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# Phytotoxicity tests with higher plants for environmental risk assessment

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## Abstract

***Phytotoxicity tests were performed to evaluate their application in environmental monitoring on different substrates, e.g. commercial detergents (D1, D2) and surface water from the dismissed Cu and Fe mine of Libiola (NW Italy), respectively.***
***Seeds of Vicia faba were grown in 0, 10, 30, 60, 100 g/l of D1 and D2. Number of germinated seeds and root length, Germination Index (GI) and growth inhibition ($EC_{50}$) were considered. The Vicia test showed no significant differences between D1 and D2, with an $EC_{50}$ of 13 g/l (11-15 95% CI) for D1 and 16 g/l (14-19 95% CI) for D2. These data were confirmed by GI<50% at 18, 26, 30, 60 and 100 g/l.***
***Onion bulbs of Allium cepa, were tested at 0, 6, 12, 25, 50 and 100% of mine water. Root length inhibition ($EC_{50}$) and Mitotic Index (MI) were evaluated. The Allium test showed a statistically significant inhibition of root growth at 50% and 100%. The root growth percentage decreased in a dose-related manner, with an $EC_{50}$ of 87%. The MI decreased significantly only at 100% (undiluted mine water).***
***Both phytotoxicity tests should be considered as valid ecotoxicological indicators for health and environment.***

## Introduction

Plant bioassays are more sensitive and simpler than most other systems to detect the phytotoxicity [1-3] and the genotoxic effects of environmental pollutants [1,4] These tests have been validated by international collaborative studies under the United Nations Environment Program (UNEP), World Health Organization (WHO), and US Environmental Protection Agency (USEPA) [5].
Different plant systems such as *Allium cepa* and *Vicia faba* have been used for mutation assays, cytogenetic tests and chromosomal aberration tests [6,7].
The tests with *Vicia faba* var. *minor* can be used to estimate phytotoxic and genotoxic effects and, in addition, to evaluate the quality of compost [8].
The *Vicia* root growth test has been used to detect the effects of dyes, commonly used in food industry, and of polycyclic aromatic hydrocarbons and heavy metals [9,10].
The *Allium* test is a fast and simple method to assess the phytotoxicity of substances, or matrices of environmental concern, based on root growth inhibition in onion bulbs. The analysis of genetic material in meristematic cells also allows to detect alterations of mitosis [11,12]. This test has been used both to assess the potential damage of chemicals hazards, as well as physical ones, such as electromagnetic fields [7 and references therein].
In the present study, we used *V. faba* seed germination and root elongation tests to evaluate the phytotoxicity of two commercial detergents. In addition, the *Allium* test was employed to study the phytotoxicity and cytogenetic effects of metal-rich mine water.
The usefulness of these ecotoxicological tests to assess environmental quality is discussed.

## Materials and methods

### *Seed germination and root elongation tests*

*Vicia faba* seeds were soaked in distilled water, and then sown in veg-boxes containing vermiculite saturated with test solutions, consisting of two different commercial detergents, D1 and D2. These solutions had the same chemical composition (vaseline oil, water, non ionic ethoxylated surfactant, alkyl C10-C16-benzyl-dimethyl ammonium chloride 50%, polyacrilamide, 2-bromo-2-nitropropane-1,3-diol), but a different scent, 10 times more concentrated in D2. Tap water was used as control. The seeds were cultured in a growth chamber under controlled conditions (T=20±1°C, 72 h, on dark). In each treatment group, 9 seeds were observed for germination. All treatments were done in five replicates. The following parameters were considered: number of germinated seeds (Gt), root length (Lt), Germination Index GI%=(GxL/G0xL0)x100, where G0 and L0 are the number of germinated seeds and mean root length of controls, respectively, while Gt and Lt are the number of germinated seeds and root length of each sample. Growth inhibition was evaluated in terms of $EC_{50}$.

*Allium test*

Commercial onion bulbs of *Allium cepa*, from biological agriculture were used. A set of five onions was used for each concentration of mine water (0, 6, 12, 25, 50 and 100%), previously centrifuged (4000 rpm, 5') to separate the colloidal fraction, and placed (60 ml) in test tubes. The experiment was performed at T=19±1°C for 72 h in the dark. At the end of the experiment the length of root bundles was measured. The growth inhibition $EC_{50}$ was expressed as the concentration of mine water causing 50% root length reduction, and calculated by plotting the percentage of root growth reduction of treated vs. control plants against the concentrations of test solution.
Slides for microscopical studies were prepared, as described in [13]. From each bulb, two root tip were taken and each of them was used to prepare 1 slide. For each water concentration, the mitotic index (number of dividing cells per 1000 observed cells) was derived from a total of 2000 cells. Slides were observed under a light microscope (Leica DM 2000, equipped with digital camera). Cells were recorded as normal or aberrant at different stages of the cell cycle, namely: interphase, prophase, metaphase, anaphase or telophase. Data analyses were performed using Statistica 8.0, StatSoft, Inc. Difference were considered significant at $p < 0.05$, and highly significant at $p < 0.01$, using one-way ANOVA and Tukey's post-hoc test.

## Results

*Seed germination and root elongation tests*

Considering the ratio between the number of germinated seeds and the total number of seeds, and the GI for D1 and D2, a hormetic effect at 10 g/l is observed. The germination rate and root elongation of D1 and D2, at different concentrations, showed a similar trend, with GI <50% at 18, 26, 30, 60 and 100 g/l. The root length showed a dose-dependent decrease, and the analysis of dose-response curves yielded $EC_{50}$ values of 13 g/l (11-15, 95% CI) for D1, and of 16 g/l (14-19, 95% CI) for D2.

*Allium test*

The *Allium* test showed a statistically significant inhibition of root growth at 50% and 100% mine water concentrations. The mitotic index also decreased in a dose-dependent way for increasing concentrations of the tested solution, and showed a significant reduction at 100% (undiluted mine water). The root growth percentage decreased in dose-related manner, with an $EC_{50}$ of 87%. In the treatment with 100% mine water only interphasic cells were observed, and some of them showed anomalies like highly condensed nuclei.
Mitotic aberrations were found in 25%, 50%, and 100% mine water: disturbed anaphases, pulverization of chromosomes, anaphasic bridges, c-metaphases, contracted nuclei with disorganized genetic material and metaphases with "sticky" chromosomes.

## Discussion

The *Vicia faba* (broad bean) root growth test is recommended by many international research programs such as the International Programme of Chemical Safety, as it represents a rapid, reproducible, and cheap experimental model for biological monitoring [14].
The data obtained with the same dilutions of the two detergents were comparable. We can assume that the scent, the only difference between the two products, does not significantly affect the D1 and D2 phytotoxicity. The results of this analysis suggest the right dilution for a correct use of these products, in order to avoid environmental risk.
The *Allium* test detects cytotoxic, genotoxic and/or mutagenic molecules, directly or indirectly dangerous to organisms [1,14,15]. This index is enough sensitive to be used in the monitoring of contaminated waters [14].
In our experiments, only the most concentrated solutions (50% and 100%) showed marked effects on onion roots, both in terms of average root elongation and MI. The cytotoxic level of a compound can be determined on the increase or decrease in the MI, which can be used as a useful parameter in environmental biomonitoring [16]. The dose-dependent decrease of the MI revealed the presence of cytotoxic substances in mine water. A chemical characterization of this water, already reported in the literature [17], highlighted the presence of heavy metals. These latter elements could affect mitosis in apical meristem cells of *A. cepa*, or inhibit DNA repair mechanisms, causing irreversible damage to genetic material [7,11,12]. The concentration of 50% mine water had a MI of 41.1% compared to control, defined as sublethal effect [18], while 100% had an MI of 15.9%, corresponding to lethal effect [19]. This finding is in agreement with the calculated $EC_{50}$.
Our results have confirmed that phytotoxicity tests with higher plants may be useful tools in the risk assessment of xenobiotics, both directed to the environment or to human health.

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