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INVESTIGATION OF THE EFFECT OF PHENOL POLLUTION ON DIFFERENT PLANTS IN POT EXPERIMENTS

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Abstract: *Soil contamination with organic pollutants is one of the most serious environmental problems that can cause serious threat to people and the environment. Phenolic compounds represent a large group of molecules and have many functions regarding the aspects of development and the behaviour of the plant. Pot experiments are inexpensive in comparison to field experiments with variety of experimental treatments without contamination of the site. It is easier to observe the possible changes because the contaminants and the material absorption are more pronounced, because the soil and root are tight, and the root cannot grow out of the polluted zone. After a 14-day test period, the physical parameters were measured, but no significant differences were found between the treatments. It has changed the vitamin C content, which is very important in the oxidative stress of plants, as it is a basic material for the plant life. For vitamin C, we have also experienced the phenomenon of hormesis. It is extremely interesting how the plant tries to respond to a certain stress effect by changing the content of vitamin C. As a result of phenol contamination, we have been able to increase vitamin C in some concentrations and decrease mostly at higher concentrations, and vice versa. The amount of vitamin C in plants was measured by HPLC. Phenol as a stress factor, increased the content of vitamin C in certain concentrations in plants. By selecting a suitable non-toxic stress factor, vitamin C content can be increased in different plants.*

Keywords: *Phenol; vitamin C; antioxidant; hormesis; pot experiment; HPLC*

INTRODUCTION

Soil pollution with organic pollutants is one of the most common environmental problems, posing a serious threat to people and the environment. Innovative solutions for the remediation of soils contaminated with organic matter deserve special attention [1]. Contaminants are taken up by the roots of plants from the soil or their leaves from the air and, if they enter the tissue, can cause serious damages [2].
Phenol and its derivatives form a large family. These include indicator molecules, pigments and flavouring substances that attract or repel or protect plants from viruses, bacteria, fungi and insects [3]. To assess human risks, it is extremely important to understand the uptake and accumulation of organic contaminants in plants. However, scientists have also observed the decomposition of these accumulated substances in plants [4]. Phenolic compounds act as stressors on plants, thus also influencing the number of antioxidants [5].
Hormesis is a dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition. Hormesis usually occurs in plant species [6], [7]. The most common form of dose-response curve is the low-dose stimulating and high-dose inhibitory effect; the inverted U-shaped curve ("A") [6]. The other common

form depicts the low-dose decrease and the high-dose enhancing effect, the U-shaped curve (“B”) [6].
In the present paper was studied the effect of phenolic pollution in the soil on various plants. Pot experiments were performed on soil samples with phenol contamination and control samples. Soil contamination was artificial and was investigated for its effects on various plants (*Lepidium sativum, Sinapis alba, Triticum aestivum*). After planting and test period, Vitamin C content in different parts of plants (root, stem, leaf) were examined. Vitamin C concentration was determined by HPLC. The results were evaluated using Microsoft Office Excel.

MATERIALS AND METHODS

Soil sampling

Soil samples were taken from the area of Óbuda University (H-1034 Budapest, Doberdó u. 6.). Soil type was Soil Ramann's brown forest soil. Soil characteristics were measured in the Soil Research Institute [8]. After soil sampling, the soil was air-dried (for approximately 72 hours). The soil was then sieved through a 2 mm diameter sieve. It was further dried at room temperature for 72 hours.

Pot experiment with artificially polluted soil (phenol)

0, 50, 100, and 150% of the soil phenol contamination limit (according to 6/2009. (IV. 14.) KvVM-EüM-FVM decree: 1 mg phenol / kg) was chosen for the pollution of the soil used [9]. The pot experiment was performed in 3 replicates. Number of seeds planted per pot was 100.
After planting, 50 ml of distilled water was sprinkled daily for a test period of 14 days.

Sample preparation for chromatographic analyses

One g plant sample was use for vitamin C determination. The plants had to be prepared very fast since vitamin C is very sensible for heat, UV light and oxygen. For the determination of vitamin C the frequently used method Furusawa [8] was applied and plant sample preparation was performed according to Lásztity and Törley [9].

Chromatographic analysis of vitamin C content

The measurement was done by HPLC (type: YL9300 HPLC System built-in Vacuum Degasser Quartenary Pump, UV/VIS Detector and 7725i injector). The measurement was carried out by HPLC-UV was at 254 nm. Vitamin C was separated on a C18 Kinetex column (5μ C18 100 A 150 x 4.6 mm and Security Guard Cartridges C18 4x3.0 mm ID). The flow rate was 1 cm3 min-1; solvent was 2% acetic acid (isocratic system). The chromatographic data processing software was YL-Clarity data system.

Plants used in the pot experiment

The following plants were used for planting: mustard, wheat (BiOrganik Online Kft.) and garden cress (Rédei Kertimag - Vetőmagkereskedelmi Zrt.).

RESULTS AND DISCUSSION

The following questions were looking for the answer: did phenol influence biochemical processes within the plant (e.g., antioxidant balance). Is phenol left in the soil? What effect did it have on the vitamin C content measured in the plant parts?

Garden cress

In the case of root: The phenomenon of hormesis [6] can be observed. Based on the dose-response curve, there was an increasing in the phenol concentration at low concentrations that inhibit root growth to the minimum of the function (0.5 mg / kg phenol in soil) and then the vitamin C content increases. At the same time, phenol at a certain concentration, reaches a maximum from which vitamin C is already reduced and a toxic effect prevails in the plant.

Stem: The phenomenon of hormesis can be observed; however, here the curve is blunter. Based on the dose-response curve [6], there was an increasing the phenol concentration at low concentrations with an inhibitory effect on stem growth to the minimum of the function (0.5 mg / kg phenol in soil) and then increasing the vitamin C content. At the same time, phenol at a certain concentration, reaches a maximum, from which the vitamin C content is already reduced, and a toxic effect prevails in the plant.

Leaf: The phenomenon of hormesis can be observed. The changes in the vitamin C content of the leaf can be plotted on the "A" curve (Figure 1) of the hormesis phenomenon [6]. Based on the dose-response curve [6], increasing the phenol concentration stimulates the vitamin C content of the leaf up to the maximum of the function (0.5 mg / kg phenol in soil), and then the vitamin C content decreases.

Mustard

Root: The vitamin C content of mustard roots decreased with increasing phenol concentration. A linear relationship can be established.

Stem: The vitamin C content of mustard stems decreased with increasing phenol concentration. A linear relationship can be established.

Leaf: Hormesis [6] is observed in the vitamin C content of the mustard leaves as the phenol concentration increases. The changes in the vitamin C content of the leaf can be plotted on the "B" curve of the hormesis phenomenon [6]. However, a blunt curve is obtained. Based on the dose-response curve, by increasing the phenol concentration, at low concentrations, it inhibits leaf growth to the minimum of the function (0.5 mg / kg phenol in soil), and then the vitamin C content increases. At the same time, phenol reaches a maximum at a certain concentration, from which the vitamin C content is already reduced, and a toxic effect prevails in the plant.

Wheat

According to the results, the vitamin C content of the plant increases if the stress affecting the plant is marginal (1.5 mg kg-1 phenol in soil), however the growth was not intensified. This response of immune reaction confirms that hormesis is measurable and that it might be generalized. This concentration dependent biochemical change is rarely studied in detail [6], in spite of the fact that over the past decade the concept of hormesis has been accepted and widely investigated [7].

Root: Hormesis is observed in the vitamin C content of wheat root with increasing phenol concentration. Changes in the vitamin C content of the root can be plotted on the "B" curve of the hormesis phenomenon [6]. Based on the dose-response curve, by increasing the phenol concentration, at low concentrations, it inhibits root growth to the minimum of the function (0.5 mg / kg) and then increases the vitamin C content (1 mg / kg phenol). The maximum of hormesis can be observed here, because after that the vitamin C content decreases. It can be concluded that the phenol concentration of 1.5 mg / kg phenol in soil no longer has a stimulating effect on wheat roots.

Stem: Hormesis is observed in the vitamin C content of wheat stems with increasing phenol concentration. Changes in the vitamin C content of the stem can be plotted on the "B" curve of the hormesis phenomenon [6]. However, the dose-response curve is quite blunt.
Based on the dose-response curve, by increasing the phenol concentration, at low concentrations, phenol has an inhibitory effect on root growth, to a minimum of function (0.5 mg / kg phenol in soil), and then the vitamin C content increases. However, we do not know the maximum of the function here. A phenol concentration of 1.5 mg / kg phenol in soil has a stimulating effect on vitamin C content.

Leaf: Hormesis is observed in the vitamin C content of wheat leaf with increasing phenol concentration. Changes in the vitamin C content of the leaf can be plotted on the "B" curve of the hormone phenomenon [6]. However, the dose-response curve is quite blunt. Based on the dose-response curve, by increasing the phenol concentration, at low concentrations [10], [11] it has an inhibitory effect on root growth to the minimum of the function (0.5 mg / kg phenol), and then the vitamin C content increases. However, we do not know the maximum of the function here. A phenol concentration of 1.5 mg / kg phenol in soil also has a stimulating effect on vitamin C content.

CONCLUSIONS AND RECOMMENDATIONS

For selected plants, we would suggest that cress and wheat are excellent for determining phenolic contamination; we would not recommend mustard and beans. Initially, we also applied the measurements to beans, but it developed at a slower rate than germination, or not at all. The phenol contamination causes oxidative stress on plants, which increases hydrogen peroxide production. Its proportion and amount are proportional to the concentration of vitamin C in the plant. We would recommend measuring hydrogen peroxide simultaneously with the measurement of vitamin C concentration measured with an HPLC device. Regarding plants, phenol can cause hormesis in plants.

Then, we have measured the inhibition of growth of certain plant parts by phenol on plants. Pot experiments are inexpensive in comparison to field experiments with variety of experimental treatments without contamination of the site.

Phenol has changed the vitamin C content, which is very important in the oxidative stress of plants, as it is a basic material for the plant life. Without vitamin C, the excess of hydrogen peroxide in the plant would kill it. For vitamin C, we have also experienced the phenomenon of hormesis.

No measurable amount of phenol was found in the soil. According to the literature, the phenol has a half-life in soil less than 5 days. The amount of vitamin C in plant samples and residual amount of phenol were measured by HPLC.

Our experimental plants are fit for human consumption and their consumption are widespread in the world also. Phenol as a stress factor increased the content of vitamin C in certain concentrations in plants. In today's world, you can hear more and more that the fruit and vegetables available in the store contain far fewer C-vitamins than before. By selecting a suitable non-toxic stress factor, vitamin C content can be increased in different plants.

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