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TOXIC INFLUENCE OF EXCESSIVE CONCENTRATIONS OF SOME HAEVY METALS UPON ANTHOCIANS, FLAVONOIDS, AND PHENOLS IN PEPPER *(Capsicum annuum)* AS A VEGETABLE

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SYNOPSIS

This paper presents the negative effects of excessive concentrations of heavy metals upon anthocians, phenols and flavonoids. For this experiment the soil cultures of pepper (capsicum annuum) were used, grown in experimental conditions. They were treated with excessive concentrations of $ZnSO_4 \cdot 7H_2 0$ with four different concentrations, while the control group was treated only with water.

The results shown clearly indicate the negative effect that heavy metals have on plant material of *Capsicum annuum*.

INTRODUCTION

Studies and research of heavy metals in ecosystems have shown that many areas near cities, urban complexes, mines or great road systems contain unusually high concentrations of heavy metals. The soil in these regions is especially contaminated by a wide range of sources of lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and other heavy metals. Nriagu (1988) wrote that we may be experiencing "a quiet epidemic of poisoning the environment with heavy metals" by the existent increased quantities of metals that are thrown up into the atmosphere.

The term „heavy metals", although not completely defined, is widely known and used. It is often accepted as the name of a group of metals that are connected with pollution and toxicity but also includes several elements that are significant for living things, but in small concentrations.

"Toxic metals" is an alternative term for "heavy metals" but it relates more to certain elements such as lead (Pb), cadmium (Cd), mercury (Hg), arsen (As), thallium (Tl), and uranium (U), but this term is not suitable for biologically important elements such as cobalt (Co), copper (Cu), manganese (Mn), selenium (Se) and zinc (Zn). The term "elements in traces" is becoming popular as a substitute for the term "heavy metals", but is till not used very often.

Among the numerous bio-elements present in living organisms a special group of these consists of those which are present in natural environment in very small quantities – “elements in traces”. Some of these (iron, manganese, and iodine) are present in minimum quantities and others (copper, zinc, molybdenum and cobalt) in even smaller ones. Some other elements in traces (vanadium, barium, silicon, nickel, strontium) are necessary only to the certain number of animal species. But some other elements in traces are especially interesting (lead, mercury, cadmium, aluminium, and solder) and they are present in plant and animal species but their biological function is unknown. Starting from this, they are not included in normal metabolitic processes of transformation and, consequently, these elements are accumulated and have cumulative and toxic effect on living organisms. It is thought that around 30 elements (chromium, nickel, cadmium, zinc, lead, copper, antimony) represent a potential danger for human health.

“Pollution” is in some ways easier to define than “heavy metals”, but it is often replaced with the term “contamination”. Although there are several interpretations of the terms “contamination” and “pollution”, the definition which is given by Holdgate (1979) is widely accepted. It shows that “pollution” is bringing a certain substance or energy by man into the environment which can cause danger to human health, damage to sources of life and ecosystems.

Pepper as an agricultural culture

Class: *Angiospermae*
Subclass: *Dicotyledones*
Order: *Polemoniales*
Family: *Solanaceae*
Genus: *Capsicum*



Family *Solanaceae* comprises annual and perennial plants that are especially common in America, in tropical and subtropical regions, and, in smaller number, also in moderate regions. A great number of vegetables as well as some medicinal, industrial and decorative species also belong here. From the bio-chemical point of view they almost all contain alkaloids and glycosides, and some of them contain saponines because of which most of them are considered poisonous plants. The genus *Capsicum* originates from the tropical regions in America and has at least 25 species four of which are domesticated. The genus *Capsicum annuum* was first domesticated in the mountanaeous areas in Mexico and includes almost all Mexican hot peppers, most of African and Asian peppers, as well as various species cultivated in the countries of the moderate zone.

Determination of the anthocianines content

The dried plant material we use for anthocianine extraction is first measured and we take 1-10 g (depending on the anthocianine content). The quantity taken is transferred into a 100 ml dish and filled to the mark with 1% of HCL solution in methanol. After the extraction is finished (30 minutes in the dark), it is filtered through filter paper. From the resulting filtrate we take 5 ml and put it into the measuring dish of 50ml and fill it to the mark with a buffer rN-1

This solution is used for filling the kivetite and the ekstinciite of the analyses are measured in relation to the control of the spectrophotometer at the wavelength of 510 nm.

The following formula is used for calculation:

$$A = \frac{E - PH_1 \bullet V_1 \bullet V_2}{m_1 \bullet m_2 \bullet V_1}$$

V_1 - volume of the filtrate
V_2 - volume of the extract
m_1 - fresh mass of the plant material
m_2 - dry mass of the plant material

Determination of phenol and flavonoid content

The extraction of phenols and flavonoids begins with macerating of the plant material with 3ml 80% methanol and is incubated 30minutes at 4°C in an ultrasonic bath. After that the extract is centrifuged for 10 minutes at 13700 rpm. After centrifuging 2 ml of the supernatant is collected into specially labelled test tube, and methanol is again added to the residue (grounds) and re-extraction and centrifuging is performed; then another 2 ml are collected. The procedure is performed after 1ml Folin−Chioclateau reagent and 800 µl 0,7 M Na_2CO_3 are added to 1 ml extract. The mixture incubates for 5 minutes at room temperature.

The absorption of the total phenols is measured at 765nm and for flavonoids at 425nm.

The solution of catehin (0,4 mg/ml) is used to prepare the standard curve.

The formula for calculating is as follows:

$$\text{A µg catehin} = \frac{\sum A_{st}}{\sum AC_{st}}$$

A µg-catehin for total phenols (765nm)=0,332
A µg-catehin for flavonoids (425nm)=0,161

Preparation of the original solution: 50ml of dry plant material is dissolved in a 25ml lab dish with several drops of 80% methanol (also possible 100%), and is filled to 25ml with methanol -FV1. 0,5ml is taken for determination. If concentration is high, it is diluted. The calculation is done according to the following formulas:

$$\text{C mg/L} = \frac{Aproba}{A(1\mu g_{katehin}) \bullet FV} \bullet DF / 1000(\mu g / mg)$$

$$DF = \frac{FV}{V}$$

RESULTS AND DISCUSSION

The results of the research analysis made on pepper concerning the concentration of anthocianines lead to the following:

Tab.1. Content of anthocians (mg/l00g) in the fruit of red pepper treated with different concentrations of $ZnSO_4 \cdot 7H_20$:

	Control	1,0 mg/kg	5,0 mg/kg	10 mg/kg	20 mg/kg
anthocians	42	96,98	84,095	45,91	47,938

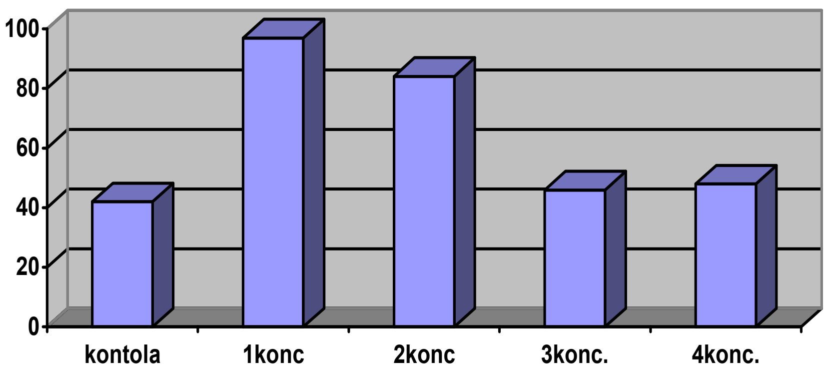

Figure 1.

Tab.2. Content of anthocians (mg/l00g) in fruit treated with different concentrations of $CuSO_4 \cdot 5H_2O$:

	Control	0,5 mg/kg	1,0 mg/kg	5,0 mg/kg	10 mg/kg
anthocians	42	255,485	1032	1302	71,265

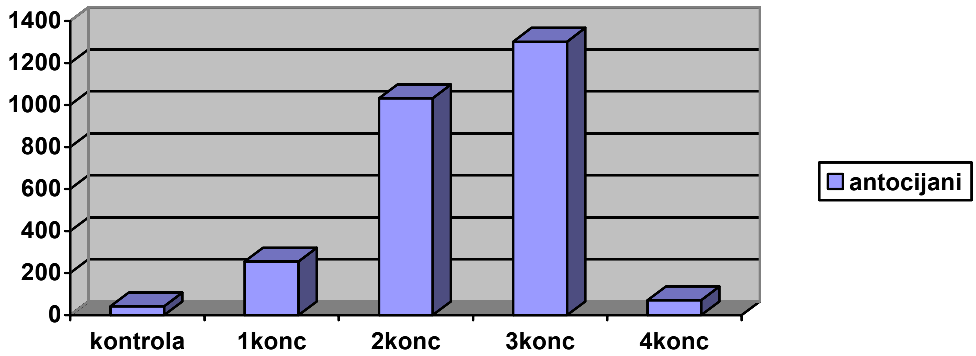

Figure 2.

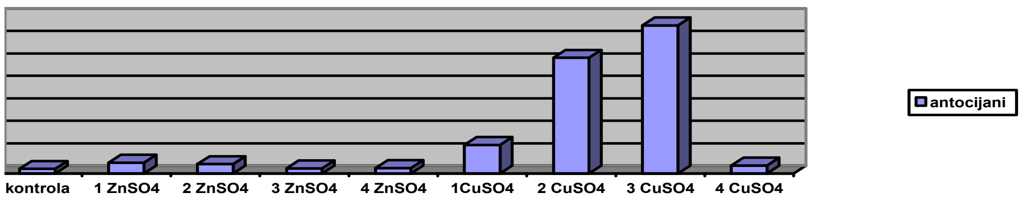

Figure 3. Comparative survey of all concentrations of anthocians got after treating with $ZnSO_4 \cdot 7H_2 0$ and $CuSO_4 \cdot 5H_2O$:

From the analyses made it can be concluded that during treatment of plants with Zn there is a reversely proportionate dependence between the applied concentration of respective heavy metals and the anthocianine content being synthesized; the least concentration leads to greatest synthesis; still, all the values are biggerthan in control plants. Copper that also enters the anthocianine synthesis increases the anthocianine synthesis much more compared to the control group of plants; however, in the last case we notice a great fall in the anthocianine content and we can come to the conclusion that the plant somehow stops fighting against the negative influence of heavy metals. When looking at the comparative results of the influence of copper and zinc on the anthocianine synthesis it can be said that Zn does not cause significant stress effect in pepper.

Anthocianines represent compounds that, among other, also show anti-oxidative characteristics, protecting the plants from the formed free radicals (Lee and Gould, 2002).Anthocianine, compared to other components, represents the best indicator of the oxidative stress resulting in plants under the influence of heavy metals. As a mechanism protecting from the toxic influence of high concentrations of Zn and Cu it is synthesized in plants in great concentrations if compared to the control plant. Andersen (2006) came up with the same effects as we did in our research.

Tab.3. Content of phenols (mg/g) and flavonoids (mol/g) in plants treated with $ZnSO_4 \cdot 7H_20$:

	Phenols mg/g	Flavonoids mol/g
Control	21,540	7,910
20mg/kg $ZnSO_4 \cdot 7H_20$	17,006	8,470

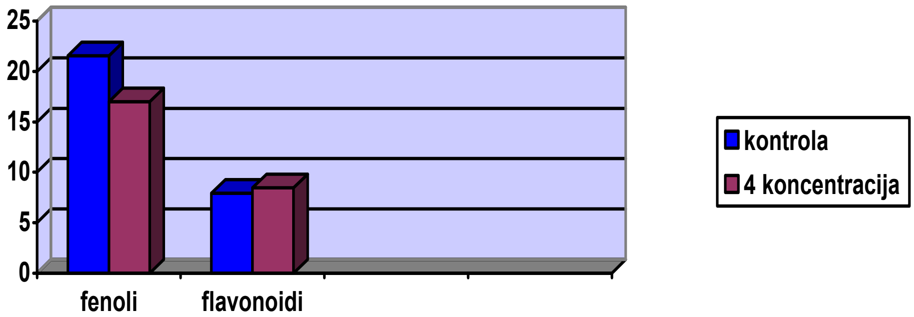

Figure 4.

Tab. 4. Content of phenols (mg/g) and flavonoids (mol/g) in plants treated with $CuSO_4 \cdot 5H_2O$:

	Phenols mg/g	Flavonoids mol/g
Control	21,540	7,910
10mg/kg $CuSO_4 \cdot 5H_2O$	18,270	8,810

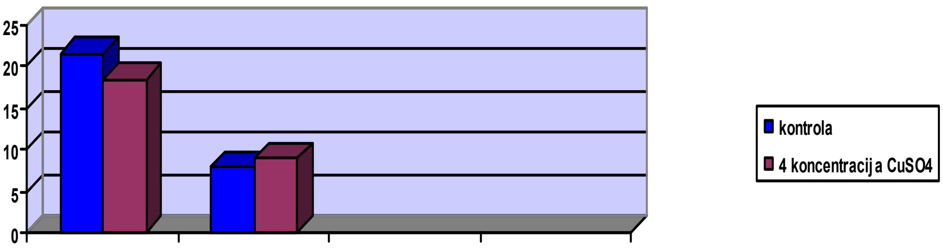

Figure 5.

Based upon these results we can state that, generally, both zinc and copper cause a fall in the total content of phenols, while the content of flavonoids and anthocianines is increased. There is a directly proportionate dependence between the concentration of heavy metals and the contents of flavonoids and anthocianines, and a reversely proportionate dependence with the total phenols.

Phenolsand flavonoids are important anti-oxidants. Szent-Györgyi, Nobel Prize winner who isolated the ascorbat demonstrated that flavonoids behave in the same manner as the ascorbat (Bentsath and assoc., 1937). Their synthesis can be induced by biotic and abiotic factors (Dixon &i Paiva, 1995). Phenols are considered tobe anti-oxidants the function of which is to help the primary ascorbat-dependent anti-oxidative system in plants (Yamasaki and assoc., 1999).

Clark (2006) came up in his analyses with the real proportionate dependence between heavy metals and the synthesis of flavonoids.

CONCLUSION

The results shown clearly indicate the negative effect that heavy metals have on plant material of *Capsicum annuum*. This leads to the following conclusions:

1. All the applied concentrations of heavy metals result in toxic symptoms in plants, and the seriousness of the damage depends on the plant type, kind of the pollutant, manner of application, concentration, etc.

2. After treating with Zn and Cu a reduction of the photosynthetic activity was noticed, i.e. the chlorophyll pigments.

3. A rapid increase of the vitamin C synthesis was noted as well as of anthocianines, phenols and flavonoids because of an anti-oxidative defence of the plant from free radicals.

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