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EXPERIMENTAL RESEARCH ON ACCUMULATION, OVER TIME, OF HEAVY METALS IN GREEN LETTUCE

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Abstract: The experiments presented in the paper consisted in the development and monitoring of a green lettuce culture for a period of time equal to 139% of the ripening or vegetation period of the crop. The culture was done in separate pots, on three categories of soil: infested with heavy metal (zinc), 1.5%, 3.0% and 4.5% respectively. For each of the three soil categories, 11 harvests were carried out at approximately equal time periods (around seven days). The whole culture was developed in the greenhouse, so the conditions of temperature and humidity were common for all plants and had small variations. The study followed the variation of some environmental conditions during the time period of the lettuce culture life as well as the variation of the heavy metal (Zn) content in time in the plant and in the soil.

Keywords: heavy metal, green lettuce, accumulations in time

1. INTRODUCTION

Plants as essential components of natural ecosystems and agrosystems represent the first compartment of the terrestrial food chain. Due to their capacity of toxic metals accumulating, when they grow on soils polluted with such metals, they represent a threat to the living beings which consume them. Also, their development and growth may be affected at high levels of metal concentration implying reduced cultures and economic loss [10].

In the paper [10], was present the connection between the heavy metals (Cu, Pb, Zn, Cd) concentration in soil and their bioaccumulation in lettuce. Lettuce is a very good bioaccumulator of heavy metals and nutrients. In general, plants manifest a certain reaction to increasing the toxic metal concentrations in soil which they are cultivated on. The differences depend on plants sensitivity, and time exposure intensity (concentration of heavy metals, cultivation length, etc.), presence or absence of nutrients and other chemical species in soil. According to the achieved results the zinc from reference soil was under the alert threshold for sensitive usage and the one from polluted soil exceeded 1.77 times for sensitive usage. Zinc accumulation was higher in the lettuce from the pots with unfertilized soil than in the lettuce with fertilized soil.

Industrial activities, mining and smelting operations, are recognized as the main sources of metal pollution of soils. Heavy metals contamination of agricultural soils and crops in the vicinity of mining and industrial areas has been regarded as a great environmental concern [3-5,11]. Soils near Pb-Zn smelter plant "Zletovo" situated in the town of Veles, Republic of Macedonia [9], are exposed to high environmental contamination related to heavy metal pollution (Cd, Pb and Zn) from waste disposal sites. It was found that the content of Cd, Pb and Zn in vegetables exceeded the maxi mum permissible concentration. The content of Zn in washed lettuce samples was in the range from 21.8 to 37.5 mg kg. Extraction order of heavy metals from the analyzed garden soils in buffered DTPA solution as follows: Cd>Pb>Zn, for Cd <27%, for Pb <10% and for Zn <7.9%. The obtained high extractability of these elements allows its high content in the vegetables produced on this land. Thus, the content of Cd, Pb and Zn were several times higher in lettuce produced in the investigated area compared with the permissible values [9].

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Zinc is not considered to be highly phytotoxic and the toxicity limit for Zn (300-400 mg/kg) depends on the plant species and its growth stage [8]. High concentrations of zinc in plants may cause loss of leaf production, where low concentrations may cause deformation of leaves. A plant foliar concentration of 100 mg/kg of Zn has been quoted by various authors [8] as a critical indicator of whether the environment is polluted with Zn. According to [7] the soil-to-plant Transfer Factor (TF) for various metals and for most common vegetable crops showed that the TF values differed significantly between locations and between plant species. TF decreased when the plants were grown in the higher soil heavy metal contamination. TF decreased in the following order: Zn>Cd>Cu>Pb. TF for different species decreases in the following order: grains < root vegetables < leaf vegetables < wild plants [7].

Zinc obtains its toxicity from essential macronutrients for plants, animals and humans. It is also found naturally in water, most frequently in areas where it is mined, it enters the environment from industrial waste metal plating and plumbing, and is a major component of sludge. Zinc causes no ill health effects excepts in very high dose, it imparts undesirable taste to water and is toxic to plants at high levels [1].

The purpose of the present study was to develop a culture of green lettuce, grown controlled (in individual pots) in soil contaminated with zinc in three concentrations (1.5, 3.0 and 4.5%) and monitored weekly from planting to seedling up to 80 days of vegetation. Weekly monitoring consisted of soil pH and moisture analysis, moisture analysis of the mass, height and diameter of the plant and determinations of zinc content in soil and in the plant.

2. MATERIAL AND METHOD

Salad seedling, Figure 1, was planted in soil infested with the following three zinc concentrations: 1.5%, 3.0%, 4.5%. The salads were planted in controlled environment using pots in which contaminated soil was added to each of the three solutions with concentrations of 1.5%, 3.0%, 4.5% zinc, prepared individually and using as a Zn sulphate reagent and distilled water. The pots in which the seedlings were planted were loaded with fertile soil (1 kg / pot) which was mixed and homogenized in turn with each of the three solutions of different concentrations (250 ml solution). The heavy metal loading was done by initially loading the soil with each of the three Zn concentrations, without further supplementation until harvesting. In parallel as reference samples, seedlings were planted in pots with uncontaminated fertile soil.



Figure 1 - Planting seedlings of lettuce

The physical-chemical properties of the fertile soil (control soil) were: pH 5.0-7.0; total nitrogen 1.68 %, total phosphorus 0.5%, total potassium 0.9 %, electrical conductibility 1.2, particle elements of over 20 mm maximum 5%, moisture 14.7 %.

The sampling of the vegetal samples was done in time up to 68 days after planting (Figure 2) and each time the salad was harvested, the soil sample was taken from the pot, after it was homogenized. The measurement, height and diameter of each salad was made with a ruler, the height was measured from the tip of the root to the end of the last leaf, and the diameter consisted of stretching left-right of all the salad leaves on both sides of the stem and measuring the size from the widest leaf on the right side of the stem to the widest leaf on the left.

The mass of the samples was determined by weighing the KERN precision electronic balance 0,001g. The humidity of the soil and the plant was made using the oven in which it dries at 105° C soil / plant to evaporate the water related to the soil / plant.

Soil pH was determined using the pH determination kit. The soil sample was taken about 20 g, dried in the oven and then it was passed through a 1 mm sieve and placed in a bowl with 100 ml of water. It was mixed for several times, for 30 minutes, then filtered. The pH paper was used, which was immersed in the filtered liquid, waited 30-60 seconds and compared the resulting color with the color sample on the lid.

The determination of zinc from the contaminated soil and from the whole lettuce plant (root and leaves) was performed by the spectrophotometric method (atomic absorption in the flame) [6].





Harvesting 7

Harvesting 9

Harvesting 10

Figure 2 ~ Pots with plants from the three types of crops harvested at several stages of vegetation. **3. RESULTS**

Variation in time of pH and of the humidity of the plant and the soil, are shown in Figure 3, 4, 5. It can be observed the relatively small interval in which these conditions were controlled.







Figure 3 - Variation in time of pH

Figure 4- Variation in time of plant humidity

Figure 5 - Variation in time of soil humidity

Maintaining the three monitored environmental parameters, at very narrow intervals, it can also be observed from their Box-plot diagrams, which shown in Figure 6. It is observed that the pH, and the humidity were maintained with a slight variation around an average value.



Figure 6 - Box-plot representations of pH distribution and moisture in the plant and soil, during the experiments

The graphical representation of the raw data recorded at each lettuce harvest is shown by categories of measured parameters, for the three crops in soil infested with zinc in the three concentrations. The variation of the mass of the plant harvested over time was represented in Figure 7. In Figure 8 are shown the variations of the heights of the collected samples over time. The variation of the diameter of the samples collected over time, in graphical form is shown in Figure 9.

Maintaining the three measured parameters (mass, height and diameter of the plant), at very narrow intervals, can be seen from their Box-plot diagrams, which appear in Figure 10.

The correlation between the variation of the mass of the samples harvested from the soil infested with Zn in the concentration 1.5% and the variation of the mass of the samples harvested from the soil infested with Zn in the concentration 3.0%, has the value 0.879, the correlation between the variation of the mass of the samples harvested from the soil infected with Zn in the





concentration 3.0% and the variation of the mass of the samples harvested from the soil infused with Zn in the concentration 4.5%, has the value 0.907, and the correlation between the variation of the mass of the samples harvested from the soil infested with Zn in the concentration 4.5% and the variation of the mass of the samples harvested from the soil infested with Zn in the concentration 1.5%, has value 0.919.



Figure 7 ~ Variation of the mass of the plant harvested over time



Figure 8 - Variation of the height of the plant harvested over time



Figure 9 - Variation of the diameter of the plant harvested over time



Figure 10 - Diagramele Box-plot Figure 10 - Box-plot diagrams for distributions of masses, heights and diameters, for the last 10 harvests

The order between the three curves is visible, which is clearly observed after approximately 14 days (28.57% of the vegetation period). The order relationship is as follows:

$$m_{4.5}(t) \le m_{3.0}(t) \le m_{1.5}(t) \tag{1}$$

where: $m_{1.5}(t), m_{3.0}(t), m_{4.5}(t)$ are the mass functions of the green salad grown in the soil with initial concentration 1.5, 3.0, respectively 4.5% Zn, time dependent functions. This means that the increase of the heavy metal content in the soil leads to the decrease of the biological mass of the plant.

The variation in time of plant mass, for the three groups of plants (after the initial concentration of Zn in soil), are very well correlated with each other. This means that the variation of masses over time is similar (the monotony intervals coincide or are very close).





The variation of the heavy metal content in plants is given in graphical form in Figure 11. The variation of the heavy metal content in the soil is given in graphical form in Figure 12.





Figure 11 - Variation of the concentration of zinc in plants for the three cases of infestation, over time



plants for the three cases of infestation, over time in soil for the three cases of infestation, over time In 7 of the 11 harvests (63.64%) the highest concentration of heavy metal was recorded in the sample grown in soil infested with 4.5% solution, followed by the sample grown in soil infested with 1.5% concentration and then by the sample grown in soil infested with 1.5% concentration solution. This behavior can also be seen on the graphs in Figure 11. Furthermore, this observation is supported by the average of the last 10 harvests, which has the value 228.993 mg / kg, for the culture with the soil infested with 1.5% concentration solution, 315.334 mg / kg, for the culture with the soil infested with the concentration solution 3.0%, 357.909 mg / kg, for soil culture infested with 4.5% concentration solution.

According to the experimental data (the temporal variations shown in Figure 12), the variation of the heavy metal concentration in the soil oscillates, showing a slight tendency to increase over time. The problem arises if the phenomenon is real or is due to errors of measurement or interpretation.

If the concentration of the heavy metal in the plant is naturally increasing, local or continuous decreases may be accepted at the end of the study time period, putting this phenomenon of decrease on account of a decrease of the rate of absorption of the metal and of the increase of the mass. For the metal concentration in the soil, such an explanation is more difficult to accept, even if some of the substances in the soil pass into the plant (the mass is considered very small compared to that of the soil).

4. CONCLUSIONS

Some general conclusions about the variation in time of the heavy metal concentration in the lettuce cultivated in the three categories of soils are obvious:

- globally, without changing the concentration of heavy metal in the soil, the accumulation of heavy metal in plants increases;
- —at the local level (on certain subintervals of time) the concentration of heavy metal may decrease in plants (obviously abstraction causing some measurement or cultivation errors) this phenomenon is interesting because, if it is real, it may indicate that the removal of heavy metal from the plant can be done naturally, remaining for future experiences determining the influential factors in this process;
- accumulation of heavy metal in plants increase from plants grown in soil least infested with heavy metal to the plants grown in the soil with the highest initial concentration of heavy metal;
- for soil infested with a higher concentration of heavy metal (4.5%), the accumulation is more intense, at least for the range of values covered by these experiments;
- the masses of the plants that grow in the three types of soils, increase until near the optimum age of vegetation (45-50 days) [2], approximately monotonous, after which they increase up to about 130.6% of the standard vegetation period, then, without exception, decrease;
- regarding the geometrical characteristics of the plants, behavior of both (height and generalized diameter), varies rapidly to an average height (in about 25% of the vegetation period), after which it varies in a narrow range around this value;
- influence of growth parameters (environment) on mass evolution (recorded parameters: pH, plant humidity and soil moisture), at least in their (relatively small) ranges of variation, it is not





obvious as it results from the correlation calculation. Specifically, pH, and humidity were maintained with as little variation around an average value (see Figure 6).

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