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Performance evaluation of *Lemna gibba* in a lagooning system : experimental and analytical approach

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Abstract : Water is an essential resource for all human activities, particularly domestic ones, and its use generates significant volumes of wastewater that require proper collection, treatment and disposal. Faced with population growth and water scarcity, Morocco has adopted an environmental policy based on sustainable development, mindful of its difficult climatic conditions and the degradation of its natural heritage. In this context, this study is part of an approach to promote wastewater treatment processes through the evaluation of the purification performance of *Lemna gibba*, an aquatic macrophyte commonly used in lagooning systems. This plant plays a key role in the phytopurification process, contributing to the elimination of certain heavy metals and nutrients present in wastewater. It is notably used as a tertiary treatment before the reuse of treated water. The main objective of this work is to evaluate the effectiveness of *Lemna gibba* in a lagooning basin under natural conditions, by monitoring several physico-chemical parameters. The results obtained demonstrate the ecological and economic benefits of this approach as a sustainable and environmentally friendly solution for wastewater treatment.

Keywords : wastewater, phytoremediation, macrophytes, *Lemna gibba*,heavy metals.

I. Introduction :

The world's growing population, and the increasing use of science and technology, are bringing us major environmental challenges. Pollution and over- consumption of water are

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among the most pressing problems. It is crucial that we take action to preserve our planet and ensure a sustainable future. Beyond being a simple resource, water is the key to life itself. In the current context, where it is becoming more and more scarce, its protection is essential to the well-being of both ourselves and the world. Consciousness of the importance of water and the introduction of sustainable management habits are essential to safeguard this precious resource. This means taking steps to reduce water pollution, recycle and reuse water [1-3]. Wastewater treatment has become an essential step in reducing the negative environmental impact of our activities and safeguarding water quality for future generations. The use of urban wastewater treatment could generate more water and fertilizer. Nevertheless, it should be noted that wastewater management for reuse and nutrient recovery needs advanced technologies and appropriate management to ensure sanitary and ecological protection. Phytoremediation is an ecofriendly green engineering technology, defined as an economic approach based on plants and solar energy. Phytoremediation is an action that involves purifying water or, in some cases, soil, i.e. removing contaminants from it, based on natural processes of biotic and/or abiotic nature set in action by plants. A wide range of floating, emergent and submerged aquatic plants, known as macrophytes, can be used, most of which can take up heavy metals from wastewater that present a risk to human health. Macrophytes take their nutrient requirements from the aquatic environment using their own organisms (roots and fronds), and contribute to the phyto-purification of aquatic areas. This effective and less expensive method is used as a tertiary treatment and is ideally associated with wastewater lagooning. The basic concept is very simple : bacteria, which need oxygen, transform organic substances into mineral substances that can be assimilated by plants; in return, the roots of these plants feed the bacteria, providing them with the oxygen they need [4,5]. The substantial influx of heavy metal pollutants into aquatic environments as a result of human activities has become a critical issue. The high toxicity, long-term durability and degradability of heavy metals not only result in considerable economic losses, but also have dramatic consequences for ecosystems and human health. With their remarkable capacity to uptake heavy metals from water, macrophytes represent a promising approach to reduce heavy metal pollution effectively, and provide considerable potential for real-world applications. This article presents an example of the remediation of heavy metal pollution in a lagoon plant using duckweed. The adsorption performance of heavy metals by the species Lemna gibba is evaluated. The results of the study also provide a better understanding of the accumulation and transfer of heavy metals through this species.

II. Materials and methods :

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1. **Description of vegetal material :**

Duckweeds are an aquatic plant family of which some 40 species have been discovered worldwide to date. They belong to the Lemnaceae family, subdivided into two subfamilies: Wolffioideae, with two genera (Wolffia and Wolfiella), and Lemnoideae, with two genera (Lemna and Spirodella). These small floating plants can absorb high quantities of nutrients such as nitrogen and phosphorus. Duckweed is already widely used in the treatment of domestic and agricultural wastewater. Duckweed is a simple plant, consisting of roots, stems and a separate leaf. The frond or leaf is elliptical, slightly obtuse at the tips, 1 to 3 millimeters long. It has a conspicuous single rib [6-14]. The geographical distribution of Lemnaceae depends on the physiological characteristics of each genus. Our study focuses on the Lemna gibba species, which is generally adapted to the Mediterranean climate.

2. Study site and sampling

Duckweed samples were taken from the pilot station's lagooning basins, and wastewater samples were collected in sterilised glass bottles and analysed on site for physicochemical parameters such as pH, biochemical oxygen demand, and chemical oxygen demand, then stored for heavy metal analysis. The pilot treatment plant is designed to treat domestic wastewater and laboratory wastewater. Given that the resident population in the Bouregreg complex is approximately 800 people, and the domestic water to be treated includes wastewater from the executive housing complex and the staff housing complex with a relatively low flow rate (86 m3/day) compared to the discharge from the treatment plant. The wastewater from the laboratory, administrative premises and restaurant has an estimated flow rate of 35 m^3/day. The study was conducted between February and July 2020, despite the disruption caused by the COVID-19 pandemic.

3.Parameters monitored and analysis of heavy metals

The treatment performance of the plant was assessed by analysing several physical and chemical parameters:

3.1. Measurement of suspended solids (SS)

The SS content was determined by gravimetric filtration:

A dry filter was weighed (M_0) to an accuracy of 0.1 mg.

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A known volume of the sample (V) is filtered (25 mL for raw water ; 500 mL for treated water). The filter is then dried for 2 hours at 105 °C, cooled in a desiccator and weighed (M_1). The SS concentration is calculated using the following formula :

SS (g/L) = ($\mathbf{M1 - M_0 / V}$) *1000

3.2. Determination of VSS (Volatile Suspended Solids)

After determining the TSS, the filters are calcined at 550 °C for 2 hours: The loss of mass after calcination corresponds to the volatile fraction of the TSS. After cooling in a desiccator, the filter is weighed again (M_2).

3.3. Determination of COD (Chemical Oxygen Demand)

COD was measured using colorimetric kits and a thermal reactor:

2 mL of sample is placed in a COD tube. The tube is heated for 2 hours at 120 °C in a reactor. After cooling, the reading is taken at 600 nm using a spectrophotometer. A blank is performed with distilled water.

3.4 Heavy metal analysis :

In order to quantify the heavy metals dissolved in wastewater (such as lead, cadmium, copper, or zinc), an acid mineralisation step was carried out on the water samples prior to the analysis, as briefly described in the following table :

Table 1 : Method of water mineralization for heavy metal analysis

Test drive	Acids used for mineralization	Quantity of acid used	Mineralization tool	Volume at the end of mineralization
25 mL	concentrate HNO_3	2,5 mL	Digest block 95◦C	≤25 mL
	concentrate HCl	2,5 mL	Warmer plate 95◦C	≤10 mL

4.Acid hydrolysis/mineralization treatment of plant material :

The Temminghoff and Houba protocol is used to perform hydrolysis [15]. This protocol aims to completely dissolve the plant sample components in acid, then concentrate the solution to

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facilitate analysis. Most of the original organic compound is oxidized by boiling nitric acid. The plants' fatty components are destroyed by hydrogen peroxide. The addition of hydrofluoric acid solubilizes the plant's silicate skeleton.

Sample preparation : Plant samples are initially dried, then ground in a crusher to obtain a finely ground, homogeneous material. A sample of at least 200 mg (0.2 g) is taken and precisely weighed. This step ensures that the sample quantity is well controlled.

Sample digestion : The weighed sample is transferred to polytetrafluoroethylene (PTFE) tubes. To moisten the sample and prepare it for digestion, 2 mL of concentrated nitric acid and 0.5 mL of concentrated hydrofluoric acid are added to the sample. These acids are commonly used to dissolve organic and inorganic samples.

Incubation : Digestion tubes are capped and left at room temperature overnight to allow the chemical reaction to take place.

Hydrolysis : after 24 h, the tubes are heated for four hours at 110°C. During this step, the tubes are slightly opened to avoid excessive pressure build-up. After 10 seconds, hydrogen peroxide is added to the solution. This step can be repeated several times. After hydrolysis, 1 mL of concentrated nitric acid is added to the tubes. The tubes are heated again, but not completely closed.

Evaporation : After 4 hours, the tube lids are removed to allow the water to evaporate until about 2 mL of liquid remains in the tubes. The residue remaining in the tubes is taken up with 2 mL dilute nitric acid and heated to a lower temperature for 5-10 minutes, without boiling. The mixture is then cooled and quantitatively transferred (make up to the mark with water) into a 15 mL tube using a special funnel.

After the mineralization step, the wastewater and plant material samples are then ready for heavy metals ICP-MS analysis.

5.Analysis of heavy metals by ICP-MS

There's no doubt that inductively coupled plasma mass spectrometry (ICP-MS) is an effective method for the quantitative analysis of heavy metals and other compounds present in liquids. It works by combining an argon (or sometimes helium) plasma with a mass spectrometer. The plasma is a source of extremely high temperature ions, and ionizes the atoms in the sample, transforming them into ions. This data can be compared with a reference standard to assess the concentration of substances present in the sample at ultra-low concentrations.

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III. Results and discussion :

1. Evaluation of the treatment efficiency of the lagooning system

Below are the results obtained from one year of monitoring the parameters used to evaluate the treatment efficiency of the pilot aerated lagooning station. The curves in the following figures show the results for COD, BOD5 and TSS evaluated during 2020. Figure III-1 shows that COD varies over the course of a year for raw water between 366 mg/L and 43 mg/L with an average of 126.5 mg/L, and for treated water between 5 mg/L and 91 mg/L with an average of 38.5 mg/L, with a reduction of 98.63%. Figure III-2 shows that the biological oxygen demand varies over the course of a year, for raw water, between 157 mg/L and 14 mg/L with an average of 47.5 mg/L, and for treated water between 6 mg/L and 45 mg/L with an average of 14.6 mg/L. The treatment efficiency or reduction rate is 96.18%. A curve showing the variation in TSS concentration is presented in Figure III-3. This shows that the TSS concentrations measured in raw water varied during 2020 between 120 mg/L and 4.5 mg/L, with an average of 36.07 mg/L, and in treated water between 4 mg/L and 70 mg/L, with an average of 13.44 mg/L. The treatment efficiency is 92.31%.

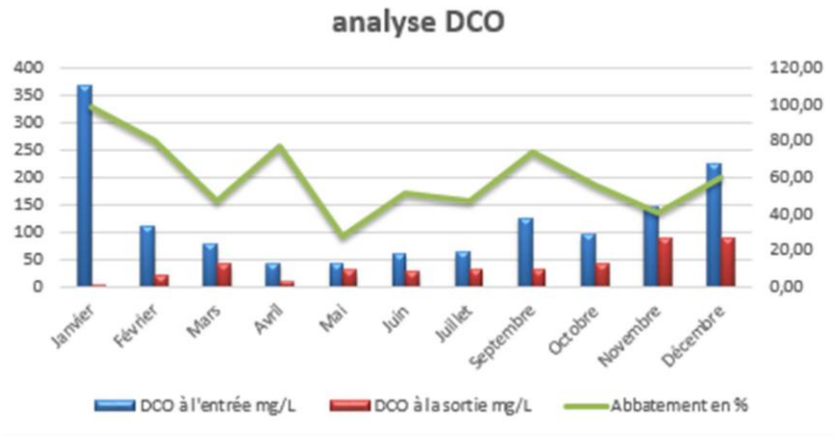

Fig. III-1 : COD variation curve for 2020 at the lagooning station

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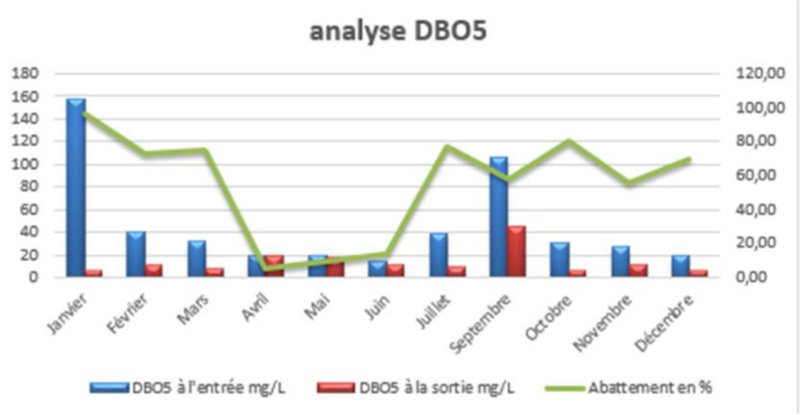

Fig. III-2 : Variation curve of BOD5 during 2020 at the lagooning station

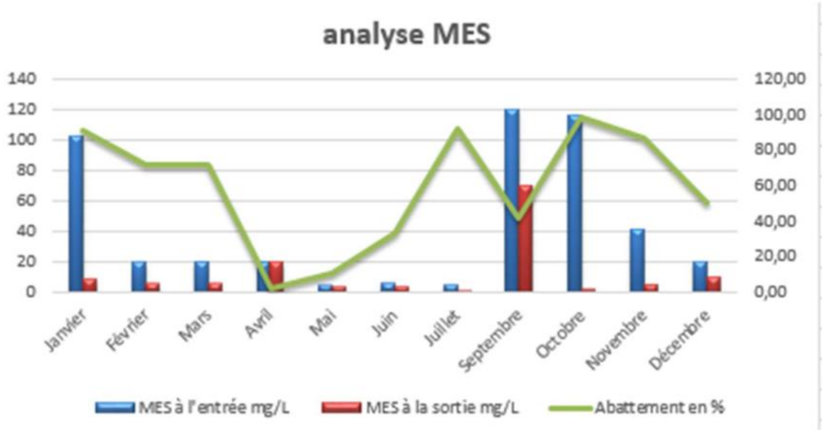

Fig. III-3 : Variation curve for MES concentrations during 2020 for the exensive lagooning system

2.Heavy metal results :

We are presenting the initial results of a preliminary study to evaluate the accumulation and purification of wastewater by the *Lemna gibba L.* results, grouped in Table 2, of analyses of some heavy metals and minerals present in the water, analysed at the Central Laboratory of ONEE's water quality control department, taken on 23/10/2020, which coincided with the period of confinement caused by covid 19. These element concentrations are presented in ppm and ppb.

Table 2 : Concentrations of chemical compounds in purified and raw water, analyzed by ICP-MS.

Sample collection	Concentrations of analyzed compounds in mg/L	
	In ppm	In ppb

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	Na	Ca	Mg	K	Si	Al	Fe	Ba	B	Zn	Mn	Cu	Cd
Lagoon input	**171,83**	**45,73**	**20,14**	**3,80**	**2,31**	**51,75**	**59,16**	**53,58**	**38,62**	**13,19**	**5,28**	**0,52**	**0,14**
Lagoon output	**103,14**	**46,83**	**19,61**	**4,62**	**2,27**	**52,42**	**46,50**	**47,30**	**38,04**	**33,45**	**53,48**	**0,54**	**0,13**

The table below (Table 3) lists some of the analysed compounds present in the *Lemna gibba* plant after mineralization of the vegetal material carried out at the (Centre National pour la Recherche Scientifique et Technique), i.e. the plant sample was collected during the same time period as the water sample.

Table 3 : Concentrations of accumulated chemical compounds in Lemna gibba, analyzed by ICP.

CNRST Division UATRS	Rapport d'Analyse ICP AES	PQ-006-008 Page 2/2
Angle Avenue Allal Fassi et Avenue des FAR, Hay Riad, B.P. 8027, 10102 Rabat, MAROC Tél. : +212 (0) 5 37 56 98 75 / 76 / 77 - Fax : +212 (0) 5 37 56 98 25 / 26 E-mail : uatrs@cnrst.ma - Site web : https://gda.cnrst.ma		Rapport N° : 7 Dossier N°: D98U2019

	Zn (mg/l)	Cd (mg/l)	Pb (mg/l)	Fe (mg/l)	Cr (mg/l)	Cu (mg/l)	Mg (mg/l)	Ca (mg/l)	Na (mg/l)	K (mg/l)
1	2.404	0.0053	<0.001	4.988	0.0748	0.103	49.89	167.41	91.905	356.39
2	1.63	0.0018	0.052	4.393	0.0613	0.0806	52.604	301.633	167.22	323.344
3	1.4859	0.00236	0.0037	3.986	0.05615	0.0827	48.947	269.298	165.126	314.732
4	1.76	0.0085	0.003	4.369	0.0692	0.0818	49.369	281.798	163.056	316.68

Comparing these two results (Tables 2 and 3) reveals that duckweed in general, especially Lemna gibba, is a bio-accumulative plant for nutrients, minerals and heavy metals in its natural environment [16]. In spite of the present state of trace amounts of some heavy metals in the water, the plant has been able to accumulate and conserve these compounds. These findings support the studies reported in the literature concerning the accumulation of heavy metals by the genus Lemna, as described by Jain and Ater [17] and [18]. For this reason, duckweed is recommended for wastewater treatment in the aforementioned first instance. This tolerance to accumulation is further evidenced by the ability to extract nutrients or heavy metals from the water through the roots (an excellent medium for filtration/absorption of suspended solids) and store them within the plant body. Duckweeds use atmospheric oxygen and carbon dioxide through photosynthesis, providing microorganisms with a means of degrading organic substances, and can also use their leaves to nitrify ammoniacal nitrogen.

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The nutrient content of the growing conditions is an important parameter influencing both the growth and composition of duckweed, which is made up of 94% water and 6% “dry” plant matter.

IV. Conclusion :

The aim of these preliminary analyses is to provide an illustration of the purification efficiency of *Lemna gibba* by measuring its capacity to remove some of the heavy metals and minerals from wastewater. This investigation was carried out in the context of the worldwide Covid 19 situation. It was therefore concluded that even in a state of traces of some minerals and heavy metals, this plant is able to strongly accumulate these extremely important compounds.

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