International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Influence of climatic conditions and aquatic environment on the growth of *Lemna gibba* in the pilot lagoon treatment plant of Bouregreg RABAT-Morocco

FARAH GOURCHANE [a,c] *, NAIF Al-JADABI [b,c], LOUBNA BENRAHMANE [c], MOHAMED El ALAMI [c], ABDLHAKIM BOUYAHYA[d], MOHAMED LAAOUAN [c], ABDERRAHIM EL HOURCH [a]

[a] Laboratory of Materials, Nanotechnologies and Environment, Department of Chemistry, Faculty of Sciences, Mohammed V University in Rabat, 4Avenue Ibn Battouta, B.P. 1014, Rabat10000, Morocco.

[b] Laboratory of Spectroscopy, Molecular Modeling, Materials, Nanomaterials, Water and Environment (LS3MN2E-CERNE2D), Department of Chemistry, Faculty of Sciences, Mohammed V University in Rabat, Avenue Ibn Battouta, B.P. 1014, Rabat10000, Morocco.

[c] National office of Electricity and Potable Water (ONEE-Branche Eau), Rabat, Morocco.

[d] Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.

Abstract

Duckweeds, *Lemna gibba* in particular, are used in Morocco as a purification additive in some extensive purification processes, for its purification efficiency and its capacity to accumulate heavy metals and different organic pollutants in wastewater. This floating aquatic plant, considered among the most used macrophytes for its easy maintenance and disposal due to its small size. In this work we want to answer the worrying problem of the vegetal carpet formation by the duckweed in the lagoons of the pilot station of Bouregreg, Rabat-Morocco, by following the evolution's growth of this species and noting the influence of the physico-chemical parameters of the aquatic environment on this growth. During the period from February to July 2020, we followed the evolution of *Lemna gibba* in two lagoons maturation ponds, with and without aeration, noting the temperature of the air and water, the pH, the conductivity and the level of dissolved oxygen. We have found that *Lemna gibba* during the winter period takes up to 35 days to completely fill a one cubic meter pond naturally, and 30 days for an additionally aerated pond. On the other hand, during the spring period, this species reaches 80% in the two ponds (ventilated and no-ventilated) for 5 days only and completely filled 15 days later, under the influence of the high temperature (37 °C). This significant growth in biomass forms a vegetation carpet. This problem can be avoided if the parameters and growth period of *Lemna gibba* are well known, as well as the adequate aquatic environment for its reception.

Keywords: wastewater, lagooning, macrophytes, *Lemna gibba*, ImageJ image processing software

Full length article **Corresponding Author*, e-mail: farah_gourchane@um5.ac.ma

1. Introduction

In the face of climate change in the Mediterranean region, water resources have been the struggle of these countries for decades. For this reason, Morocco has given a major priority to the water sector. On the one hand, it has been able to introduce sanitation into its culture as an essential step in the water cycle. On the other hand, it is planning to be among the African countries that will soon resort to the reuse of wastewater after treatment as a sustainable alternative source [1,2]. The treatment of wastewater by lagooning is a technology adapted to socio-economic conditions and to the African climate, because it is a classic treatment and a less expensive method [3]. Lagooning can be microphyte-based, i.e. the purification is done naturally by microbial activity in exchange with the atmosphere, in shallower ponds. To optimize the residence time, aerators can be added to stir the air. For an efficient performance, aquatic plants can be added to purify the water; this is a macrophyte lagoon [4].

These macrophytes are generally classified into three types: emergent, submerged and floating [5]. In this study we are particularly interested in floating plants, more precisely duckweeds. The latter belong to the Lemnaceae family, which has about 40 species worldwide, divided into 4 genera: *Spirodela, Lemna, Wolffiella and Wolffia* [6]. These asexual Lemnaceae occur by budding or colonies, each mother frond produces during its lifetime about 10 generations of daughter fronds from two pockets on each side of the frond tip, and each daughter frond becomes a mother by detaching from its own mother. [7]. *Lemna Gibba* was chosen as the most suitable species for the Mediterranean climate [8] because of its great capacity to accumulate chemicals, especially heavy metals [9-13], in the aquatic environment through the lower part of the frond and store them in its tissues [13,14]. It is chosen as a promising source of protein [15] for future generations.

This study consists of evaluating the behaviour of the species *Lemna gibba* with respect to the different physico-chemical parameters of the environment and to give an idea of its development within the lagoons.

2. Materials and methods

2.1. Location of the study site:

The *Lemna gibba* duckweeds studied are present in the aerated lagoons of the pilot wastewater treatment plant of the Bouregreg complex located at (33°56'33.6"N 6°48'00.5"W) upstream of the Bouregreg valley and downstream of the large reservoir of the Sidi Mohamed Ben Abdallah dam (Fig.1) at the International Institute of Water and Sanitation at the National Office for Electricity and Drinking water (ONEE- Branche EAU), in the city of Rabat in the north-west of Morocco. This experimental pilot treatment plant is the subject of several research and development activities and is designed to treat domestic wastewater (offices and restaurant).

2.2. Experimental study of the growth of Lemna gibba under natural conditions:

The experimental part of the study was between February and July 2020 despite the disruption caused by the covid-19 pandemic. The experiment consists of studying the growth of *Lemna gibba* in lagoons under natural conditions. In two aerated and non-aerated maturation ponds, two tanks (ponds) of one square metre in size and one cubic meter in volume each were set up (Fig. 2). Growth monitoring was carried out by image processing software [16,17] using ImageJ-Win64 (ImageJ software for multidimensional image processing and analysis). The monitoring by taking digital photos was every 5 days according to the retention time of the plant [18]. These photos are taken by a smartphone of the brand Huawei Honor 8X with photo resolution (20Mpx 5120x3840(4:3)) by simultaneously noting once or twice a week the influence of climatic conditions and physico-chemical parameters of the aquatic environment (water and air temperatures, pH, dissolved oxygen level, conductivity) [7,8,18] on the growth of *Lemna gibba*. The tests were carried out in mostly sunny weather throughout the study period. The pH, conductivity and temperature of the water were measured on site with a multimeter (Multi 3430 SET G, 2FD47G made in Germany), dissolved oxygen was measured with an oximeter (YSI 52 DISSOLVED OXYGEN METER) and the air temperature was measured with a laser thermometer (C.A 871 Infrared Thermometer) at midday in the shade.

2.3. Study of the growth of Lemna gibba by image processing:

The pictures of duckweed *Lemna gibba* taken every 5 days in the aerated and non-aerated lagoons are processed by ImageJ software. The instrumentation of this software made it possible to estimate the approximate percentage of duckweed growth during the winter and spring period (vegetation period) [19]. The calculation of the surface area is given in pixels based only on the green colour of the duckweed and determining the mean, min and max value (statistical processing of the data) by using half area of the pond.

The growth percentage of *Lemna gibba* is given by the formula below:

% Growth: $C = \frac{A}{A\ total} \times 100$ [17,20]

A: area occupied by the plant in half a pond in pixels

A total: total area of the plant in half a pond in pixels

C: calculated growth percentage

On the result of this, the growth graph was drawn: the relationship between the growth period (in days) and the growth percentage.

3. Results and discussion

The duckweed present in the station and being observed between February and July 2020 in natural conditions of temperature and sunlight (table 1) has revealed a very important growing behavior with respect to the measured physico-chemical parameters (fig. 3). The above measured temperature and pH values (Table 1) are similar to the growing conditions of duckweed identified by WILLIAM S. HILLMAN mentioning that Lemnaceae reproduce in a pH up to 8.5 and a temperature between 20 and 30 °C [7]. In addition, the influence of temperature, light and running water remain essential for gibbous production in *Lemna gibba* [21]. The growth evolution of duckweed was processed by ImageJ software. However, the performance of this software is limited because it treats the images on a colour basis only.

Fig. 1: Geographical location of the study site

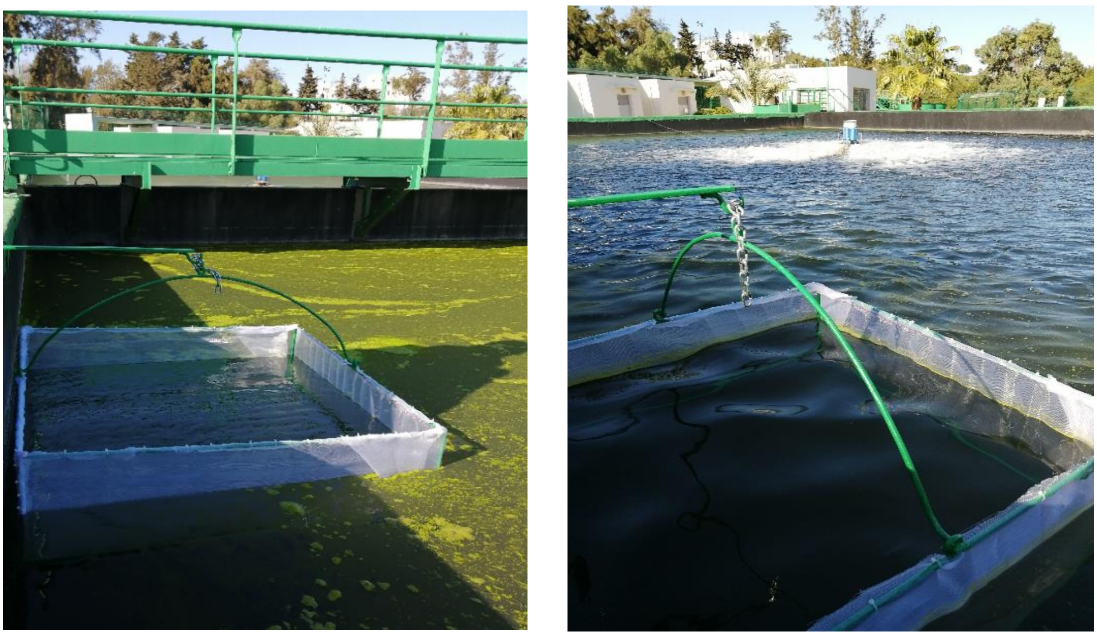

Fig. 2: The set-up ponds in the aerated and non-aerated lagoons of the pilot lagoon plant

Table 1. Water physico-chemical parameters measured in the aerated and non-aerated maturation pond

Physico-chemical parameters	Non-aerated lagoon	Aerated lagoon
Water's temperature in (°C)	20.73 ± 6.45	21.03 ± 5.70
Air's temperature in (°C)	20.77 ± 12.65	20.77 ± 12.65
pH	8.14 ± 0.86	8.56 ± 1.37
Dissolved oxygen levels in (mg/L)	3.46 ± 2.94	6.39 ± 4.18
Conductivity in (µs/cm)	95.93 ± 242.50	1058.60 ± 218.50

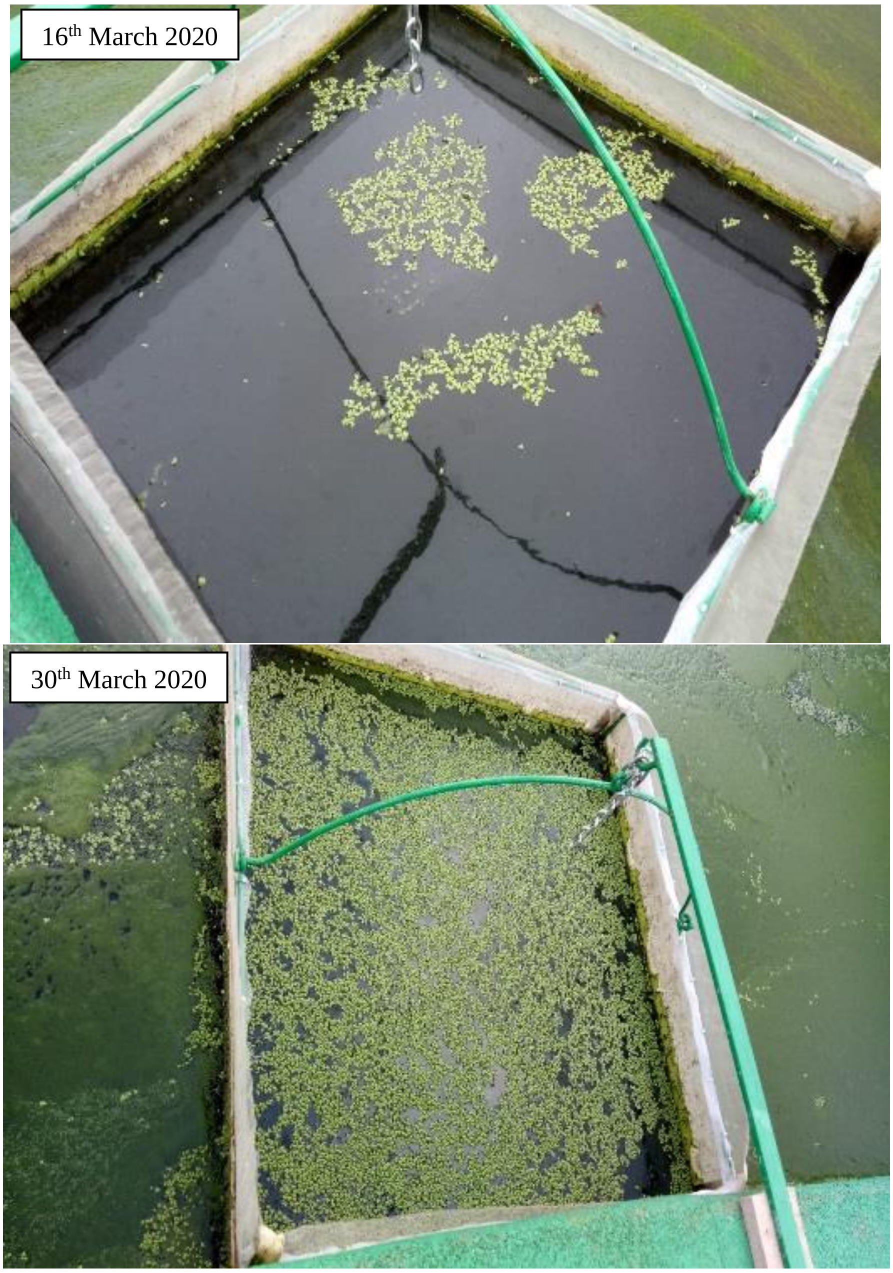

Fig. 3: The growth evolution's pictures of *Lemna gibba* in the aeration pond during 14 days.

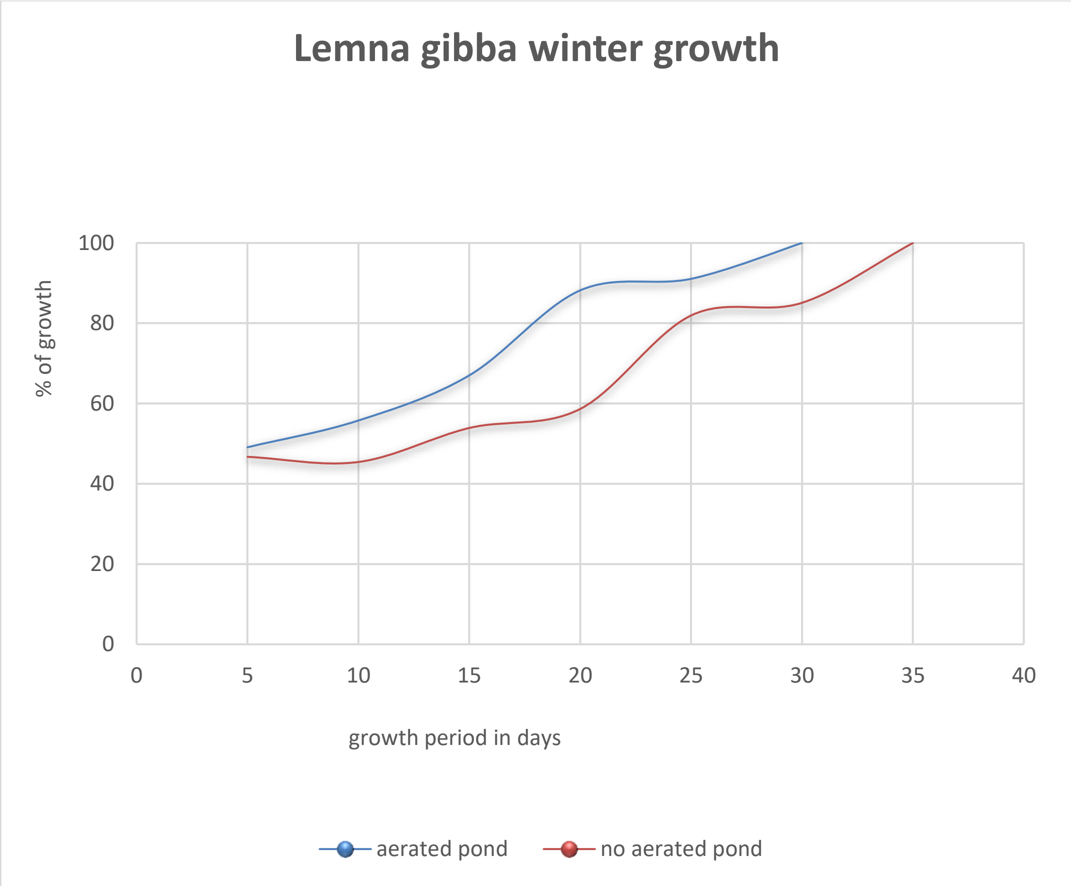

Fig. 4: growth evolution graphs of Lemna gibba in aeration and no aeration ponds in the winter

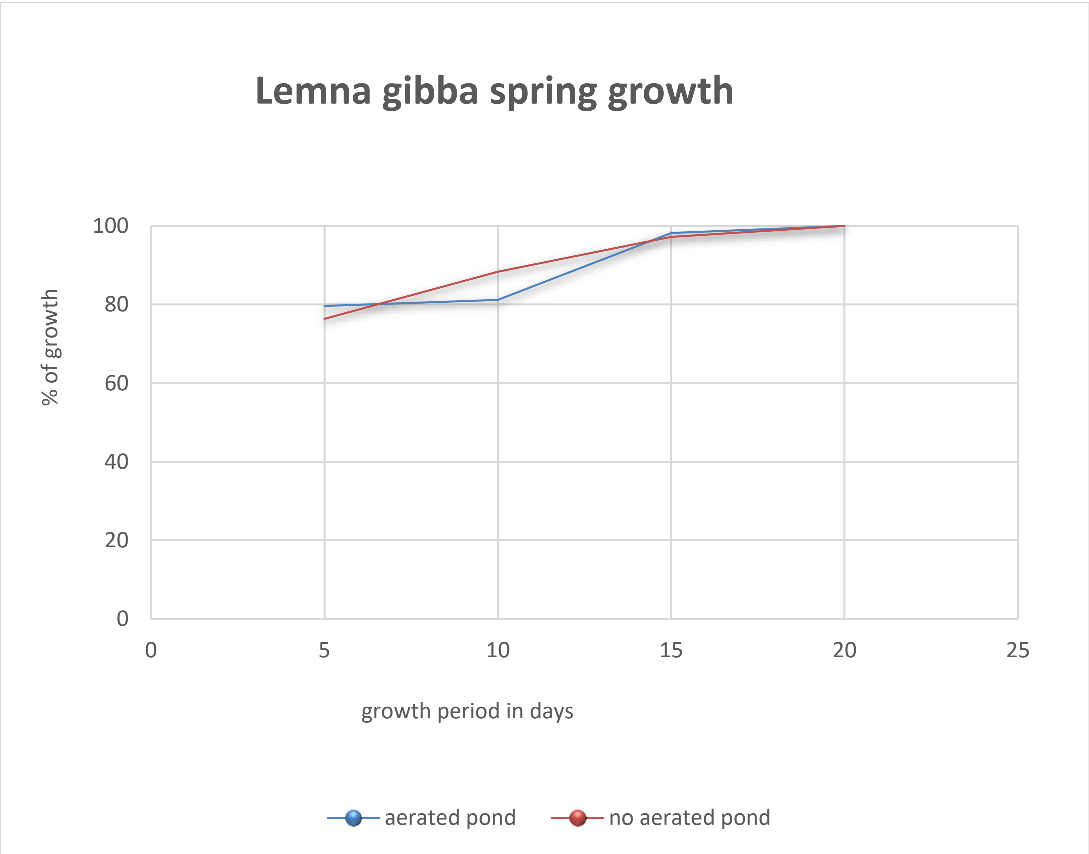

Fig.5: growth evolution graphs of *Lemna gibba* in aeration and no aeration ponds in the spring

During the first 5 days the area occupied by duckweed was not estimated because of its small size, which left a strong uncertainty about the area occupied by duckweed and wastewater. After 5 days the biomass of *Lemna gibba* became significant, the software was able to detect the colour of the duckweed and make a distinction between it and the wastewater. During the winter period *Lemna gibba* in the aeration pond were 49.1% during the first 5 days, those in the pond without aeration were 46.71%. After 20 days, the area occupied by duckweed in the aeration tank had reached 88.19% of the total area, contrary to the other which was just 58.64% of the total area.10 days later, the aeration tank was totally occupied by duckweed, the one without aeration required 35 days in total to achieve 100% (fig. 4). This means that the aeration or the additional quantity of dissolved oxygen in the water helped to minimize the time's duration of growth of *Lemna gibba* despite the low temperature and the winter low sunlight intensity.

The high spring temperature and sunlight intensity had a significant effect on the growth of the lentils. Within 5 days the area occupied by *Lemna gibba* reached 79.64% in the aerated pond and 76.36% in the non-aerated pond. 5 days later the non-aerated pond quickly became 88.37% (fig.5). For 20 days both ponds were completely full of duckweed. It is clear that the high air temperature (37°C) has a more significant influence on the growth of the duckweed than the additional aeration. It can be concluded that during the spring season the growth duration is limited to 20 days, but the winter season was 30 to 35 days.

This growth monitoring has given us an idea of the behaviour of this aquatic plant towards the environment in which it may multiply naturally, and this has helped us to determine the temperature factor as a key element for its development.

4. Conclusions

Lemna gibba, the duckweed most recommended for the Mediterranean climate, for its extraordinary purifying capacity can be another element of pollution if it is not looked after in the best conditions. This small floating aquatic plant can form a carpet of invasive duckweed on the water surface. According to the study conducted in the pilot plant of the aerated lagoon, the growth conditions of *Lemna gibba* were checked, and it was found that *Lemna gibba* during winter could reach 100% growth in 35 days and only 20 days in spring. For this reason, we recommend using it in no-aerated lagoons in the coldest cities of Morocco, in order to facilitate its maintenance and reduce its proliferation.

References

[1] E. ZEINO-MAHMALAT, A. BENNIS. (2012). Environnement et Changement Climatique au Maroc Diagnostic et Perspectives. Konrad-Adenauer-Stiftung E.V., Bureau Au Maroc Association RIBAT Al Fath Pour le Développement Durable. 1-90.

[2] M. Bzioui. (2004) Rapport national 2004 sur les ressources en eau au Maroc.94.

[3] M. SEIDL et J. M. MOUCHEL. (2003). Valorisation des eaux usées par lagunage dans les pays en voie de développement Bilan et enseignements pour une intégration socio-économique viable. Rapport final Action A10 du programme gestion durable des déchets et de l'assainissement urbain. 1-40.

[4] M. A. MOREL, M. KANE. (1998). Le lagunage à macrophytes, une technique permettant l'épuration des eaux usées pour son recyclage et de multiples valorisations de la biomasse. Sud-Sciences et Technologies., (1) 5-16.

[5] C. Boutin et A. Dutartre. (2014). Note. Des macrophytes pour épurer les eaux. Sciences Eaux & Territoires. (15). 70-73.

[6] W. Wang. (1990). Literature Review on Duckweed Toxicity Testing. Environmental Research., 52 (1) 7-22.

[7] W. S. Hillman.(1961). The Lemnaceae, or duckweeds a review of the descriptive and experimental literature. Botanical Review., 27 (2) 221-287.

[8] A. Ennabili, J. Ezzahri, et M. Radoux. (2019). Performance of Lemna gibba bioreactor for nitrogen and phosphorus retention, and biomass production in Mediterranean climate.
Journal of Environnemental Management. 252.1-9.

[9] M. Ater, N. A. Ali, et H. Kasmi. (2006). Tolérance et accumulation du cuivre et du chrome chez deux espèces de lentilles d'eau : Lemna minor L. et Lemna gibba L. Revue des Sciences de l'eau. 19 (1) 57-67.

[10] L. Weltje, A. H. Brouwer, T. G. Verburg, H. Th. Wolterbeek, et J. J. M. de Goeij. (2002). accumulation and elimination of Lanthanum by duckweed (Lemna Minor L) as influenced by organism growth and Lanthanum sorption to glass. Environmental Toxicology and Chemistry. 21 (7). 1483-1489.

[11] S. K. Jain, G. S. Gujral, N. K. Jha, et P. Vasudevan. (1988). Heavy Metal Uptake by Pleurotus sajor-caju from Metal-Enriched Duckweed Substrate. Biological Wastes. 24 (4) 275-282.

[12] R. Upadhyay et S. K. Panda. (2010). Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed Spirodela polyrhiza L. Journal of Hazardous Materials. 175 (1-3) 1081-1084.

[13] C. L. Duxbury, D. G. Dixon, et B. M. Greenberg. (1997). Effects of simulated solar radiation on the

bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed Lemna Gibba. Environmental Toxicology and Chemistry. 16 (8). 1739-1748.
[14] J. J. Cheng et A.-M. Stomp. (2009). Growing Duckweed to Recover Nutrients from Wastewaters and for Production of Fuel Ethanol and Animal Feed. CLEAN journal- Soil Air Water.37 (1) 17-26.
[15] M. F. A. de Beukelaar, G. G. Zeinstra, J. J. Mes, et A. R. H. Fischer. (2019). Duckweed as human food. The influence of meal context and information on duckweed acceptability of Dutch consumers. Food Quality and Preference.71 76-86.
[16] T. T. Tabou, D. T. Baya, M. N. D. Liady, D. M. Eyul'Anki, et J.-L. Vasel.(2014). Apport du traitement d'images dans le suivi de l'influence des teneurs en nutriments sur la croissance des lentilles d'eau (Lemna minor). Biotechnologie Agronomie Société et Environnement., 12.
[17] T. Kuznetsova, N. Politaeva, Y. Smyatskaya, et A. Ivanova. (2019). Lemna Minor Cultivation for Biofuel Production. IOP Conference Series: Earth and Environmental Science. 272, 1-8.
[18] G. Oron, D. Porath, et H. Jansen.(1987). Performance of the Duckweed Species Lemna gibba on Municipal Wastewater for Effluent Renovation and Protein Production. Biotechnology and Bioengineering. 29 (2)258-268.
[19] M. Radoux et D. Kemp. (2005). Rôle de la fréquence des prélèvements de la biomasse produite sur les capacités épuratrices de Lemna minor L., Revue des Sciences de l'eau, 5 (1) 55-68.
[20] T. Kuznetsova et al. (2019). Lemna minor cultivation in the climatic conditions of Saint Petersburg.IOP Conference Series: Earth and Environmental Science. 3371-8.
[21] François Van Horen. (1869). Observations sur la physiologie des Lemnacées. Bulletin de la Société Royale de Botanique de Belgique. Van K. Bot. Ver.8(1) 76.