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STUDY ON THE EFFECTS OF PHYTOTOXICITY OF SOME XENOBIOTICS ON AQUATIC PLANTS

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ABSTRACT
The presence of toxic substances in wastewater and natural water resources, as well as in other environments, is an important ecotoxicological problem. Phytoremediation is an environmentally friendly, cost-effective technology that uses plants to remove, transform or stabilize a variety of contaminants located in water, sediment or soil. Aquatic macrophytes have been widely used to remedy pollutants in wastewater and aquatic environment effluents in the last 20 years, mainly using plants of the Lemnaceae family, the genus Lemna being considered to have some of the most effective macrophytes that have been applied for remediation studies. These plants and the highlighting of their testing capacity constitute the main interest of the paper, the highlighting being made by looking for the most relevant information about the evaluated parameters, tested substances and their effects. This paper is based on the study of the aquatic phytoremediation potential of the floating macrophytes genus Lemna, based on statistics applied to specialized articles resulting from the introduction of keywords in the Google Scholar search engine. The search covered data in the form of articles describing Lemna's ability to remove naturally occurring pollutants, classified into review or research articles. Scores were made according to the relevance of the parameters influenced in the phytoremediation processes and also the contaminants tested were classified according to the nature of the substance. Following the results, Lemna showed, as expected, strong potential for phytoremediation of heavy metals, agrochemicals, pharmaceuticals and personal care products, nanoparticles and synthetic dyes. The morphological and physiological peculiarities, but especially the sensitivity of this macrophyte make it an aquatic model for testing toxicity.
KEY WORDS*:* *phytoremediation, Lemna, chemical pollutants, phytotoxicity, xenobiotics.*

INTRODUCTION

The presence of toxic substances in wastewater and natural water resources, as well as in other environments, is an important ecotoxicological problem. Water quality is subjected to natural degradation, eutrophication processes and the impact of anthropogenic activities. Natural or man-made wastewater and surface water may be unsuitable for consumption and irrigation, or for the health and proliferation of naturally occurring freshwater organisms, precisely because of the presence of excessive amounts of macronutrients and toxic heavy metals, as well as the presence of organic xenobiotic compounds. Phytoremediation is one of the serious efforts for sustainability. Phytoremediation is an emerging, environmentally friendly and cost-effective technology that uses plants to remove, transform or stabilize a variety of contaminants located in water, sediment or soil. Both terrestrial and aquatic plant species have been intensively exploited for application in phytoremediation technology, as they have a huge potential for detoxification, degradation and / or elimination of environmental contaminants. The main categories of phytoremediation include phytoextraction, phytotransformation / phytodegradation, phytostabilization, phytovolatilization and rhizofiltration (Alexan & Ianovici, 2018; Luchian et al, 2019; Datcu et al, 2020; Boboescu et al, 2020; Ianovici et al, 2020).

Macrophyte-based wastewater treatment systems have several potential advantages compared to conventional treatment systems. Free-floating plants are partially superior to submerged aquatic macrophytes, as their removal does not require any complex filtration equipment nor do they cause significant disturbances to water accumulation (Priya et al. 2011).

Aquatic macrophytes have been widely used to remedy pollutants in wastewater and aquatic effluents over the past 20 years, mainly using plants of the Lemnaceae family, with the "common" representative, *Lemna minor*, one of the most effective macrophytes that have been applied for remedial studies. *Lemna* has shown strong potential for phytoremediation of organic pollutants, heavy metals, agrochemicals, pharmaceuticals and personal care products, radioactive waste, nanoparticles, dyes, toxins and associated pollutants.

Aquatic macrophytes, such as those mentioned above, are becoming increasingly popular because they have rapid growth rates and achieve high levels of nutrient elimination. Moreover, they can be harvested easily, are relatively tolerant to low temperatures and appear to suppress algae growth (Priya et al. 2011). Wastewater treatment systems using *Lemna* have been studied for effluents with pollutants from the dairy industry, raw and diluted sewage, treated wastewater, ponds polluted with sewage water and ponds dedicated to fish farming.

The morphological and physiological characteristics of the Lemnaceae family are well known because they have been intensively researched. Their small size and rapid reproduction, predominantly vegetative, forming genetically uniform clones, make them valuable research bodies for studies in plant physiology, genetics, ecology and environmental monitoring.

Lemna species are used as test organisms for water quality assessment as well as for ecotoxicological studies on the adverse effects of xenobiotics due to their high sensitivity to organic and inorganic substances. Internationally regulated protocols and guidelines have been designed and improved for water quality testing or testing of chemicals: ISO 20079: 2005 Water quality - determination of the toxic effect of water and wastewater constituents in *Lemna* (*Lemna minor*) - inhibition test and the OECD Test Guideline no. 221, *Lemna* (*L. minor* and *L. gibba*) growth inhibition test, 2006.

MATERIAL AND METHODS

In order to highlight the role and importance of the use of the floating *Lemna* macrophyte in the phytotoxicity tests of the aquatic environments in which it is found and the harmful compounds in them, a search was made for scientific articles on this topic, following several points of interest, such as the relevance of the articles, morphological and physiological parameters investigated in *Lemna* plants in standardized and non-standardized tests, as well as the nature of the tested contaminants. Thus, keywords were entered in the Google Scholar search engine, choosing a specific interval of time: from 2000 to the present, in 2020 (Fig. 1).

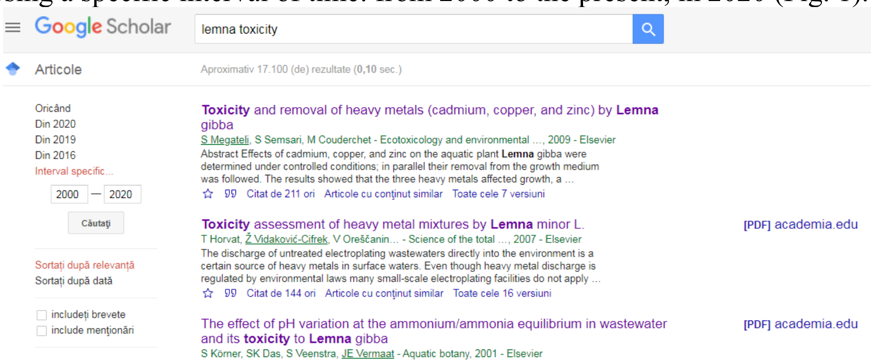

FIG. 1. Search for scientific articles based on keywords in Google Scholar (Personal Print Screen).

The first area of interest was the type of article showing the use of *Lemna* to establish the presence of toxicity. The aim was to make a comparison between the number of review articles, which merge complex information on the subject, investigating, in turn, a multitude of articles written over many years of research and research articles, which tests and demonstrates new hypotheses related to the subject, through well-studied protocols and optimized by previous attempts. For this comparison, were used the key formulations: "*Lemna* toxicity review" and "*Lemna* toxicity xenobiotics review" for the review type articles, while the research ones were searched using the words "*Lemna* toxicity research" and "*Lemna* toxicity xenobiotics research." The word "xenobiotics" has been added to restrict the number of results, for greater relevance of the information.

The second category of classification of the articles was made based on the parameter on which the testing was focused, a parameter which was followed by morphological or physiological changes when applying the contaminants to be tested. We looked for all the articles that considered the punctual investigation of the parameters using the words "*Lemna* toxicity parameters", after which, the search for each parameter was individualized, to establish their importance and preference for observing some parameters after exposure to contaminants more often than others. Therefore, articles were searched for: *Lemna* growth rate toxicity, *Lemna* fronds number toxicity, *Lemna* fronds area toxicity, Biomass resulting from the accumulation of contaminants ("*Lemna* biomass content toxicity"), the amount of chlorophyll affected by exposure to toxicity ("*Lemna* chlorophyll content toxicity"), the amount of carotenoid pigments ("*Lemna* carotenoids toxicity"), the length of the root ("*Lemna* root length toxicity"), the fresh mass of the plant ("*Lemna* wet mass toxicity") and the dry mass ("*Lemna* dry mass toxicity").

The last category of searches took into account the nature of the substance whose toxicity was tested using *Lemna*. Due to the widespread use of heavy metals in aquatic environments and the popularity of *Lemna* as their bioaccumulator, we started with the keywords "*Lemna* toxicity heavy metals". The individualization of the search for the bioaccumulation of each metal was continued, in order to compare the results and their action. The following structures were used: "*Lemna* copper bioaccumulation", "*Lemna* cadmium bioaccumulation", "*Lemna* zinc bioaccumulation", "*Lemna* nickel bioaccumulation", "*Lemna* lead bioaccumulation" and "*Lemna* mercury bioaccumulation".

To the classification according to the nature of the test substance were added searches of the articles in which nanoparticles are used in researching their effects on *Lemna*: "*Lemna* nanoparticles toxicity". After the general search, the influence of silver nanoparticles toxicity was emphasized, as well as that of oxides formed with metals such as copper, aluminum and zinc: "*Lemna* copper oxide nanoparticles toxicity", "*Lemna* aluminum oxide nanoparticles toxicity", respectively „*Lemna* zinc oxide nanoparticles toxicity".

Also of interest were substances such as synthetic dyes, pharmaceuticals and cosmetics. "*Lemna* synthetic dyes toxicity" were the keywords for searching synthetic dyes in the manufacturing industries, the pharmaceutical products being highlighted by the structure "*Lemna* pharmaceuticals toxicity", insisting on searching for tests that use antibiotics on aquatic macrophytes ("*Lemna* antibiotics toxicity"). For cosmetics were introduced the keywords "*Lemna* cosmetics toxicity".

It was wanted to obtain information on the influence of phytotoxicity tests as well for chemical contaminants used in the agricultural industry. The number of articles looking at the action of the presence of pesticides in *Lemna* test environments was searched by introducing the words "*Lemna* pesticides toxicity".

Depending on the class of pests that the substances attack, articles on the toxicity of herbicides ("*Lemna* herbicides toxicity"), fungicides ("*Lemna* fungicides toxicity") and insecticides ("*Lemna* insecticides toxicity ") were searched.

RESULTS AND DISCUSSIONS

1. Relevance of articles. By entering the keywords in the Google Scholar search engine to separate the review articles from the research ones, between 2000 and June 2020, the following results were obtained (See chart 1):

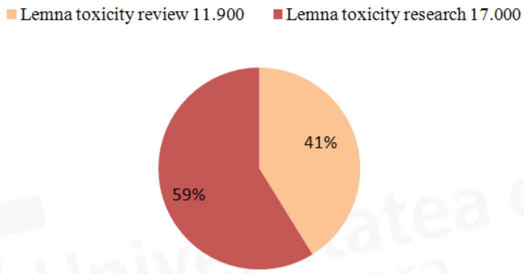

CHART 1. Percentage comparison between the number of review articles and research articles.

Given that the research articles outnumber the reviews on testing for toxicity using *Lemna*, there is a real interest in improving, to the point of perfecting, the working methods used in tests for phytotoxicity. Also, research papers are more numerous due to the variety of contaminants that can be tested through this aquatic macrophyte and the numerous attempts to test as many substances as possible, phytoremediation being a reliable method for sustainability.

The addition of the keyword "xenobiotics" to this comparative search significantly reduced the number of results recorded, from 3,620 for "*Lemna* toxicity xenobiotics research" to 3,120 using "*Lemna* toxicity xenobiotics review". In this case, the relevance of the content of the articles on the treatment of toxicity caused by xenobiotics increased, but the research articles remained more numerous than the reviews. The difference between the two percentages narrowed, being 46% of the total results in the case of reviews and 54% allocated to research articles.

The importance of this macrophyte is reflected in the large number of articles describing the presence of toxicity and its improvement using Lemnaceae and it can be concluded that there is an increased interest in them, both in terms of reviews that accumulate information on phytoremediation processes and of ongoing research.

2. Parameters evaluated in phytotoxicity tests using *Lemna sp*. Knowledge of the most investigated parameters in such tests provides information on the most important morphological and physiological changes of aquatic macrophytes and indicates which organ or physiological process of the plant (or a combination of them for more accurate results) should be monitored to determine the sensitivity of macrophytes to contaminants. specific and establishing their exact effects in polluted environments. Also, the knowledge of these parameters allows future studies to improve the protocol and to focus on certain structural and functional changes of *Lemna*, which can also lead to the improvement of standardized tests.

The result of searching the total number of articles containing data on the parameters evaluated using the structure "*Lemna* toxicity parameters" was 15,800 articles, a number close even to the total number of research articles, which were 17,000. This considerable number reflects the great interest given to the changes in *Lemna* due to the toxicity to which it is subjected.

Regarding the individualization of the parameters and the scientific interest offered to each of them, the results from 2000 to the present have been the following (See chart 2):

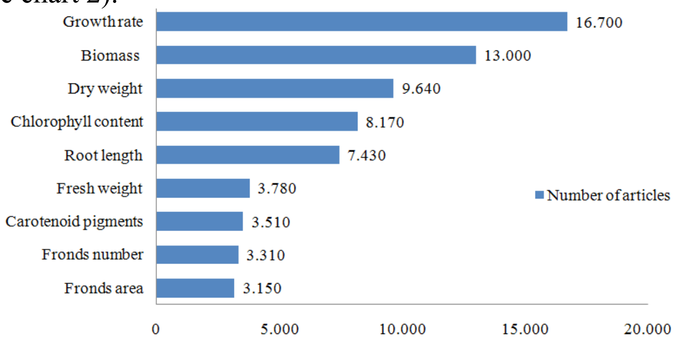

CHART 2. Search results according to the evaluated parameter.

Given the results obtained, the growth rate is the most monitored and evaluated parameter when it comes to the effects of contaminants on *Lemna*. Macrophyte growth rate is a sensitive parameter, easily influenced by fluctuations of the tested substances or their concentrations.

OECD standardized test no. 221, *Lemna* growth inhibition test (*L. minor and L. gibba*), from 2006, aims to inhibit growth, expressed as a logarithmic increase of the measurement variable (average specific growth rate) during the exposure period. (OECD Test Guideline No. 221, 2006).

In 2007, Drost and colleagues stated that testing the growth rate of *Lemna minor* allows for convenient studies on the time dependence of aquatic toxicity of chemicals, as it fluctuates as soon as the concentrations of environmental contaminants change.

Mitsou et al. (2006) evaluated the effects of propanyl herbicide toxicity on *L. minor* L., based on a 24-day monitoring of inhibition of *Lemna* crop growth. The results obtained showed that the growth rate of *Lemna* was affected by the pesticide.

Biomass, present as a parameter analyzed in 13,000 scientific articles, is also related to the growth of *Lemna*, indicating the mass accumulated following the exposure to contaminants. This is an important parameter, because it expresses the phytoextraction capacity of the macrophyte and its measurement determines the bioaccumulation power that *Lemna sp*. for each substance tested. The accumulated biomass can also be used for bioenergy production (Wang et al. 2014). Wang et al. (2014) also investigated the effect of high NH_4^+ concentration on biomass accumulation of *Lemna minor*, using ammonium as the only source of nitrogen. It was found that the biomass production was inhibited by high concentrations of this compound, exposed for 7 days.

Rofkar et al. (2013) evaluated the tolerance of *Lemna minor* to arsenic, copper and silicon, simultaneously, by measuring biomass growth, but also focusing on another parameter very present in the search for scientific articles, the chlorophyll content (8,170 results). Copper had a negative impact on *L. minor*, leading to a 60% decrease in biomass production and a 45% reduction in chlorophyll content, but exposure to silicon attenuated the effect of copper.

Chlorophyll content is a parameter that reflects the potential to capture light and underlies the photosynthetic capacity of *Lemna* as a photoautotrophic organism. In 2015, Fekete-Kertész et al. examined the chlorophyll content in a series of contact experiments for 7 days. The duckweed was exposed to caffeine, benzophenone, bisphenol A, 3,4-dichlorophenol, metamizole sodium, diclofenac, acetochlor, atrazine, diuron, metazachlor and metolachlor to find a sensitive response to the chemicals tested. The diversity of substances tested (composed of pharmaceuticals, chemicals used in the materials industry and many pesticides) reflects the versatility of the sensitivity of this parameter. The chlorophyll content was most affected by the herbicides, most of which were inhibited by photosynthesis.

The relatively large difference between the number of articles focusing on the measurement of dry mass and that of fresh mass is due to a preference in finding dry mass, being considered more relevant in terms of substances accumulated in phytotoxicity tests. The dry or fresh weight must be determined at the beginning of the test from a sample of inoculation culture representative of what is used at the beginning of the test and at the end of the test with the plant material from each test and control vessel. If the surface of the fronds is not used as a relative parameter, the dry weight is preferred to the fresh one (OECD Test Guideline No. 221, 2006).

Gopalapillai et al. (2014) suggest that similar to terrestrial plants, the average root length (RL) of aquatic plants would be an optimal and relevant parameter. The results demonstrate that RL is the ideal assessment point based on 3 criteria: accuracy (toxicological sensitivity to contaminants), accuracy (varies slightly) and ecological relevance. The roots play a major role in the absorption of nutrients, including in low nutrient conditions, thus having ecological relevance for fresh water in polluted regions. The root length was the most sensitive and precise parameter of their study in which the water chemistry varied a lot, in order to match the effluent environments in the mining areas.

Parameters targeting changes to fronds, such as number, or surface area, are well integrated into most internationally regulated protocols, which explains the low number of new research articles on these morphological features. In most research articles it is possible to observe the simultaneous monitoring of several parameters, for an increased relevance of the results. The more sensitization of several biomarkers is observed, the more the level of efficiency of *Lemna* as a model plant can be established in phytotoxicity tests, the effects of contaminants being obvious. For example, Naumann et al (2007) used the test conditions of the ISO 20079 protocol to investigate the effect of 10 heavy metals. By using the growth rates derived from the number of fronds (FN), fresh weight (FW), dry weight (DW), chlorophyll content and carotenoids, classifications of the concentration-response curves for all heavy metals were performed, according to all parameters, which led to the detection of a series of phytotoxicity of the respective metals.

3. The nature of the tested substances and their influence on *Lemna sp*. Regarding the nature of the substance subjected to phytotoxicity tests to determine its influence on aquatic plants, *Lemna* is known for its potential to accumulate a wide variety of contaminants in its own tissues. In some cases, *L. minor* was more efficient than the normal wastewater treatment plant for remediation of municipal effluents (Ekperusi et al. 2019). The results of numerous scientific articles that consider the testing of several categories of contaminants with the help of *Lemna sp.* prove its capacity and can be seen in the following graph:

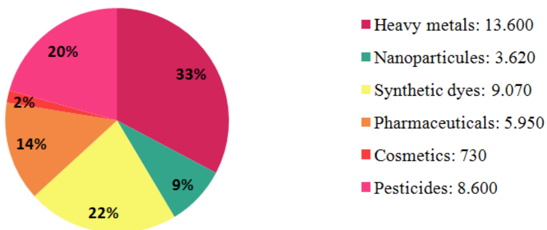

CHART 3. Search results according to the nature of the test substance.

3.1. Heavy metals. As expressed by the results of the bioaccumulation of heavy metals by *Lemna*, they form one of the largest categories of contaminants that are effectively eliminated by aquatic plants (Dhir et al. 2009). The potential of free floating plant species *Lemna* for the removal and accumulation of heavy metals has been intensively studied over the last 20 years.

Most authors test and describe the effects on *Lemna* of several toxic metals, in order to draw up a series of phytotoxicity depending on the substance. An example is the experiment carried out by Naumann and collaborators in 2007, where 10 heavy metals were tested under the standardized conditions of the ISO 20079 protocol. Based on the accumulated mass of each compound, the following series of phytotoxicity was detected:

Ag^{+}>Cd^{2+}>Hg^{+}>Ti^{+}>Cu^{2+}>Ni^{2+}>Zn^{2+}>Co^{2+}>Cr(VI)>As(III)>As(V).

Li and Xiong investigated the responses of *Lemna paucicostata* (wild type) to seven heavy metals, using a hydroponic culture. The results showed that six of the metals could induce the macrophyte to release daughter leaves from the mother frond before maturity, leading to colony rupture. This phenomenon is unique and the metals responsible for it were copper, cadmium, nickel, zinc, mercury and chromium, although the sensitivity and response of the plant varied depending on each compound.

Daud and colleagues (2018) investigated the phytoextraction capacity of *L. minor* for zinc, copper, lead, iron and nickel from waste leachate, every 3 days, for a period of 2 weeks. The results of this study showed that *L. minor* significantly reduced the concentration of heavy metals in the aquatic environment. The removal efficiency of *L. minor* for all metals in the waste leachate was higher than 70%, with the maximum value for copper (91%).

Khellaf and Zerdaoui (2009) evaluated the tolerance and effect of heavy metal pollution on *Lemna minor* species, exposing them to different concentrations of Cu, Ni, Cd and Zn, at pH = 6.1, daily, in the light for 16 hours. Cd and Zn decreased by 50% the fronds growth at high concentrations. The toxicity result depending on the level of damage was: Cu> Cd> Ni> Zn. It was concluded that *Lemna minor* is very sensitive to Cu and Cd pollution.

The effects of some compounds were tested to monitor their impact depending on environmental conditions. The boron metalloid (B) was tested on *Lemna minor* by Liu et al (2018) to evaluate the potential of the macrophyte to remove the compound under saline stress, which is often present in high salinity waters. The results suggested that *Lemna* is suitable for the accumulation of B when the NaCl concentration is below 100mM.

To associate the interest given to each contaminating metal with the number of studies that have been done to determine the capacity of *Lemna* to accumulate and eliminate it, or to simply observe its sensitivity to one of the compounds, the results of individual searches can be found in the graph below:

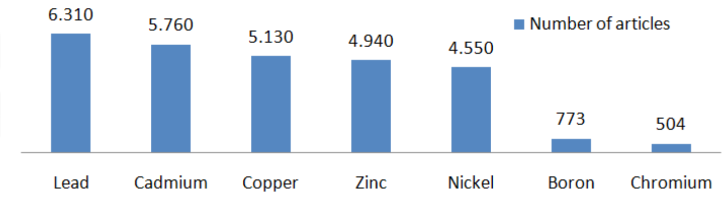

CHART 4. Search results for each metal.

The multiple suggestions of scientific articles offered by the search engine for each compound indicate the importance of testing contaminants by different methods, in different combinations and mixtures of heavy metals, but also individually, at different concentrations. Toxic metals and environmental stressors decreased the functional capacity of macrophytes when such stressors exceeded the tolerance threshold of plants.

The metals that produce the highest damage level of *Lemna* test plants are found in most articles (Pb, Cd, Cu), because testing their impact demonstrates the capacity and identity of *Lemna* as a bioaccumulative organism, a model for testing the toxicity of heavy metals in aquatic environments. Also, the results of the order of thousands for most of the sought metals (those considered the most relevant) show interest in testing them also for ecological reasons. The more toxic substances are present in the natural environment, the greater the need and desire to research ways to eliminate them, which is reflected in the continuous improvement of protocols.

3.2. Nanoparticules. Nanoparticles have many applications in modern society, especially in medicine, but also in industry, production, agriculture and environmental management (Ekperusi et al. 2019). Because their use has gained so much interest, the concern about the fate of nanomaterials in the environment has adapted to this interest quite recently, which is reflected in the low number of articles found in the last 20 years, compared to other classes of compounds. The narrowing of the search for articles in the last decade has shown an increase in interest in testing the toxicity of nanoparticles using *Lemna*: 3620 articles from 2000 to 2020, of which, 3050 only between 2010-2020.

Narrowing the search for the most tested nanoparticles, there was found interest in silver nanoparticles (2200 results for "*Lemna* silver nanoparticles toxicity") and for metal oxide nanoparticles such as Al, Cu and Zn: 1160 articles for "*Lemna* aluminum oxide nanoparticles toxicity", 2600 for "*Lemna* copper oxide nanoparticles toxicity", respectively 2240 for searching "*Lemna* zinc oxide nanoparticles toxicity".

In 2013, Oukarroum and co. tested the effect of silver nanoparticles toxicity on the growth and cell viability of the aquatic plant *L. gibba*, exposed for 7 days to increasing concentrations (0, 0.01, 0.1, 1 and 10 mg / L of AgNPs). Inhibition of growth was demonstrated by a significant decrease in the number of fronds, depending on the concentration of AgNPs. The authors' results suggested that the accumulation of AgNP in an aquatic environment may be a potential source of toxicity and a risk to the viability of *Lemna*. Also, Üçüncü et al. (2014) investigated the effect of laser-ablated silver nanoparticles on the development of *L. minor*. Different concentrations of AgNPs were tested for 7 days under simulated natural conditions. *L. minor* has proven to be a successful bioremediation agent for AgNPs and has shown higher removal rates even when AgNP doses were increased.

Cu (II) oxide nanoparticles are widely used and eventually reach a body of water through wastewater. Ecotoxicological studies on the effects of this type of NPs on hydrophytes are very limited in the present. *L. minor* was exposed by Song et al. (2016) in environments with different concentrations of CuONPs. The plant has accumulated many reactive oxygen species in this type of environment. The concentration of 50 mg / L of CuO nanoparticles in the culture media caused severe damage to *Lemna* plant cells. The toxicology of CuONP on aquatic macrophytes must be taken in consideration, as these are the basic elements of the aquatic ecosystem (Song et al. 2016).

In 2011, Juhel et al. found a stimulating effect regarding the testing of Al oxide nanoparticles on *L. minor* species, such an effect not being previously reported. In this study, the effects of aluminum oxide nanoparticles on macrophyte growth, morphology and photosynthesis were quantified. Al_2O_3 nanoparticles were found to substantially increase the biomass accumulation of *L. minor.* Improved biomass accumulation was supplemented with morphological adjustments, such as increased root length and number of fronds per colony and increased photosynthetic efficiency.

ZnO nanoparticles have been reported to be toxic to many aquatic organisms, although it is debated whether this is caused by nanoparticles per se, or rather by dissolved Zn (Chen et al. 2016). Chen et al (2016) investigated the role of dissolved Zn in the toxicity of zinc oxide nanoparticles for *Lemna minor*. The results showed a rapid and total dissolution of ZnO nanoparticles in the medium (pH 4.5). The conclusion was that the toxicity of nanoparticles is, in fact, caused by Zn dissolved in aquatic environments.

In a paper, Ergen and Tunca investigated the toxic effect of zinc oxide nanoparticles (ZnONPs) and analyzed their removal from the aqueous environment by the association between the crustacean *Daphnia magna* and *Lemna minor*. They observed that *Lemna* was more successful than *D. magna* in the process of removing ZnONP in high concentration and that the addition of *L. minor* in test groups containing only *D. magna* increased the efficiency of the elimination.

3.3. Synthetic dyes. Water pollution due to effluents from the textile dyeing industry is a matter of serious concern, which can be deduced from the result of 9070 scientific articles addressing this ecotoxicological problem. Dye detection techniques are expensive and useless, as dyes undergo chemical changes under environmental conditions, and transformation products can be more toxic and carcinogenic than the parent molecule. Therefore, instead of detecting each chemical individually, it is advisable to study the toxic effect of effluents on different living organisms (Ratna & Padhi, 2012).

The increased interest reflected in the number of research articles on the treatment of water polluted with synthetic dyes is due to the fact that colored industrial effluents, which have a significant impact on the environment due to the content of several pollutants are largely the result of dyeing processes (Uysal et al. 2014).The most important parameter of this wastewater is the color and there are a lot of physical and chemical methods to remove it from the wastewater. In a 2014 study, Uysal and colleagues investigated the potential of *L. minor* to remove dyes from industrial effluents. According to the results, the percentage of color removal from the laboratory system has continuously decreased over time.

Azo dyes are the largest class of synthetic dyes (Brüschweiler & Merlot, 2017) and are used in several industries. Imron et al (2019) investigated the removal of methylene blue dye (MB) using *L. minor*. *L. minor* (2 g) was exposed to 50 mg / L MB dye for 24 hours. Absorption values were measured at 0, 0.5, 1, 2, 3, 4, 5, 6 and 24 h with maximum at a wave length of 665 nm. It has been established that *L. minor* has the potential as a phytoremediation agent for the removal of dyes from wastewater.

Movafeghi et al. (2012) tested the potential offered by *Lemna minor* for the bioremediation of the azo dye "Basic Red 46" (BR46). The results revealed the remarkable ability of the plant to biodegrade BR46, however, dependent on various operational parameters, such as the initial dye concentration, pH, temperature and amount of the plant.

Balarak et al. monitored, in 2015, the use of a combination of *Lemna minor* together with algae to remove the dye "Acid Red 88" (AR88) from an aqueous solution. The application of *Lemna minor* removed 98% of AR88 from aqueous solutions, showing that this aquatic macrophyte can be used as a high-efficiency and low-cost adsorbent for the treatment of effluents from the textile industry.

3.4. Pharmaceuticals and cosmetics. Many pollutants are generated annually from the pharmaceutical and personal care industries to combat human disease and increase the well-being of society. Chemical waste generated by this industry, whose behavior in the environment is poorly understood, adds to the burden of pollution and degradation of water resources (Ekperusi et al. 2019). Several authors reported the significant removal of medicinal products from various effluents, the 5950 articles reflecting the importance of knowing the possibility of eliminating them with *Lemna sp*.

The results obtained by using the search engine, in terms of testing cosmetics, were significantly lower, the emphasis being on different drugs, personal care products being studied in the same class as them. Therefore, the individualized articles with the keywords "*Lemna* cosmetics toxicity" were only in the hundreds, with a percentage of 2% of the total resulting articles.

The fate of 4 antimicrobial substances (cefadroxil-CFD; trimethoprim-TRI; metronidazole-METRO and sulfamethoxazole-SMX) was studied in *Lemna minor* systems by Iatrou et al. (2017) and the role of different mechanisms in their elimination was evaluated. All micropollutants were significantly removed in the static tests, the highest removal was observed for CFD (100% in 14 days), followed by METRO (96%), SMX (73%) and TRI (59%) during 24 days of the experiment.

Also in 2017, Amy-Sagers and co-workers measured and detected sucralose (an artificial sweetener), fluoxetine (an antidepressant) and other pharmaceuticals and personal care products in the Portneuf River in Idaho, USA, where the *Lemna minor* species was also present. They performed laboratory ecotoxicological evaluations for a wide range of sucralose and fluoxetine concentrations on the physiology and photosynthetic function of *L. minor*. Sucralose significantly increased leaf area and photosynthetic capacity at environmentally relevant concentrations. Unlike humans, who cannot decompose and use sucralose, the authors documented that *L. minor* - being myxotrophic - can use sucralose as a sugar substitute to increase its leaf area and photosynthetic capacity. Fluoxetine significantly decreased root growth, daily growth rate and vegetative reproduction. This study supported the idea that *L. minor* may be useful in bioremediation of pharmaceuticals and cosmetics in wastewater.

Acetaminophen is, globally, one of the most prescribed drugs due to its antipyretic and analgesic properties (Nunes et al. 2014). However, it is very toxic when the dose exceeds the detoxification capacity of an exposed organism. To address the ecotoxicity of acetaminophen, Nunes and colleagues exposed *L.gibba* and *L.minor* species to this compound. The results showed big differences between the two species. Acetaminophen caused a significant decrease in the number of fronds in *L. minor*, but not in *L. gibba*. No effects were reported in both species for the parameters indicative of chlorophyll content and total biomass. The general conclusions indicated the appearance of an oxidative stress scenario for *L. minor*.

At the time of individualizing the search for articles using pharmaceuticals by the keywords "*Lemna* antibiotics toxicity", the result of ecotoxicological testing of antibiotics was a number of 4,070 articles, a number close to the total of those related to drugs in general. Complex studies have been conducted on this class of drugs widely used all over the world.

Brain et al. (2004) evaluated 25 pharmaceuticals, including 22 antibiotics, for phytotoxicity using *L. gibba*. A static renewal test was used for 7 days, and the plants were treated with 0, 10, 30, 100, 300 and 1,000 mg / L of growth media containing pharmaceuticals. 12 different classes of antibiotics were evaluated; however, only members of the antibiotic classes fluoroquinolone, sulfonamide, and tetracycline showed significant phytotoxicity. The most toxic members of each class tested were: lomefloxacin, sulfamethoxazole and chlorotetracycline. The symptoms of plant damage were relatively uniform and consistent between chemical classes, while the degree of phytotoxicity varied considerably.

In 2017, Aubakirova conducted a scientific study of the toxicity effect of five antibiotics: amoxicillin, clarithromycin, azithromycin, sulfamethoxazole and oxytetracycline hydrochloride, using *L. minor*. Overall, the results of the study showed that *Lemna* was sensitive to all selected compounds, although sulfamethoxazole was the most toxic to these species. The author considered the study significant because people consume antibiotics in huge quantities. Their occurrence in environmental factors has been highlighted worldwide, and their effects on the environment and living organisms are not yet fully studied. Recent studies show that, in most cases, they have adverse effects on aquatic organisms.

3.5. Pesticides. Once they serve their useful purpose in agriculture, agricultural chemicals add additional effects to environmental pollution. Every year, several tons of agrochemicals are produced, such as fertilizers, pesticides, herbicides and fungicides. A considerable amount of these chemicals applied to agricultural land and aquaculture eventually accumulate in the aquatic environment without treatment applied in advance. Searching for articles on "*Lemna* pesticides toxicity" gave a total of 8600 results regarding the use of pesticides in *Lemna* toxicity tests. The following results were obtained when classifying the substances according to the pests they eliminate: the highest number of articles, 6590, at the introduction of "*Lemna* herbicides toxicity" keywords, followed by 3550 results for insecticides - "*Lemna* insecticides toxicity" and finally 2570 results on fungicides - "*Lemna* fungicides toxicity". Frequent testing the effects of herbicides reflects their ability to inhibit physiological mechanisms and degrade the morphological features of other plants, increasing interest in their actions when it comes to a free floating plant in the aquatic environment, which is not the direct target of the pesticide but has to suffer due to the environment in which most plant protection products accumulate.

Burns et al. (2015) studied the potential of *L.minor* and *L. gibba* species to recover from a 7-day exposure to different concentrations of the herbicide diuron.

There was a significant inhibition of biomass production and growth after the initial 7-day exposure. After being transfered to a clean environment, recovery was possible for primary producers at environmentally relevant concentrations, considered significant in the ecological risk assessment.

Böttcher and Schroll (2007) examined the degradation, bioaccumulation and volatile loss of the herbicide isoproturon in a freshwater microbiome with the species *Lemna minor* during a 21-day exposure. Only a minor amount of herbicide was completely metabolized, probably by rhizosphere microorganisms and released as CO_2. In total, approximately 9% isoproturon was removed from the aquatic environment for 21 days. Most of the pesticide was eliminated by bioaccumulation by *L. minor*.

Dosnon-Olette et al. (2011) also focused on the toxicity and phytoremediation potential of aquatic plants to remove plant protection products from contaminated water. They investigated the ability of *L. minor* to remove isoproturon and glyphosate from their environment. After an incubation time of 4 days, the removal yields were 25% for isoproturon and 8% for glyphosate.

A comparison of a test performed exclusively in laboratory conditions and one in field conditions of *Lemna* treated with atrazine, indicated that none of the configurations reflected any difference in exposure toxicity and absorption to atrazine (Dalton et al. 2013). This was unexpected, as factors in the natural environment are expected to differ from a simulated configuration in a laboratory microcosm and should influence the results.

Duckweed can respond differently in the presence of two or more agrochemicals in the environment. Regarding fungicides, Megateli et al. (2013) reported different responses for the removal rate of copper and dimethomorph at the same concentration (1000 µg / L) on *L. minor.* The plant was more effective in reducing copper compared to dimethomorph. Both inhibited its growth, the dimetomorph degrading the plant extremely hard. The previous results are in line with those obtained by Olette et al. (2008) in a study where several fungicides were applied, the dimethomorph being found to be the most toxic, followed by copper sulfate and flazasulfuron.

Regarding insecticides, Souza et al. (2011) tested diflubenzuron. Parasitosis in fish farming is treated by applying pesticide formulas commonly used in agriculture or veterinary medicine. Diflubenzuron is used by direct application in water to control ectoparasites, which cause economic losses for fish production. However, this practice can intoxicate non-target species. This research aimed to determine the acute toxicity of diflubenzuron to *Daphnia magna*, *Poecilia reticulata* and *L. minor*. The results of the study showed diflubenzuron to be extremely toxic to *D. magna*. However, no significant toxicity was observed in *P.*

reticulata and *L. minor*. The bioavailability of this insecticide has been significantly reduced by the presence of sediments in the water.

The proposed models for the risk assessment of chemical mixtures do not involve interactions between chemicals. However, there are studies that indicate that some organophosphorus insecticides can inhibit the detoxification of other plant chemicals, thus enhancing their effect. Munkegaard et al. (2011) investigated whether interactions between organophosphorus insecticides and herbicides can occur in the aquatic algae *Pseudokirchneriella subcapitata* and in the aquatic macrophyte *L. minor*. For both species, binary mixtures of organophosphorus insecticides were tested: malathion, endosulfan and chlorpyrifos together with the herbicides metsulfuron-methyl, terbutyrazine and bentazone. Tests on *Lemna* did not show any indication of synergy for any of the combinations, on the contrary, significant antagonism was found for several of the mixtures. The study did not show any indication of synergistic interactions between the tested pesticides, which confirmed previously proposed models for chemical mixtures.

CONCLUSION

Lemna species are model organisms for water quality assessment, as well as for ecotoxicological studies on the adverse effects of xenobiotics in aquatic environments. Elimination of pollutants and remediation of aquatic contamination by *Lemna sp.* is reflected in the accumulation of biomass. The phytoaccumulation of toxins by *Lemna* in its own tissues may sometimes have no effect on the plant, but may inhibit its growth, and in some cases, may even increase the growth of certain parameters, depending on the compound and its concentration. The more parameters evaluated at the same time, the more relevant the results of the phytotoxicity tests. Due to their sensitivity to a wide variety of compounds (heavy metals, nanoparticles, pharmaceuticals and personal care products, synthetic dyes and various pesticides), *Lemna* floating macrophytes are extremely useful in bioremediation of contaminated effluents.

Despite promising efforts so far, there are still some limitations to demonstrate the effectiveness of *Lemna* in phytoremediation of chemical pollutants, and further extensive studies on chemicals challenging conventional remediation methods are needed to establish more accurate ecotoxicological testing protocols and strategies.

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