ACUTE PHYTOTOXICITY OF FOUR COMMON PHARMACEUTICALS ON THE GERMINATION AND GROWTH OF *Lactuca sativa* L.

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Abstract. The pharmaceutical industry has provided an extraordinary variety of drugs that have improved human health, yet the incorrect final disposal of drugs has been released into the environment. In this research, the phytotoxic effect of four pharmaceutical products exposed to five concentrations was evaluated, using *Lactuca sativa*, and the percentage of germination, stem, and root length were evaluated. The four pharmaceuticals used in this bioassay caused inhibitory effects on root growth, elongation of hypocotyls, and germination of *Lactuca sativa* seeds. The greatest inhibition effect occurred from 10 to 1000 mg L^{-1}, likewise significant differences, and interaction ($p \leq 0.05$) were observed between concentrations and drugs. Ranitidine was the drug that had the greatest phytotoxic effect on the inhibition of germination and root growth of *Lactuca sativa*. The results obtained in this study could contribute as a reference to future chronic studies with *Lactuca sativa*, as well as to prevent or reduce the impact on ecosystems and agricultural soils.
Keywords: *drugs, toxicity, ranitidine, bioassay, contaminants*

Introduction

Currently, the consumption of pharmaceutical products has been increasing due to the increase in the world population as a basic need for the improvement of human health, however, the incorrect final disposal of medicines has been released into the environment (Arnold et al., 2013; Vasquez et al., 2014; Christou et al., 2019). Moreover, pharmaceuticals are generally discharged into the environment through contaminated wastewater and biosolids in different environmental compartments such as rivers, water tables, coastal areas, as well as in agricultural soils (Singh et al., 2018). In addition, pharmaceutical residues in rainy seasons can be transported by terrestrial runoff that reaches surface waters or can also infiltrate the soil, thus generating the dispersion of the pharmaceutical pollutant in other environmental compartments of the subsoil (Edwards

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et al., 2009; Bártíková et al., 2016; Hurtado et al., 2016). Most research conducted on ecotoxicology has focused on aquatic organisms (e.g., phytoplankton, marine bacteria, algae, crustaceans, or fish) and very few studies on soil-dwelling organisms (e.g., invertebrates, plants, soil bacteria, and fungi). There are other studies, but only for phytoremediation purposes or to determine possible human exposure through trophic transfer (Carvalho et al., 2014). However, the effects of toxicity documented with pharmaceuticals on land plants are rare (Hillis et al., 2011; Carvalho et al., 2014; Bártíková et al., 2016). Some studies conducted with pharmaceutical products such as paracetamol were classified as harmful to the copepod *Tisbe battagliai* (Trombini et al., 2016) and exhibited toxicity to a variety of other aquatic species such as the unicellular algae *Pseudokirchneriella subcapitata,* the cyanobacterium *Cylindrospermopsis raciborskii* (Nunes et al., 2014). Ibuprofen can accumulate in the soil and cause toxic effects on plants in their development (Schmidt, 2015) or inhibition of root growth (González and Boltes, 2014), depending on the type of species. On the other hand, amoxicillin has been determined to stimulate photosynthetic activity and microcystin production of *Microcystis aeruginosa* (Liu et al., 2016). This pharmaceutical product was also able to induce oxidative stress in zebrafish (*Dania rerio*) (Oliveira et al., 2013). Amoxicillin has also shown toxicity to terrestrial organisms affecting photosynthetic processes in wheat (*Triticum* aestivum) (Opri et al., 2013).

The *Lactuca sativa* seed toxicity bioassay is a 120-hour static acute toxicity test, which evaluates the phytotoxic effects of pure compounds or complex mixtures on the process of seed germination and seedling development in the first days of growth (Bowers et al., 1996). The success or fitness of a seedling to settle in a certain environment is of great importance to ensure the survival of the species. The evaluation of the development of the radicle and hypocotyl are representative indicators to determine the establishment and development capacity of a plant (Priac et al., 2017). The use of bioassays with plants represents a fast and economical method for the characterization of the toxicity of environmental samples (Chan-Keb et al., 2018). Therefore, the objective of this study is to determine the acute toxic effects of the exposure of four pharmaceutical products to different concentrations in the germination and elongation of the radicle and hypocotyl of lettuce (*Lactuca sativa* L.).

Materials and methods

Characterization of pharmaceutical products and experimental design with Lactuca sativa

The experiment was conducted at the Faculty of Chemical Biological Sciences of the Autonomous University of Campeche, Mexico. The sowing of the lettuce seeds and the analysis of the samples were carried out in February 2019.

For the realization of the bioassay, *Lactuca sativa* seeds (Vita-Los Molinos) were used, four pharmaceutical products were used (bezafibrate, hydrochlorothiazide, losartan, and ranitidine) of Sigma Aldrich, the physicochemical properties are shown in *Table 1*. For this bioassay, 1 liter of 1000 mg L^{-1} (w/v) stock solution of each of the four drugs was prepared with distilled water.

For the determination of acute toxicity, 5 treatments per drug were performed with concentrations of 0.01, 0.1, 1, 10, and 1000 mg L^{-1} that were obtained from successive dilutions in triplicate and for the negative growth, control distilled water was used, for a total of 80 experimental units.

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Table 1. *Physicochemical properties of the four drugs*

Chemical group	Compound	Drug class	Formula molecular	Molecular weight (g mol^{-1})	PKa	CAS Registration Number	ATC
H2 Antagonist	Ranitidine	Anti-ulcer agents	$C_{13}H_{22}N_4O_3S$	314.404	7.8	66357-35-5	A02BA02
PPAR-alpha agonist	Bezafibrato	Lipid-lowering agents	$C_{19}H_{20}ClNO_4$	361.8	3.83	41859-67-0	C10AB02
Diuretics; Sodium chloride symporter inhibitors	Hydrochlorothiazide	Antihypertensive agents;	$C_7H_8ClN_3O_4S_2$	297.7	7.9	58-93-5	C03AA03
Angiotensin II receptor blockers type 1	Losartan	Antiarrhythmic agents; Antihypertensive agents	$C_{22}H_{23}ClN_6O$	422.9	5.5	114798-26-4	C09CA01

ATC: For its acronym in English refers: Anatomy, Therapeutics, Chemical Classification System

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For each unit, 25 seeds of *Lactuca sativa* (Vita - Los Molinos) were placed in a polyethylene container (petri dish) 90 mm in diameter, with filter paper (Whatman® No. 3) at the bottom as support. Subsequently, 10 ml of each of the concentrations of the aqueous solution were applied. All units were kept at a controlled room temperature of 24 ± 1°C for 120 hours (5 days) as set by the EPA for phytotoxicity testing. At the end of the exposure period, the number of germinated seeds was counted and the length of the root and hypocotyls was measured. As response variables, the mean and standard deviation of radicle length and hypocotyls were determined in the negative controls and each treatment (exposure concentration) (*Figure 1*). In addition, the percentage of germination (*%G*) of the seeds for each concentration concerning the negative control was determined according to the equation of Chan-Keb et al. (2018), *Eq. (1)*.

$$\% G = (\text{No. of sprouted seedlings in each concentration} * 100) / \text{No. of germinated seedlings in the control} \quad \text{(Eq.1)}$$

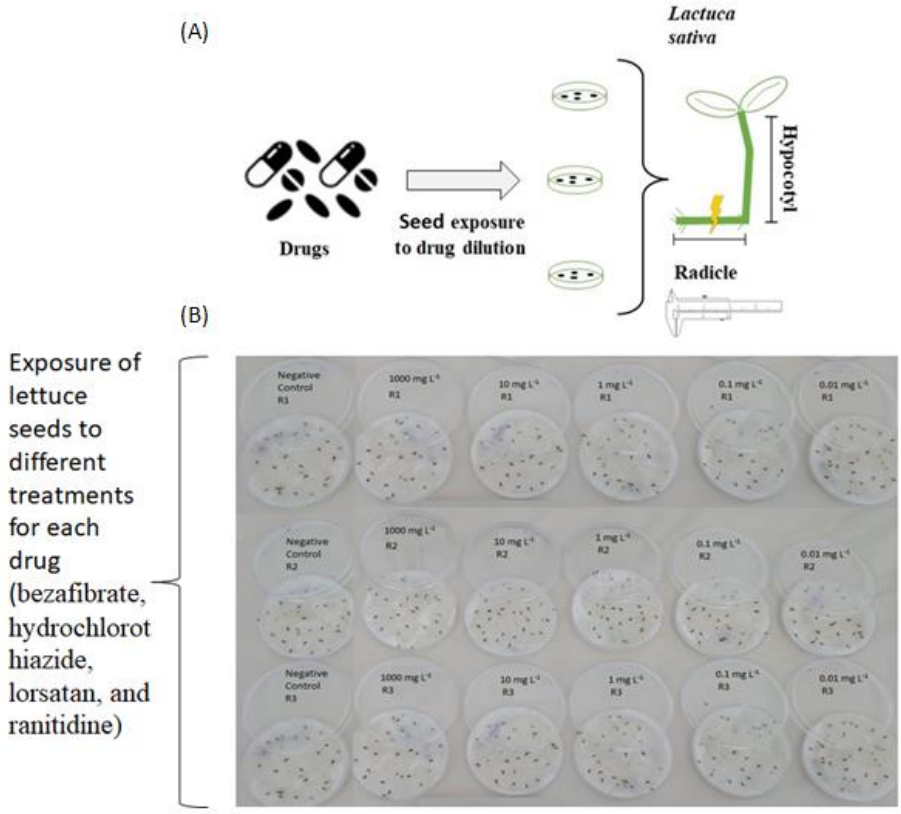

Figure 1. *(A) Experimental diagram of the exposure of Lactuca sativa seeds to drug dilutions; (B) Exposure of Lactuca sativa seeds to different treatments for each drug*

Statistical analysis

To evaluate the toxicity effect of pharmaceutical products on the morphometric variables (seed germination, root elongation, and hypocotyl) in *Lactuca sativa*, we compared the treatments and the four drugs exposed, to determine the variation or interaction of these two factors, applying a two-way ANOVA. Before this comparison

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analysis, the normality of the variables was validated with the method of Shapiro and Wilks (1965), with a significance level $\alpha = 0.05$, not complying with the assumption of normal distribution, the data were transformed using the Box-Cox method so that the variables will present the assumptions of normal distribution (Zar, 2010). *Post-hoc* analysis was realized with least significant difference (LSD) Fisher test. All statistical analyses were performed with the STATISTICA V.12 program (©Copyright StatSoft, Inc., Palo Alto, CA, USA, 1984–2014).

Results

Figure 2 shows the data of the percentage of germination of lettuce seeds concerning the negative control with distilled water. Seeds germinated in the negative control were considered 100% germination. In the treatments, the phytotoxic effect was observed when drugs exposure concentration increased. The low germination was statistically significant in all treatments with regard to control ($p \leq 0.05$, *Table 2*). For bezafibrate, hydrochlorothiazide and losartan the effect on decrease germination was noticeable from the exposure of 10 to 1000 mg L^{-1}, in addition, ranitidine shown the lowest germination to concentrations of 0.01 to 1000 mg L^{-1}, where decrease of 61 to 41% are observed when increasing the concentration of exposure (*Figure 2*).

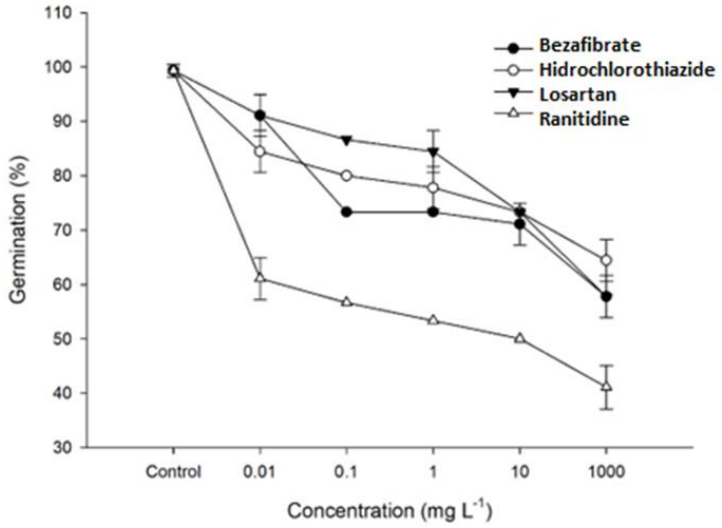

Figure 2. *Average germination of Lactuca sativa seeds between the concentrations of the 4 drugs exposed. The error bars represent the standard deviation*

Exposure to different concentrations of the 4 drugs also showed a decrease in the development of root length and the hypocotyls of lettuce (*Figure 3A and 3B*). When performing a 2-way ANOVA, significant differences were observed between drugs and exposure concentrations in the average development of root length and hypocotyls of *Lactuca sativa* (*Table 2*). From the above, considering all the concentrations of the 4 drugs, a decrease in the elongation of the radicles were observed concerning the control, being the treatment at 1000 mg L^{-1} the greatest effect was presented (*Figure 3A*); it was also observed in the treatments exposed with ranitidine they were notorious in terms of the decrease in root elongation.

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Table 2. *Two-way analysis of variance concerning germination, root length, and hypocotyls of Lactuca sativa between concentrations (Control, 0.01, 0.1, 1, 10, and 1000 mg L^{-1}) and exposure to 4 drugs (bezafibrate, hydrochlorothiazide, losartan, and ranitidine), with a significance level of p<0.05*

Parameter	Factor	Gl	F	P
Germination (%)	Concentration	5	258.09	<0001
	Drug	3	174.89	<0001
	Concentration * Drug	15	10.42	<0001
	Error	48		
Root length (mm)	Concentration	5	1420.09	<0001
	Drug	3	11.99	<0001
	Concentration * Drug	15	2.43	<0.010
	Error	48		
Hypocotyl length (mm)	Concentration	5	2569.93	<0001
	Drug	3	119.14	<0001
	Concentration * Drug	15	9.92	<0001
	Error	48		

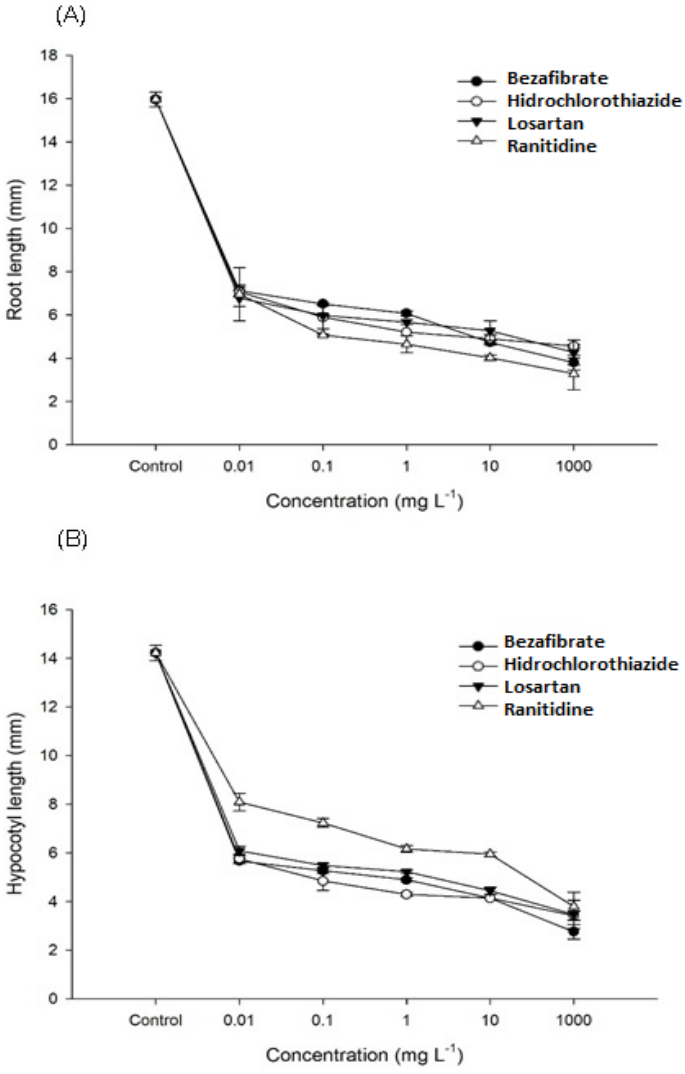

Figure 3. *Average growth in (A) root length; (B) length of the hypocotyl of Lactuca sativa between the concentration exposed to 4 drugs. The error bars represent the standard deviation*

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In *Figure 3B* see that the bezafibrate, hydrochlorothiazide, and losartan shown significative less hypocotyls length that ranitidine effect in concentrations of 0.01 to 100 mg L^{-1}, however, for 1000 mg concentrations do not significate differences were observed among all drugs.

Discussion

In general terms, germination, root length, and hypocotyls of *Lactuca sativa* showed a decrease concerning control, this is due to the phytotoxic effect of the 4 drugs to which they were exposed different concentrations, which were absorbed through the roots. The results in the germination of *Lactuca sativa* seeds show that there was a greater phytotoxic effect caused by ranitidine when the exposure concentration increased compared to the 3 drugs (bezafibrate, hydrochlorothiazide, and losartan) and control, this may be due to the sensitivity of lettuce seeds to ranitidine. Similar findings have been reported by authors such as Rade et al. (2019) where they performed an individual toxic evaluation and the mixture of three pharmaceutical products (paracetamol, ibuprofen, and amoxicillin) in the germination and growth of seeds in *Lactuca sativa*, where they determined that the lowest amount of germination occurred in the drug ibuprofen and the mixture of the 3 drugs (Rade et al., 2019). Moreover, authors such as Biruk et al. (2017) found that lettuce seeds were more sensitive to extracts containing inorganic elements than organic extracts.

The phytotoxic effect on the radicle and hypocotyl of lettuce was greater at concentrations of 10 to 1000 mg L^{-1} in the four drugs exposed. This is due to the absorption of drugs that are bioavailable in aqueous solution, through the roots, as the main route for transfer and direct contact, where the influx of nutrients and water in lettuce is also regulated, that is, the morphology, anatomy, and biomass production of the roots is also associated with the effect on the absorption of drugs (Rodriguez-Ruiz et al., 2015), as presented in this study.

When comparing the effects of ranitidine on radicular and hypocotyl length in lettuce, it was observed that the toxic effect was greater in the roots than in the length of the hypocotyls, therefore, we described that the root was more sensitive to aqueous solutions of ranitidine, due to direct contact in the absorption of the drug generating physiological stress. In this regard, Gómez-Oliván et al. (2014) mention that the presence of isolated drugs in the soil can induce oxidative stress in plants and influence their antioxidant defenses giving rise to different responses (phytotoxic effects), this will depend on the drug, its concentration and the sensitivity of the plant species (Carvalho et al., 2014; Marsoni et al., 2014; Minden et al., 2017). On the other hand, Christou et al. (2019) mention that the bioaccumulation of contaminants in plant tissues depends on the type of soil, the physicochemical properties of the contaminants, and the interactions of these factors, as well as the type of species.

Based on the results obtained from this research, more future studies can be carried out on chronic phytotoxic effects in *Lactuca sativa* considering other types of indicators to be evaluated, different concentrations of drugs or similar to those found in the environment, to contribute to the environmental regulations that allow establishing the permissible limits of discharge of residual drains that contain drug residues and thus be able to prevent or mitigate short-, medium- and long-term impacts on coastal areas, rivers, soils, and crops.

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Conclusions

This study describes the phytotoxic effect of four drugs where 3 are commonly used (bezafibrate, hydrochlorothiazide, and losartan) and one (ranitidine) that was banned from use and excluded since 2019 for marketing and consumption by the U.S. Food and Drug Administration (FDA), due to its classification as a carcinogen. Phytotoxicity caused by the drugs was related to inhibitory effects on a radicle, hypocotyl elongation, and seed germination in *Lactuca sativa*. Exposure to ranitidine aqueous solution exerted a greater effect on seed germination and root elongation in lettuce. Based on the results of this study, the phytotoxic effect of ranitidine that it can cause in a plant species such as *Lactuca sativa* is verified and described for the first time. The test used allowed the identification of different levels of phytotoxicity in the samples of drugs in aqueous solution, in addition, the bioassay with *Lactuca sativa* proved to be efficient, sensitive, economical, fast, and reproducible. Likewise, the results of our research could contribute as reference information for future chronic studies with *Lactuca sativa* and other drugs.

REFERENCES

[1] Arnold, K. E., Boxall, A. B. A., Brown, A. R., Cuthbert, R. J., Gaw, S., Hutchinson, T. H., Jobling, S., Madden, J. C., Metcalfe, C. D., Naidoo, V., Shore, R. F., Smits, J. E., Taggart, M. A., Thompson, H. M. (2013): Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. – Biol. Lett. 9.

[2] Bártíková, H., Podlipná, R., Skálová, L. (2016): Veterinary drugs in the environment and their toxicity to plants. – Chemosphere 144: 2290-2301. https://doi.org/10.1016/j. chemosphere.2015.10.137.

[3] Biruk, L. N., Moretton, J., Fabrizio de Iorio, A., Weigandt, C., Etcheverry, J., Filippetto, J., Magdaleno, A. (2017): Toxicity and genotoxicity assessment in sediments from the Matanza- iachuelo river basin (Argentina) under the influence of heavy metals and organic contaminants. – Ecotoxicol. Environ. Saf. 135: 302-311.

[4] Bowers, N., Pratt, J. R., Beeson, D., Lewis, M. (1997): Comparative evaluation of soil toxicity using lettuce seeds and soil ciliates. – Environ. Toxicol. Chem. 16: 207-213.

[5] Carvalho, P. N., Basto, M. C. P., Almeida, C. M. R., Brix, H. (2014): A review of plantpharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. – Environ. Sci. Pollut. Res. 21: 11729-11763.

[6] Chan-Keb, C. A., Agraz-Hernández, C. M., Perez-Balan, R. A., Gómez-Solano, M. I., Maldonado-Montiel, T. D., Ake-Canche, B., Gutiérrez-Alcántara, E. J. (2018): Acute toxicity of water and aqueous extract of soils from Champotón river in *Lactuca sativa* L. – Toxicology reports 5: 593-597.

[7] Christou, A., Kyriacou, M. C., Georgiadou, E. C., Papamarkou, R., Hapeshi, E., Karaolia, P., Michael, C., Fotopoulos, V., Fatta-Kassinos, D. (2019): Uptake and bioaccumulation of threewidely prescribed pharmaceutically active compounds in tomato fruits andmediated effects on fruit quality attributes. – Sci. Total Environ. 647: 1169-1178.

[8] Edwards, M., Topp, E., Metcalfe, C. D., Li, H., Gottschall, N., Bolton, P., Curnoe, W., Payne, M., Beck, A., Kleywegt, S., Lapen, D. R. (2009): Pharmaceutical and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids to an agricultural field. – Sci. Total, Environ. 407(14): 4220-4230.

[9] Gómez-Oliván, L. M., Neri-Cruz, N., Galar-Martínez, M., Islas-Flores, H., García-Medina, S. (2014): Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen, and acetylsalicylic acid and these pharmaceuticals in isolated forminduce oxidative stress on Hyalella azteca. – Environ. Monit. Assess. 186: 7259-7271.

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DOI: http://dx.doi.org/10.15666/aeer/2005_37373746
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[10] González-Naranjo, V., Boltes, K. (2014): Toxicity of ibuprofen and perfluorooctanoic acid for risk assessment of mixtures in aquatic and terrestrial environments. – International Journal of Environmental Science and Technology 11(6): 1743-1750.
[11] Hillis, D. G., Fletcher, J., Solomon, K. R., Sibley, P. K. (2011): Effects of ten antibiotics on seed germination and root elongation in three plant species. – Arch. Environ. Contam. Toxicol. 60: 220-232.
[12] Hurtado, C., Domínguez, C., Pérez-Babace, L., Cañameras, N., Comas, J., Bayona, J. M. (2016): Estimate of uptake and translocation of emerging organic contaminants from irrigation water concentration in lettuce grown under controlled conditions. – J. Hazard. Mater. 305: 139-148.
[13] Liu, Y., Chen, S., Zhang, J., Gao, B. (2016): Growth, microcystin-production and proteomic responses of Microcystis aeruginosa under long-term exposure to amoxicillin. – Water Res. 93: 141-152.
[14] Marsoni, M., De Mattia, F., Labra, M., Bruno, A., Bracale, M., Vannini, C. (2014): Uptake and effects of amixture of widely used therapeutic drugs in *Eruca sativa* L. and *Zea mays* L. plants. – Ecotoxicol. Environ. Saf. 108: 52-57.
[15] Minden, V., Deloy, A., Volkert, A. M., Leonhardt, S. D., Pufal, G. (2017): Antibiotics impact plant traits, even at small concentrations. – AoB Plants 9: plx010.
[16] Nunes, B., Antunes, S. C., Santos, J., Martins, L., Castro, B. B. (2014): Toxic potential of paracetamol to freshwater organisms: a headache to environmental regulators? – Ecotoxicol. Environ. Saf. 107: 178-185.
[17] OECD: Guideline for the Testing of Chemicals. Proposal for Updating Guideline 208. – Available online: http://www.oecd.org/hemicalsafety/testing/33653757.pdf. Accessed November 2017.
[18] Opris, O., Copaciu, F., Soran, M. L., Ristoiu, D., Niinemets, U., Copolovici, L. (2013): Influence of nine antibiotics on key secondary metabolites and physiological characteristics in *Triticum aestivum*: leaf volatiles as a promising new tool to assess toxicity. – Ecotoxicol. Environ. Saf. 87: 70-79.
[19] Priac, A., Badot, P.-M., Crini, G. (2017): Treated wastewater phytotoxicity assessment using Lactuca sativa: focus on germination and root elongation test parameters - Évaluation de la phytotoxicite'eaux de rejets via Lactuca sativa: paramètres des tests de germination et d'e´longation. – Comp. Rend. Biol. 340(3): 188-194.
[20] Rede, D., Santos, L. H., Ramos, S., Oliva-Teles, F., Antão, C., Sousa, S. R., Delerue-Matos, C. (2019): Individual and mixture toxicity evaluation of three pharmaceuticals to the germination and growth of Lactuca sativa seeds. – Science of the total environment 673: 102-109.
[21] Rhaul, S., McDonough, S., Ladewig, J. C. L., Soares, A. M. V. M., Nogueira, A. J. A., Domingues, I. (2013): Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio). – Environ. Toxicol. Pharmacol. 36(3): 903-912.
[22] Rodriguez-Ruiz, A., Etxebarria, J., Boatti, L. Marigómez, I. (2015): Scenario-targeted toxicity assessment through multiple endpoint bioassays in a soil posing unacceptable environmental risk according to regulatory screening values. – Environ. Sci. Pollut. Res. 22: 13344-13361.
[23] Schmidt, W., Redshaw, C. H. (2015): Evaluation of biological endpoints in crop plants after exposure to non-steroidal anti-inflammatory drugs (NSAIDs): implications for phytotoxicological assessment of novel contaminants. – Ecotoxicol. Environ. Saf. 112: 212-222.
[24] Shapiro, S. S., Wilks, M. B. (1965): An Analysis of Variance Test for Normality. – Biometrika 52(3/4): 591-611.
[25] Singh, V., Pandey, B., Suthar, S. (2018): Phytotoxicity of amoxicillin to the duckweed Spirodela polyrhiza: growth, oxidative stress, biochemical traits and antibiotic degradation. – Chemosphere 201: 492-502.

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DOI: http://dx.doi.org/10.15666/aeer/2005_37373746
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[26] Trombini, C., Hampel, M., Blasco, J. (2016): Evaluation of acute effects of four pharmaceuticals and their mixtures on the copepod *Tisbe battagliai*. – Chemosphere 155: 319-328.
[27] Vasquez, M. I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D. (2014): Environmental side effects of pharmaceutical cocktails: what we know and what we should know. – J. Hazard. Mater. 279: 169-189.
[28] Zar, J. H. (2010): Biostatistical Analysis. – 5th Edition, Pearson Prentice-Hall, Upper Saddle River, NJ.

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