Carpathian Journal of Earth and Environmental Sciences, August 2020, Vol. 15, No. 2, p. 369 – 376; Doi:10.26471/cjees/2020/015/137

LUDWIGIA GLANDULOSA (WALTER, 1788) AS A POTENTIAL BIO INDICATOR OF WATER QUALITY

Ioana-Claudia MOROȘAN[1], Andrei LOBIUC[2], Lăcrămioara Carmen IVĂNESCU*[1], Ștefan Mihăiță OLARU[1], Naela COSTICĂ[1] & Maria Magdalena ZAMFIRACHE[1]

[1]Universitatea „Alexandru Ioan Cuza" din Iași, Facultatea de Biologie, B-dul Carol I, 700505 A, Iași, România
[2]Laboratorul CERNESIM, Universitatea „Alexandru Ioan Cuza" din Iași, Lab. L_2, B-dul Carol I, 700505 A, Iași, România Corresponding author: *ivanescu@uaic.ro*

Abstract: This article aims to determine how saline pollutants affect plants from the aquatic environment and to observe the changes in *Ludwigia glandulosa* morphology, anatomy, physiology and biochemistry in order to assess this species as a potential bioindicator of water quality with focus on saline stress. Several individuals of *L. glandulosa* were submerged in solutions of various concentrations of salts (sodium chloride, nitrites and nitrates). An influence on morphometric indices of plant organs was observed, while anatomical sections of stems and leaves revealed plasmolysis, reduction of photosynthetic activity, excessive accumulation of starch, high numbers of open stomata and raphides and variations of vascular bundles positioning, which could reflect a response to saline stress. These results were further sustained by values of leaf chlorophyll fluorescence, and of chlorophyll, total anthocyanins and polyphenols contents. Changes in the organic/inorganic contents of roots, stems and leaves were noted. Following these results, *Ludwigia glandulosa* growth parameters may reflect changes in water quality, especially in the presence of salts in high concentrations.

Keywords. cylindric fruit primrose-willow, saline stress, nitrates, nitrites, morpho-anatomy, photosynthetic apparatus.

1. INTRODUCTION

Plants represent the most important primary producers, capable of synthesizing organic matter and oxygen, therefore their significance within ecosystems is remarkable. Through their apparently simple mechanisms of survival, they have managed to spread across all types of habitats, terrestrial or aquatic, and became essential within the trophic chains, some of them even involved in the life cycle of other living organisms (Hiscock, 2003). Since the majority of aquatic life is dependent on underwater vegetation, either for feeding, hiding, reproduction or nursing their offsprings, plants growing in this type of habitat prove themselves as indispensable. Under optimal conditions, aquatic plants could reduce even nitrite or nitrate levels in waters through their leaves and roots, using them as nutrients. Nitrites and nitrates from water can be assimilated and converted by plants into organic nitrogen-containing compounds like proteins and nucleotides. Under normal conditions, nitrate represents one of the major sources of nitrogen in plants for their physiological processes (Barker & Bryson, 2016). However, even the most well - adapted aquatic or semi-aquatic plants are prone to suffer from anthropogenic stress. Constant use of chemical fertilizers, ill-planned and untreated sewage, factories effluent disposal and landfill by domestic waste (Singh, 2013; Khan et al., 2015; Tallar & Suen, 2015: Nicula et al., 2017) containing nitrites, nitrates and other salts are just some of the pressures these plants face. Such compounds would accidentally reach aquatic habitats, primarily perturbing the plants' metabolism, leading to changes in the chemical composition or even death. Nitrites and nitrates are not simply externally loaded into waters, but also continually circulated through internal biogeochemical processes (assimilation, remineralization, nitrification and denitrification) (Sebilo et al., 2003; Sutyla et al., 2009; Gruca-Rokosz et al., 2009 in Koszelnik, 2014).

A heavy nutrient level in waters favors the growth of some aquatic plants and may induce negative effects on water quality by accelerating the growth of algae creating a rapid imbalance that is unfavorable for other organisms. Sodium chloride, when dissolved in water in high-concentrations, can displace other vital minerals or nutrients in some plants, disrupting normal functions. Plants absorb the chloride and sodium instead of the needed nutrients such as potassium and phosphorus, leading to some deficiencies. Chloride ions can be transported to the leaves where they interfere with the photosynthetic activity and chlorophyll production or accumulation of this substance can reach toxic levels, causing leaf burn or die-back (Gruttadaurio et al., 2013).

The uptake and accumulation of nutrients and/or toxic elements in plants depend on their genetic properties (Singh et al., 2003: Györi et al., 2014), so the choice of plants is an important issue because they have to survive the potential toxic effects of wastewater (Laabassi et al., 2015). *Ludwigia glandulosa* was selected for this experiment as a potential bio indicator of water quality due to its amphibious adaptive mechanisms and uncommon color given by anthocyanins which belong to the flavonoid family of polyphenols. Such compounds are produced in plants with the purpose of protecting them against parasites or unfavorable conditions, thus represent an important part in determining the plant's state and response to various factors. Chlorophyll fluorescence and chlorophyll pigment content are also relevant indicators of a plant's photosynthetic activity and general state. The objective of this experiment was to determine how and to what extent the *L. glandulosa* plants are affected by solutions of salts of various concentrations and to observe if they are suitable for being used as bio indicators of water quality.

2. MATERIALS AND METHODS

Plants of *Ludwigia glandulosa* were obtained from commercial sources. A total number of 21 plants were distributed in 5 cm diameter x 5 cm height sponge-coated pots, resulting 4-5 plants per pot. Pots were submerged in glass jars filled with tap water. The plants were maintained in a growth room in the laboratory. Growth conditions were kept constant throughout the experiment (21 days), 24^{o} C and a 16 h/day photoperiod. In order to achieve better acclimation, the tap water was enriched with PROFLORA JBL 7 Balls (clay + insoluble Fe) and Tetra PlantaMin 0.5 ml/l (Fe, K, Mg) as nutrients for aquatic plants. After 14 days, plants were redistributed to 3 plants per treatment. The treatments consisted in control (tap water) and solutions of various concentrations of salts, corresponding to the maximum and 2x maximum concentrations allowed by European legislation, as shown in Table 1. Subsequent analyses were repeated on a periodic basis, 3 times at 7 days intervals.

Table 1 – Pollutants concentrations used for the experiment

Salts	**Maximum allowed quantity**	**2 x Maximum allowed quantity**
NaCl	0.8 g/1000ml	1.6 g/1000ml
$NaNO_2$	0.016 g/1000ml	0.032 g/1000ml
KNO_3	0.04 g/1000ml	0.08 g/1000ml

Morphometric measurements (width and length of the leaves, height of the stem) of each plant were made using a scale ruler. For measurements, 3 leaves per plant, each one from different parts of the stem were selected.

At the end of the experiment, fragments of stems and leaves were kept in 70^{o} ethanol for 48 h and cut in sections using microtome, dyed with iodine green and ruthenium red and afterwards examined under optical microscope (Andrei & Paraschivoiu, 2003).

Chlorophyll fluorescence analysis was conducted using a Hansatech FMS2 fluorometer on 3 leaves from each plant, a total of 9 leaves per treatment. The content of chlorophyll pigments, was assessed using an Opti-Sciences CCM-200 plus device on 5 leaves from each plant, a total of 15 leaves per treatment.

The method described by Lee et al. (2005) was followed in order to analyze the content of anthocyanin pigments. The polyphenol and flavonoid content were determined according to the method described by Herald et al., (2012).

The organic and inorganic content of the plants submerged in solutions of various concentrations was determined for stem, leaf and root through gravimetric method. Organic matter and water content were assessed after drying the plant material at 60^{o} C, until constant weight, while inorganic content (ash) was assessed after incinerating at 550^{o}C.

3. RESULTS AND DISCUSSIONS

After weekly height measurements, an increase of about 0.5 cm was observed after the first week (all concentrations), slightly decreasing to 0.4 cm the next week. The most visible growth was in the plants submerged in $NaNO_2$ 0.032 g/l and in KNO_3 0.04g/l solutions (Fig. 1). Measurement of the length and width of leaves revealed that the plants submerged in $NaNO_2$ 0.032 g/l and in KNO_3 0.08 g/l solutions have the highest foliar growth rate (Fig. 2).

Transversal sections of the stem of *L. glandulosa* control plants revealed one layer of radial-elongated epidermis cells, with thin, cellulose-pectin walls and without stomata or trichomes. The cortex contains 3 sub regions: external, with 2-3 layers of compact cells, medium, with 5 aerenchyma channels, and internal, which ends with a primary endodermis (Caspary). The vascular bundles are circularly arranged (a characteristic of aquatic plants), comprising, from outside to inside, external phloem, xylem and internal phloem (these merge into a bicollateral fascicular bundle, characteristic of *Onagraceae* family). The external phloem is circularly arranged, whereas the internal phloem is fascicular. The xylem consists of few tubes, but with numerous libriform fibers. The pith is parenchymal (Fig. 3). In leaf longitudinal section of *L. glandulosa* control plants, epidermis cells with straight walls, several stomata and cells with raphides were observed (Fig. 4). Several differences appeared in the stems of plants submerged in NaCl 0.08g/l and in KNO_3 (0.04g/l and 0.08 g/l) solutions: numerous starch cells in the pith and in the internal cortex (Fig. 5). Excessive starch reserves at this level could appear as a response mechanism to saline stress (Gupta & Huang, 2014), but also could precede the reproduction phase of the plant, which deposits supplementary reserves of nutrients in order to reproduce. Also, an increase in the number of raphides in cells was observed (Fig. 6). Phloem isles appeared in the center of the pith in plants submerged in NaCl 1.6g/l and in $NaNO_2$ 0.032g/l solutions (Fig. 7). Longitudinal sections on the leaves of *L. glandulosa* plants submerged in saline solutions showed a strong flexuosity of the epidermis cell walls and an increase in the number of raphides in cells (Fig. 8), which could indicate plasmolysis, and an opening of stomata, which could represent a collapse of the stomatal apparatus as a consequence of a high concentration of salts in the leaf (Gupta & Huang, 2014).

Saline stress produces abscisic acid synthesis in plants, which closes the stomata from the moment it is transported in the guard cells of the stomatal apparatus, and an excess of Na^+ in plants could cause a deficit in the K^+ content, since Na^+ has a strong inhibitive influence over the radicular absorption of K^+. Thus, K^+ being responsible for maintenance of the turgor of cells, membrane potential and enzymatic activity, the plant growth and development is inhibited. Also, in photosynthetic tissues, the Na^+ accumulation affects the photosynthetic compounds, such as enzymes, chlorophyll and carotenoid pigments, which are decreased (Zhu 2007).

ΦPSII represents the photochemical efficiency of the photosystem II. The diagram below (Fig. 9) shows that the photochemical efficiency of the plants submerged in NaCl 1.6g/l, $NaNO_2$ 0.016g/l, $NaNO_2$ 0.032g/l and KNO_3 0.08g/l solutions have registered a decrease after the first week, followed by an increase. The ΦPSII of plants from KNO_3 0.04g/l solution seems to increase after the first measurement, but decreases through the last measurement. The plants from NaCl 0.8g/l and control solutions slightly increase in photochemical efficiency and maintain its values constant throughout the experiment. As well as in ΦPSII, plants from NaCl 0.8g/l and control solutions show an increase in the electron transfer rate (ETR). Plants from $NaNO_2$ 0.016g/l solution also registered an increase in ETR. However, the plants submerged in KNO_3 0.04 g/l, KNO_3 0.08 g/l, NaCl 1.6 g/l and $NaNO_2$ 0.032 g/l indicate a decrease in ETR during the first 2 measurements, followed by an increase (Fig. 10). The redox state of electron transporters is important in understanding the events that lead to changes in photochemical efficiency of PSII. When the leaf is exposed to an optimum illumination after a period of light absence, a transitory increase in chlorophyll fluorescence occurs, as a result of a decrease in the number of electron transporters in thylakoid membranes. After an increase in the levels of chlorophyll fluorescence, a reduction follows (extinction). The extinction appears as a consequence of photosynthetic activity and opening of stomata, which increases the availability for CO_2 (Lawson et al., 2012).

The density of chlorophyll pigments content indicates that the plants that have been submerged in low salt concentration solutions (NaCl 0.8 g/l, $NaNO_2$ 0.016 g/, KNO_3 0.04 g/l) appeared to benefit from the presence of salts, thus increasing the quantity of chlorophyll pigments. On the other hand, plants from NaCl 1.6 g/l, $NaNO_2$ 0.032 g/l, KNO_3 0.08 g/l solutions showed a decrease in the chlorophyll pigment content (Fig. 11), which could indicate a decline in the general photosynthetic activity. However, the levels of chlorophyll pigments are higher in plants that have been submerged in high concentration nitrate and nitrite solutions in comparison with those in low concentration solutions.

Figure 12 shows variations in the anthocyanin pigments content in *L. glandulosa* plants that have been submerged in salt solutions. All plants (except from $NaNO_2$ 0.032 g/l, where the anthocyanin pigments levels were low) showed an increase in the anthocyanin pigments content, which induces a crimson-red color of the leaves. On the other hand, the control plants showed a decrease in anthocyanin pigment levels.

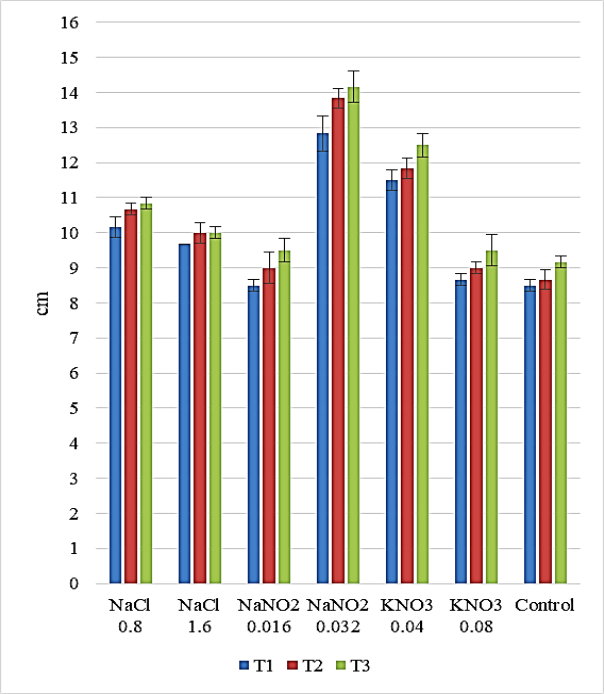

Figure 1. Height variations of *L. glandulosa* plants under experiment (T represents the week of measurement)

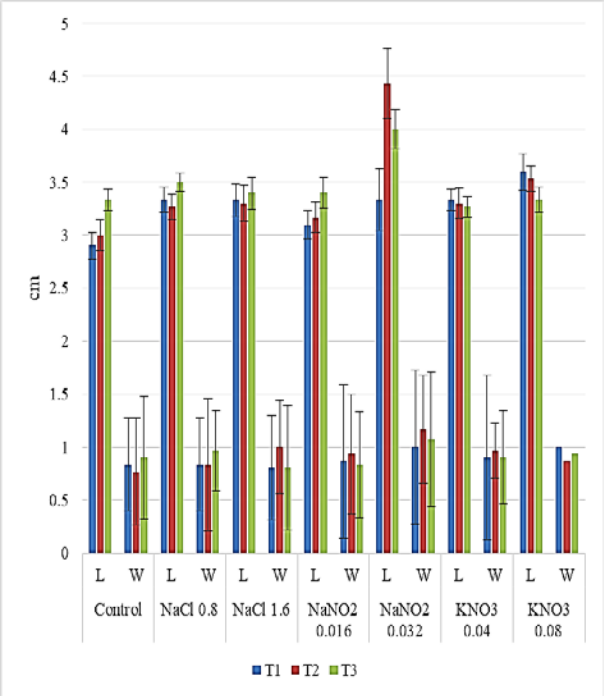

Figure 2. Length and width of *L. glandulosa* leaves (under experiment)

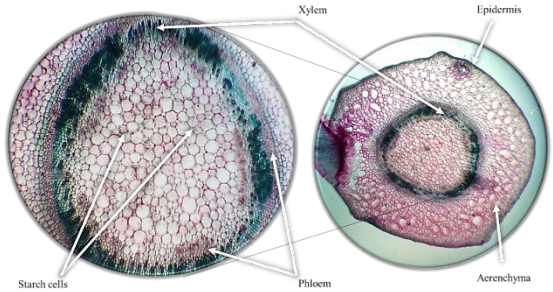

Figure 1. Stem transversal section - *L. glandulosa* (Control) (original photo)

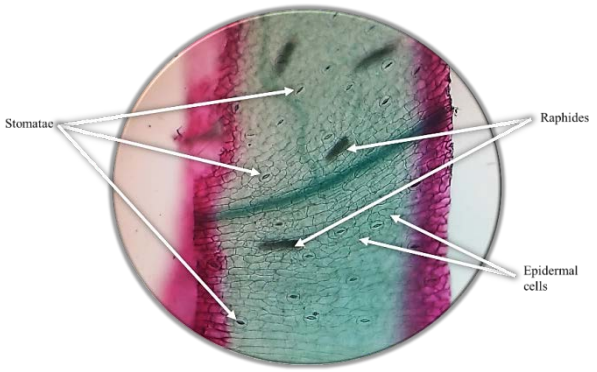

Figure 2. Leaf front view section - *L. glandulosa* (Control) (original photo)

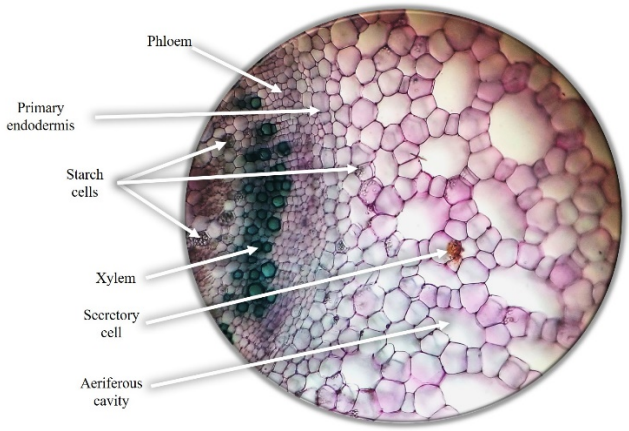

Figure 3. Stem transversal section - *L. glandulosa* (NaCl 0.8g/l) (original photo)

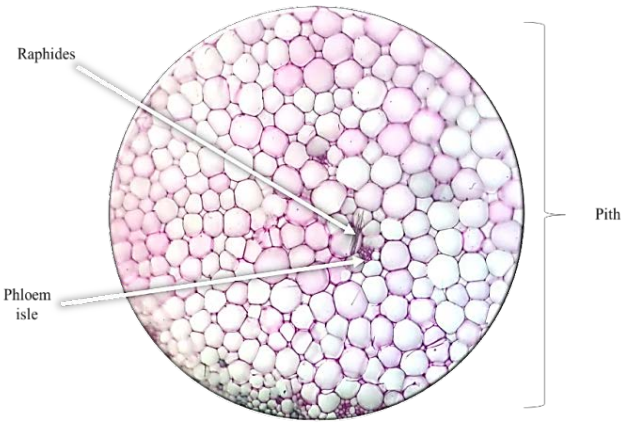

Figure 4. Stem transversal section - *L. glandulosa* (NaCl 1.6 g/l) (original photo)

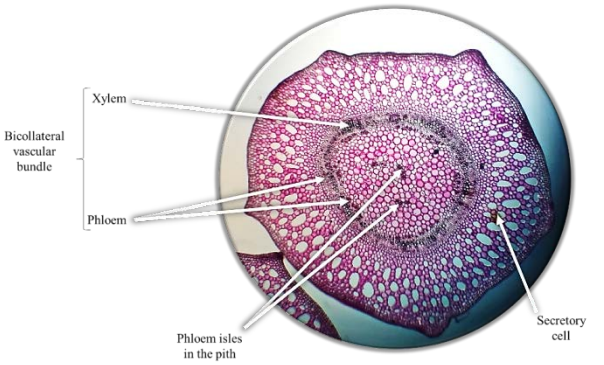

Figure 5. Stem transversal section - *L. glandulosa* ($NaNO_2$ 0.032g/l) (original photo)

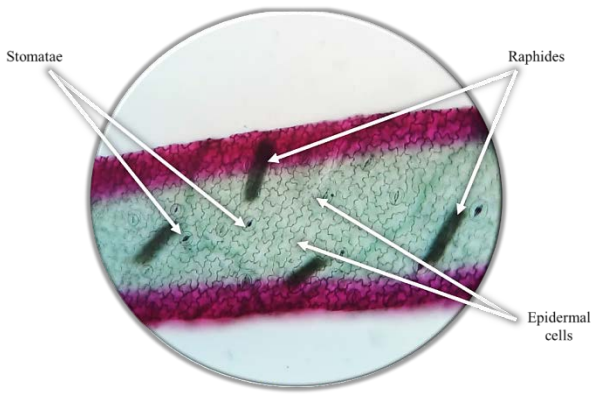

Figure 6. Leaf front view section - *L.glandulosa* (all concentrations) (original photo)

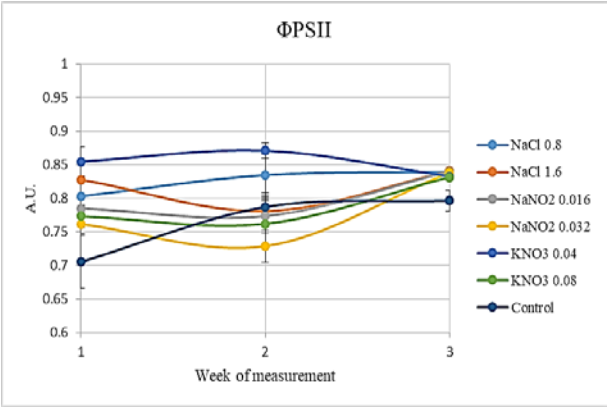

Figure 7. Photochemical efficiency of PSII in *L. glandulosa* plants under experiment

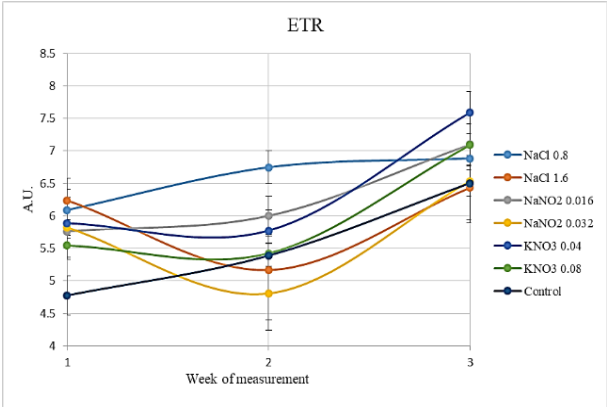

Figure 8. Electron transfer rate in *L. glandulosa* plants under experiment

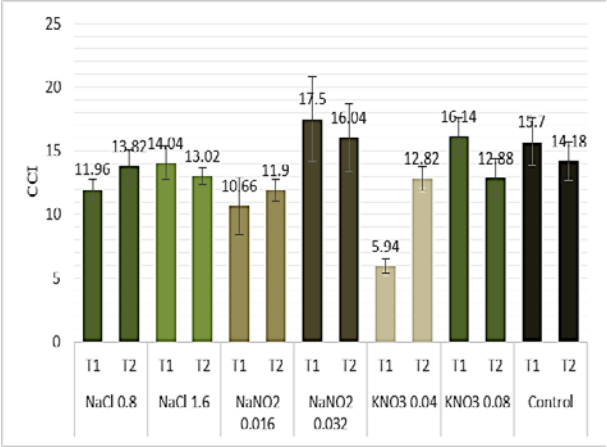

Figure 9. Variations in the content of chlorophyll pigments of *L. glandulosa* leaves

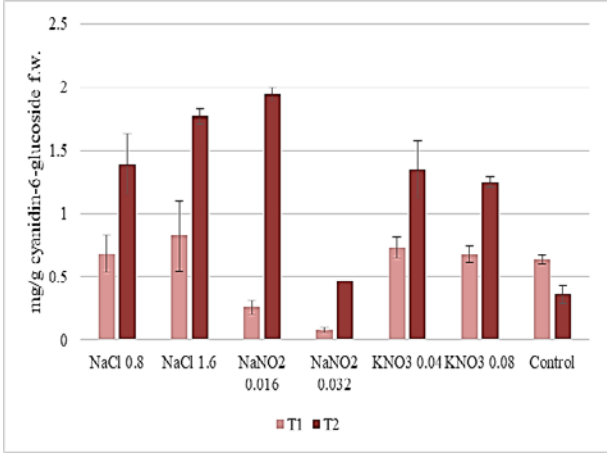

Figure 10. The anthocyanin pigments content in *L. glandulosa* plants submerged in salt solutions

Figure 13 shows a difference between the initial and the final polyphenol content. *L. glandulosa* plants that have been submerged in NaCl 1.6 g/l, $NaNO_2$ 0.032 g/l, KNO_3 0.04 g/l and control solutions have suffered a slight decrease in the polyphenol content, followed by an increase. In the case of plants from NaCl 0.8 g/l and $NaNO_2$ 0.016 g/l, the increase was linear and constant, whereas the plants from KNO_3 0.08g/l showed a substantial increase in the first part of the experiment. Major variations were observed in plants from NaCl 1.6 g/l, $NaNO_2$ 0.032 g/l and KNO_3 0.08 g/l solutions. Figure 14 indicates an initial decrease in the flavonoid content in all *L. glandulosa* plants, followed by an increase (especially in plants cultivated in KNO_3 0.08 g/l solution). The importance of polyphenols in plants is represented by their antioxidant activity, which protects them against negative effects produced by reactive oxygen and

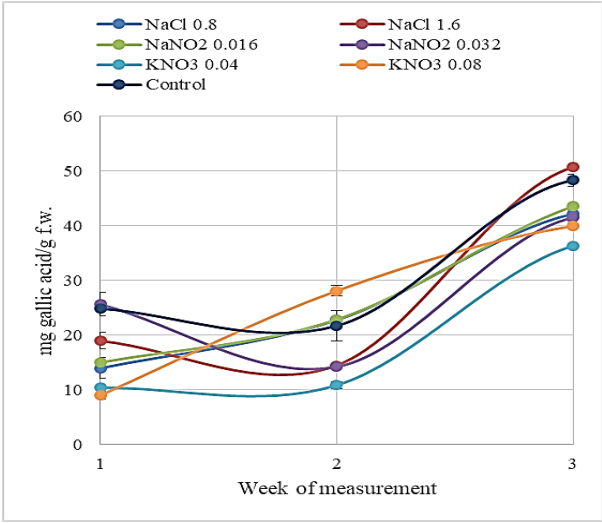

Figure 11. Variations in the polyphenols content of *L. glandulosa* plants under experiment

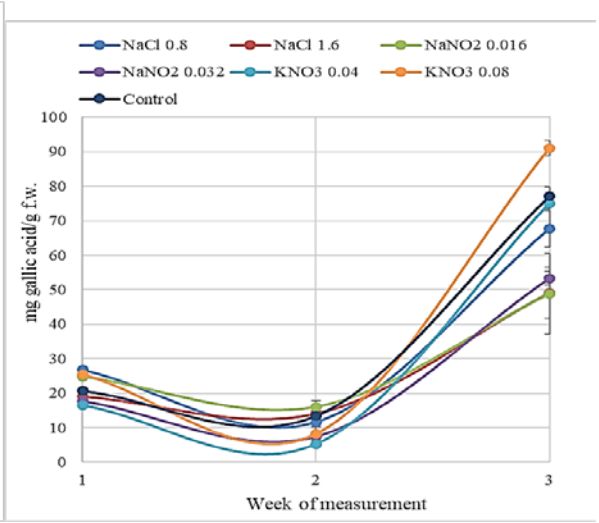

Figure 12. Variations in the flavonoid content of L. glandulosa plants under experiment

nitrates (Galili & Hovav, 2014). Polyphenols are the most versatile secondary metabolites, plants being capable to respond promptly to various stress factors due to these compounds. Also, polyphenols such as phenolic glucosides, hydroxycinnamic acid derivatives and flavonoids are involved in the thickening of the secondary cell walls (Gunnaiah et al., 2012), thus mechanically increasing the resistance of the tissues. This property is correlated anatomically with the draught tolerance and the increase in the resistance to oxidative stress (Bennet et al., 1996; McLusky et al., 1999; Agati et al., 2012). It has also been observed that the biosynthesis of polyphenols could increase as a response to saline stress (Di Ferdinando et al., 2013). Flavonoids are involved in different biological functions, including protection against UV rays and phytopathogens, reproduction functions, physiological regulation of the plant cell, intervention in the response reaction of the plant to diverse environmental factors, cytotoxicity and their ability to interact with enzymes, forming protein complexes (Falcone Ferreyra et al., 2012). Flavonoids are also involved in the polar transport of the auxins (hormones which are fulfilling an important role in the plant response to stress through control on the opening of stomata and on the distribution of nutrients during unfavorable conditions (Peer & Murphy, 2007; Kuhn et al., 2011; Lewis et al., 2011).

Table 2 shows certain changes in the organic/inorganic matter and water content in *L. glandulosa* plant organs: the NaCl 1.6 g/l solution affected the H_2O content in leaves and roots (decreased), whereas organic matter content in these organs increased; NaCl 0.8 g/l solution did not have a strong impact on plants; the $NaNO_2$ and KNO_3 solutions mainly affected the roots, where a raise in H_2O content occurs, in contrast with the decrease in organic and inorganic matter content.

Table 2 - H_2O, organic and inorganic content in *L. glandulosa* plants under experiment

	Sample	H_2O (%)	Inorg. Subst. (%)	Org. Subst. (%)
NaCl 0.8	**Stem**	92.5	1.22	6.28
	Leaf	91.27	1.2	7.53
	Root	92.78	1.5	5.72
NaCl 1.6	**Stem**	92.68	1.2	6.12
	Leaf	88.96	1.44	9.59
	Root	87.8	1.69	10.51
$NaNO_2$ 0.016	**Stem**	94.93	1.11	3.96
	Leaf	92.48	1.03	6.49
	Root	97.98	0.29	1.73
$NaNO_2$ 0.032	**Stem**	92.79	1.01	6.2
	Leaf	93.22	0.91	5.87
	Root	98.2	0.39	1.41
KNO_3 0.04	**Stem**	93.49	1.05	5.45
	Leaf	92.56	0.82	6.62
	Root	96.94	0.5	2.56
KNO_3 0.08	**Stem**	91.64	1.16	7.2
	Leaf	92.58	0.82	6.6
	Root	95.36	2.04	2.59
CONTROL	**Stem**	92.72	1.1	6.19
	Leaf	92.62	0.85	6.52
	Root	93.71	2.12	4.17

4. CONCLUSIONS

Ludwigia glandulosa is a plant with a distinctive appearance, in which the anthocyanin and chlorophyll pigments contents are in close correlation

with the photochemical efficiency, since the color of leaves is directly affected by light conditions. It requires warm temperatures, phosphates and nitrates in its environment, and plenty of light in order to grow well, as, unlike its related species from the genus *Ludwigia*, it is not an invasive species and grows with difficulty in most environments. It is a threatened or even endangered species in some regions of North-Eastern United States, even though it is widely commercialized as an aquarium plant.

Our study showed that, besides its utility as an ornamental plant for aquariums, *Ludwigia glandulosa* can withstand high concentrations of salts and could be an indicator of water quality not only through its morphological and color changes, but also when examined at an anatomical and physiological level, where a strong flexuosity of epidermis cell walls, an increase in number and density of stomata and an instability of the photochemical efficiency of photosynthetic system II indicate saline stress. Morphological changes may show a positive effect of salts on *Ludwigia glandulosa* plants for a short period of time, however, the prolonged action of salts in high concentrations could produce negative effects. By comparing these changes, it can be observed that salts induce saline stress of various impacts which trigger different responses in all *L. glandulosa* treated plants.

Following the results of this experiment, *Ludwigia glandulosa* might be considered a suitable organism to cultivate as a bio indicator of water quality in aquariums, and to collect (from its natural habitat, where it is not threatened or endangered) and analyze in order to detect potentially hazardous substances in water that could exert negative effects on the natural ecosystem.

Acknowledgements

We would like to acknowledge the collaboration between CERNESIM research Centre and Faculty of Biology, University 'Alexandru Ioan Cuza' of Iași which has made this experiment possible.

REFERENCES

Agati, G., Azzarello, E., Pollastri, S. & Tattini, M., 2012. *Flavonoids as antioxidants in plants: location and functional significance*, Plant Sci., 196, pp. 67–76, DOI: 10.1016/j.plantsci.2012.07.014.

Andrei, M. & Paraschivoiu, R. M., 2003. *Botanical microtechnique* (*Microtehnică botanică)*. Ed. Niculescu, Bucharest.

Barker, A.V. & Bryson, G.M., 2016. *Nitrogen*. In Handbook of Plant Nutrition. CRC Press. pp. 22-43.

Bennet, M.H., Gallagher, M., Fagg, J., Bestwick, C., Paul, T., Beale, M.H. & Mansfield, J.W., 1996. *The hypersensitive reaction, membrane damage and accumulation of autofluorescent phenolics in lettuce cells challenged by Bremia lactucae*, Plant J, nr. 9, pp. 851–865, DOI: 10.1046/j.1365-313X.1996.9060851.x.

Di Ferdinando, M., Brunetti, C., Agati, G. & Tattini, M., 2013. *Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas*, Elsevier B.V, DOI: 10.1016/j.envexpbot.2013.09.012.

Falcone Ferreyra, M.L., Rius, S.P. & Casati, P., 2012. *Flavonoids: biosynthesis, biological functions, and biotechnological applications*, Frontiers in Plant Science, Volume 3, Issue 222, DOI: 10.3389/fpls.2012.00222.

Galili, S. & Hovav, R., 2014. *Determination of Polyphenols, Flavonoids, and Antioxidant Capacity in Dry Seeds*, Chapter 16, Polyphenols in Plants – Isolation, Purification and Extract preparation, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, pp. 305-323, DOI: 10.1016/B978-0-12-397934-6.00016-4.

Gruca-Rokosz, R., Tomaszek, J.A. & Koszelnik, P., 2009. *Denitrification in the sediment of a eutrophic reservoir as measured by the isotope pairing technique*. Oceanological and Hydrobiological Studies, 38, pp. 75-81, DOI: 10.2478/v10009-009-0005-9.

Gruttadaurio, J., Mattson, N., Petrovic, M. & Brewer, L., 2013. *Soluble Salts in Soils and Plant Health*. Cornell University. Cooperative extension and Department of Horticulture. https://cpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/f/575/files/2015/12/Soluble-Salts-May-6-2013-o36voh-2evtxtu.pdf.

Gunnaiah, R., Kushalappa, A.C., Duggavathi, R., Fox, S. & Somers, D.J., 2012. *Integrated metabolo-proteomic approach to decipher the mechanisms by which wheat QTL (Fhb1) contributes to resistance against Fusarium graminearum*, PLoS ONE 7(7): e40695, DOI: 10.1371/journal.pone.0040695.

Gupta, B. & Huang, B., 2014. *Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization*. International journal of genomics, 701596, DOI: 10.1155/2014/701596.

Györi, Z., Filep, T. & Lehoczky, E., 2014. *Trace element content of several weed seeds.* Carpathian Journal of Earth and Environmental Sciences, May 2014, Vol. 9, No. 2, pp. 251 – 257.

Herald, T.J., Gadgil, P. & Tilley, M., 2012. *High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour*, Journal of the Science of Food and Agriculture, Volume 92, Issue 11, DOI: 10.1002/jsfa.5633.

Hiscock, P., 2003. *Encyclopedia of Aquarium Plants*, Interpret Publishing, pp. 12-13.

Khan, I.U., Khan, N.U., Khan, M.Q., Ullah, K., Bibi, Z., Rahman, H.U. & Gangohi, M.S., 2015. *Prolonged wastewater irrigation and heavy metal accumulation in soils, vegetables and fodder crops*.

Carpathian Journal of Earth and Environmental Sciences, February 2015, Vol. 10, No. 1, pp. 211 – 221.

Koszelnik, P., 2014. *Investigating the source of nitrate in Poland's San River water by analyzing the nitrogen and oxygen isotopic ratio*, Carpathian Journal of Earth and Environmental Sciences, August 2014, Vol. 9, No. 3, pp. 85 – 96.

Kuhn, B. M., Geisler, M., Bigler, L. & Ringli, C., 2011. *Flavonols accumulate asymmetrically and affect auxin transport in Arabidopsis*. Plant Physiol. 156, pp.585–595, DOI: https://doi.org/10.1104/pp.111.175976.

Laabassi, A., Harzallah, D. & Boudehane, A., 2015. *Performances of a constructed wetland treating planted with emergent and floating macrophytes under algerian semi-arid climate*. Carpathian Journal of Earth and Environmental Sciences, November 2015, Vol. 10, No 4, pp. 65 – 74.

Lawson, T., Kramer, D.M. & Raines, C.A., 2012. *Improving yield by exploiting mechanisms underlying natural variation of photosynthesis*. Current Opinion in Biotechnology 23, pp. 215–220, DOI: https://doi.org/10.1016/j.copbio.2011.12.012.

Lee, J., Durst, R.W. & Wrolstad, R.E. 2005. *Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study*, Journal Of Aoac International, Volume 88, Issue 5.

Lewis, D. R., Ramirez, M. V., Miller, N. D., Vallabhaneni, P., Ray, W. K., Helm, R. F., Winkel, B. S. & Muday, G. K., 2011. *Auxin and ethylene induce flavonol accumulation through distinct transcriptional networks*. Plant Physiol. 156, pp. 144–164, DOI: https://doi.org/10.1104/pp.111.172502.

McLusky, S.R., Bennet, M.H., Beale, M.H., Lewis, M.J., Gaskin, P. & Mansfield, J.W., 1999. *Cell wall alterations and localized accumulation of feruoyl-3'-methoxytyramine in onion epidermis at sites of attempted penetration of Botrytis alii are associated with actin polarization, peroxidase activity and suppression of flavonoid biosynthesis*. Plant J. 17, pp. 523–534, DOI: https://doi.org/10.1046/j.1365313X.1999.00403.x.

Nicula, A.M., Roba, C., Piștea, I. & Roșu, C., 2017. *Assessment of water quality from Brăteni lake, Bistrița-Năsăud county*. Carpathian Journal of Earth and Environmental Sciences, July 2017, Vol. 12, No. 2, pp. 365 – 370.

Peer, W. A. & Murphy, A. S., 2007. *Flavonoids and auxin transport: modulators or regulators?*, Trends Plant Sci, 12, pp. 556–563, DOI: 10.1016/j.tplants.2007.10.003.

Sebilo, M., Billen, G., Grably, M. & Mariotti A., 2003. *Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system*. Biogeochemistry, 63, pp. 35-51, DOI: https://doi.org/10.1023/A:1023362923881.

Singh, A.L., 2013. *Nitrate and phosphate contamination in water and possible remedial measures*. Environmental Problems and Plants. Springer, pp.44-46.

Singh, O. V., Labana, S., Pandey, G., Budhiraja, R. & Jain, R. K., 2003. *Phytoremediation an overview of metallic ion decontamination from soil*. Appl. Microbiol. Biotechnol., 61, pp. 405–412, DOI: 10.1007/s00253-003-1244-4.

Sutyła, M., Tomaszek, J. A. & Masłoń, A., 2009. *Anaerobic conversions of ammonium nitrogen in aquatic ecosystems*. Environment Protection Engineering, 35(2), pp. 105-111.

Tallar R. & Suen J., 2015. *Identification of waterbody status in Indonesia by using predictive index assessment tool*, International Soil and water Conservation Reserch, 3 (2015), pp. 224-238, DOI: https://doi.org/10.1016/j.iswcr.2015.06.009.

Zhu, J.K., 2007. *Plant Salt Stress*. Encyclopedia of Life Sciences, John Wiley & Sons Ltd, DOI: https://doi.org/10.1002/9780470015902.a0001300.pub2.

Received at: 06. 06. 2020
Revised at: 18. 07. 2020
Accepted for publication at: 26. 07. 2020
Published online at: 29. 07. 2020