THE CYTO- AND GENOTOXIC EFFECTS INDUCED BY SULPHATES IN *Allium cepa* L.

Elena DOROFTEI[*], Maria-Mihaela ANTOFIE[**]

[*] *Faculty of Natural and Agricultural Science, „Ovidius" University, Constanţa, Romania*
[**] *Faculty of Agricultural Sciences, Food Industry and Environmental Protection, University "Lucian Blaga", Sibiu, Romania*
Corresponding author: Maria-Mihaela Antofie, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, University "Lucian Blaga", 5-7 Ioan Raţiu Str., 550027, Sibiu, Romania, email: mihaela_antofie@yahoo.com

Abstract. The scope of this paper is to assess the cyto- and genotoxic effects of sulphate on *Allium cepa* mitosis for root meristem. Three different concentrations of sodium sulphate (i.e. 0,1%; 1% and 5%) have been used, in which the onion bulbs were immersed for different periods of time such as 6, 24 and 72 hours. In the end of the experiment the harvested root tips were prepared according to Feulgen's squash technique and using Schiff reagent. The cytotoxic effects of sulphate were investigated by calculating the mitotic index and also through the analysis of chromosomes alterations during the mitosis. The phase ratio of cells undergoing mitosis in all the phases is estimated for all variants. For microscopy investigations it was used a Novex Holland B microscope with digital camera included. The cytological analysis realized on *Allium cepa* revealed a strong decrease in the mitotic index due to sulphate treatments which is more intense with the time of exposure. Moreover this phenomenon is associated with the appearance of different chromosomal complement alterations including the appearance of highly condensed chromatin. The mitotic index and genotoxic observations over the chromosomes can also be correlated with phase ratio of cells undergoing mitosis.

Keywords: *Allium cepa*, mitosis, phase ratio, sodium sulphate, mitotic index.

INTRODUCTION

Sulphur compounds are widely distributed and among major compounds found today in nature it also can be added sulphites and sulphates which may reach the natural environment due to the sulphur cycle in which human activity have a great impact [26] based on which today the sulphate concentration reach 2700 mg/L in the see and ocean waters [20]. Moreover, the human activity is constantly generating huge sulphur compounds residues, the major economic activities responsible for this type of pollution being the steel industry, metallurgy, mining, pulp and paper industry, acid gas processing industries, fossil fuels, etc [24] Therefore, monitoring sulphates in polluted soils and waters is a must [25] considering that sulphates play an important role in maintaining human health. In humans the sulphur deficiency is responsible among others of gallbladder miss functioning, oedema, congestion of the neck and / or head. For those who are susceptible or suffering from diabetes, providing the necessary sodium sulphate is critical, as it affects the pancreas physiology which is the provider of insulin in the human body [3]. The natural sources for sodium sulphate are among others: red beets, spinach, cauliflower, radishes, cucumbers, onions, pumpkin [9].

The toxicity symptoms of different mutagenesis chemical agents based on sulphur compounds are the results of detrimental effects over physiological processes that in plants include the inhibition of respiration and photosynthesis, the alteration of plant-water equilibrium which induces the decrease in membrane permeability of the root cells and adverse effects on the entire metabolic activity [7, 14, 23, 24, 26].

The scope of this paper is on one hand to analyse cytotoxic effects and mutagenic potential induced in *Allium cepa* by sulphate and on the other hand the results to be further used in developing a rapid reliable method for analysing mitotic index and chromosomes complement alterations in order to investigate the genotoxic limits of sulphate concentrations present in different polluting sources including the classical beverage industry [13, 15]. Phase ratio of cells undergoing mitosis is also discussed and correlated with mitotic index and chromosomes alterations.

MATERIAL AND METHODS

Plant material. In this experiment bulbs of onion as chives (*Allium cepa* L.) have been used, the Romanian cultivar "Diamant". Before starting the experiment the onion chives (small bulbs) of 1 cm in diameter where selected based on their appearance and uniformity in size and health. Berzelius jars of 250 ml were used filled with 10 ml boiled and cooled tap water in which the small bulbs of onion have been placed. The water was changed daily and the rooting process was stimulated under a photoperiod of 16h light/8 h dark at 18-20°C.

Experiment description. Only bulbs with roots between 1.5 and 2 cm in length have been selected for testing the sodium sulphate effect. Three series of sodium sulphate solutions have been prepared as following 0.1%, 1% and 5% which according to the time of action (6, 24 and 72 hours) determined the scheme of the experiment according to data included in table no. 1. It was used the anhydrous sodium sulphate produced by Merck: no 106643. The control was represented by root maintained in the boiled and cooled tap water.

The rooted bulbs were placed in 10 ml solution of sodium sulphate as mutagenic agent and maintained for 6-, 24 and 72 hours to interact, starting with the 10:00 hour in the morning. In this experiment the Test Levan (1938) was applied as it is economic, requires short time and gives the possibility to work on excellent prepared slides. Moreover, the chromosomes from *Allium cepa* are large enough, in a low number (2n=16) making easily their observation under the microscope and the root meristems contains a huge numbers of cells under division [2, 6, 12].

In the end of experiment for each variant, according to the time of exposure the roots tips are taking out and

fixed in a solution of absolute ethylic alcohol and anhydrous acetic acid in a volume report of 3:1 for 16 hours at refrigerator followed by a gentle acidic hydrolysis using HCl 1 N for 5 min at 60°C. The roots tips colouring was realized following the Feulgen technique and using Schiff reagent for 90 min followed by water immersion for 20 min.

Table 1. Experimental variants used in the treatment of onion roots tips with sodium sulphate solutions

Sodium sulphate solution concentration	Variant	Exposure time
0,1%	V_1	6 h
1%	V_2	
5%	V_3	
0,1%	V_4	24 h
1%	V_5	
5%	V_6	
0,1%	V_7	72 h
1%	V_8	
5%	V_9	

The ready coloured root tips, of 5 mm in length where squashed on slides and used for microscopy analysis under a Novex Holland B microscope with digital camera included. The mitotic index was calculated based on each slide analysis and observations have been made for each mitosis stage regarding the number of cells for each phase and chromosomes' abnormalities.

In order to determine the mitotic index there have been counted a minimum 500 cells for each of five replica at the meristem level of each experimental variant. It should be noted that the total investigated cells include cells in interphase and mitosis and under x1000 magnification have been investigated for chromosomal alterations.

The mitotic index (MI) was calculated by using the classical formula:

$$MI = \frac{\text{no of cells in mitosis}}{\text{no of total investigated cells}} \cdot 100$$

Five repetitions for each variant have been analysed and in each slide. Phase rate is calculated based on dividing the cell numbers for specific mitosis phases to the lowest number of cells for a phase.

Statistical analysis. The MI was compared using analysis of variance (ANOVA) to confirm the variability of the data and validity of results. The differences between variants have been analyzed for their significance based on Duncan's multiple range (DMRT) test (pb0.05) and differences between corresponding controls and exposure treatments were considered statistically significant at pb0.05.

RESULTS

For more than 30 years, the effects of chemical substances on the living organisms are studied for their effects on the plant chromosomes especially following treatments of the roots tips as they are easily produced during seed germination, the experiments may be conducted all over the year and are not costly [1, 8, 16, 21]. Five replica of each variant have been investigated, covering an entire slide, and cells in interphase or in mitosis have been counted. Cells undergoing mitosis have been separately counted for each phase: prophase, metaphase, anaphase, telophase and cytokinesis. The results of these investigations are presented in table no.2 in which the mitotic index is also calculated.

Mitotic index and cell cycle. Analyzing the obtained results presented in table no 1, it is obviously that the mitotic index is decreasing compared to the control in all experimental variants and in direct correlation with the concentration and the exposure time to the sodium sulphate as it can also be observed in fig. no 1. This decrease showed a statistically significant difference between all variants and the control for all exposure times. Still, the highest statistically significant differences were counted in case of sodium sulphate treatment with concentrations of 0.1% for 72 hours; 1% for over 24 hours and 5% for all treatments. Thus, if for the control in onion root tips the mitotic index was 43.2, for all variants treated with sodium sulphate the lowest mitotic index was for the highest concentration in the mutagenic agent and for the longer exposure of 72 hours. Under these conditions it should be underlined that the treatment for 6 hours with sulphate induced a mitotic index of 21,6, which is half of the control. The MI for 24 hours was17.2 which means more than 2.5 times lower compared to the control and for 72 hours MI was 12.0 which is more than 3.6 times lower compared to the control. In other words it is obvious that by increasing the concentration and time of exposure the mitotic index will be decreased more in onion and this follows a linear impact on this.

Table 2. The effect of sodium sulphate on the mitotic index and cell division in *Allium cepa*

Experimental variant	Total cells in interphase	Total cells in mitosis	Total cells in prophase	Total cells in metaphase	Total cells in anaphase	Total cells in telophase	Total cells in cytokinesis	Mitotic index (MI)
Control	284±4.3	216±4.3	93±2.01	32±3.11	29±2.65	34±2.45	28±2.35	43.2±0.21
V_1	332±2.7**	168±2.7	78±2.13	23±2.80	14±2.89	28±2.98	25±2.65	33.6±0.32*
V_2	353±3.1*	147±3.1	70±2.23	17±2.78	12±1.99	24±2.76	24±2.98	29.4±0.31*
V_3	392±2.5**	108±2.5	58±1.98	10±2.65	4±1.98	19±2.89	17±3.02	21.6±0.23**
V_4	359±3.3*	141±3.3	71±2.34	17±1.89	11±1.78	23±2.23	19±2.34	28.2±0.33*
V_5	389±4.1*	111±4.1	64±1.99	11±1.89	5±1.98	17±3.45	14±4.18	22.2±0.28**
V_6	414±6.2*	86±6.2	58±1.78	5±2.31	2±1.86	12±5.21	9±5.32	17.2±0.19**
V_7	388±2.8**	112±2.8	54±2.21	14±2.87	11±1.56	20±3.01	13±3.28	22.4±0.25**
V_8	412±3.5*	88±3.5	50±1.89	6±2.23	5±1.62	17±3.12	10±4.12	17.6±0.16**
V_9	443±2.1**	57±2.1	42±1.75	0	0	9±3.33	6±2.32	12.0±0.15**

* Significantly different from control: $P < 0.05$; ** Significantly different from control: $P < 0.01$.

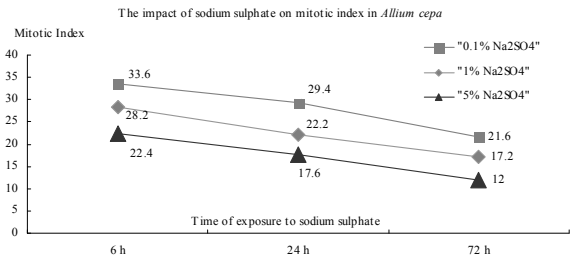

Fig. 1. Correlations between mitotic index and sodium sulphate concentrations and time of treatments in *Alium cepa*

As it was underlined that the lowest mitotic index calculated was 12 and it was registered for the highest concentration in the treatment with sodium sulphate for the longer period of time (i.e. 72 h) which supports the idea that at this concentration it was obtained the highest statistically significant inhibiting effect in mitosis for onion root tips.

Cell division. In order to support the mitotic index analysis it was also investigated the proportion or ratio between cells undergoing different mitosis phases based on the results presented in table no 1. In this regard the base line in calculating *phase ratio* (PR) indicator is provided by cells number in anaphase which is constantly the lowest for all experimental variants. Based on this it can be observed that the control expresses a net proportion between all cells undergoing divisions such as 3:1:1:1:1 which is prophase (3): metaphase (1): anaphase (1): telophase (1): cytokinesis (1). This can be considered as a control PR which is dramatically changed for the onion root tips exposed to sodium sulphate even the largest proportion of cells are in the prophase which is increased compared to the control in all variants. Even for the lowest concentration of sodium sulphate and the shorter time of exposure the proportion of cells in prophase is increasing with 1.16 times compared to control. Considering the PR between investigated cells we underline that for the variant 1, this changed as following: 5.5:1.5:1:2:1.8. Based on this result the cells in prophase appear to dramatically increase as a ratio as well as the cells in telophase and cytokinesis. The PR trend is almost the same for 24 hours of treatment in variant V2 which changed in V3 for 72 hours of action where this is significantly changed:14.5: 2.5: 1: 4.7: 4.2. Thus, the cells in prophase appear to be blocked in their fate to continue cell division. By increasing the concentration of sodium sulphate 10 times at the shorter exposure time in variant V4 the PR among the cells is slightly following the same pattern such as: 6.5: 1.5: 1: 2: 1.7. For 24 hours of exposure in variant V5 the cell ratio is almost reaching the pattern for 72 hours in 0.1% sodium sulphate solution of variant V3: 12.8: 2.2: 1: 3.4: 2.8 and the cells ratio in prophase is doubled compared to variant V4 and four times higher compared to control. Increasing the time of exposure to sodium sulphate the ratio is dramatically changed: 29: 2.5: 1: 6: 4.5. The blockage of cells in prophase is increased for almost 10 times. Very interesting is the ratio of the variant V7 when the concentration of sodium sulphate is increased 50 times compared to V1: 5: 1.3: 1: 1.5: 1.2. By prolonging the time of exposure the ratio is changing more: 10: 1.2: 1: 3.4: 2 which means that the cells blocked in prophase are doubled. For variant 9 the ratio is as following 42: 0: 0: 9: 6 where it is obvious that even metaphases and anaphase are blocked this time.

Cytological observations. During these microscopy investigations for sodium sulphate effects on the onion root cells division it was also observed different changes in the normal mitosis related to chromosomes and chromatin. After a six hours treatment of onion root tips, the sodium sulphate produces a series of cytotoxic effects. For the concentration of 0,1% the mutagenic agent is inducing an apparent accumulation of cells in prophase even there is a lower number compared to the control associated with normal and longitudinal cytokinesis. Increasing the concentration of sodium sulphate at 1% for the same time period it is increased the proportion of longitudinal cytokinesis (fig. 5) and at 5% this process is associated too with the appearance of small highly condensed chromosomes (fig. 6). After 24 hours of treatment with sodium sulphate it was observed that the cells in prophase are dominant aside cells in normal or longitudinal cytokinesis no matter of concentration (figs. 7, 8, 9). Following the treatment for 72 hours with sodium sulphate it was also observed the dominance of cells in prophase or in normal and longitudinal cytokinesis no matter of used concentrations (figs. 10, 11, 12).

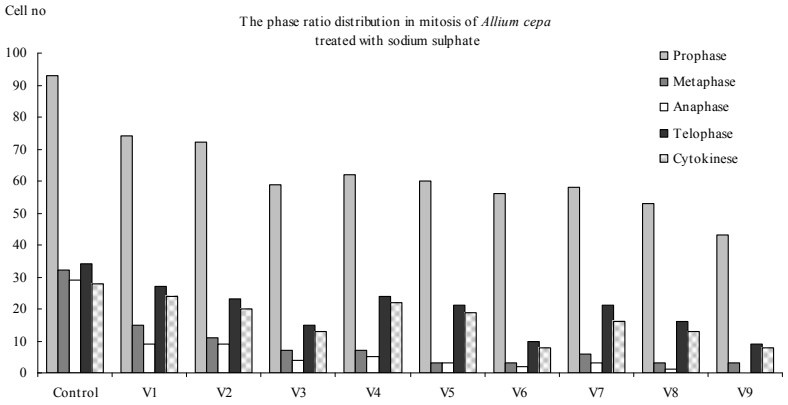

Fig. 2. The phase ratio distribution in the root tips of *Allium cepa* after sodium sulphate treatment

After 24 and 72 hours of exposure it was also observed the frequent plasmolysis of investigated cells and also the stronger condensation of chromatin which is responsible for shrinking more the chromosomes and also nuclei volume. Very often in the microscopy fields were identified either prophases as the early stage for preparing cell entering into division either the later stages such as telophases and cytokineses and no intermediary stages were observed such as metaphases or anaphases. The nuclei appear to be bigger compared to control which is associated with hypertrophy, the membranes appear to be less organized and the chromatin appear to become roughly and highly heterogeneous in size and shape. Again another peculiar observation is for cytokinesis in which the division plane is rather longitudinal and very seldom transversal.

At a concentration of de 5% of sodium sulphate for 24 or 72 hours the cytotoxic effects includes among others the appearance of star-like metaphases and anaphase's bridges, the presence of laggards and cells with abnormal shape of nuclei associated with heterochromatin and also with the increase in vacuoles' size.

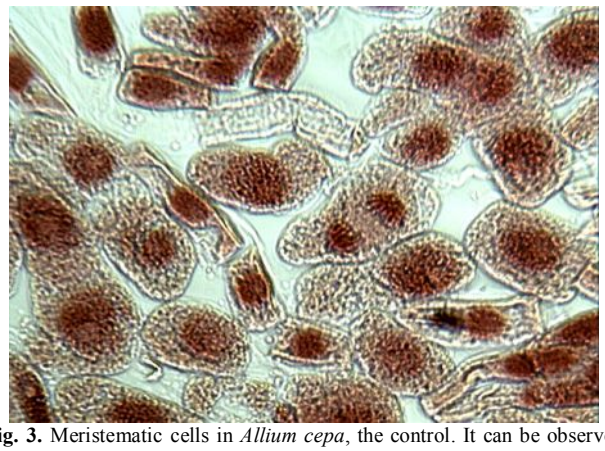

Fig. 3. Meristematic cells in *Allium cepa*, the control. It can be observed numerous prophases, central is a late anaphase and a telophase (400x)

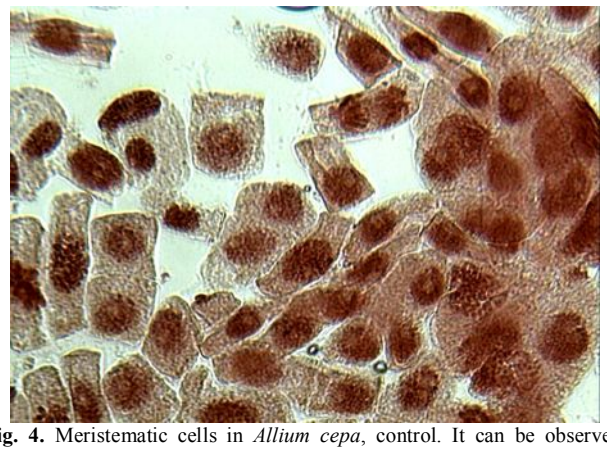

Fig. 4. Meristematic cells in *Allium cepa*, control. It can be observed numerous prophases, two metaphases, one anaphase, one telophase and one cytokinese (400x)

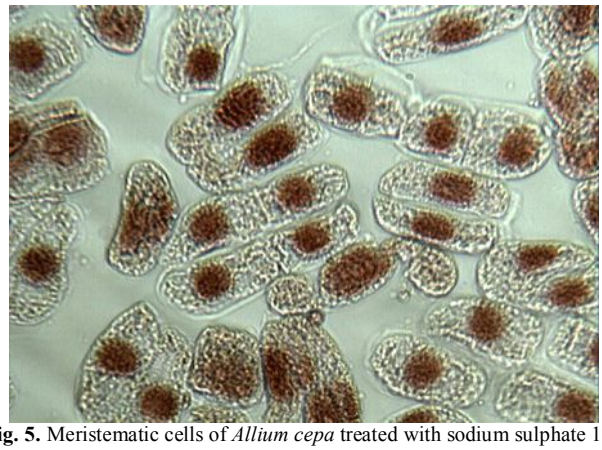

Fig. 5. Meristematic cells of *Allium cepa* treated with sodium sulphate 1% for 6 hours. It is observed the dominance of prophases and normal and longitudinal cytokineses (400x)

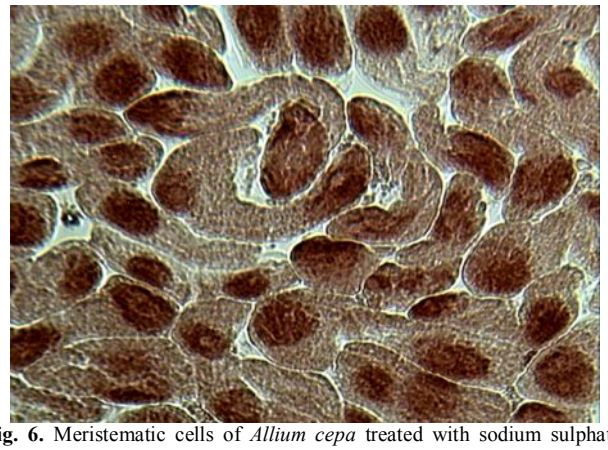

Fig. 6. Meristematic cells of *Allium cepa* treated with sodium sulphate 5%, 6 h. It is observed the dominance of prophase, metaphase and anaphase with small chromosomes (400x)

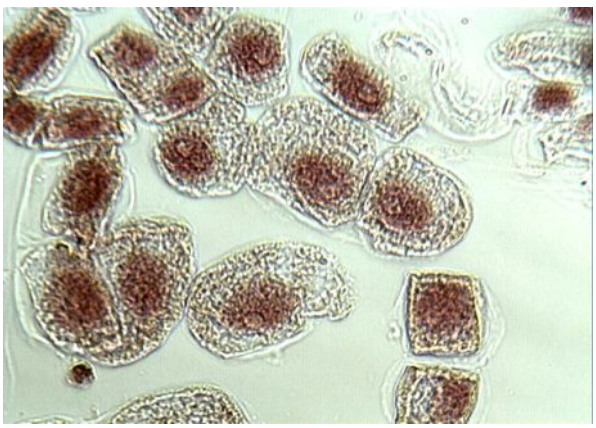

Fig. 7. Meristematic cells of *Allium cepa* treated with sodium sulphate 0,1% for 24 hours. It is observed the dominance of prophase and normal cytokinese (400x)

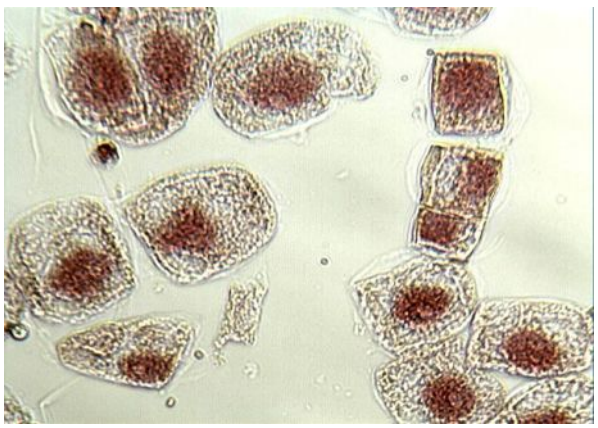

Fig. 8. Meristematic cells of *Allium cepa* treated with sodium sulphate 1%, 24 hours. It is observed the dominance of prophase and normal and longitudinal cytokinesis (400x)

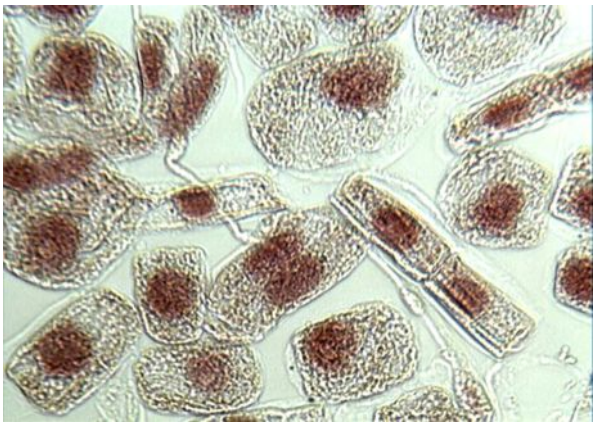

Fig. 9. Meristematic cells of *Allium cepa* treated with sodium sulphate 5%, 24 hours. It is observed the dominance of prophase, normal and longitudinal cytokinses (400x)

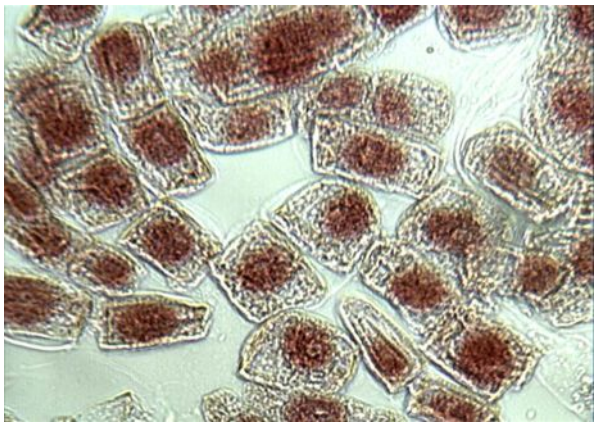

Fig. 10. Meristematic cells of *Allium cepa* treated with sodium sulphate 0,1%, 72 hours. It is observed the dominance of prophase and normal cytokinese (400x)

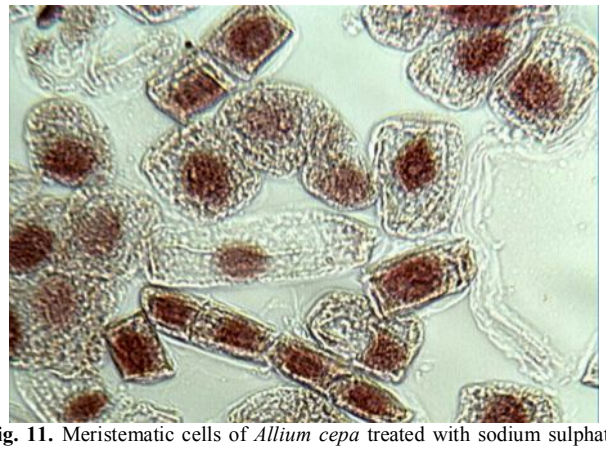

Fig. 11. Meristematic cells of *Allium cepa* treated with sodium sulphate 1%, 72 hours. It is observed the dominance of prophase and normal cytokinese (400x)

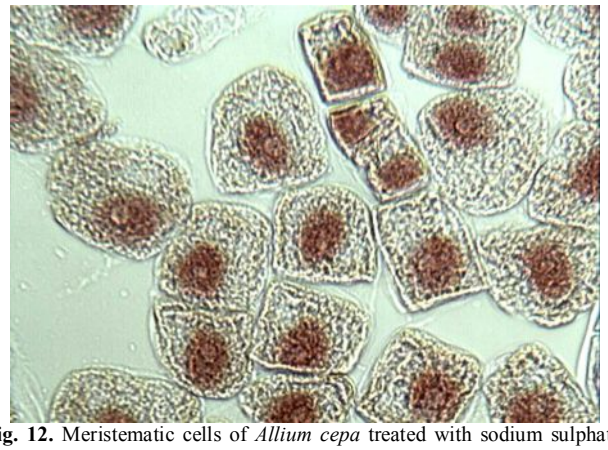

Fig. 12. Meristematic cells of *Allium cepa* treated with sodium sulphate 5%, 72 hours. It is observed the dominance of prophase, normal and longitudinal cytokineses (400x)

DISCUSSIONS

Sodium sulphate is considered as a chemical substance inducing in overdoses both cytotoxic and genotoxic effects in living organisms and therefore it is studied for their effects on the plant chromosomes especially following treatments of the roots tips as they are easily produced, all over the year and are not costly [1, 8, 17, 22].

Applying *Allium* test in the laboratory for investigating effects of potential mutagens on mitosis index and cell cycle it was proved by previous studies that it is a reliable method in terms of time and expenses. In this regard it was preferred to be used only fragments of 5 mm in length surrounding the root meristems which is slightly modifying also the mitotic index which for the meristematic zone become higher, almost double compared to previous results [18, 21]. This choice may be grounded with the fact that for industrial purposes it is easier to investigate on a focused area for a shorter time when there are working experimented researchers multiple samples. Considering the evaluation of results presented in table no. 2, the mitotic index is dramatically decreasing under the treatment of all used sulphate concentrations for all three periods of time and this is in line with other scientists' results [18]. Counting the cells undergoing mitosis for the effect of sodium sulphate treatment it clear appears that it is seriously affected compared to the control. Still, the highest cells number in prophase, excluding control, are found in the variant V1 (i.e. 0.1% sodium sulphate for 6 hours) with 78 cells and the lowest in variant V9 (i.e. 5% sodium sulphate for 72 hours) with 42 cells. Moreover, the highest cells number for telophase is also in variant V1 with 28 cells and the lowest in variant V9 with 9 cells, following the same pattern. Furthermore, for cytokinesis the highest cells number is found for variant V1 with 25 cells and the lowest is variant V9 with 6 cells. In the case of metaphase analysis 23 cells are found as the highest cell number for variant V1 and this was absent in variant V9. In the same variant V1 it was found that the highest number of cells in anaphase with 14 cells which are absent in variant V9. Realizing a comparative analysis the highest inhibiting effect on cell mitosis of the sodium sulphate is for 5% and 72 hours but it should be accepted that all variants induced dramatic changes in the proportion of cells in different stages of cell division and particularly of cells undergoing anaphase.

Thus, by counting the cells in different mitosis phases gives the opportunity to analyse the phase ratio. In this case the number of cells in anaphase is considered as a reference number as it was constantly the lowest. Applying this methodology it becomes obviously that the normal ratio between cells residing

the meristematic area and undergoing mitosis, is 3:1:1:1:1. This ratio is modified under the sodium sulphate treatment at 6-, 24 and 72 hours for all three used sulphate concentrations: 0.1%, 1% and 5%. Still, in numeric terms, if in the control a mean of 284 cells were observed in interphase and 216 in mitosis for 500 counted cells, under the effect of sodium sulphate, the number of cells in interphase increased up to 443 at a concentration of 5% and for 72 hours exposure and as a consequence the number of cells in division decreased to 57 (table no 1, figs. 1 and 2) in a similar manner like that described by Rencüzogullari and collaborators working on *Allium cepa* [19, 27] and our previous results [4].

By analysing the phase ratio among the investigated cells into the root tip, as a tissue marker, it is obviously that the mutagenic agent sodium sulphate is blocking the cells in prophase and it dramatically decreases the evolution of the cells undergoing mitosis to cytokinesis through a constant decreasing the cell ratio in anaphase and even in metaphase for the highest concentration and longer time of exposure. This may be related not only to the cell response to the mutagen but it should also be correlated with an over answer of the whole root tissues which are in direct contact with this chemical. In supporting this it is significant to underline that among the cells in division, in all experimental variants, the largest number was counted for prophase (preparing cells for division) followed by telophase and cytokinesis compared to the lowest cell number for anaphase. This may be correlated with the direct impact of the mutagen on the cell division progression at the tissue level. Thus, anaphase appears to be dramatically affected in its evolution which is correlated with the blockage in prophase and low contribution of cells in metaphase. Under these circumstances we may conclude that the time frame for anaphase might be shortening also under the effect of this mutagen and further supporting the research results of Eastmond and Tucker in 1989 [5]. The cytotoxic effect of sodium sulphate is stronger and it is associated with the genotoxic effect at a concentration of 1% for 72 hours and 5% for more than 24 hours when were observed the strong condensations of chromatin which is responsible for shrinking more the chromosomes. Another interesting observation is for cytokinesis which is peculiar, the division plane being rather longitudinal and very seldom transversal; star-like metaphases and anaphase's bridges, the presence of laggards and abnormal shape of nuclei associated with heterochromatin and also with the increase in vacuole size. Such results are supporting the idea that sulphates in higher concentrations are negatively influencing cell division [10, 11, 18, 20].

Moreover, *Allium* test is a reliable method for laboratory investigations which can be further developed. The phase ratio into the onion root tip may be used as a very sensitive indicator of the tissue' answer to the chemicals and it can be added for supporting the results such as mitotic index and genotoxic effects. In case of sulphate no matter of used concentration or the time of exposure on the cells in the root tips of *Allium cepa,* it produces dramatic decreases of mitotic index associated with changes in the ratio of cells undergoing division. This compound is increasing the incidence of the cells in interphase and when they are in division than the ratio is moved to an increased proportion of cells in prophase, telophase and cytokinesis and a constant reduced ratio of cells in anaphase for all variants and therefore it was chosen as a reference base for calculating the phase ratio for cells undergoing mitosis in *Allium* test. Such studies should be continued to further investigating direct correlations between cell ratio, mitotic index and the frequency for chromosomes abnormalities appearance.

REFERENCES

[1] Adeyemo, O.A., Farinmade, A.E., (2013): Genotoxic and cytotoxic effects of food flavor enhancer, monosodium glutamate (MSG) using *Allium cepa* assay. African Journal of Biotechnology, 12(13): 1459-1466.

[2] Bateman, A.J., (1977): Handbook of mutagenicity - Test procedures. Edited by B.J. Kilbey, M. Legator, W. Nichols, C. Ramel. Ed. Elsevier, North Holland, Amsterdam, 325 p.

[3] Dawson, P., (2013): Role of sulphate in development. Reproduction REP-13-0056, doi: 10.1530/REP-13-0056 First published online 30 May 2013.

[4] Doroftei, E., Antofie, M.M., Sava, D., Arcuş, M., (2010): Cytogenetic effects induced by Manganese and Lead microelements on germination at *Allium cepa*. Botanica Serbica, 34(2): 115-121.

[5] Eastmond, D.A., Tucker, J.D., (1989): Identification of aneuploidy - inducing agents using cytokinesis - blocked human lymphocytes and an antikinetochore antibody. Environmental and molecular mutagenesis, 13(1): 34-43.

[6] Fijesko, G., (1985): The Allium test as a standard environmental monitoring. Hereditas, 91: 169-178.

[7] Fisjekö, G., (1982): Evaluation of short term tests of toxicity and mutagenity with special reference to mercury and selenium. Hereditas, 95: 155-162.

[8] Gomes, K.M.S., Oliveira, M.V.G.A.D., Carvalho, F.R.D.S., Menezes, C.C., Peron, A.P., (2013). Citotoxicity of food dyes sunset yellow (E-110), bordeaux red (E-123), and tatrazine yellow (E-102) on Allium cepa L. root meristematic cells. Food Science and Technology (Campinas), 33(1): 218-223.

[9] Hossain, M.M., Hosono-Fukao, T., Tang, R., Sugaya, N., van Kuppevelt, T.H., Jenniskens, G.J., Uchimura, K., (2010): Direct detection of HSulf-1 and HSulf-2 activities on extracellular heparan sulfate and their inhibition by PI-88. Glycobiology, 20(2), 175-186.

[10] Jürgens, G. (2005): Cytokinesis in higher plants. Annual Review of Plant Biology, 56: 281-299.

[11] Jürgens, G. (2005): Plant cytokinesis: fission by fusion. Trends in cell biology, 15(5): 277-283.

[12] Kapustka, L.A., (1997): Selection of phytotoxicity tests for use in ecological risk assessment. In Plants for environmental studies. Wang, Gorsuch and Hughes (Ed) Lewis Publisher, 563 p.

[13] Kayraldiz, A., Basri, H., Topaktafi, M., (2001): The Cytogenetic Effects of Sodium Metabisulfite, a Food Preservative in Root Tip Cells of *Allium cepa* L. Turkish Journal of Biology, 25: 361-370.

[14] Kristen, U., (1997): Use of higher plants as screens for toxicity assessment. Toxicology *in vitro*, 11: 181-191.

[15] Kumar, L.P., Panneerselvam, N., (2007): Cytogenetic studies of food preservative in *Allium cepa* root meristem cells. Medicine and Biology, 14(2): 60-63.
[16] Leme, D.M., Marin-Morales, M.A., (2009): *Allium cepa* test in environmental monitoring: A review on its application. Mutation Research/Reviews in Mutation Research, 682(1): 71-81.
[17] Levan A., (1938): The effect of colchicines in root mitosis in Allium, Hereditas, 24: 471–486.
[18] Mueller, S.O., Schmitt, M., Dekant, W., Stopper, H., Schlatter, J., Schreier, P., Lutz, W.K., (1999): Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. Food and chemical toxicology, 37(5): 481-491.
[19] Rencüzogullari, E., İla, H.B., Kayraldiz, A., Topaktaş, M., (2001): Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfite, a food preservative. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 490(2): 107-112.
[20] Risueño, M.C., Giménez-Martín, G., López-Sáez, J.F., (1968): Experimental analysis of plant cytokinesis. Experimental cell research, 49(1): 136-147.
[21] Sampson, J., Guilbeault, K., (2013): Baseline physicochemical investigations on waters from three blue holes, San Salvador Island, Bahamas. Studia UBB, Geologia, 58(1): 11-19.
[22] de Souza Pohren, R., da Costa, T.C., Vargas, V.M.F., (2013): Investigation of Sensitivity of the *Allium cepa* Test as an Alert System to Evaluate the Genotoxic Potential of Soil Contaminated by Heavy Metals. Water, Air, & Soil Pollution, 224(3): 1-10.
[23] Vangronsveld, J., Clijsters, H., (1994): Toxic effects of metals. pp. 150-177. In Farago, M.E. (ed): Plants and the chemical elements, VCH, Weinheim, New York, Basel, Cambridge, Tokio.
[24] Vennitt, S., Parry, J.M., (1984): Mutagenicity testing: a practical approach. IRL Press, Oxford, Washington DC., 334 p.
[25] Wang, X., Li, Z., Lei, L., (2013): Model study of sulfite oxidation in seawater flue gas desulfurization by cylindrical wetted-wall corona-streamer discharge. Chemical Engineering Science, 97: 7-15.
[26] Wu, N., Farquhar, J., (2013): Metabolic rates and sulfur cycling in the geologic record. Proceedings of the National Academy of Sciences, 110(28): 11217-11218.
[27] Yi, H., Heng, Z., (2003): Genotoxicity of hydrated sulfur dioxide on root tips of Allium sativum and Vicia faba. Mutation Research, 537: 109-114.

Received: 2 August 2013
Accepted: 18 September 2013
Published Online: 20 September 2013
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589