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Prof. Dr Snežana Šerbula

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THE EXAMINATION OF ECOTOXIC EFFECT OF FOLIC ACID BASED CARBON DOTS ON MAIZE

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Abstract

Carbon dots (CDs) are popular or emerging nanomaterial which found application in many fields such as drug delivery, optoelectronic, and imaging due to their high solubility, low cost and easiness of their functionalization. Their effect on plants is not sufficiently investigated, so it is necessary to investigate their ecotoxicity. In this research, CDs prepared from folic acid was used for the treatment of maize plants at two different concentrations. The treatment was performed during the plant growth in hydroponics. ICP method was used for the analysis of macronutrients (Ca, K, Mg, P, S) uptake in plants from the hydroponic medium, which was used for the plant growth. The obtained TPC results demonstrated low oxidative stress proportional to the used concentration, which was not significant. The similar trend was observed in TAA where the only significant increase was in plant shoots after the treatment at 500 μg L^{-1}.

Keywords: carbon dots, maize, phenolics, antioxidant activity, ecotoxicity

INTRODUCTION

Carbon dots (CDs) are metal free nanomaterial with an inner graphitic core [1]. High solubility, chemical stability, stable luminescence, photo-bleaching resistance, low cost and easiness of their functionalization are advantages of these nanoparticles in comparison with conventional heavy metals based quantum dots and organic dyes [2]. Their applications have been tested in printing inks [3], drug delivery [4], imaging [5], photocatalysts [6], fingerprint [7] and optoelectronics [8]. Their elemental content (in carbon, oxygen, and heteroatoms) and graphitization degree significantly depends on the type of starting materials and the synthetic route, giving an expanding gallery of photoactive materials [9]. The literature data about the effect of CDs on plants are scarce, so there is a need to examine their ecotoxic effect.

In this study, CDs were synthesized from folic acid (FA) as starting material and their ecotoxic effect was examined on maize at 167 and 500 μg L^{-1} during 7-day exposure in hydroponics. The main objective of this study is to examine if CDs have an impact on

macronutrient concentration, total phenolic content (TPC) and total antioxidative activity (TAA), as indicators of plant response to the nanoparticles.

MATERIALS AND METHODS

Folic acid (≥ 97%,), H_3PO_4 (85% wt%, 99.99%), NaClO, $Ca(NO_3)_2{\cdot}4H_2O$, KNO_3, $MgSO_4{\cdot}7H_2O$, KH_2PO_4, HNO_3, H_2O_2, K_2HPO_4, KH_2PO_4, Folin-Ciocalteu reagent (2N), CH_3OH, Na_2CO_3, gallic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), horseradish peroxidase (HRP) type II (150-250 units per mg solid) and cellulose membrane for dialysis were supplied from Sigma-Aldrich Química S.A. (Spain) were used. All reagents were obtained from Sigma Aldrich (St. Luis, USA). Ultrapure Millipore water and further reagents of analysis quality were used throughout all experiments.

Synthesis of CDs from FA

CDs were obtained by adding 10 mg of FA to 5 mL phosphoric acid solution (7.31 M) and heated under reflux at 100ºC for 1 h. Then, 500 μL of the previous solution was diluted with deionized water and was dialyzed *vs* water for 1 h [1].

Experimental design for plant treatments

Maize (*Zea Mays*, L.) seeds (cv. VA35; Maize Research Institute "Zemun Polje", Serbia) were surface-sterilized with 4% NaClO for 2 min and rinsed with distilled water (2-3 times for 1 min each).

Four replicates of 20 maize seeds were germinated in a filter paper moistened with 10 mL of distilled water. After germination, 20 seedlings per each treatment (control, 167 μg L^{-1} and 500 μg L^{-1}) were transferred to the 17.5 cm height-plastic vessels containing 2.5 L half-strength nutrient solution in the presence of CDs nanoparticle. The seedlings were grown for the next 7 days under 16h/8h photoperiod and the nutrient solution was aerated by bubbling. After 7 days, the shoots and roots of 10 plants (2 plants per replicate) were collected, frozen in liquid nitrogen and kept at -80˚C until the determination of TPC and TAA. Concentrations of macronutrients (K, Ca, Mg, P and S) were determined in the remaining 10 plants.

Determination of micronutrients

Dry pulverized plant materials were digested with conc. HNO_3 and 30% H_2O_2 (1:4) in the Tecator digestion system [10]. After cooling to the room temperature, the solutions were filtered using Whatman filter paper and volume was adjusted with MilliQ water to 25 mL. The concentrations of macronutrients (excluding N) in the plant samples and nutrient solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany).

Extraction of phenolics and determination of TPC

In order to obtain phenolic extracts, roots and shoots of ten plants (2 per sample, in 5 replicates) were separately homogenized in a mortar with liquid nitrogen. Then, homogenates were resuspended in 80% methanol in the 1:10 (m:V) ratio and stirred for 60 minutes at room temperature. The extracts were centrifuged at 10000 rpm for 5 minutes, and extracted phenolics were obtained in the supernatant.

For determination of TPC in the samples, Folin-Ciocalteu's spectrophotometric procedure [11] was used. Phenolic extracts were mixed with Folin-Ciocalteu reagent in 1 mL of total volume. After 3 min sodium carbonate solution was added and the mixture was incubated for 60 min at 25°C. Gallic acid was used for the construction of the standard curve (0.1 - 2.0 mM). Absorbance was read at 724 nm (2501 PC spectrophotometer, "Shimadzu", Japan) and the results were expressed as micromoles of gallic acid equivalents per gram of fresh weight.

Determination of TAA

ABTS/HRP endpoint method was used for measuring of TAA in the samples, according to the modified procedure of Cano et al. [12]. In brief, the reaction mixture contained 2 mM ABTS, 15 μM H_2O_2, 0.25 μM horseradish peroxidase (HRP) type II and 20 μL of 80% methanol extract of the samples in 50 mM potassium-phosphate buffer, pH 7.5, in 1 mL of total volume. The assay was performed at the temperature 25°C, in 5 replicates per treatment. The reaction was monitored at 730 nm (2501 PC spectrophotometer "Shimadzu", Japan) until a stable absorbance, due to ABTS radical ($ABTS^{\cdot+}$) formation in the reaction with HRP. After adding methanol extracts of plant, the decrease of absorbance due to $ABTS^{\cdot+}$ depletion was used for calculation of TAA from the standard curve obtained with ascorbic acid (0.1 - 1 mM) as a universal antioxidant. The TAA was expressed as micromoles of ascorbic acid equivalents per gram of fresh weight.

Statistical analysis

The raw data (macronutrients' concentration, dry biomass, TPC and TAA in root and shoot parts of maize plants treated with different concentrations of CDs) were used as input variables. Exploratory and data analysis were performed using the IBM SPSS Statistics 20 software (IBM, USA). A non-parametric Kruskal-Wallis test for independent samples was used to test the differences in TPC and TAA (n=5), as well as in concentrations of macronutrients (n=3) measured in root and shoot parts in plants under the different treatments. Post hoc inter-group comparisons of variables (between different treatments and control) were performed by the non-parametric Mann-Whitney test at the level of the significance $p<0.05$.

RESULTS AND DISCUSSION

Uptake of macronutrients

The treatment of plants with CDs did not lead to any visible signs of tissue damage such as chlorosis or necrosis. Interestingly, CDs even increased shoot dry biomass in comparison with the control (Table 1).

Table 1 *Dry biomass of shoot and root per individual plant*

Treatment	Dry biomass of shoot per individual plant	Dry biomass of root per individual plant
Control	0.0215 ± 0.0021	0.0092 ± 0.0019
167 CDs	0.0414 ± 0.0039	0.0196 ± 0.0024
500 CDs	0.0351 ± 0.0041	0.0085 ± 0.0026

Figure 1 shows the concentration of macronutrients in maize roots and shoots after the treatment with CDs. K showed the highest uptake, while the lowest uptake had Mg. According to the concentration of macronutrients in roots and shoots, it can be concluded that root-to-shoot translocation is similar for all macronutrients.

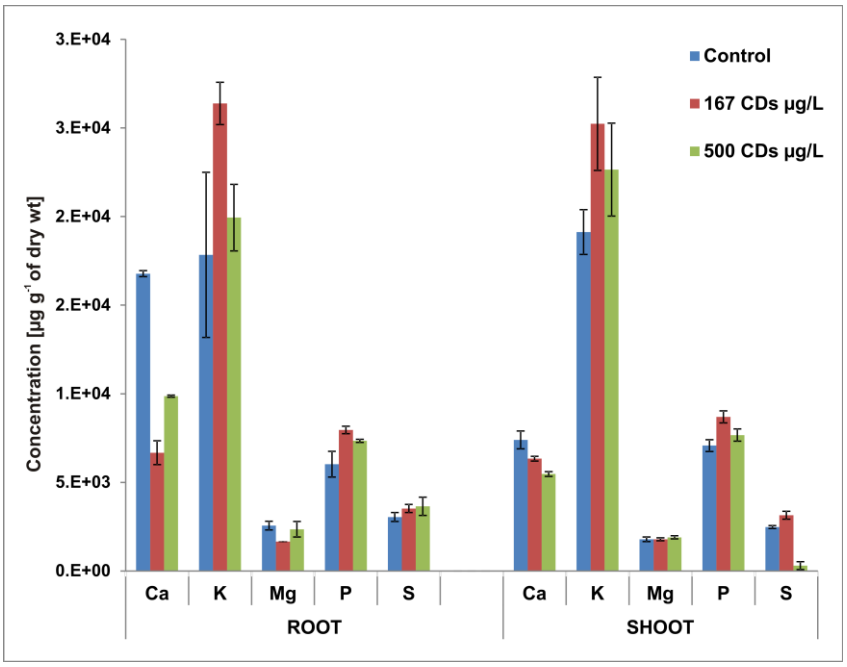

Figure 1 *Concentration of macronutrients in maize after the treatment with different CDs concentrations*

Effect of CDs on TPC

In order to investigate the potential for oxidative stress due to CDs treatment of plants, we measured phenolic content, as an indicator of the plant defense capacity. The effect of CDs treatments on TPC in maize roots and shoots is presented in Figure 2.

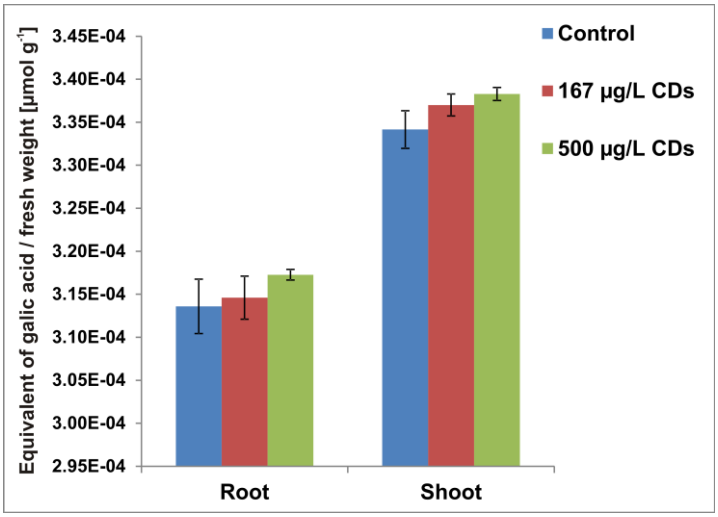

Figure 2 *Effect of CDs on TPC in roots and shoots in maize. Values are shown as mean ± SE; * indicates statistically significant differences in comparison with the corresponding control, $p<0.05$*

Although TPC was increased in both parts of plants after treatment with CDs at both concentrations, this effect is negligible because it is not significant. It can be concluded that CDs does not cause significant oxidative stress in treated plants.

Effect of CDs on TAA

Since TAA may be an indicator of metabolic disorder in plants, it was analyzed in phenolic extracts of maize shoots and roots after treatment with CDs (Figure 3). Results suggested that TAA was significantly increased compared to the control only after the treatment with 500 μg L^{-1} CDs. This can be explained with the ability of CDs to induce excessive production of ROS [13], which activates plant defense system leading to the increase of TAA in plants.

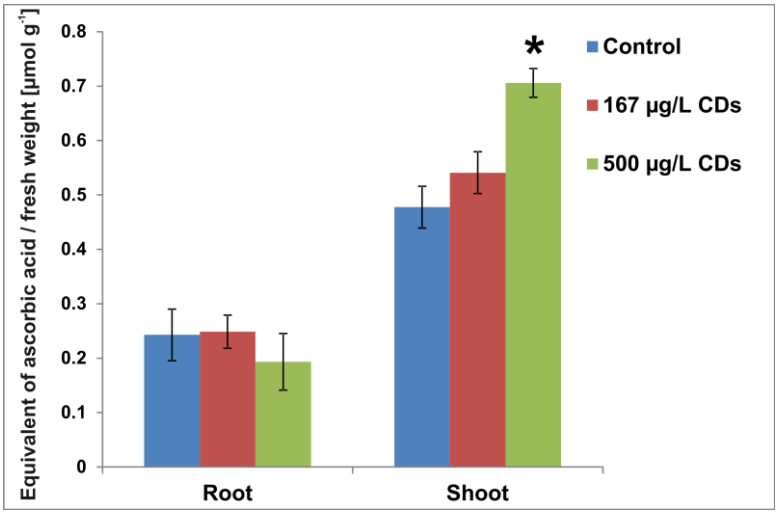

Figure 3 *Effect of CDs on TAA in roots and shoots in maize. Values are shown as mean ± SE; * indicates statistically significant differences in comparison with the corresponding control, $p<0.05$*

CONCLUSION

The treatment of maize plants with CDs did not any phytotoxic symptoms at both tested concentrations. CDs at both concentrations significantly decreased leaf Ca accumulation while 500 μg L^{-1} significantly increased TAA. This may indicate the phytotoxic effect of CDs at higher concentration, which remains to be examined in future research.

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