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MAGNETIC ENVIRONMENTAL POLLUTION: EXPERIMENTAL SIMULATION OF ENGINEERED MAGNETIC NANOPARTICLES IMPACT ON *ZEA MAYS* VEGETAL EMBRYOS

MIHAELA RĂCUCIU, HOREA OLOŞUTEAN

"Lucian Blaga" University of Sibiu, Faculty of Sciences, Applied Ecology Research Center,
Environmental Sciences and Physics Department,
Dr. I. Ratiu Street, no. 5–7, 550024, Sibiu, Romania,
E-mail: mihaela.racuciu@ulbsibiu.ro

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Abstract. The engineered nanoparticles release into the environment raises important ecological worldwide problems. To evaluate the effects of the magnetic nanoparticles on *Zea mays* vegetal embryos, we have accomplished experimental studies focused on the cell proliferation in the root tips tissue during the seeds germination under the influence of magnetic nanoparticles (MNPs) coated with different layers. Mitotic division process appears to be stimulated than controls and chromosomal aberrations occurred under MNPs presence. The experimental results have been comparatively analysed by means a statistically soft in order to obtain mathematical models to describe the MNPs influence on mitotic index and aberrations index.

Key words: magnetic nanoparticles, chromosomal aberrations, statistical analysis, mathematical modelling.

1. INTRODUCTION

Nanotechnology is a rapidly growing industry with global economic importance. The new technologies exploiting the characteristics of nanoscale materials have developed for use within the biomedical, industrial or environmental directions. The environmental impact of nanotechnology consists in the possible effects that the nano-sized particles may have on the environment. The fast development of nanotechnology at this moment generates an increased concern regarding the nanoparticles impact on the environmental elements, like soil, water, air, microorganisms, plants or animals. Since the availability of nano-toxicology information is scarce, any scientific contribution about environmental risks of nanoparticles should help to regulate the use and production of engineered nano-materials. The analysis of toxic effects of nanoparticles and their potential accumulation in plants or microorganisms are current research topics, which take

into account the impact of nanoparticles on the environment. Some studies demonstrates possible toxic effects of metal oxide nano-materials on plants, which underscores the need for ecologically responsible disposal of wastes containing metal oxide nanoparticles and asks for further experimental studies on the potential impacts of manufactured nanoparticles on environmental systems. Different nanoparticle types have induced different effects in plant development in early ontogenetic stages [1]. Lee *et al.* reported that Fe_3O_4 exerted inhibitory effects on root elongation of *Arabidopsis thaliana* at all nanoparticles concentrations [2]. The iron nanoparticles presence on the culture medium of cucumber and lettuce seeds produced a significant negative effect on the germination index and root index [3]. Significant uptake, translocation, and accumulation of Fe_3O_4 in the roots and leaves of pumpkin have been reported without any effect on growth and development of the test species [4]. Giordani *et al*. [5] analyzed the Fe_3O_4 nanoparticles effects on tomato seedling growth observing that these were absorbed by the roots and after translocated to the hypocotyl. Li *et al.* [6] studied the magnetic iron oxide nanoparticles (Fe_2O_3) influence on watermelon seeds observing clear effects on seeds germination, seedlings growth and physiological function. El-Temsah *et al*. [7] observed complete inhibition of barley seeds germination in presence of zero-valent iron nanoparticles. Dhoke *et al.* [8] studied effects of different nanoparticles type on the growth of mung and concluded these were able to affect the development process of the plants.

In this paper, we follow to evaluate the effects of the magnetic nanoparticles on *Zea mays* vegetal embryos by assessing the mitotic index and the chromosomal aberrations percentage in the root tips tissue developed under the presence of different nanoparticles type and concentrations level. The experimental results were comparatively analysed by means a statistically soft in order to obtain mathematical models to describe the magnetic nanoparticles influence on mitotic division process and chromosomal aberrations appearance.

2. MATERIALS AND METHODS

Three experimental sets were accomplished, using three magnetic nanoparticles types, coated with different layer, by adding of same quantity in the germination culture medium for the same number of *Zea mays* seeds per sample. The MNPs suspensions used in this experimental study, about their influence on the cellular division rate and the percentage of chromosomal aberrations induced in the root meristematic tissue, consisted of nano-sized iron oxide nanoparticles coated with citric acid ($C_6H_8O_7$) and dispersed in water (A_1 type), coated with betacyclodextrine and dispersed in water (A_2 type) and respectively, coated with tetramethylammonium hydroxide and dispersed in water (A_3 type). The magnetic nanoparticles (magnetite Fe_3O_4) were prepared by co-precipitation of $FeCl_3$ and

$FeCl_2$ in an alkali ammonia solution (NH_4OH 25%) and further stabilized with different coating substance (citric acid, beta-cyclodextrine, TMAOH) according to [9–11]. Citric acid is a natural organic acid found in many fruits and vegetables. Also, citric acid is found on the ingredient list of many food products. Beta-cyclodextrine is an inoffensive food additive, generally recognized as safe and thus used in food, pharmaceutical, agriculture and environmental engineering. Tetramethylammonium hydroxide (TMAOH) is a quaternary ammonium salt. It has numerous and diverse industrial and research applications. The tetramethylammonium hydroxide is toxic, affects nerves and muscles, causing difficulties in breathing, muscular paralysis. Hence, due to the coat substance implication and considering the biocompatibility of magnetite, we tested two biocompatible magnetic nanoparticles and one with toxic action.

The physical characteristics of the magnetic nanoparticles (MNPs) types used in these experimental studies are presented in Table 1.

Table 1

The physical characteristics of magnetic nanoparticles (MNPs): d_{TEM} is the physical size of nanoparticles obtained by TEM, d_{magn} is the magnetic core size of nanoparticles obtained by magnetization measurements, M_s is the static magnetization value of nanoparticles sample at normal temperature, N is the number of nanoparticles present in the original nanoparticles sample used to obtain different concentration of nanoparticles number

MNPs type	**Coating substance**	d_{TEM} **(nm)**	$d_{magn.}$ **(nm)**	M_S **(kA/m)**	N **(part/m^3)**
A1	citric acid	7.47±2.94	5.59	33.00	$7.52 \cdot 10^{23}$
A2	beta-cyclodextrine	12.3±1.77	6.72	2.80	$0.36 \cdot 10^{23}$
A3	tetramethylammonium hydroxide	7.97±3.97	6.70	9.80	$1.30 \cdot 10^{23}$

Considering its economic importance for agriculture and food industry, the popcorn (*Zea mays*) seeds were chosen as biological material. In order to diminish the putative genophond variations, seeds from a single plant with vigorous biological features from an experimental micropopulation were used in each experimental study. For every experimental sample we used ten seeds that germinated in the presence of 10 ml aqueous magnetic nanoparticles suspensions on porous paper support in Petri dishes, in darkness and controlled temperature (24 ± 0.5^oC) and humidity. For every type of magnetic nanoparticles, water solutions with six different concentrations of nanoparticles were added in the culture medium of *Zea mays* seeds germination (Table 2). Quantitative cytogenetic parameters have been used to evaluate MNPs influence at cellular level. Mitotic index (M.I.) and chromosomal aberration index (A.I.) were find following: M.I. [%] = $\boldsymbol{D}_c \times 100/T_c$; A.I. [%] = Ab × $100/T_c$ (T_c = total number of analyzed cells; Ab = abnormal cells number, $\boldsymbol{D}_c$ = divided cells number). For distinctive

chromosomal aberrations were taken microphotographs using a digital photo-camera (FUJI–FinePix S5100 model).

Table 2

The nanoparticles solution concentrations (μg/ml) and total number of magnetic nanoparticles added in culture medium of seeds germination for every experimental sample

Sample	**nanoparticles solution concentrations (μg/ml)** / number of nanoparticles added in culture medium of the seeds germination		
	A1 magnetic nanoparticles type	A2 magnetic nanoparticles type	A3 magnetic nanoparticles type
CONTROL	0	0	0
P1	**8.197** / $3.27 \cdot 10^{12}$	**1.753** / $3.08 \cdot 10^{10}$	**1.721** / $1.57 \cdot 10^{11}$
P2	**40.980** / $16.35 \cdot 10^{12}$	**8.764** / $15.40 \cdot 10^{10}$	**8.611** / $7.86 \cdot 10^{11}$
P3	**81.968** / $32.70 \cdot 10^{12}$	**17.528** / $30.80 \cdot 10^{10}$	**17.221** / $15.72 \cdot 10^{11}$
P4	**122.952** / $49.05 \cdot 10^{12}$	**26.292** / $46.20 \cdot 10^{10}$	**25.816** / $23.58 \cdot 10^{11}$
P5	**163.936** / $65.40 \cdot 10^{12}$	**35.057** / $61.60 \cdot 10^{10}$	**34.422** / $31.44 \cdot 10^{11}$
P6	**204.920** / $81.75 \cdot 10^{12}$	**42.821** / $77.00 \cdot 10^{10}$	**43.028** / $39.3 \cdot 10^{11}$

From the experimental results obtained for MNPs influence on mitotic division process and chromosomal aberrations occurrence at meristematic level, we tried to obtain mathematical models for biological effect – cause dependences and we performed a statistical analysis between the experimental results given by the MNPs different types influence. Experimental data were used for statistical analysis performed using the Kruskal-Wallis one-way analysis of variance [12] in Systat v.13 [13], in order to highlight the differences in action of the various MNPs layer. In order to develop mathematical models for the dependence functions between the Mitotic Index values and MNPs concentrations, we tested several polynomial functions using the Non-linear Estimation package from Statistica v.7.0 [14] and we considered the ones with the most suitable statistical parameters. The same procedure was used for the relation between the values of the Aberrations Index and MNPs concentrations.

3. RESULTS AND DISCUSSIONS

Following this experimental study we observed that various MNPs types have different effects on mitotic division process and on chromosomal aberrations occurrence. The presence of MNPs in the seeds germination medium induced various type of chromosomal aberrations development: simple, such as: inter-chromatin bridges, lagging chromosomes, ring chromosomes, chromosome fragments and micronucleus; or combination of multiple chromosomal aberrations.

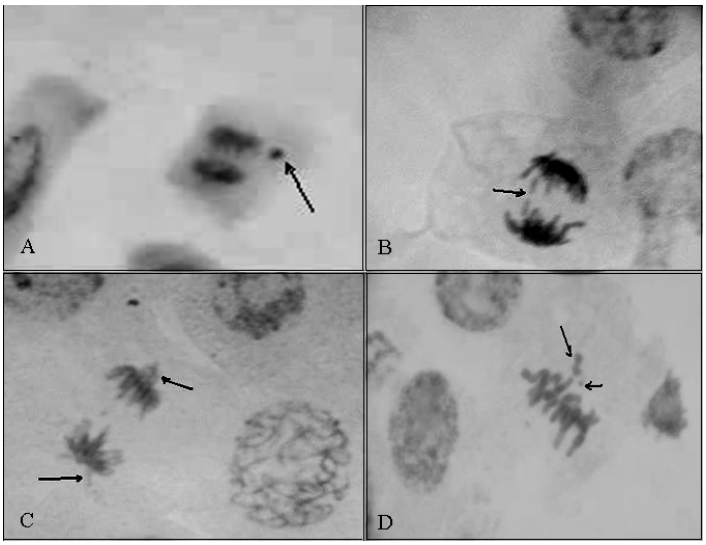

Fig. 1 – Some example of chromosomal aberrations found in root tips cells of seeds germinated on MNPs presence: (A) ana-telophase with micronucleus (MNPs-A1 type, 204.92 μg/ml), (B) anaphase with broken bridge (MNPs-A1 type, 81.968 μg/ml); (C) anaphase with star effect, ring chromosome and lagging chromosomes (MNPs-A1 type, 122.952 μg/ml), (D) metaphase with lagging chromosome and chromosome fragment (MNPs-A1 type, 163.936 μg/ml). Arrows indicate particularly, the specific chromosomal aberrations.

The specific action of each layer type used for the MNPs stabilisation is confirmed by the statistical analysis (Table 3), where the number of aberrations from each phase of the cellular mitotic division is compared.

Table 3

Statistical significance of the differences on chromosomal aberrations occurence for the three different layer of nanoparticles used in the experiment Kruskal Wallis test (CA – citric acid, TMA – tetramethylammonium hydroxide, bCD – beta-cyclodextrine; - – not significant; * – significant; *** – very significant)

MNPs layer	**Division phase of chromosomal abberations**			
	Prophase	**Metaphase**	**Anaphase**	**Telophase**
CA vs. TMA	0.001 -	0.179 ***	0.084 *	0.004 -
CA vs. bCD	0.004 -	0.047 -	0.035 -	0.338 ***
TMA vs. bCD	0.001 -	0.002 -	0.001 -	0.001 -

If the differences between the two synthesized chemical layers, beta-cyclodextrine and tetramethylammonium hydroxide, seem to be not significant, citric acid layer presents significant differences from the number of aberrations induced in the different phases of mitotic division with both the other nanoparticles

types (with different layer), namely a higher number of aberrations in the metaphase and anaphase, as opposed to tetramethylammonium hydroxide layer, and a higher number of aberrations in the telophase, as opposed to beta-cyclodextrine layer.

Since nanoparticles coated with tetramethylammonium hydroxide layer and citric acid layer have roughly the same size, but different effects on aberrations occurrence at different phases of the cellular mitotic division, while nanoparticles coated with beta-cyclodextrine layer and tetramethylammonium hydroxide layer, with significantly different sizes, present no significant differences in aberrations occurrence on different phases of the mitotic division, it can be concluded that the MNPs size is not an important factor on the chromosomal aberrations occurrence to the *Zea mays* root tips tissue, and most likely the chemical particularities of the MNPs layer are those responsible for the amount of chromosomal aberrations and the moment inside the cellular mitotic division process when they appear.

As regards the MNPs influence on the mitotic division process by mitotic index (MI) evaluation, for A1 and A2 type nanoparticles it was observed that the increase of MNPs concentrations exhibited a stimulating influence, while for A3 type nanoparticles, higher concentrations of MNPs added to the germination seeds medium, induced a very slight decrease of the MI.

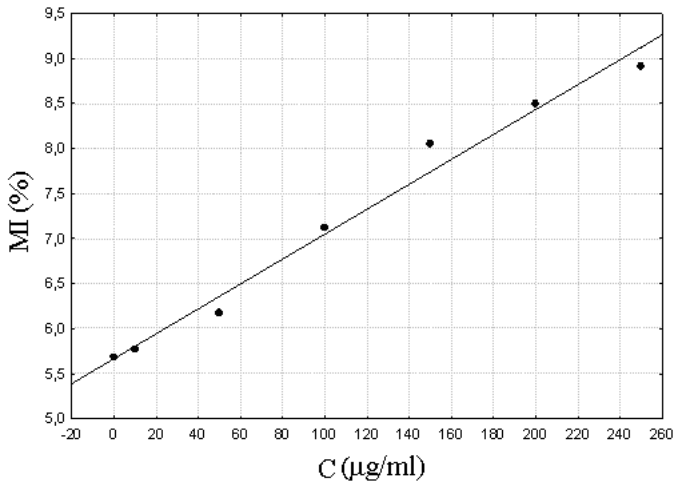

Fig. 2 – Dependence between the Mitotic Index MI [%] and the nanoparticles concentration level *C* in the case of seeds germinated in the presence of A1 type nanoparticles.

According Fig. 2 in the case of A1 type MNPs added in the seeds germination medium, it was obtained a linear dependence between the mitotic index (MI) and MNPs concentrations (*C*) (MI = 5.66823 + 0.013824 · $\boldsymbol{C}$, R^2 = = 0.991, $p = 0$).

The dependence function between the MI and A2 type MNPs concentrations obtained by mathematical modelling software is an polynomial function of the third degree ($MI = 7.95252 + 0.018333 \cdot \boldsymbol{C} - 0.9 \cdot 10^{-4} \cdot \boldsymbol{C}^2 + 0.3 \cdot 10^{-6} \cdot \boldsymbol{C}^3$, $R^2 = 0.998$, $p = 0$) (Fig. 3). As seen in Fig. 3, the mitotic division process was stimulated by the presence of the A2-MNPs in the seeds germination medium.

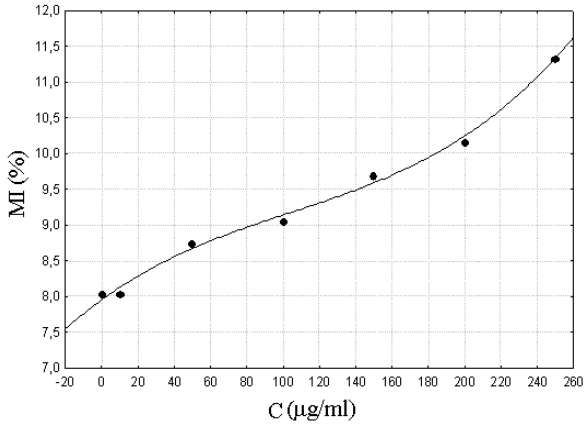

Fig. 3 – Dependence between the Mitotic Index MI [%] and the nanoparticles concentration level C [µg/ml] in the case of seeds germinated in the presence of A2 type nanoparticles.

In the case of seeds germinated in the presence of A3 type MNPs, the relation between the mitotic index and MNPs concentration is presented in Fig. 4, the model being a polynomial function of the fifth degree ($MI = 4.36328 + 0.151637 \cdot \mathbf{C} - 0.00372 \cdot \boldsymbol{C}^2 + 0.3 \cdot 10^{-4} \cdot \boldsymbol{C}^3 - 0.14 \cdot 10^{-6} \cdot \boldsymbol{C}^4 + 0.197 \cdot 10^{-9} \cdot \boldsymbol{C}^5$, $R^2 = 0.969$, $p = 0$).

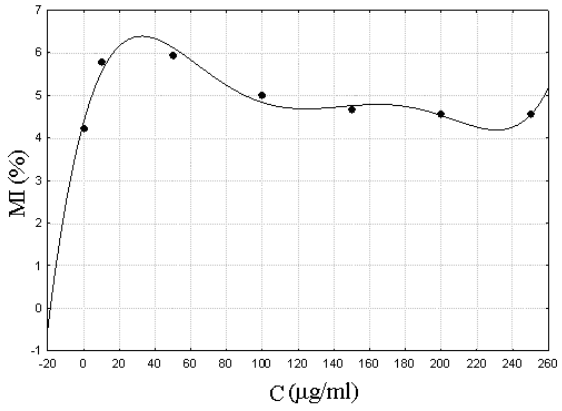

Fig. 4 – Dependence between the Mitotic Index MI [%] and the nanoparticles concentration level C [µg/ml] in the case of seeds germinated in the presence of A3 type nanoparticles.

Lower concentrations of A3 type MNPs in the germination medium induced a slight increase of the MI values, while higher concentrations are responsible for MI values comparable to the control sample, slightly decreasing along with the increase of MNPs concentrations.

It was observed that for each type of MNPs layer, the relation between the mitotic index and the MNPs concentration has a specific model, indicating that each of the chemical substances used as layer for the nanoparticles stabilisation conduct to own specific cytological effect of the MNPs type.

The relation between the Aberration Index and MNPs concentration for seeds germinated in the presence of A1 type nanoparticles is presented in Fig. 5, the mathematical model best fitting relation being a polynomial function of the third degree ($AI = 4.51427 + 0.106269 \cdot \boldsymbol{C} - 0.00056 \cdot \boldsymbol{C}^2 + 0.94 \cdot 10^{-5} \cdot \boldsymbol{C}^3$, $R^2 = 0.925$, $p = 0$). As seen, the chromosomal aberrations percentage induced by the A1 type MNPs increased for lower MNPs concentrations and had been stabilized around the same value at higher MNPs concentrations.

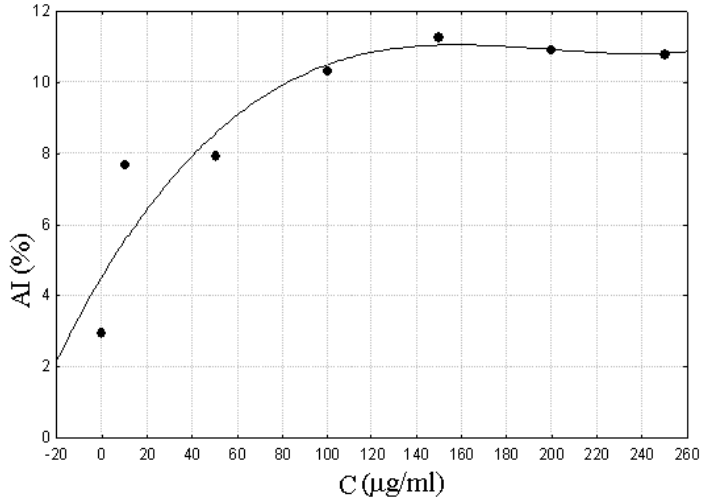

Fig. 5 – Dependence between the Aberrations Index AI [%] and the nanoparticles concentration level C [µg/ml] in the case of seeds germinated in the presence of A1 type nanoparticles.

In Fig. 6 is presented the dependence between the AI (%) and A2 type MNPs concentration and the most suited mathematical representation is a polynomial function of the sixth degree ($AI = 2.45399 + 0.973438 \cdot \boldsymbol{C} - 0.03557 \cdot \boldsymbol{C}^2 + 0.5 \cdot \cdot 10^{-3} \cdot \boldsymbol{C}^3 - 0.4 \cdot 10^{-5} \cdot \boldsymbol{C}^4 + 0.142 \cdot 10^{-7} \cdot \boldsymbol{C}^5 - 0.\ 2 \cdot 10^{-10} \cdot \boldsymbol{C}^6$, $R^2 = 1$, $p = 0$). The chromosomal aberrations percentage induced by the presence of A2 type MNPs in the seeds germination medium is roughly the same for all MNPs concentrations, with values slightly higher than the control sample ones.

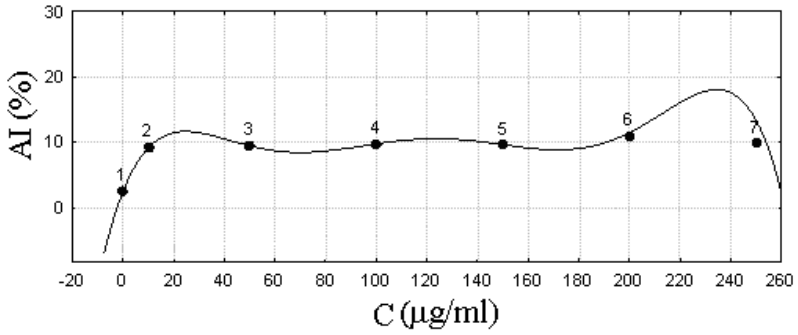

Fig. 6 – Dependence between the Aberrations Index AI [%] and the nanoparticles concentration level C [µg/ml] in the case of seeds germinated in the presence of A2 type nanoparticles.

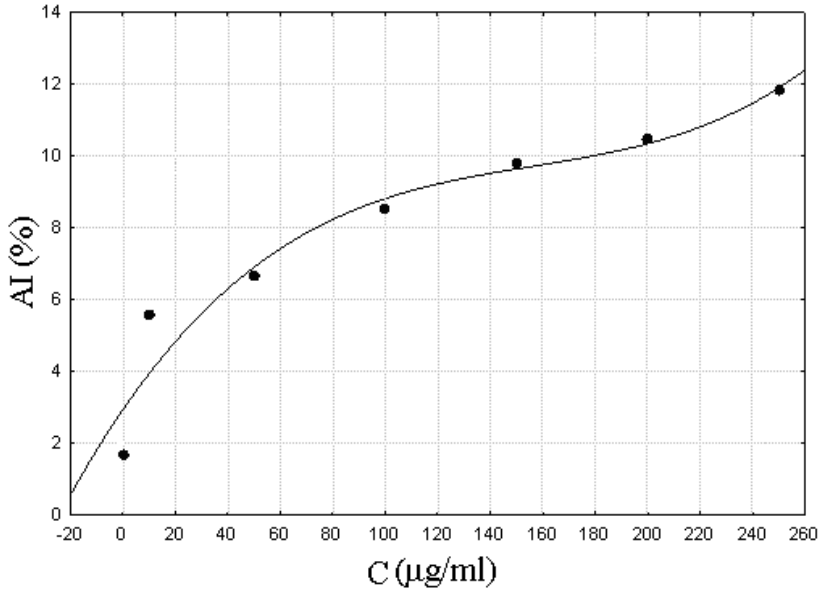

Fig. 7 – Dependence between the Aberrations Index AI [%] and the nanoparticles concentration level C [µg/ml] in the case of seeds germinated in the presence of A3 type nanoparticles.

Also, the chromosomal aberrations induced by the presence of A3 type MNPs in the germination medium presents a slight increase with the MNPs concentration, following a polynomial function of the third degree – $AI = 2.93444 + 0.106264 \cdot \boldsymbol{C} - 0.6 \cdot 10^{-3} \cdot \boldsymbol{C}^2 + 0.129 \cdot 10^{-5} \cdot \boldsymbol{C}^3$, $R^2 = 0.968$, $p = 0$ (Fig. 7). Similar to the situation observed on the Mitotic Index, the relation between the Aberration Index and MNPs concentration is different for each MNPs layer, confirming that each of the coatings induce to its own particular bioeffect.

4. CONCLUSIONS

From the results obtained on MNPs influence on mitotic division and chromosomal aberrations occurrence at meristematic level, we obtained mathematical models of the MNPs interaction and we statistically compared the influences of different types of MNPs studied. It can be concluded that the size of the coated MNPs is not an important factor of chromosomal aberrations appearance at cellular level of *Zea mays* seeds, and most likely the chemical particularities of the MNPs layer are those responsible for the amount of chromosomal aberrations and the division phase inside the cellular mitotic division process when they appear. The mathematical models of the dependences between both the Mitotic Index and the Aberration Index and the MNPs concentrations are different for each of the MNPs type stabilised with different layers, indicating that the chemical structure of the layer is responsible for the influence way of the MNPs at the cellular level. There are also significant differences between the mathematical models of MNPs influence on the Mitotic Index and the Aberration Index for each of the layer used, indicating that most likely processes responsible for mitotic division and the chromosomal aberrations induced are influenced by the chemical structure of the MNPs layer in specific ways.

REFERENCES

1. E. Masarovicova, K. Kralova, Ecological Chemistry and Engineering S **20**(*1*), 9-22 (2013).
2. C.W. Lee, S. Mahendra, K. Zodrow, D. Li, Y.C. Tsai, J. Braam, P.J. Alvarez, Environ. Toxicol. Chem. **29**(*3*), 669-675 (2010).
3. R. Barrena, E. Casals, J. Colón, X. Font, A. Sánchez, V. Puntes, Chemosphere **75**(*7*), 850-857 (2009).
4. H. Zhu, J.Han, J.Q. Xiao, Y. Jin, J. Environ. Monitor. **10**(*6*), 713-717 (2008).
5. T. Giordani, A. Fabrizi, L. Guidi, L. Natali, G. Giunti, F. Ravasi, A.Cavallini, A. Pardossi, Environmental Quality **8**, 27-38 (2012).
6. J. Li, P.R. Chang, J. Huang, Y. Wang, H. Yuan, H. Ren, J. Nanosci. Nanotechnol. **13**(*8*), 5561-5567 (2013).
7. Y.S. El-Temsah, E.J. Joner, Environmental Toxicology **27**(*1*), 42-49 (2012).
8. S.K. Dhoke, P. Mahajan, R.Kamble, A. Khanna1, Nanotechnology Development **3**(*e1*), 1-5, (2013).
9. M. Răcuciu, D. E. Creangă, A. Airinei, Eur. Phys. J. E. **21**, 117-121 (2006).
10. M. Răcuciu, D. E. Creangă, V. Bădescu, A. Airinei, Journal of Optoelectronics and Advanced Materials **9**(*5*), 1530-1533 (2007).
11. M. Răcuciu. D. E. Creangă, V. Bădescu, N. Suliţanu, J. Magn. Magn. Mater. **316**(*2*), e772-e775 (2007).
12. W. Kruskal, W.A. Wallis, J. Am. Stat. Assocn. **47**(*260*), 583-621 (1952).
13. StatSoft, Inc., *Electronic Statistics Textbook*, *Tulsa, Oklahoma*, 2010.
14. Systat Software, *Systat 12 Getting Started*, *San Jose, California*, 2009.