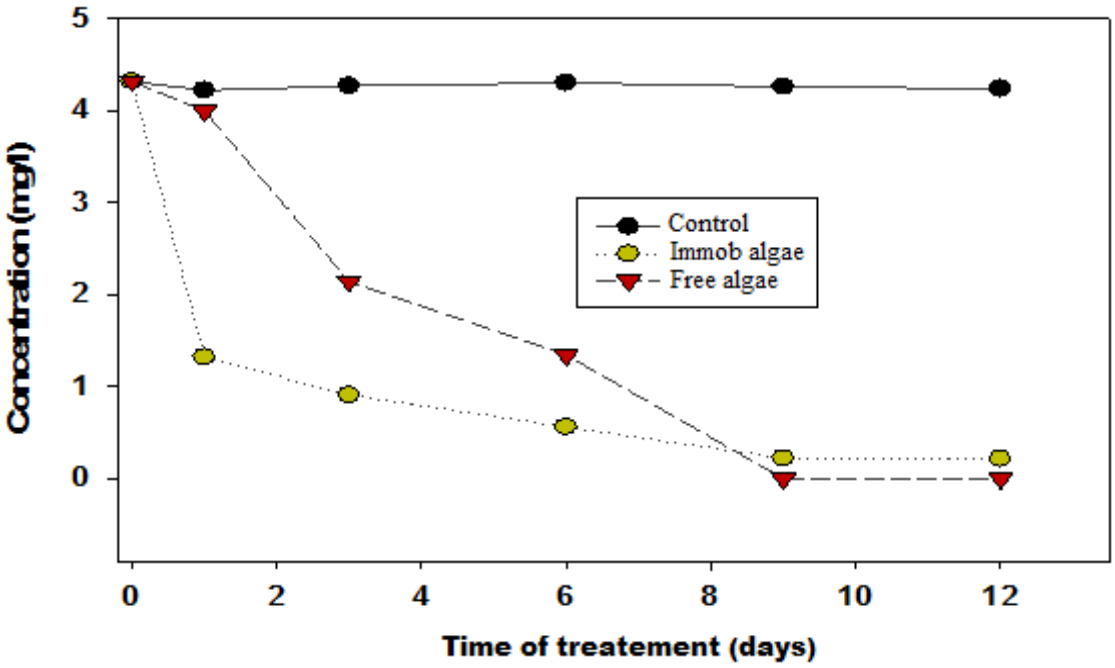


Fig.4.29. Removal of Alpha-Estradiol by free and immobilized in Na-alginate blue-green algae *Spirulina platensis* strain in Zarrouk medium

The equilibrium of Alpha-Estradiol decrease in samples of immobilized algae is performed on the 9th day when its concentration is reduced almost totally. The concentration of same molecules in the sample with free algae reduces total to the 9th day (Fig.4.29.).

IV.5.2. Bioremediation of PAH's by blue-green algae *Nostoc gelatinosum* in two types of medium (wastewater and synthetic medium)

Bioremediation of PAH's by blue-green algae *Nostoc gelatinosum* in two types of medium (non-diluted treated wastewater and Drew medium) were used throughout the bioremediation experiment and the *Nostoc gelatinosum* species were exploited as PAH-degrading species. Among the 8 targeted PAHs, only the results related to the removal of four PAHs (fluorene, phenanthrene, pyrene and fluoranthene) is shown in the present study because the results obtained of the others were not conclusive. The percentages of abiotic

losses of PAHs at the end of 22-days of incubation were not high (<15%) so abiotic losses due to photo-degradation were, therefore, ignored in the present study.

The amount of PAHs remained in the medium decreased significantly from 0 to 3 days of incubation indicating that fluorene, phenanthrene, pyrene and fluoranthene were biodegraded or bio-transformed by the algal culture whatever the type of medium was used. According to Fig.4.30., the mechanism involved in the removal of PAHs by *Nostoc gelatinosum* includes a rapid initial removal by passive physicochemical adsorption and followed by a slow active adsorption/accumulation/degradation as also reported elsewhere [188]. At the end of the treatment, the residual amount of the targeted PAHs seems depending on the recalcitrance of the PAHs molecules.

The low molecular weight (3-rings PAHs) fluorene and phenanthrene were removed better than pyrene and fluoranthene. Their depletion rate ranged from 52 to 70% and from 47 to 52% for phenanthrene and fluorene respectively. For phenanthrene, its appreciable removal seems achieved when using the treated wastewater as culture medium suggesting that nutrient element in this medium may be appropriate for the algal cells development leading them to be more effective on biodegrading this molecule. For fluorene, its uptake rates were almost at the same magnitude as in the non-diluted treated wastewater culture medium than in the Drew one (47, 52 and 50% for Jar n°1, n°3 and n°5 respectively). And pyrene and fluoranthene were more recalcitrant and their depletion rates were less than 45% (Fig.4.30 (A, B)). Indeed, they are categorized as high molecular weight PAH (4-ring PAHs) thus stable and less available for the algae. Our experiments show that the degradations were more effective in the treated wastewater than in the Drew medium.

Anyway, PAHs uptake by algal cells has been reported dependent on the algal species, the incubation time and PAH treatments [189]. PAHs removal by micro-algal species was also been reported directly related to the initial cell density employed; that higher cell density or biomass provides more surface area and cell volume for adsorption and absorption of pollutants, leading to better removal in a shorter period of time [190]. In our study, this fact is similar only for the case of fluoranthene (Fig.4.30.(A)) where its degradation rate was higher using the treated wastewater medium added by a higher amount of biomass (Jar n°3).

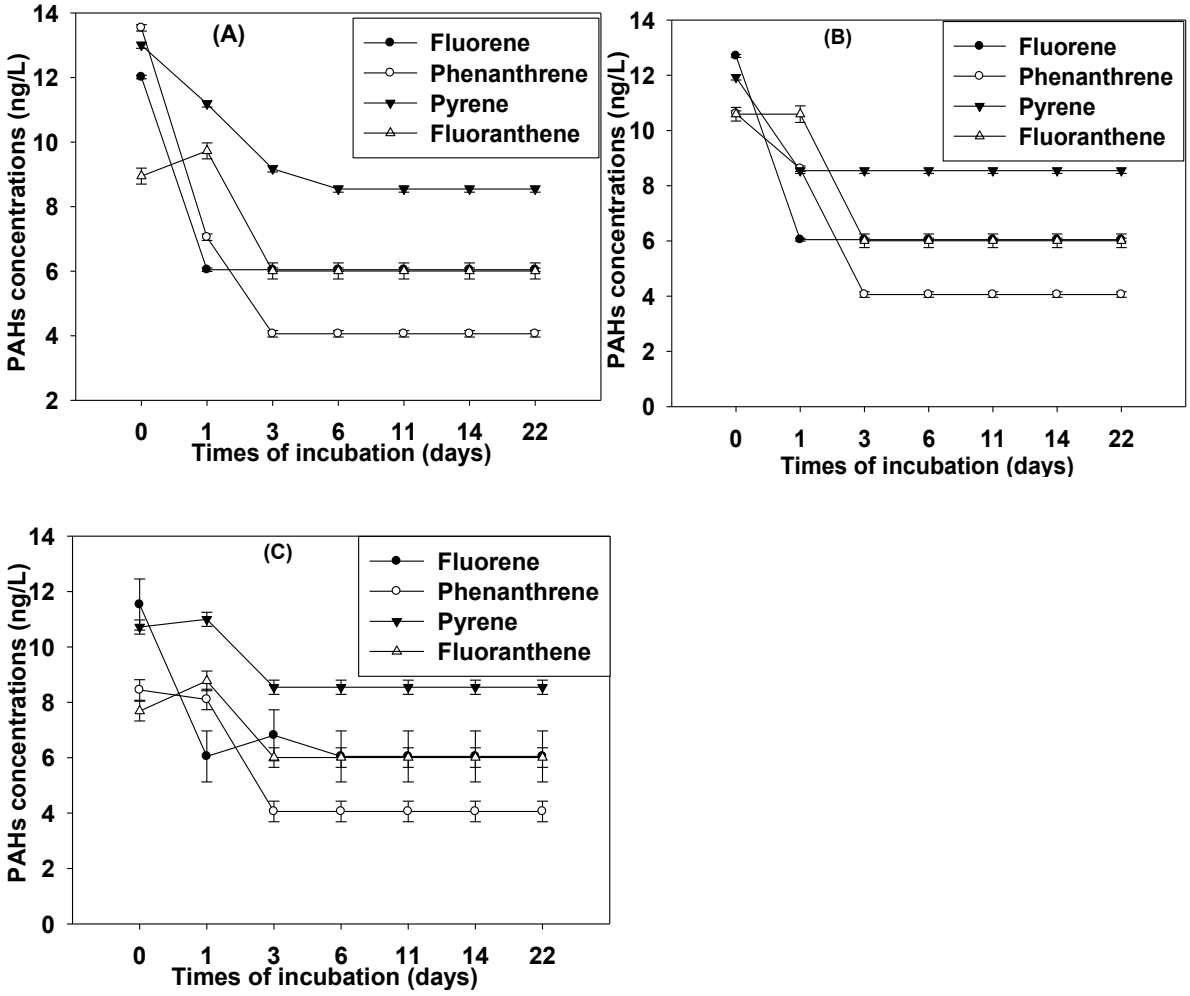


Fig.4.30. Comparison of the 4 PAHs removal using 100% treated wastewater (A, B) and Drew (C) as algal medium.

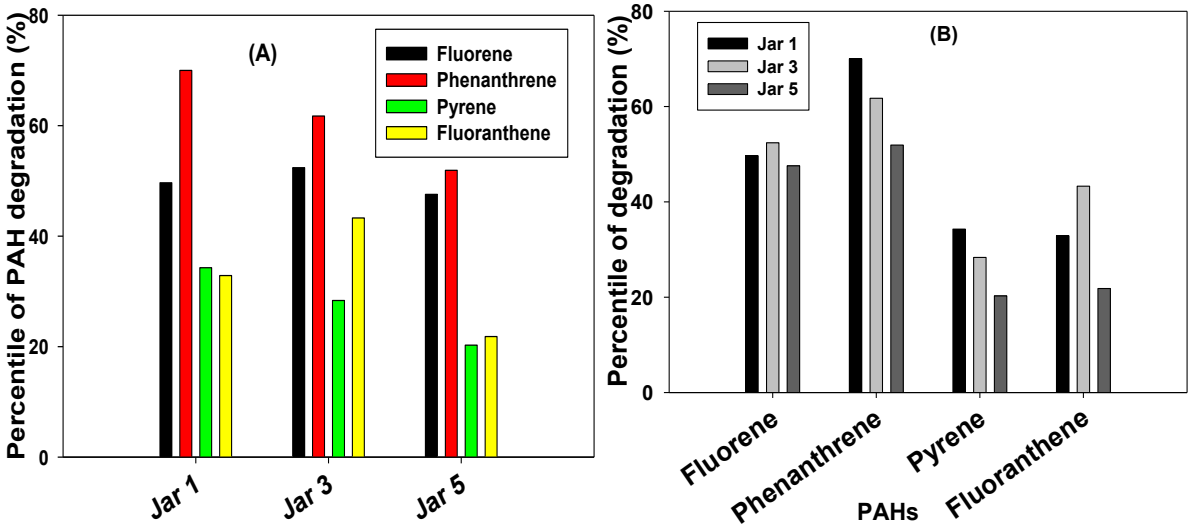


Fig.4.31. The percentile of the PAHs remaining in the medium during the 22 days of incubation.

The biodegradation metabolites of fluoranthene have been investigated using *Scenedesmus subspicatus* as the test organism [191]. The results of that study indicated that fluoranthene generated the highest toxicity to algae, whereas its metabolites displayed relatively lower toxicity. The toxicity of fluoranthene towards cladocera, fish, and benthos has also been reported [192]. When algae are exposed to fluoranthene, the chlorophyll and protein content of the algae will generally decrease rapidly and the inorganic constituents of the cells will change to a certain degree, resulting in an increase in carbohydrates and lipids [193]. Owing to their toxicities and high concentrations, the removal and degradation of PAH in marine environments has become one of the primary concerns in investigations into environmental pollution [194]. At present, the degradation of PAH occurs mainly through light oxidation, chemical oxidation, and biological methods. It is known that there are varieties of algal species that exist and grow rapidly in the marine environment that are sensitive to the toxicity of toxic substances [195] but also possess the ability to degrade PAH. The degradation processes of PAH by algae can be influenced by a number of factors [196].

IV.6. Metals analysis, Adsorption in function of pH medium

IV.6.1. Effects of pH on biosorption of Macronutrients and ETM

pH is an important parameter affecting the biosorption process. Cadmium (II) uptake by immobilized *Spirulina platensis* is also a function of pH solution, as observed in the other similarly works [182]. At low pH values the cell wall ligands were closely associated with the hydronium ions H_3O^+ and restricted the approach of metal cations as a result of the repulsive force. As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface [180]. Crist et al. (1981) [181] also suggested that zero-point charge, or isoelectric point, would be found at pH 3.0 for the algal biomass. Above this algal cells would have a net negative charge. The ionic state of ligands will be such as to promote reaction with metal ions. This would lead to electrostatic attractions between positively charged cations such as cadmium (II) and negatively charged binding sites, hence the rapid rise binding efficiency. It is for this reason that our choice has to work at this range of pH in buffered wastewater solutions. In order to track the ad(ab)sorption or other essential metabolic processes mechanisms in a not disturbed system we decided to keep one naturally range of water pH values which is between 7,8 and 8,3 values.

IV.6.2. Preparation of *Spirulina platensis* algae-alginate beads

Alginate beads with *Spirulina platensis*, last one extracted alive from the exponential phase of the Zarrouk synthetic medium of growth were synthesized. In this way, 3g wet weight of *Spirulina platensis* alive, removed from the upper layer during the centrifugation were immobilized in 2 g of sodium alginate (AppliChem, PanReac) in the same kind of experimental water. The algae-alginate beads are then shaken and allowed overnight in the solution 0.5 M $CaCl_2$. Once removed from the solidifying solution, the balls of algae-alginate compounds are rinsed 3 times in abundance with MQ water and are then rinsed with a solution of 0.5M of HCl diluted acid to remove ETM presented in the matrix content. After that it is again rinsed with MQ water to not allow the degradation of the living algae cells of the blue-green algae *Spirulina platensis* involved in our research.

IV.6.3. Preparation of cadmium II solutions for biosorption

Cadmium (II) solutions were prepared by dilution of stock cadmium (II) solution 1.0 g/L obtained by dissolving weighed quantity of anhydrous cadmium (II) nitrate (Merck) in ultrapure Milli-Q water.

IV.6.4. The preparation of pH buffer solutions

pH buffer are the solutions that maintains the pH around a stable value throughout an experiment. Five liters of wastewater solutions from the exit of the Villeneuve d'Ascq treatment plant, Nord-Pas-de-Calais region, France was utilized. The water was filtered through 0.45 µm acetate cellulose and passed to UV light for 30 min before using in our experiments. We prepared 4L of different pH buffer solutions, range from pH 5 to pH 8 included, with adding the following salts in each in 1L of solutions:

pH 5- CH_3COOH (96%) - 5.9 ml and NaOH (1M) - 68 ml in 1L of wastewater
pH 6- MES-Na (Acros Organics) -2.9 g and HNO_3 (1M) - 61.9 ml in 1L of wastewater
pH 7 –HEPES-Na (Acros Organics) -26.03 g and HNO_3 (1M)- 50 ml in 1L of wastewater
pH 8 – H_3BO_4 (Merck Pro Analysis) – 6.1 8g and NaOH (1M) – 5.5 ml in 1L of wastewater
pH 9 –NaOH (1M) – 10 ml in 1L of wastewater

A natural non-buffered wastewater is also prepared for experiments.

IV.6.5. Experience with Spirulina platensis blue-green algae in a wide of pH ranges

The experiments were conducted in 125 ml Nalgene Erlenmeyer flasks containing each 50 ml of wastewater buffer to pH 5, 6, 7, 8 and natural non-buffered wastewater was contaminated with 25/50 µg of cadmium, in duplicate. At the contaminations time of water solutions with cadmium, the flasks were agitated on a shaker at constant shaking rate (200 rpm) for 2 h to attain the equilibrium. The blue-green *Spirulina platensis* algae immobilized in Na-alginate beads using the above presented method were washed in abundance with MQ water and drained through a mesh plastic that we use and further. After draining the algae-alginate beads were weighed by 0.5 g wet weight. As a control, an empty alginate beads without *Spirulina platensis* algae in natural wastewater from the exit of the treatment plant was utilized in our experience. After which the first 5 mL samples (at t=0 time) were taken, we mix the biosorbent immobilized algae and cadmium (II) ion previously prepared solutions. At pre-determined time intervals (10 min, 20 min, 40 min, 1h, 1h30, 2h, 3h, 6h, 9h and 24h) we take samples for the determination of residual metal ion concentration in solution.

IV.6.6. Metal Desorption and Biosorbent Reactivation

For the durability economic reasons an adsorption-regeneration cycles experiments were subjected. Following initial biosorption of metals, the metal-laden beads were soaked in 25 ml 0.5 M HCl for more than 24 h for desorption of metals, rinsed three times with Milli-Q water and the water was changed. The soaking process was repeated several times until the pH of the soaked water reached 3.5 [197]. Immobilized algae beads weight, were added to the same content (50 ml) of solutions with cadmium (II) contaminated in the same range of buffered pH 5, 6, 7, 8 and natural non-buffered wastewater for the second cycle. The same range of initial concentration 25 and 50 µg/L at ambient temperature (23.0 ± 1°C) was maintained.

Before the next biosorption experiment the soaked water is desorbed using a plastic mesh placed on paper filter support at the ambient temperature. The initial metal concentration and that after equilibrium were assayed for, each time, and the biosorbent beads were desorbed and weighed using analytical balance after the second reuse cycles. The concentrations corresponding to 25 and 50 µg/L of cadmium in each different pH samples containing living immobilized *Spirulina platensis* in alginate beads and empty alginate beads

noted down: pH (5, 6, 7, 8 or N) S.P. and the blanks with the same range of cadmium concentrations containing empty beads of alginate noted pH (5, 6, 7, 8 or N) Alginat.

IV.6.7. Analysis of Macro-elements and ETM in the wastewater, Analysis of Cadmium II ions

The concentration of un-adsorbed cadmium (II) ions was determined by an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) using Ultrasonic Nebuliser with the detection limit of 0.5 µg/L at the specifics wave-lengths [4]. A standard solution was always run for every 20 samples. An average of concentration for all wave-lengths of each element with standard deviation is proposed. The analyzed samples were performers in triplicate with a RSD–10%. The concentration of others un-adsorbed ions presents in important amounts or in traces was determined by the same ICP AES (Inductively Coupled Plasma Atomic Emission Spectroscopy), above described.

IV.6.7.1. Effect of pH on cadmium (II) biosorption

To better understand the behavior of major elements and ETM in water a different pH, the major physical and chemical processes of retention on the solid phase must be studied. This series of experiments was conducted to better understand the stability of new compounds after co-precipitation or re-adsorption phenomena in a wide range of pH. The pH may intervene on the interaction between free metal cations in the solid phase in the non-specific adsorption. This study was established by multi-element sorption experiments using different pH solutions (synthetic and natural) ranks 5 to 8 and to cadmium contaminated of 50 µg/L, in the presence of fresh blue-green algae *Spirulina platensis* ad(b)sorbent immobilized in a Na-alginate matrix.

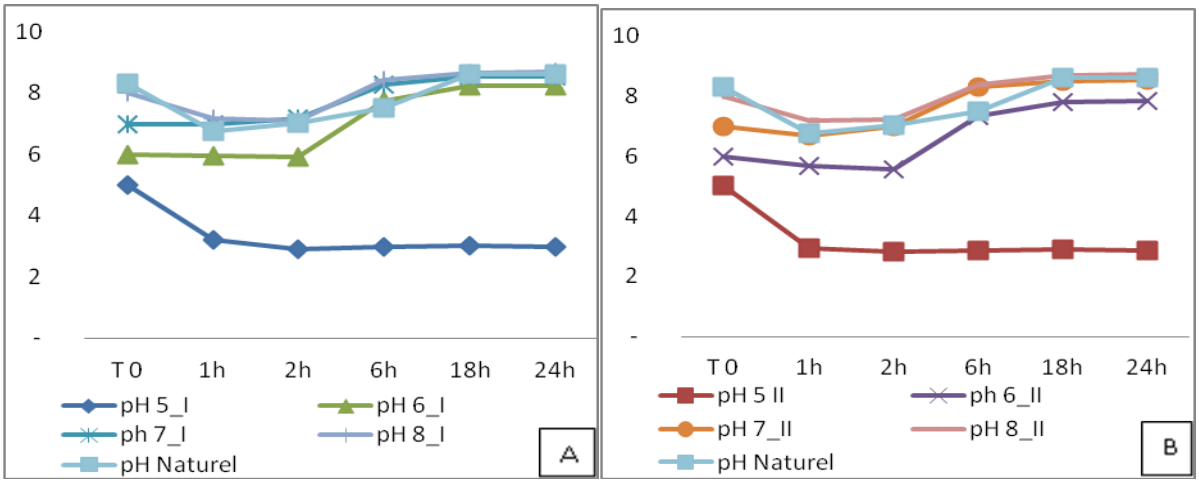


Fig.4.32. Evolution of pH values in cycle I (A) and cycle II after regeneration (B) in the presence of blue-green algae *Spirulina platensis* immobilized in Na-alginate for the fives analyzed solutions

Comparison of concentrations for both re-suspension cycles emphasizes that all metals maintain stable values in second cycle than in the first cycle of adsorption. The results presented in the figures below, allow the classification of metals in several categories:

- The metal category that does not change their adsorption potential between the two cycles. It is the case of cadmium, calcium, copper, iron, magnesium, manganese, potassium, sodium, silicon and zinc.
- The category of metals that the adsorption capacity increases in a wide range of pH (6-8) and the interaction with the adsorbent is within 30 min after inoculation in both cycles of adsorption. This is the case of cadmium, magnesium and zinc
- The category of metals that the adsorption capacity is not visible to high concentrations of the metal in any pH level. This is the case of sodium.

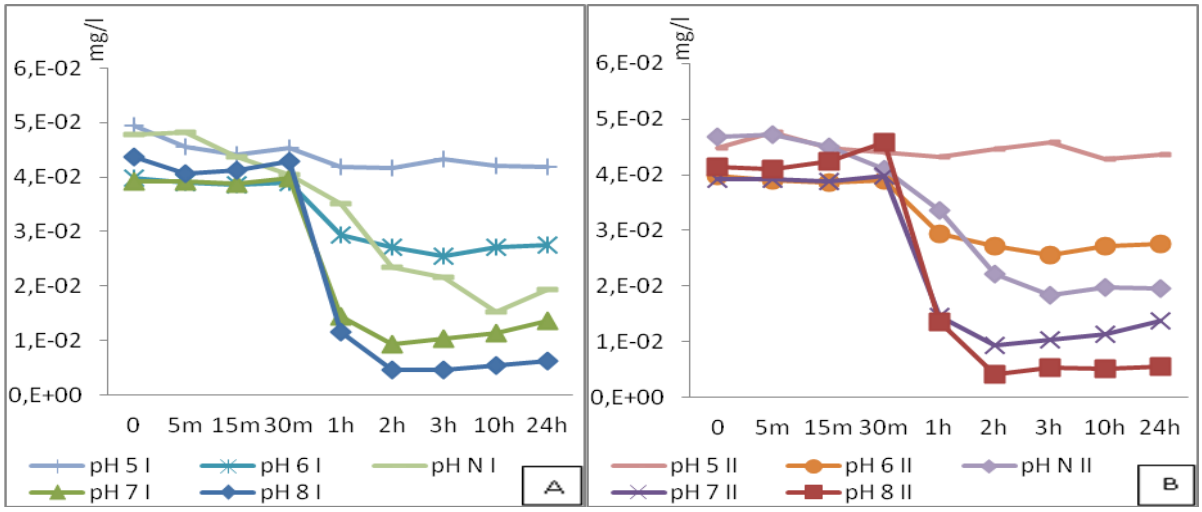


Fig.4.33. Evolution of cadmium ions concentration in cycle I (A) and cycle II after regeneration (B) in the presence of blue-green algae Spirulina platensis immobilized in Na-alginate for all 5 pH analyzed solutions.

Thus the cadmium concentration in both cycles describes important dynamics, similar, in chosen adsorption on the support. Depending on the pH and time these concentrations vary sharply. Thus begins from first hour experience sudden decrease cadmium concentrations in all variants pH (pH out of 5) equilibrium starting with 2h experience. That suddenly decrease mentioned above is specified as mentioned above and other metals (Mg and Zn). The best ad(b)sorption of cadmium is observed in solution at pH 8 in both cycles ad(b)sorption (Fig.4.33.(A, B)).

However, the cadmium concentration decreases from 41 ± 2 to 14 ± 1 µg/L in the first cycle of adsorption and from 44 ± 3 to 12 ± 1 µg/L in the second round of adsorption after regeneration in the first hour after contact with immobilized algal matrix in Na-alginate. The adsorptions continue until 2h of adsorption when equilibrium is reached (5 ± 1 µg/L) in both cycles. Sample of natural un-fixed pH of the wastewater describes a decrease more balanced reaching her balance after 3h of experience. The decrease started with the first 15 minutes of experience. The smallest decrease in the concentration of cadmium it has in sample solution of pH5 where cadmium decreased by only 1 ± 0.05 µg/L from initial 45 ± 1 µg/L to 44 ± 0.5 µg/L in the second cycle and 7 ± 0.5 µg/L from 49 ± 0.5 to 42 ± 1 µg/L after 24 hours of experience in first cycle, respectively. Cadmium concentration is resting quasi-constant during the two cycles of adsorption after that equilibrium is fixed at 3 hours of experience, which is valuable from zinc and magnesium.

IV.6.7.2. Effect of pH on Magnesium biosorption

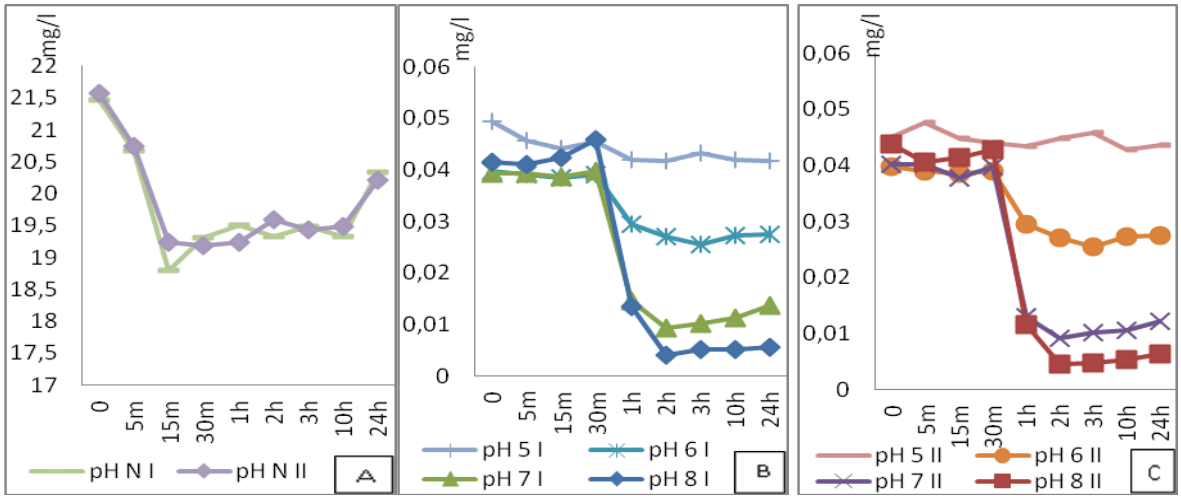


Fig.4.34. Evolution of magnesium (Mg) concentration in two cycles of natural pH wastewater solution(A), cycle I(B) and cycle II(c) for four pH synthetic solutions, contaminated with 50 µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate

The concentrations values of the magnesium in the four experimental sets of synthetic pH described in their dynamics similar decreases trends for both cycles to that seen in cadmium mentioned above (Fig.4.34.(B), (C)), and Fig.4.33.(A), (B)). However, the tendencies of magnesium concentrations in the sample of wastewater from natural un-fixed pH differs somewhat from that of cadmium. The Fig.4.34 (A) shows the decrease in the concentration of Mg starting from inoculation over a period of 15 minutes of exposition, decreased from 21.45 ± 0.84 to 18.8 ± 1.01 mg/L in un-fixed natural pH of wastewater sample. That important decrease due to absorption that occurs inside of algal cell followed by an equilibration reaching after 15-30 min or a slight re-solubilisation of Mg in the wastewater solution until the end of the 24h of experience, as we can see in Fig.4.34.(A).

IV.6.7.3. Effect of pH on Zinc biosorption

The evolution of Zn concentrations in the synthetic pH fixed solutions shows the same trend as in the case of Mg and Cd (Fig.4.34 (B) and (C)). However, the concentration of Zn decrease in solution of natural non buffered pH wastewater describes specific dynamics compared to others (Fig.4.35 (A)).

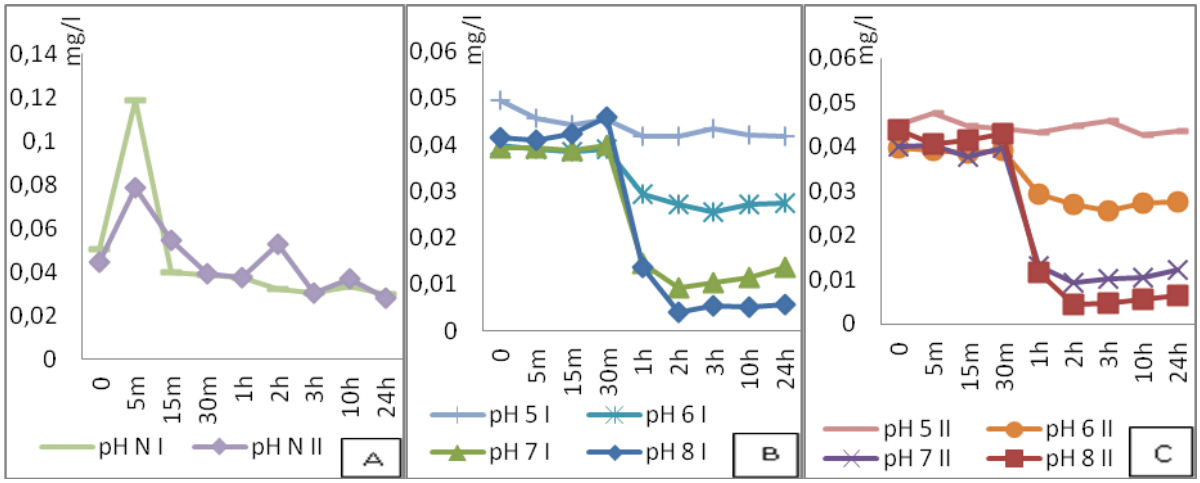


Fig.4.35. Evolution of zinc(Zn) concentration in two cycles of natural pH wastewater solution(A), cycle I(B) and cycle II(C) for four pH synthetic solutions, contaminated with 50 µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate

Therefore, the linear decrease of the Zn concentration in solution is similar in the two cycles of adsorption, decreasing from 50 1 µg/L to 30 µg/L in the first cycle of adsorption and from 44.0±1 µg/L to 28±2 µg/L in the second cycle (Fig.4.35).

IV.6.7.4. Effect of pH on Calcium biosorption

The evolution of calcium concentration in the samples cannot be analyzed in the samples with different pH values synthetically obtained because for obtained this solution it not be used any source of calcium. However, we chose to demonstrate in Fig.4.36. (B and C) that algal biomass plays an important role in re-mobilization of calcium in solution. These calcium ions coming from algal cells can be re-mobilized in solution starting with 30 minutes of experience especially in weak pH solutions (for example pH 5).

The Fig.4.36 shows that the calcium concentrations range from 95 ± 5 to 178±10 mg/L in the first cycle and from 129±7 to 251 ± 10 µg/L in the second round of adsorption. Mobilizing in the solution is valid also for other ranges of pH with values that can reach 246 ± 15 µg/L for pH 6 and even 756 ± 15 µg/L for pH 8 of synthetic solutions treated with algae. It is possible that this remobilization in solution to be based on competition between this major element and ETM such as cadmium.

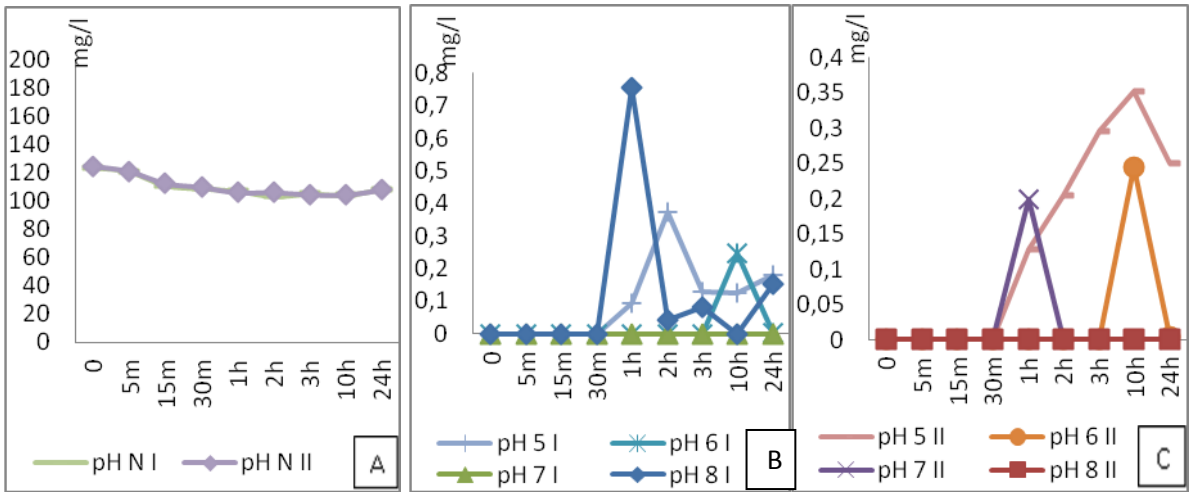


Fig.4.36. Evolution of calcium concentration in natural pH wastewater samples for two cycles (A); cycle I(B) and cycle II (C) are the synthetic pH solutions in presence of blue-green algae *Spirulina platensis* immobilized in Na-alginate of four pH analyzed solutions.

The calcium concentration in the synthetical samples with different pH values was low because of the absence any source of calcium. However, we chose to demonstrate in Fig.4.36 (B and C) that algal biomass plays an important role in re-mobilization of calcium in solution. These calcium ions coming from algal cells can be re-mobilized in solution starting within 30 minutes of experience especially in weak pH solutions (for example pH 5). The calcium concentrations ranged from 95±5 to 178±10 µg/L in the first cycle and from 129±7 to 251±10 µg/L in the second round of adsorption in treated wastewater. Mobilizing in the solution is valid also for other ranges of pH with values that can reach 246±15 µg/L for pH 6 and even 756±15 µg/L for pH 8 of synthetic solutions treated with algae. It is possible that this remobilization in solution to be based on competition between this major element and ETM such as cadmium.

IV.6.7.5. Effect of pH on Cuivre biosorption

Lack of copper ions from work solutions (synthetic and natural) from both adsorption cycles analyzed by us do not give us some important kinetic information on this toxic metal. However, as we see from Fig.4.37. (A), (B) and (C) that in samples with synthetic solutions as well as in wastewaters from the exit of Villeneuve d'Ascq treatment plant, at slightly acidic (pH 5) pH values, the copper initial ions concentrations vary from 10.0 ± 0.5 µg/L to

inoculation of the *Spirulina platensis* algae to 7.0 ± 0.5 mg/L at the end of our accumulation experience.

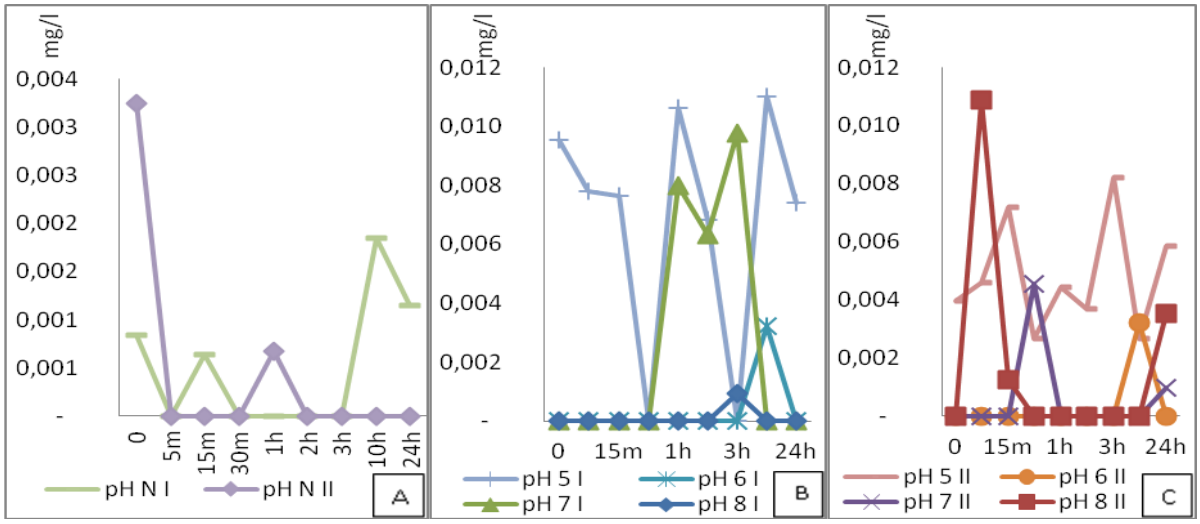


Fig.4.37. Evolution of copper ions concentration in cycle I and II for natural pH wastewater(A), cycle I(B) and cycle II(C) for four synthetic pH solutions, contaminated with 50µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate

This fact confirms us that as in the case of calcium, the possibility of re-mobilization in solution of ETM to low pH values (in our case pH5). The same situation is in case of slightly basic pH-values (pH 7 and pH 8) where a remobilization of copper in solutions but they are milder, possibly because competitions of the elements present in solution for adsorption sites.

IV.6.7.6. Effect of pH on Iron biosorption

Tendency of Fe ion accumulation is more visible in all 4 pH sets in both cycles of adsorption, which is related to the inability of algae siderophors to bind the iron ion, the last being recycled to the extracellular medium by a pump efflux. Therefore, as we see in Fig.4.38 in the synthetic solutions with controlled pH, iron increases from 5 ±0.07 to 23 ± 6.82 µg/L after ion efflux from algal cells can be noted.

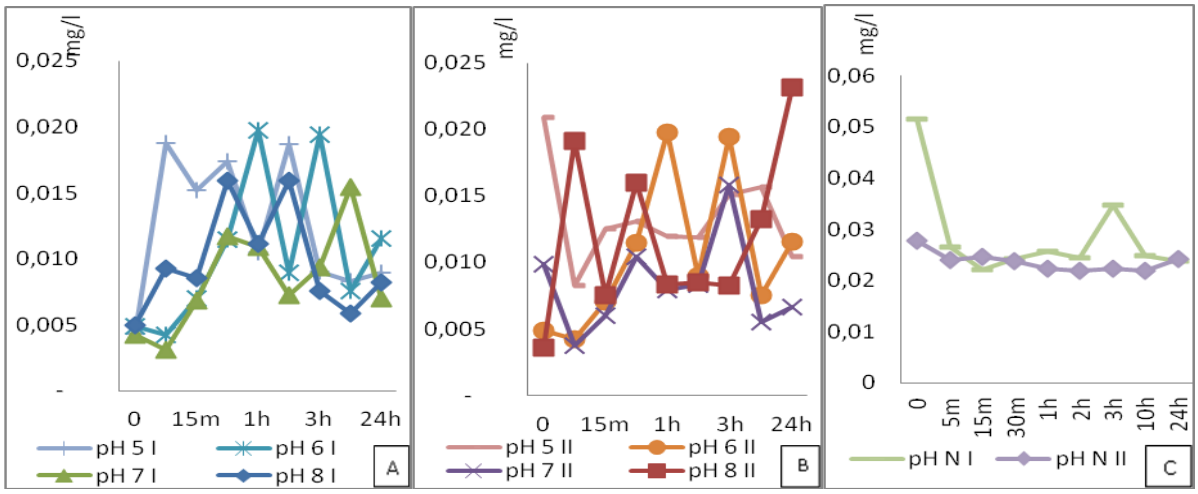


Fig.4.38. Evolution of iron (Fe) concentration in cycle I (A) and II (B) for synthetic pH solutions and natural pH of wastewater solution(C), contaminated with 50µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate.

In treated wastewater solutions the concentration of iron was 51.6 ± 6.0 µg/L in the first cycle and 27.7 ± 2.1 µg/L in the second adsorption cycle. After that the inoculation with blue-green algae *Spirulina platensis* occurs, an siderophors algal activation in conditions of basic pH (pH 8.3) and constant aeration (due to stirring) the same as Hunter said in 1972 that, microalgae, particularly at the alkaline pH and aerobic conditions in the photic zone, should possess a siderophors-mediated iron uptake system [198].

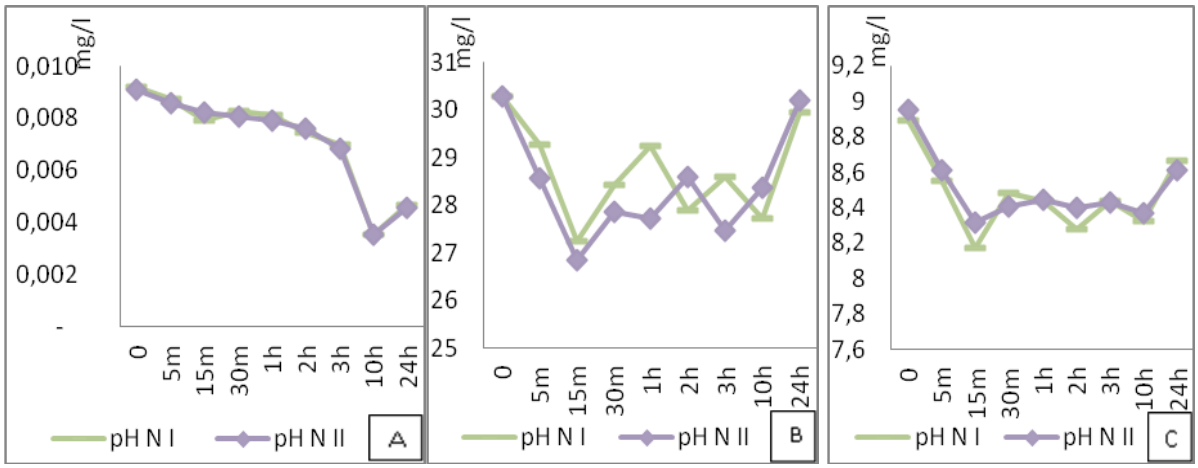


Fig.4.39. Evolution of manganese(A), potassium(B) and silicon(C) concentration in two regeneration cycles of natural wastewater solution at natural pH solutions, contaminated with 50 µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate

In batches with synthetically prepared stable pH solutions have not been seen traces of Mn, K or Si while in wastewaters from the exit of the Villeneuve d'Ascq treatment plant at pH 8.3 they are detected and adsorbed by algal biomass immobilized in Na-alginate. The Fig.4.39 (A) shows the initial amount of the Mn ion in the first and second adsorption cycle is 9.19 ± 0.24 and 9.11 ± 0.11 μg/L respectively. This is effective reduced during the 10h of experience until minimal values up to 3.55 ± 0.19 μg/L in both cycles. A slight remobilization up to 4.60 ± 0.05 μg/L of Mn occurs in both cycles of adsorption to the end of experience. The evolution of potassium (K) and silicon (Si) ions have a similar tendency during the both cycles of our experience with adsorption evident tendencies in the first 15 minutes of contact with blue-green algae fresh immobilized in Na-alginate. Starting from the initial values, which reached 30.28±1.20 mg/L for potassium and 8.95±0.21 mg/L for silicon in wastewaters, they got to attend the lowest values in an interval time of 15 minutes. The minimum value of the potassium concentration is 27.23±0.45 mg/L and the silicon concentration decreased to 8.17±0.89 mg/L in the both adsorption cycles.

IV.6.7.7. Effect of pH on Sodium biosorption

Once with the preparation of the synthetic solutions for pH-fixing to the desired value a wide range of Na^+ concentrations were established due to the addition of different quantities of compounds with Na^+ in MQ water. The evolution of sodium concentration varies depending on the pH and ionic strength. For each pH value, we have a maximum and a minimum value. The Fig.4.40 shows that most sodium adsorption takes place in pH 5. In both adsorption cycles of sodium ions are corresponding a minimum of 1.22 ±0.01 g/L after adding balls with immobilized algae in Na-alginate to initial concentrations 1.31±0.02 g/L. This represents a 6.6% decrease of very high content of Na ions.

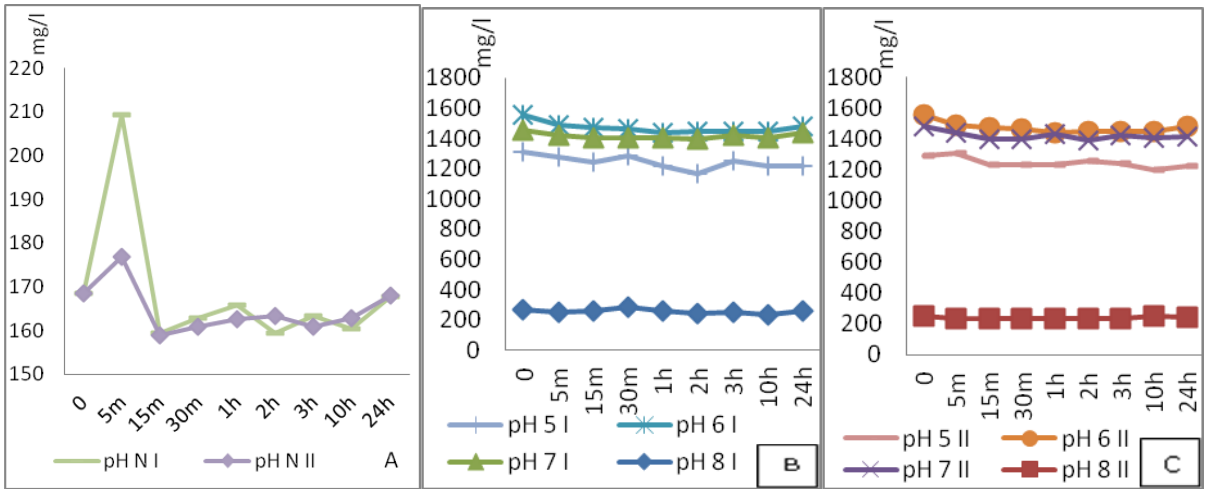


Fig.4.40. Evolution of sodium(Na) concentration in two cycles of natural pH wastewater solution(A), cycle I(B) and cycle II(c) for four pH synthetic solutions, contaminated with 50µg/L of cadmium, in presence of fresh blue-green algae *Spirulina platensis* immobilized in Na-alginate.

Similar decreases of 5%, 3% and 4.3% occurring in samples with pH 6, pH 7 and pH 8 where the sodium content attend also very high peaks of 1.48 ± 0.01 g/L in samples with pH 7. In the un-fixed pH wastewater sample treated with blue-green algae immobilized in Na-alginate, the initial 168.46 ± 2.46 mg/L concentration of sodium is not reduced during the 24 hours of analysis at initial pH of 8.3. It remained about at the same initial values for both adsorption cycles. Therefore, we can conclude that sodium is an essential macro-element with a significant physiological role for algal cell which can be consumed in large scale proportions in different concentrations and from different types of environment.

Conclusion and perspectives

The mineral and organic micro-pollutants, toxic to living organisms can be found in various aquatic environments. The organic contaminants can affect the reproductive systems by disrupting the endocrine system while mineral micro-pollutants affect particularly the functioning of nervous and renal systems.

The obtained results in the bioremediation studies with blue-green algae's show a greats levels of decontamination of certain organic micro-pollutants molecules. Very fasts bioremediation of Progesterone, Testosterone and Bisphenol G occurs in the first day of algae inoculation in solution. As examples progesterone degradation with immobilized *Spirulina platensis* leads to 85% of progesterone elimination in the water in a single day.

In three days' important amounts of PAH in wastewater solutions were removed using blue green algae. The amount of PAHs remained in the medium decreased significantly from 0 to 3 days of incubation indicating that fluorene, phenanthrene, pyrene and fluoranthene were biodegraded or bio-transformed by the algal culture whatever the type of medium was used. The low molecular weight (3-rings PAHs) fluorene and phenanthrene was observed better removed than pyrene and fluoranthene. Their depletion rate ranged from 52 to 70% and from 47 to 52% for phenanthrene and fluorene respectively. A great increase in algal productivity (about 18 times) on the wastewater mediums was observed at the same time reducing of nutrients in the media analyzed. The evolution of nutrient concentrations tends to a major reduction in the concentration of anions. Nitrate and phosphate species reduces the most obvious. Phosphate concentration is reduced to nil in the first day while the nitrates gradually until the 21 day. The pH increase significant from 8 to 10.5 in the algae samples, a basification that is a witness of good growth and physiological states of algal cell.

The rapid basification of algae-solution system enhances an increase in cadmium uptake process simultaneously with increased pH values. They could be repeatedly used in multiple adsorptions–regeneration cycles. Metal sorption slowly decreases after the first desorption cycle and shrink more obvious after fifth sorption cycle but sorption capacity was fairly smooth.

The Pseudo-first and pseudo-second order kinetic models used to fit the experimental data for analyzing the bio-sorption kinetics indicate that Cd^{2+} ion, once displaced the alkaline-earth ions from the cell sheath tended to occupy all sites available on biomass surface. Being natural, abundant, and cheap algae, the biomass of blue-green algae such as *Nostoc*

gelatinosum can be utilized successfully in selective removal of toxic metal ions from metal contaminated or other kind of wastewaters.

The trend of bioaccumulation of heavy metals in the primary producers as well as contamination and toxicological impact in the entire aquatic ecosystem by these emerging pollutants, watch, via the results shown, worrying levels of amplification in concentrations of the micro-pollutants molecules and ions in the algae biomass. Although there is no rejecting standard concerning the bisphenols and drug residues, it is preferable to conduct more detailed studies.

In this connection, the results obtained during the elimination experiments of these harmful molecules biologically were very satisfactory. These harmful molecules could be removed by the bioremediation method using few blue-green algae strains such as *Spirulina platensis* and *Nostoc gelatinosum* in freely or immobilized in matrices of Na-alginate state.

In this context, it can be suggested to continue the study in more depth accompanied by a field monitoring by consulting the following actions:

- Faced with this problem the analysis should be followed by deeper studies of the aquatic ecosystem on the algae interaction with suspended matter and sediment. The knowledge of nature and the contamination levels will help us to confirm the pollution of ecosystems studied and their origins. Adopting a rigorous legislation that aims to deal with unauthorized use of organic micro-pollutants.

- Promote consumer awareness about the harmful effects of heavy use of drugs the use of paints containing heavy metals as well as unreasonable waste of pharmaceuticals seriously damages to the human health and on entire ecosystems.

In addition, it is important to solve the problem at the source, namely find appropriate techniques to better eliminate these micro-pollutants before discharge into the environment. We can suggest additional studies in greater depth and large scale concerning disposal feasibility of these harmful molecules for optimal processing conditions.

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ANNEXES

THE NUTRIENT MEDIA USED FOR THE CULTIVATION OF ALGAE

Chu nutrient medium - 10 (g / l)

Ingredient	Quantity, g / l
$Ca(NO_3)_2$	0.04
K_2HPO_4	0.01
$MgSO_4*7H_2O$	0.025
Na_2CO_3	0.02
$Na_2SiO_3*9\ H_2O$	0.025
$FeCl_3*6\ H_2O$	0.0008

Beneche nutrient medium

Ingredient	Quantity, g / l
$MgSO_4*7H_2O$	0.1
$Ca(NO_3)_2$	0.5
K_2HPO_4	0.2
$FeC_6H_5O_7$	0.0033
$C_6H_8O_7$	0.0033

Nr.6 nutrient medium (Gromov B.V., Titanova N.N.)

Ingredient	Quantity, g / l
KNO_3	1
$K_2HPO4*3H_2O$	0.2
$MgSO_4*7H_2O$	0.2
$CaCl_2$	0.15
$NaHCO_3$	0.2
Micronutrients solution	1 ml
pH	After sterilization is adjusted to 7.5-8 using soil. NaOH (10%)

Trace elements solution for the nutrient medium Nr.6 (Gromov B.V., Titanova N.N.)

Ingredient	Quantity, g / l
$ZnSO_4 * 7H_2O$	0.22
$MnSO_4$	1.81
$CuSO_4 * 5H_2O$	0.08
$NaBO_3 * 5H_2O$	2.63
$(NH_4)_6Mo_7O_{24} * 4H_2O$	1
$FeSO_4 * 7H_2O$	9.3
$CaCl_2$	1.2
$Co(NO_3)_2 * 6H_2O$	0.02
EDTA	10

Gromov nutrient medium

Ingredient	Quantity, g / l
KNO_3	0.01
K_2HPO_4	0.0667
$MgSO_4$*7H2O	0.0333
$ZnSO_4$*7H2O	0.000022
$MnSO_4$	0.00181
$CuSO_4*5H_2O$	0.000079
$NaBO_3*4H_2O$	0.00263
$(NH_4)_6Mo7O_{24}*4H_2O$	0.001
$FeSO_4$*7H2O	0.0093
$CaCl_2$	0.0012
$Co(NO_3)_2H_2O$	0.00002
EDTA	0.01

Drew nutrient medium

Ingredient	Quantity, g / l
K_2HPO_4	0.2
$MgSO_4*7H_2O$	0.2
$CaCl_2$	Traces
$FeCl_3$	Traces

Zarrouk nutrient medium

Ingredient	Quantity. g / l
$NaHCO_3$	16.8
$NaNO_3$	2.5
$K_2HPO_4*3H_2O$	0.5
K_2SO_4	1
NaCl	1
$MgSO_4*7H_2O$	0.2
$CaCl_2*6H_2O$	0.04
$FeSO_4$	0.01
EDTA	0.08
Micronutrients solution	4 ml

A trace elements solution for nutrient medium Zarrouk

Ingredient	Quantité, g / l
H_3BO_3	2.86
$MnCl_2*4H_2O$	1.13
$ZnSO_4*7H_2O$	0.222
$NaMoO_4*5H_2O$	0.39
$Co(NO_3)_2*6H_2O$	0.049
$CuSO_4*5H_2O$	0.079

Z-8 nutritive medium

Is prepared by adding a volume of 10 ml solution I + 10 ml solution II + 10ml solution III + 1 ml solution IV brought to one liter of distilled water and then autoclaved. PH is entre 6-7.

Sol I pour le milieu Z 8

Ingredient	Quantity, g / l
$NaNO_3$	46.7
$Ca(NO_3)_2*4H_2O$	5.9
$MgSO_4*7H_2O$	2.5

Sol II for Z8 medium

Ingredient	Quantity, g / l
K_2HPO_4	3.1
Na_2CO_3	2.1

Sol III for Z8 medium

Solution III for the Z8 medium is prepared by adding 10 ml of Sol A + 9.5 ml of solution B, after adjusted to 1 liter with distilled water.

Solution A is prepared as follows: 2.8 g of FeCl3 was dissolved in 100 ml of 0.1N HCl.

Solution B is prepared as follows: 2.9 g EDTANa2 was dissolved in 100 ml of 0.1 N NaOH

Sol IV for the Z 8 medium

Ingredient	Quantity, g / 100ml
$Na_2WO_4*2H_2O$	0.033
$(NH_4)_6Mo_7O_{24}*4H_2O$	0.880
KBr	1.2
KI	0.83
$ZnSO_4*7H_2O$	2.87
$Cd(NO_3)_2*4H_2O$	1.55
$Co(NO_3)_2*6H_2O$	1.46
$CuSO_4*5H_2O$	1.25
$NiSO_4(NH_4)_2SO_4*6H_2O$	1.98
$Cr(NO_3)_3*9H_2O$	0.41
V_2O_5	0.089
$KAl(SO_4)_2*12H_2O$	4.74
H_3BO_3	31
$MnSO_4*7H_2O$	1.6 g/l