Effect of Liquid Detergents on the Biochemical Parameters of Some Plantlets

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This article reports an environmental study concerning the liquid detergent contamination and its impact on some plantlets, e.g. Phaseolus vulgaris and Petroselinum crispum. In this context, this study focuses on specific modifications of the physical-chemical and biochemical characteristics of some plantlets induced by liquid detergents present in the soil, both bio-based and without environmental compatibility. The results of this study showed that a decrease (over 60%) in total chlorophyll per fresh weight could be solely due to the presence of non-biodegradable liquid detergents in soil, in various concentrations: 0.125%, 0.25% and 0.5% (wt/wt), calculated on water mass. On the other hand, the action of the detergents on the plantlets has revealed a decrease in the catalase activity in the case of both types of detergents.

Keywords: biodegradable, chlorophyll, enzymatic activity, isothiazolinones, without environmental compatibility

Man causes the anthropic pollution through various activities in industry, agriculture, transport as well as household activities. One can easily notice while reading specialised literature of a high scientific level that the anthropic pollution has been amplified in the last decades as compared to the natural pollution. Pollutants display the property of acting differently upon the living cell. The pollutant's nature, its concentration, the reciprocal influences of the pollutants and their action time frame are only some of the factors that influence the final effect. Some pollutants are neither metabolized in a food chain, nor eliminated; therefore, they may accumulate in organisms producing the phenomenon of biological amplification [1, 2].

Many of the chemical substances present in the products for house floor cleaning are characterized by a certain degree of toxicity. Unfortunately, most of the studies in the specialized literature have focused on characterizing the ingredients of certain cleaning products (such as those intended for washing or floor cleaning) which can generate serious toxic effects upon human health [3-5] without taking into account the fact that the effects of these cleaning products (through the existing components in their composition) upon the environment can be at least equally damaging. As a rule, detergent pollution may be encountered in rural dwelling areas where the development of the sewing and household water cleaning infrastructure is either diminished or completely missing. Special attention is required when approaching this topic if we take into account that Leiva-Salinas et al. [6] highlight the fact that no limits have been set for the some components concentration in industrial products and directive 2005/42/EC of the European Commission authorizes the use of some preservatives e.g. methylisothiazolinone in household products up to 100 ppm with mandatory labeling. However, many producers have recently focused on selling eco-friendly cleaning products, including household surface cleaners and laundry products. As a consequence, the impact of detergents, especially that of liquid detergents, on plants is a complex problem, their effect being dependent on various factors such as the pollutant's concentration, the soil's characteristics and, the last but not least, on the species e.g. common beans and the parsley. Common beans (*Phaseolus vulgaris*) is a herbaceous plant which is grown on a large scale for food purposes, being an essential source of nutrients, such as proteins, vitamins (A, B6, C and K), magnesium, calcium, folic acid, iron, zinc, potassium, and phosphorus. Moreover, another plant grown for food purposes is the parsley (*Petroselinum crispum*). This is a biannual plant completely free of hairs on its entire surface, with a specific scent [7].

Although in the specialized literature there are a few studies about the impact of various detergents in terms of the damaging nature of some of their components (methylisothiazolinone, methlychloroisothiazolinone and 2-bromo-2-nitropropane-1,3-diol etc.), to the best of our knowledge there is no article about their impact on the plantlets metabolism. Due to their specific bio acid properties, the isothiazolinones have to be considered as possible eco-toxicological risks when released into the environment. Thus, the present research may contribute to the clarification of the negative effects of the preservatives present in the liquid washing detergents, keeping in mind that in the current context there is little information about the appearance and behavior of the isothiazolinones within the environment and their negative eco-toxicological properties [8]. Therefore, this article reports an environmental study concerning the liquid detergent contamination and its impact on some plantlets, e.g. *Phaseolus vulgaris* and *Petroselinum crispum*. This study focuses on specific modifications of the physical-chemical and biochemical characteristics of some plantlets induced by liquid detergents present in the soil, both bio-based and without environmental compatibility. Also, the relationship between contamination level of the soil with liquid detergents and plantlets samples in the research area is evidenced.

Experimental part

Materials and methods

Plant material

The present study has used a biological material consisting of various experimental models of vegetal

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physiology: bean plantlets (Phaseolus vulgaris) originating from their embryos and parsley (*Petroselinum crispum*). The bean and parsley plantlets were grown and monitored in a green-laboratory at Dunarea de Jos University of Galati, Romania. The recipients of the plants which grown into (plastic beakers: 0.18 L capacity, 1 plant per beaker) were kept at 20 ± 1°C at a relative humidity of under 70%. For germination, the seeds of the witness samples and the plantlets generated from them were exposed to natural sun light for 10h every day for the entire duration of the study. During the experiments (21 days), we used indirect light coming from a window facing north. The biological material used in the experiments consisted of samples of vegetal tissue from leaves, stem and root (in order to monitor the enzymatic activity), and respectively from leaves (in order to monitor the chlorophyll).

Detergents

Detergents (biodegradable vs. non-biodegradable) and topsoil (*p*H = 7; 29-60% organic matter, 0.35-0.75% mineral salts, 25-50 ppm mineral nitrogen, 52-85 ppm potassium, 32-55 ppm phosphorus) for planting of the vegetal material were purchased in local supermarkets from Galati (Romania). Each plant sample was administered a volume of 4mL of detergent solution based on the ratio of 1m^2 that is supposed to be polluted by 1L of detergent solution. After 14 days of detergent treatment, young leaves that appeared after detergent solution application and roots were harvested and used for physico-chemical and biochemical analyses.

Chlorophyll and carotenoid analyses

Chlorophyll content was determined by spectrophotometric means according to Lichtenthaler and Buschmann [9] with some modifications (for the optimization of the extractions the ultrasonication for 3 min was used). Sodium carbonate, used to inhibit the effect of chlorophyllase (0.15g Na_2CO_3/1g plant material), normally present in plants, which can easily degrade chlorophylls during the extraction [10] was obtained from Merck (Germany).

Catalase activity assay

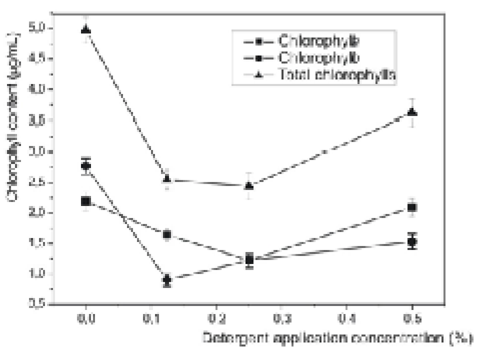
Catalase (CAT, MW~240 kDa) activity was determined according to Artenie et al. [11] and Bergmeyer [12]. The catalase activity unit was considered to be a quantity of enzyme that decomposes a micromole (mmol) of oxygenated water (0.034 mg) within a minute. All chemical reagents used in thise study were of the highest analytical purity grade.

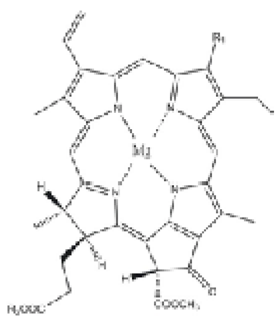
Results and discussions

Chlorophylls and total carotenoids analysis

According to Krystian and Stanislaw [13] in vivo, chlorophylls (the most common green pigments) play a key role in the process of photosynthesis. So, in the case of the bean samples treated with biodegradable and non-biodegradable detergents (fig. 1A and fig.. 1B respectively) the impact the detergents had upon the bean plantlets is significant and it depends on their type and concentration. Initially, we noticed a very high content of chlorophyll *b* in the witness sample as compared to chlorophyll *a*.

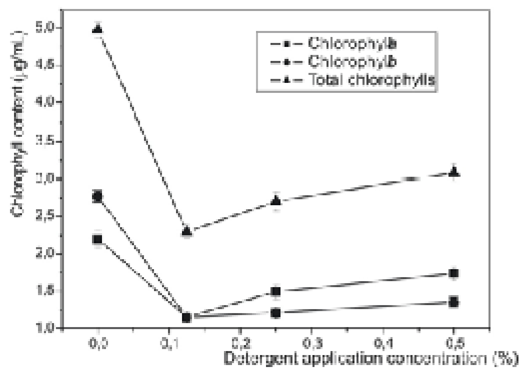
The samples treated with biodegradable detergent displayed a massive decrease of chlorophyll *b*, in the case of the sample with the lowest concentration of detergent solution (0.125%), where chlorophyll *a* decreased by 28.12%, and chlorophyll *b* with 58.93%, as compared to the witness sample. An interesting situation appeared in the case of the sample treated with the highest concentration of biodegradable detergent (0.5%), where the chlorophyll's biosynthesis was less inhibited than in the case of the minimum concentration of detergent, the total quantity of chlorophyll decreasing by only 27.26% as compared to the control sample; however, the quantity of chlorophyll *b* decreased by 44.76% as compared to the control sample.





Compounds	R_1	R_2
Chlorophyll *a*	CH_3	$C_{20}H_{39}$
Chlorophyll *b*	CHO	$C_{20}H_{39}$
Chlorophyllide *a*	CH_3	H
Chlorophyllide *b*	CHO	H

(A)



(B)

Fig. 1. Change in total chlorophylls content, content of chlorophyll *a* and content of chlorophyll *b* in extracts from *Phaseolus vulgaris* plantlets materials treated with biodegradable detergent (A) and detergent without environmental compatibility (B). The data were presented as the mean ± SE (n = 3). Also, is presented (Fig. 1A, right) the chemical structures of chlorophyll *a*, chlorophyll *b* and chlorophyllide *a* and chlorophyllide *b*, respectively (after Cubas et. al. [10])

If we refer to the samples treated with non-biodegradable detergent, the data present in figure 1B show that in the case of the sample treated with the lowest concentration of detergent (0.125%) the quantity of chlorophyll *b* was most affected decreasing by 63.53% as compared to the witness sample. As in the case of the biodegradable detergent, the chlorophyll *a* biosynthesis was less inhibited for the highest concentration of non-biodegradable detergent (0.5%), when the total quantity of chlorophyll decreased by 37.83%. However, chlorophyll *b* decreased by 44.76%. These different types of behavior are most probably due to the different viscosity of the detergent solutions used in this study. This means that in the case of low concentrations of detergent, the latter reaches the roots faster and thus the short term absorption capacity of the plantlets is higher. Essentially, all bean samples treated with detergents showed that chlorophyll *b* is more affected than chlorophyll *a*. On the other hand, on the basis of the data obtained after analyzing the chlorophyll content for *Petroselinum crispum* plantlets, one may say that a considerable decrease in the quantity of chlorophyll *a* and chlorophyll *b* was noticed in all samples treated with detergents, especially in the case of lower concentrations (fig. 2 and fig. 3).

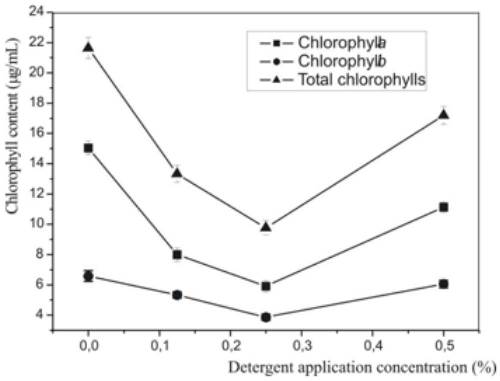


Fig. 2. Change in total chlorophylls content, content of chlorophyll *a* and content of chlorophyll *b* in extracts from *Petroselinum crispum* plantlets materials treated with biodegradable detergent The data were presented as the mean ± SE (n = 3)

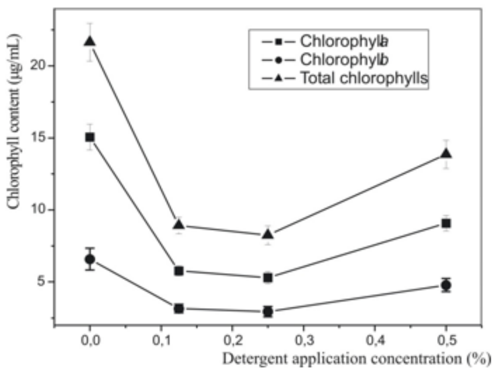


Fig. 3. Change in total chlorophylls content, content of chlorophyll *a* and content of chlorophyll *b* in extracts from *Petroselinum crispum* plantlets materials treated with detergent without environmental compatibility. The data were presented as the mean ± SE (n = 3)

In the case of the samples treated with biodegradable detergent, the most affected sample was that treated with a detergent solution of 0.25% concentration, the quantity of chlorophyll *a* decreasing by 64.87%, and the quantity of chlorophyll *b* also decreased by 55.31%, as compared to the witness sample.

The sample treated with the non-biodegradable detergent solution (0.25%) was the most affected, the quantity of chlorophyll *a* decreasing by 60.82%, and the quantity of chlorophyll *b* decreased by 41.33%, as compared to the witness sample.

Therefore, one may talk about a major impact of diluted detergents with a concentration of 0.25% upon the parsley samples, especially in the case of the non-biodegradable one. Moreover, we noticed a much larger influence upon the biosynthesis of chlorophyll *a* than the one of chlorophyll *b*, although the former is to be found in a much larger quantity in the analyzed plants than chlorophyll *b*. This shows that in the case of parsley chlorophyll *a* is much more sensitive to the contact with the organic compounds present in the composition of detergents.

Enzymatic activity analysis

The study of the plants' catalase activity is generally interesting due to its importance in the plants' metabolism, plants' defense mechanisms and the response signals towards external factors [14]. Yet, no report on the interactions of CAT with liquid detergents has been described. In this context, the data in figure 4 and figure 5 illustrate the considerable influence exerted by the analysed detergents upon the development of *Phaseolus vulgaris* and *Petroselinum crispum* plantlets.

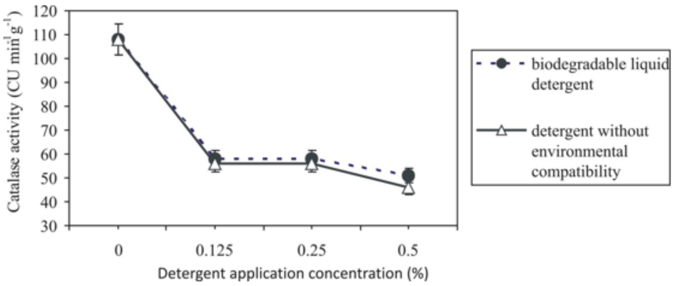


Fig. 4. Variation of catalase (CAT) activities changed of *Phaseolus vulgaris* with detergents-exposure concentrations. Vertical bars represent standard deviation of the mean (n = 3)

The action of the detergents on the plantlets has revealed a decrease in the CAT activity in the case of both types of detergents. From the study of enzymatic activity variation with detergent concentrations is notes the decrease in the CAT activity for *Phaseolus vulgaris* with 52.77% corresponding to final concentration (0.5%) of the biodegradable detergents added across the soil. In the case of non- biodegradable detergents the decrease of CAT activity was 57.41 %.

Also, results from conducted experiments with *Petroselinum crispum* leaves showed that average enzymatic activity during biodegradable detergent treatment was higher (28 ± 3.3 CAT unit/min/g) than in the cases of the detergent without environmental compatibility (6 ± 0.9 CAT unit/min/g). In the case of treating the *Petroselinum crispum* samples with biodegradable detergent we noticed that the activity of the catalase decreases, when exposed to low concentrations of detergent (0.125%), by 64% as compared to the control sample, while for the non-biodegradable detergent it decreases by 67%. The same variation holds valid in the case of *Phaseolus vulgaris* plantlets as well (46.3% and, respectively 48.2%). This phenomenon is explained by the fact that, probably, the presence of certain chemical compounds in the composition of detergents (methyl-isothiazolinone, methlychloroisothiazolinone and 2-bromo-2-nitropropane-1,3-diol etc.) inactivate the enzymes of the plantlets, aspect which can be emphasized through a

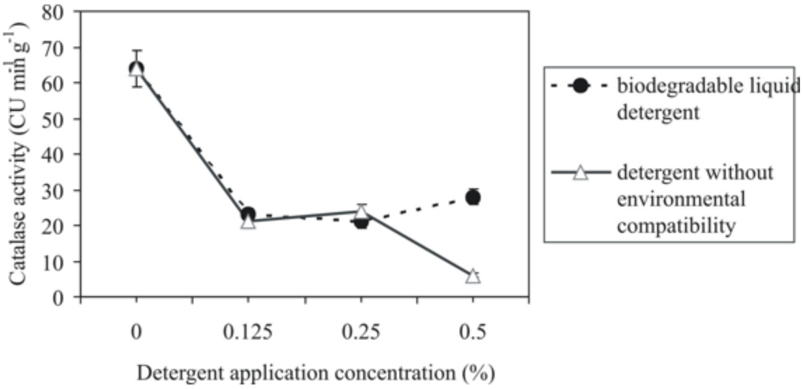


Fig. 5. Variation of catalase (CAT) activities changed of *Petroselinum crispum* with detergents-exposure concentrations. Vertical bars represent standard deviation of the mean (n = 3)

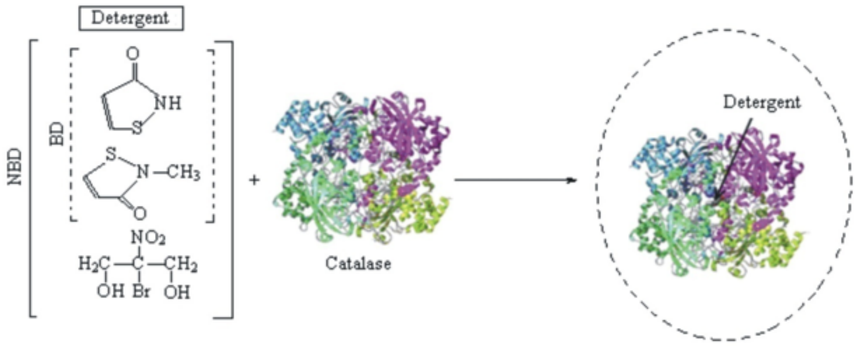


Fig. 6. The proposed mechanism of binding ability vs. some compound structures (isothiazolinone, methylisothiazolinone and 2-bromo-2-nitropropane-1,3-diol respectively) of detergents to catalase (monofunctional catalase/typical). The result is the enzymatic inactivation. CAT (structure after Balwinder et. al. [15], Diaz et al. [18]) consists of four identical subunits each with a heme situating in the active site which is accessible from the CAT surface through a main channel about 25–55Å in length [19] and the main channel leads the ligand to the active site

mechanism suggested in this paper and presented in figure 6.

This enzymatic inactivation, as a result of the impact with the chemical compounds present in the pollutant agent, that may represent potential enzyme binding centers, can be the result of the catalase binding with the hydrophilic part by means of weak bindings of the hydrogen and van der Waals type. Moreover, the chemical compounds present in detergents interact with the enzyme probably through the haem group. This is present between the internal walls of the beta barrel and several helices and it is responsible for the enzymatic activity [15-17]. On the other hand, in the case of samples treated with non-biodegradable detergent we noticed a decrease in the catalase activity of 90.62% for parsley and of 57.41% for bean plantlets, at the highest concentrations in the study. Therefore, we can talk about a higher decrease in the catalase activity when using non-biodegradable detergents than in the case of biodegradable ones. On top of this, according to the experimental results, bean displays a higher resistance to the action of detergents than parsley.

Conclusions

The results of this study show that exposure of the plantlets under scrutiny to the action of liquid detergents may induce significant physical-chemical and biochemical modifications at structural level. All these results show that, in terms of chlorophyll biosynthesis, the bean plantlets display a better behavior under the action of the environment incompatible detergents than that of the parsley plantlets. Furthermore, in present study as well, the enzymatic activity proves to be an efficient bio-indicator in terms of the aggressive action of certain pollutants, such as liquid detergents, on the plantlets. These results indicate that the lower values for the enzymatic activity would make it possible to show the stress and damage to some plantlets under liquid detergents impact. The CAT activity of *Phaseolus vulgaris* and *Petroselinum crispum* is believed to be associated with the presence of liquid detergents in soil especially the detergent without environmental compatibility which diffuse into and damage cell membrane structures and thus affects the metabolism of both plants. The manner in which the plantlets' enzymatic activity manifests when they are under the impact of detergents emphasizes the *Phaseolus vulgaris* species which presented a more positive response to the action of the aggressive factor. On the basis of the experimental results, it is clear that beans have a higher resistance to the action of the studied detergents than parsley.

Both detergents (bio-based and without environmental compatibility) were responsible for significant decreases in catalase enzymatic activity. However, results showed that liquid detergent impact on *Petroselinum crispum* was least aggressive when detergent dilutions were lower during the period of this study. Certainly, the biochemical characteristics of studied plants depend on many factors, such as, e.g. structure of the soil and exposure time. Thus, it could be useful to know their impact on metabolic responses to various liquid detergents.

The results of this study show that people should never throw the water used with detergents to wash the floors over cultivated areas of soil.

Taking into consideration the changes that might appear at the plantlets level under the impact of biodegradable detergents and, more importantly, environment incompatible ones, it is obvious that the future research of environment pollution will pay special interest to aspects regarding the enzymatic activity as a bio-indicator of the environment pollution degree.

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