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# PRELIMINARY RESEARCH REGARDING THE STRESS INDUCED IN LEMNA SP. AND IN FOLSOMIA SP. BY SOME CHLORINE COMPOUNDS USED DURING THE COVID PANDEMIC

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***Key words:*** *Lemna sp., Folsomia sp., chlorine compounds*

## INTRODUCTION

The chlorine-base compounds traded as solutions, gel, or tablets, widely used as disinfectants of surfaces, of clothes, or as a sanitizer, may be toxic for the environment, to some extent. This instantly affects the organisms in direct contact with the chlorine products, and the long term chronic effect may cause significant shifts to the impacted ecological niche (Parveen N., et al 2022).

The Covid 19 pandemic years increased the demand of chlorine disinfectants worldwide. This fact triggered a greater necessary amount of marketed products, relying on the data reported by the Market Research Report, published in 2021 (www.fortunebusinessinsights.com/industry-reports/chlor-alkali). The use of chlorine disinfectants manufactured by the Romanian company Chimcomplex S.A. (located in Oneşti and in Râmnicu Vâlcea) boosted more usually in the urban areas (as a street sanitizer), and in the rural regions, as well, increased the risk of environmental pollution. Nevertheless, the use of various concentration chlorine-based disinfectants during the Covid pandemics was recommended both by the WHO (World Health Organization) and by the EPA (Environmental Protection Agency) as a perfect solution for surfaces' sanitizer against the coronavirus contamination (https://romchimica.ro/chimcomplex).

Acknowledging the above-mentioned premises, our survey on the urban population (Bacău city) and on the rural population (from Cleja village) questioned the post-pandemic use of chlorine products and its dynamics.

This present study aims to assess the effects of two chlorine disinfectants commercialized by two known retailers in our country: Profi – Hipoclorit (sodium hypochlorite) and Lidl – chlorine tablets. The results evinced an enhanced use of sodium hypochlorite, compared to the solid product (the chlorine tablets).

The effects of various concentrations of the two chlorine base products were assessed by means of two test organisms: the common duckweed (*Lemna sp.*) (OECD no 221) mainly used to detect the toxicity level of a certain compound in the aquatic environment, and the springtails (*Folsomia sp*. – OECD no 232) – for the terrestrial environment.

Recommended by the OECD (European Organization for Cooperation and Development), the colembola type test organisms, such as the soil invertebrate *Folsomia sp.*, and the common duckweed *Lemna sp*. rapidly evince the impact of chemicals on the environment and on living organisms.

The common duckweed is a valuable plant species for aquaculture, waste management, animal feed and more. It can be cultivated in farms, used as quality forage with great results on the broiler and on the laying hens, a cost-effective and good quality fodder (Leng.1995; Ahammad M.U. et al., 2003; Akter M., et al., 2011 ). The common duckweed is considered an efficient phytoremediation agent detecting low amounts of heavy metals: Cu, Pb, Zn, As (Goswami C., et al., 2014; Iatrou E.I., et al.,2017; Sazmaz M., et al., 2017), as well as for the pharmaceuticals (Gatidou G., et al., 2017; OECD, 2003).

Numerous tests ran on the ethanol, hexane, chloroform, and aqueous common duckweed extracts evinced an antimicrobial (Gulcin I., et al., 2010; Gonzales-Renteria M., et al., 2020), an antioxidant (Petrova- Tacheva V., et., 2020; Dogan .Y., et al., 2022), or a phytotoxic effect (Velichkova K., et al., 2018).

The antimicrobial and antioxidant properties recommend this species as a valuable biomass for the food and pharmaceutical industry (Falah S.M., et al., 2022, Al Snai A.E., et al., 2019).

Common duckweed's use to assess the ecotoxicity of the organic and the anorganic pollutants, the waste water's toxicity level, the contaminated water and leachate within landfills of waste, is based on a facile in vivo and in vitro monitoring of the morpho-physiological parameters (Nika M., et al., 2020).

The general protocol and the working methodology are described by the OECD guidelines (OECD, 2003; Test no 221, 2006). Since the 1990's the springtails (*Folsomia sp.*, Willem, 1902) are used as test organisms to assess the degree of soil damage under the influence of various chemicals (Axelsen J.A., et al. 1998; Fountain M.T., et al., 2001; Greenslade P., et al. 2003; Fountain M.T., et al., 2004).The scientific classification of the springtails is: Phylum Arthropoda; Subphylum Hexapoda; Class Collembola; Suborder Entomobryomorpha; Family Isotomidae; Subfamily Proisotominae; Genus Folsomia Willem, 1902; Species Folsomia candida Willem, 1902 (NODC Taxonomic Code, database version 8.0, 1996). They are parthenogenetic invertebrates easily adaptable to lab conditions and experiments, that favour their use for the soil ecotoxicity assessment.

Included in the ISO standards as a test species (ISO-1999; ISO-2011), these isotomides (springtails) not only assess the effect of pollutants on soil (Greenslade P., et al. 2003; Fountain M.T., et al., 2004), but the impact of various compounds added in the compost, as well (Crouau Y., et al., 2002; Goldan E., et al., 2023). Many scientific studies describe to what extent the pollutants impact the reproduction of the springtails, the impact of the extreme environmental factors (temperature, humidity, excessive pasturage) on their proliferation and the consequences of soil alteration or its microfauna (Greenslade P., et al. 2003; Fountain M.T., et al., 2004; Goldan E., et al., 2023).

## MATERIALS AND METHODS

The materials (Fig. 1) used in this study were: hypochlorite and chlorine tablets, traded by two retailers from Romania: Profi and Lidl; the test species: *Lemna sp*.; *Folsomia sp*.; distilled water, sample vials of 30mL), Petri dishes, Sanyo growth chamber, Novex stereomicroscope.

The tested chlorine products' concentrations for our laboratory tests were 5% and 10%. The tested concentrations were chosen according to the chlorine concentrations stated in the standards for the drinking water (STAS 1342-1991).

The method we used to assess the effect of the two chlorine products on the common duckweed *Lemna sp*. follows ISO 2011 and OECD protocols. These perennial herbs belong to the Araceae family, Lemnoideae subfamily. They are natant or submerged hydrous plants. Their stem is reduced to a floating leafless disk called frond. The root is singular or there are a few non-vascular roots, deprived of absorbant hairs and of a root cap, as well. The duckweed (*Lemna sp.*) is very frequently found in various aquatic ecosystems, has an oval frond and a filiform root (Gurău M., 2007; USDA, NRCS, 2016).The plants tested in our experiments were collected from the ponds nearby the Gherăieşti storage lake (Bacău) and subsequently acclimatized in the lab. the suitable culture medium for the acclimatization and proliferation is the Steinberg medium, comprising: 6,7g/L NaCl, 1g/L KCl0, 0,14g/L $Ca(NO_3)_2$, 0,32 g/L $MgSO_4$, and Tris solution (Tris(hydroxymethyl)aminomethane ) 4,6 mL; the final pH value ranges from 6.8 to 7± 0.2.

The lab tests were run three times. The biologic material (*Lemna sp*. individuals) was maintained in a lab aquarium, exposed to daylight kept at $25^0$C. The stock medium solution was refreshed every fortnight or even sooner, according to the biomass amount of newly-formed duckweed (Park J., et al., 2021).

The working method for the assessment of the chlorine products's effect on the springtails (*Folsomia sp.*) is adapted following the OECD no232 protocol for the accute tests. There were run three trials for the laboratory tests.

There were used lab vials of 25 mL, each containing 10 springtails on charcoal substrate. The three experimental trials for the toxicity tests were kept in a Sanyo climate chamber at $20 \pm 2^0$ C, and a 16:8 h light:dark cycle with a light intensity of 800 lux. The juvenile springtails' number was assessed using the ImageJ software.

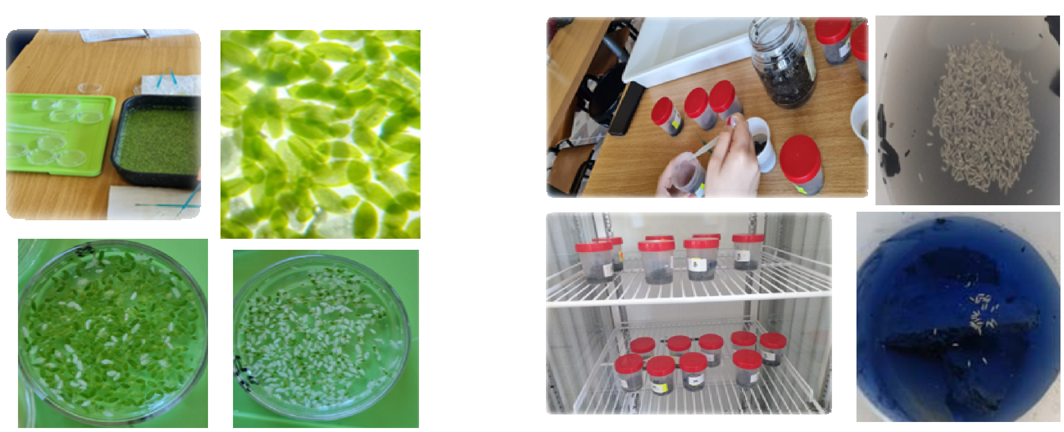


Fig. 1. Materials and results illustrating the experiments for the test-organisms *Lemna sp*. and *Folsomia sp*

## RESULTS AND DISCUSSIONS

The two Covid 19 pandemic years lead to an excessive use of chlorine products by the population, that we evaluated by means of a survey applied both in the urban (Bacău county), and in the rural areas (Cleja village).

The survey is an useful tool in the analysis of certain facts, implemented in the socio-humanistic sciences, and in other research areas, as well (Kumar R., 2018). The premises of this present study rely on the identification of the immediate or of the long-term consequences for the planet of the additional chlorine products use within the households.

The survey comprising five questions was applied to the target population. Nevertheless, the survey provided answers regarding the use of chlorine base products, of the commercial brands and mechanism of action, dosage and frequency.

Of a total number of 113 investigated subjects, 90 % use at least one of the two chlorine products (40 % - bleaching and sanitizing laundry, 40 % for surface disinfectation, 20 % as common household disinfectant products, and none uses chlorine-base compounds to sanitize fountain water or water in any domestic reservoir.

Following the analysis and interpretation of the results on the frequency and on the amounts of chlorine products, it was evinced that 45 % of the interrogated people use the products on daily basis, 33 % every week, and 22 % seldom use them.

Regarding the compliance with the label indicated dosage, 56 % of the respondents comply, and 44 % do not follow the recommended dose.

**The ecotoxicity test results in *Lemna sp*.**

The ecotoxicity test results in case of the common duckweed (*Lemna sp*.) were based on its growth and proliferation, on the number of fronds, and their aspect one week afterwards. It was noticed that the fronds were entirely chlorotic for a 10 % concentration of the tested chlorine product, and of a 63 % frond alteration induced by the 5 % concentration of the chlorine compound (Fig. 2).

The chlorotic processes affecting the fronds will be followed by a decrease of the duckweed biomass, and of frond number and size, compared to the control samples, in which the frond number doubled.

An assessment was made on other experimental variants (Fig. 3) three days afterwards (a 72 hours evaluation), aimed to evince the toxicity level of the tested products, by observing the root regrowth length in *Lemna sp*.

And of their morpho-histologic aspect. A 10 % concentration of chlorine products stops the root regrowth. To make the future tests more relevant and significant, a factorial analysis of the concentrations will be made and it will display more complex results.

**The ecotoxicity test results in *Folsomia sp.***

The influence of the toxic products tested on the springtails is displayed in fig. 4. A significant decrease in the number of juveniles was noticed, 60% less (compared to the control sample test). These results were evinced for both of the chlorine products (10 % concentration).

These studies will be followed by future, more thorough tests, to observe their proliferation influenced by the tested chlorine products. For this present study there were used the 5% and the 10 % concentrations of the chlorine solutions, that impregnated the charcoal substrate (one 5 ml/vial application). The springtails were fed and aired twice during the experiment.

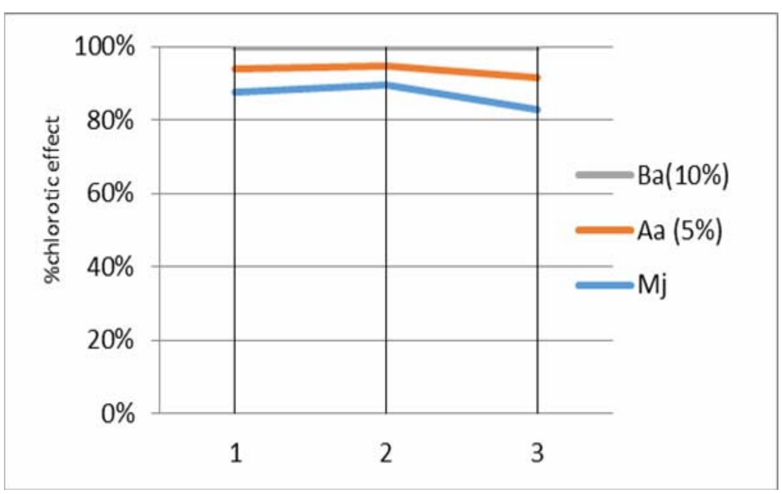
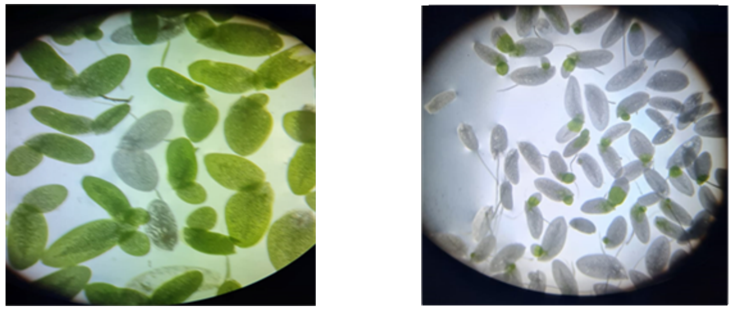



Fig. 2. The influence of the chlorine base compounds on the experimental trials in duckweed



A B

Fig. 3. The morphological aspect of the duckweed after 72 hours since the contact with the tested chlorine products (A (5%) and B (10%))

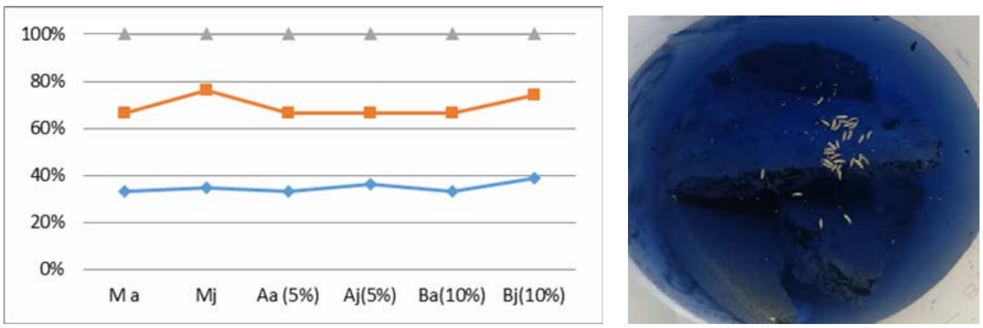



Fig. 4. Juvenile springtails proliferation dynamics during various lab tests (in the end of all the experiments)

## CONCLUSIONS

During this scientific study there were observed some test organisms, as a screening tool for the chlorine compounds from their environment.

They are characterized by significant properties due to which they may prevent environmental pollution and further changes and alterations.

Both test species (*Lemna sp*. and *Folsomia sp*.) can be tested in the laboratory at low costs. Either the common duckweed , or the springtails represent sensitive and accurate tools that can display the alterations induced by some allochtonous compounds within an ecosystem.

These ecotoxicity lab research and scientific observations rely on the easy maintenance and growth of the two test organisms in a laboratory, where their reproductive dynamics can be evaluated (based on their short life cycle), as well as their behaviour induced by the contact with the tested substances.

The toxicity impact of the chlorine products on the environment should be assessed and monitored both in situ (within lab conditions), and ex situ (in various ecosystems) in order to draw thorough conclusions on this topic.

This present study comprises preliminary results, that will be continued by a factorial analysis of other chlorine compounds concentrations and their impact reflected by the juvenile sprintails□ number, by the number of altered *Lemna sp*. fronds, and the root regrowth.

The data provided by the lab tests and observations displayed a high degree of alteration in the two species of test organisms induced by the 10 % concentration of the chlorine products.

## ABSTRACT

The chlorine-base compounds (traded as solutions, gel, or tablets, widely used as disinfectants of surfaces, of clothes, or as a sanitizer, may be toxic for the environment, to some extent). This instantly affects the organisms in direct contact with the chlorine products, and the long term chronic effect may cause significant shifts to the impacted ecological niche

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