Scientific report on the obtained results

The research teams from the two institutions (INCDTIM and JINR) have participated together in the project, entitled: "**The impact assessment of metal / metal oxide nanoparticles on wheat**", in the year 2019, having number 57 (order 397), number 55 (order 396), number 30 (order 395) (according to the list approved by JINR).

Within the project was followed:

- The synthesis and characterization of nanoparticles based on Ti and Cu;
- The germination and growth of wheat in the presence of nanoparticles;
- Impact assessment of the nanoparticles on bioactive compounds from wheat;
- The determination of the elemental content and nanoparticles accumulation in plants.

TiO_2 nanoparticles were obtained starting from $TiCl_4$ to which the bidistilled water was added. The mixture was stirred for 30 minutes at room temperature, then heated to 150°C and kept until the nanoparticles were formed entirely.

CuO nanoparticles were obtained from copper sulfate, ascorbic acid and cetyltrimethylammonium bromide brought to 6.5 with the help of NaOH, after which it is heated to 85°C and stirred at 530 rpm until the color of the brick red is obtained. The second and third types of CuO nanoparticles were obtained from copper sulfate and celandine extract, respectively, blackthorn extract, synthesis being performed under the same conditions as above. The CuO NPs considered for the present study were abbreviated as follows: NPs synthesized by chemical method (CuO-NP), NPs biologically synthesized using celandine extract (CuO-NP-cel), or blackthorn extract (CuO-NP-bth).

The formation of nanoparticles was confirmed by X-ray powder diffraction, scanning/transmission electron microscopy and Fourier-transform infrared spectroscopy measurements.

After the analysis of the plant tissues it was observed that:

- For plants grown in the presence of CuO nanoparticles, there was a decrease of about 8-25% in the amount of total chlorophyll a, 10-26% in the amount of total chlorophyll b and 5-18% in the amount of carotenoids. For plants grown in the presence of NiO_2 nanoparticles, the decrease of the amount of total chlorophyll a was 35%, of the amount of total chlorophyll b was 56 and of the amount of carotenoids was 57%.
- Compared to the control plants, the amount of total polyphenols increased in the case of wheat grown in the presence of CuO-NP and CuO-NP-bth and decreased in the case of wheat grown in the presence of CuO-NP-cel and TiO_2.
- Plants grown in the presence of CuO and TiO_2 nanoparticles showed higher antioxidant activity compared to control plants; the highest antioxidant activity is shown by the wheat grown in the presence of CuO–NP-bth.
- Analyzing the wheat tissue through transmission electron microscopy was observed that only the leaves of the plants treated with CuO-NP seemed to be negatively affected. The significant difference occurred in the chloroplasts, which had no thylakoids, grana, or starch granules and the stroma had an amorphous distribution. The roots of the plants had normal ultrastructure. Electron-dense accumulations were observed inside the cells of CuO-NP plants and EDX analysis confirmed the presence of CuO. The ultrastructure of the leaves of TiO_2-NP plants was normal, but Ti was confirmed by EDX analysis in the electron-dense accumulations observed inside the cells.

- In wheat control sample were determined 34 elements (Na, Mg, Al, Cl, K, Ca, Sc, Cr, Mn, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Zr, Mo, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Tm, Yb, Hf, Ta, Th, U), but only 14 of them were identified in the exposed wheat samples.
- 12 elements (Na, Mg, Al, Cl, K, Ca, Mn, Zn, Br, Rb, Sr, Ba) were determined in all wheat samples, including the control wheat. The other 18 elements (Sc, Cr, Co, Ni, Zr, Cs, La, Ce, Nd, Sm, Eu, Tb, Tm, Yb, Hf, Ta, Th, U) were identified in control wheat, only. Mo, As and Sb were detected in all samples except that treated with CuO NP.
- Ti was not detected in any of wheat samples, even in those grown on the soil amended with TiO_2 NP. This indirectly suggests that probably the diameter of the applied TiO_2-NPs was more significant than 36 nm, that's why the root to shoot transfer didn't take place.

From the elementary soil analysis the following were counted:

- In soil doped with CuO-NP the element content decreases in the following order: Ca>Mg>Al>Fe>K >Na>Ti>Cl>Mn>Cu>Ba>Sr>Zn>Zr>Rb>V>Cr>Ce>Br>Ni>La>Nd>As>Co>Sc>Sb>Th>W>Hf>Sn>Sm>Cs>U> Mo>Yb>Ta>Tb>Eu>Tm>Au. Eight elements have significant decline of concentration level (Ca, Ti, V, Mn, As, Sn, Sb, Cs, Ba, W, U) and eight (Na, Cl, Sc, Cr, Fe, Co, Br, Hf) significant increase.
- In the soil amended with CuO-NP-cel the element content decreases as follows: Ca>Al>K>Mg>Fe> Na>Ti>Cl>Mn>Ba>Cu>Sr>Zn>Zr>Rb>V>Cr>Ce>Nd>Ni>La>Br>As>Co>Sc>Th>Sb> Hf>Sn>Sm>Cs>W>U>Mo>Yb>Tb>Ta>Eu>Tm>Au.
- In soil modified with CuO–NP-bth the elemental content decline as follows: Ca>Mg>Al>Fe>K>Na >Cl>Ti>Mn>Ba>Cu>Sr>Zr>Zn>Sb>Rb>V>Cr>Ce>Ni>Nd>Br>La>As>Co>Sc>Th>Hf>Sm>Cs>U>Yb>W>Mo >Ta>Eu>Tb>Tm>Au. The level of nine elements (Na, Al, Ti, V, Co, Zn, Br, Cs, Au) significantly decreased compared to control soil values and six (Mg, Cl, K, Fe, Sb, Eu, Yb) show higher concentration values.
- The soil amended with TiO_2 NP the element content declines as follows: Ca>Mg>Al>Fe>K>Na>Ti> Cl>Mn>Ba>Sr>Zn>Zr>Rb>V>Cr>Ce>Br>Ni>La>Nd>As>Co>Sc>Sb>Th>W>Hf>Sn>Sm>Cs>U>Mo>Yb>Ta >Tb>Eu>Tm>Au. Only three elements (Mg, Al) have significant decline of concentration level and nine elements (Cl, K, Fe, Co, Zn, As, Sr, Sn, Sb, Au) significant positive increase.

In order to asses the element soil to plant transfer, the mobility ratio was calculated. The plants can acts like accumulators (MR>1), like excluders (MR<1), or they can have an indifferent behavior to some elements (MR ~ 1).

All plant samples have a very high accumulator behavior for Cl 4,28 <MR< 34,72. The highest value belongs to the control wheat and the lowest to the wheat grown on the soil amended with CuO-NP-bth.

In the case of K, all wheat samples grown on amended soils have very significant accumulator behavior while the control wheat seems to be indifferent to K.

The control wheat accumulates Fe, but the wheat samples grown on amended soils behave like excluders (CuO-NP-bth and TiO_2 NP) of Fe. In the wheat samples CuO-NP-cel and CuO-NP the Fe content was under the detection limit.

There is interesting to mention that in the case of CuO-NP, was detected Cu behaves like excluder in case of this metal.

The wheat control and TiO_2 NP behave like excluders for Ti while CuO-NP-bth like accumulator and CuO-NP-cel and CuO-NP are indifferent to it.

The control wheat excludes Br and CuO-NP-bth and TiO_2 NP are indifferent to it, but CuO-NP-cel and CuO-NP have evident accumulator behavior up to MR>8 for the last one.

The control wheat together with CuO-NP and CuO-NP-bth accumulate Rb while is TiO_2 NP indifferent to it and CuO-NP-cel even excludes it. At the same time, the MR values for all these samples are very low, spread from 0,76 up to 1,54.

The TiO_2 NP accumulates Mo, while CuO-NP-cel and CuO-NP-bth are indifferent to Mo and the control wheat excludes it. Mo was not detected in CuO-NP.
We detected Cs in CuO-NP-cel and control only. The control wheat is indifferent to Cs, while CuO-NP-cel excludes it.
Comparing the results obtained for the types of nanoparticles whose effect was evaluated on wheat plants, it was found that CuO nanoparticles have a more significant influence on plants than TiO_2 nanoparticles.
The results obtained within the project were communicated at the 18th International Conference "Life Sciences for Sustainable Development", which took place between September 26–28 at Cluj-Napoca, Romania. The paper presented was "*The influence of nanoparticles based on Cu and Ti on bioactive compounds from wheat*", authors: I. Lung, O. Opriș, O. Culicov, A. Stegărescu, A. Ciorîţă, S. Pintea, S. Guțoiu, I. Zinicovscaia, N. Yushin, K Vergel, M.L. Soran.
Also, the results obtained regarding the effect of CuO nanoparticles on the growth of wheat (*Triticum aestivum*) plants, the impact assessment on bioactive compounds from plants and their elemental content are contained in a manuscript in preparation