Article

Allium cepa test: An evaluation of genotoxicity

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Abstract

The importance of *Allium cepa* test contributes knowledge in preventing toxicity in the environment. This test widely used in determining genotoxic and cytotoxic substances found in the water system. In this study, the genotoxic effects of H_2O_2 (Hydrogen Peroxide) and CH_2O (Formalin) were determined using *Allium cepa* root tip cells while the water samples collected from the mining areas of Sorex Barobo, Surigao del Sur and Rosario, Agusan del Sur were also tested for genotoxicity. One-Way ANOVA shows that water samples treated with H_2O_2 were statistically significant ($P<0.05$) when compared to CH_2O while the water sample collected from the mining areas shows statistically non-significant. A concentration-dependent increased were observed among the dividing cells and aberrant cells of the treated and collected water samples. The observed abnormalities were seen into its roots morphology indicating genotoxicity. Thus, the obtained results in this study show that *Allium cepa* test is useful as bio-indicator to detect genotoxicity.

Keywords *Allium cepa*; genotoxicity; chromosomal aberration; Agusan del Sur; Surigao del Sur.

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1 Introduction

The importance of *Allium cepa* test contributes knowledge in preventing toxicity in the environment. Onion (*Allium cepa* L.) a potential biomarker of genotoxic studies (Firbas and Amon, 2013). Widely used as a bioindicator of genotoxicity from the different aquatic environs. This test helps to evaluate mutagens and detecting toxic substances found in the environment (El-Shahaby et al., 2003). Also, it is known as a fundamental biomarker to evaluate environmental pollution (Bagatini et al., 2009; Leme and Marin-Morales,

2009). Relatively, *Allium cepa* is one of the many methods for detecting and measuring the degree of alterations in the system subjected to carcinogens/mutagens or chemical causing damage and allow to describe the effects of these damages by observing chromosomal aberrations (Tedesco and Laughinghouse, 2012).

In addition, *Allium* test, generally used for analyzing the quality of drinking water and water-causing pollution (Fiskesjo, 1985; Rank, 2003). Indeed, it is an efficient approach for chemical screening and in situ monitoring of the genotoxicity effect of environmental contaminants (Fiskesjo, 1985, 1993; Barbérico et al., 2009; Siddiqui et al., 2011; Nunes et al., 2011). This test widely used to study the toxicity and genotoxicity of many dangerous contaminants, such as pesticides, azo dyes, food preservatives and hydrocarbons (Riffat and Ahmad, 2006; Mittergger et al., 2007; Feretti et al., 2007; Turkoglu, 2007; Leme and Marin- Morales, 2009; Mustafa and Arikan, 2008; Ashraf and Husain, 2010), where all tests have shown that *A. cepa* is more sensitive for detecting toxicity and genotoxicity than other tests. Furthermore, this mechanism has been known to identify the presence of pesticides in foods as well as in the environments (Abusalama et al, 2014 and Bakadir et al., 2016). This application plays an important role in bio-monitoring since roots of onions were sensitive for any toxic materials. Furthermore, plant roots are extremely useful in biological testing because root tips are the first to be exposed to toxicants dispersed in soil or in water (Fiskesjo, 1988). Moreover, the root tip chromosomal aberration assays constitute rapid and sensitive methods for bio-monitoring from the extent of pollution and to evaluate the effects of toxic and mutagenic substances in the natural environment (Matsumoto, et al., 2006; Rodriguez-Ruiz et al., 2014).

In this study, two chemicals H_2O_2 (Hydrogen Peroxide) and CH_2O (Formalin) were used as a potential transporter of chromosomal aberration while the water samples collected from the mining areas of Sorex Barobo, Surigao del Sur and Rosario, Agusan del Sur, were also tested for genotoxicity. At present the areas are undertaking large and small scale mining activities where their runoffs directly contained into the water system. The cyanide is one of the genotoxic substances being used for cyanidation to extract gold from the ore. In this way, frequent using of the chemical likely affects the condition of the waterways and to the health of the community. This study aims to describe various chromosomal aberrations in the root cells of the onion (*Allium cepa* L.) which functions as the biomarker for the several types of environmental contaminants.

2 Study Area and Methodology

2.1 Study site

The study area was part of Caraga Region located in Northern Mindanao. Geographically lies between $8^0$31’20.41”N 126^0 07’43.4” E (Barobo Surigao del Sur) and $8^0$19 22.25”N $126^0$07’37.50” E (Rosario, Agusan del Sur), Philippines.

2.2 Water sample collection and treatment

The water samples for the study were collected in the month of June 2016. It was done using washed and sterilized plastic containers after running water to waste for 4-5 minutes. In both cases, samples were taken in triplicates from each sampling point aseptically into plastic containers and kept in an ice chest. Each of the positive control was treated by H_2O_2 (Hydrogen Peroxide) and CH_2O (Formalin) with the concentrations of 1, 2 and 3% respectively and diluted with distilled water. While the collected water samples from the mining areas were also subjected to different concentrations of 25, 50 and 75% and diluted with distilled water. The tap water serves as the negative control with the same concentrations of 25, 50 and 75% respectively.

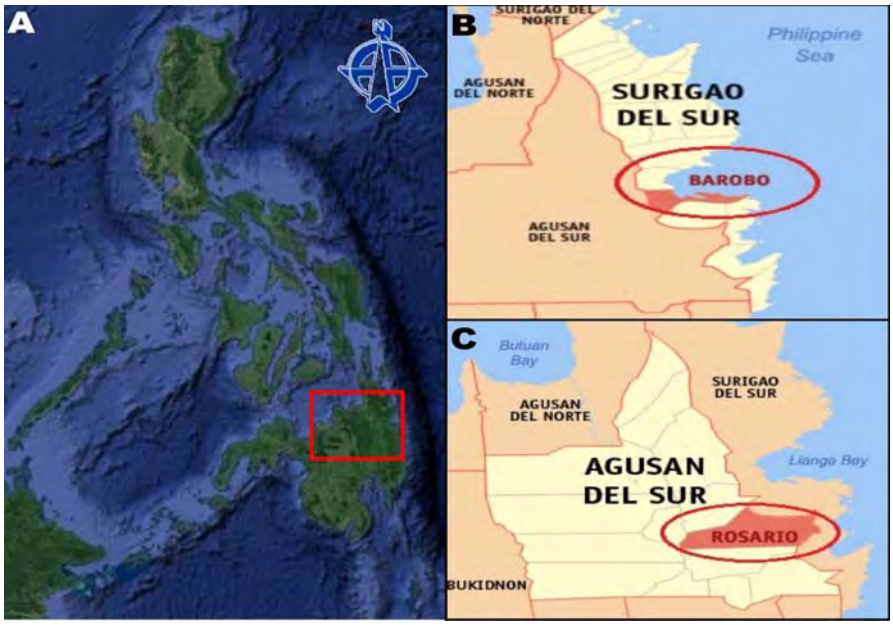

Fig. 1 Map of the study areas. **A**. Philippines **B**. Sorex, Barobo, Surigao del Sur, **C**. Rosario, Agusan del Sur, Philippines.

2.3 Plant material and treatment

The purple color varieties of onion (*Allium cepa* L.) in average sized were utilized in this study. The plant medium (*Allium cepa)* were sun-dried for two weeks and the dried roots present at the base of the onion bulbs were carefully shaved off with a sharp knife to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primordial cells from drying up. The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water. To account for a number of bulbs in the population that would be naturally slow or poor growing, three replicate bulbs were used for each test sample and control (tap water) and the best two bulbs were chosen at the approximate time for examination (Rank and Nielsen, 1993).The base of each of the bulbs was suspended on the water sample in the 25 ml test tubes in the dark for 12 days. At the end of the exposure period, the roots with the best growth were removed with a forceps and utilized for chromosomal preparation.

2.4 Maceration of the root tips and preparation for microscopy

After the exposition, the plant with the poorest root growth was excluded. Two onion bulbs were utilized from each water samples for chromosomal preparation. About 5 root tips per plants were cut using forceps at a length of 10 mm and placed into a petri dish with 2 ml acetic acid and Hydrochloric acid solution. The roots tips were then heated for 5 minutes at 500C. Hereby, the root cells become fixated and macerated. The heated root tips were placed on a petri-dish saturated with safranin solution for staining procedure and takes about 5-10 minutes. Thereafter, the saturated root tips were removed and placed on glass slides covered with a cover slip. The root tips were then squashed by pressing slightly down with a thumb and ready for microscopy.

2.5 Data analysis

Data was presented as mean ± standard error mean (SEM) and was calculated using Paleontological Statistics and Software (PAST).One-way Analysis of Variance (ANOVA) was also used for testing the significant difference of the concentrations between the treated and collected water samples.

3 Results and Discussion

The result of induced chemicals on the root growth of *Allium cepa* L. was presented in Table 1. The concentration of H_2O_2 (Hydrogen Peroxide) shows statistically significant ($P<0.05$) when compared to CH_2O (Formalin). Maximum root growth was observed in the control (1.367 ± 1.072) and there were no morphological deformities found. The roots were whitish in color, unbroken and straight. However, at tested concentrations, 1% was obtained the highest root growth from Hydrogen Peroxide (0.547 ± 0.012) and (0.167 ± 0.017) in Formalin. It also recorded the highest dividing cells and fewer in a number of aberrant cells. On the other hand, the 2% concentration attained the second highest root growth from Hydrogen Peroxide (0.413 ± 0.085) and Formalin (0.139 ± 0.001). It results to the second highest number of dividing cells and fewer numbers of aberrant cells. While the 3% tested concentrations show the poorest root growth from Hydrogen Peroxide (0.167 ± 0.033) and Formalin (0.1 ± 9.813) also displays the greatest number of aberrant cells with a lesser number of dividing cells. Thus, the root growth is manifestations of an arrest of a cell division. This indicates that root growth inhibition relatively due to the action of apical meristematic activity and cell elongation in the process of differentiation (Wierzbicka, 1988; Webster and Mcleod, 1996). Indeed, the suppression of mitotic activity constantly caused by genotoxicity and cytotoxicity (Bianchi, 2016). Consequently, mitotic index is also a result of mito depression during cell division (Mesi and Koplikua, 2013). The response of root growth identifies genotoxic substances that possibly the factor affecting chromosomal aberration. This explains that aquatic environment containing toxic substances could affect its condition as well as the organisms. Evidently, at great concentrations, this chemical will cause negative effects on the water system that may not suitable to uphold aquatic lives.

Table 1 *Allium cepa* L. root length and cytological effects of induced chemicals.

	Hydrogen Peroxide (H2O2)				**Formalin (CH2O)**			
Concentration (%)	**Mean Root Length ± S.E (cm)**	**No. of dividing cells**	**No. of aberrant cells**	**Mitotic Index (%)**	**Mean Root Length ± S.E (cm)**	**No. of dividing cells**	**No. of aberrant cells**	**Mitotic Index (%)**
0	1.367 ± 1.072	-	-	-	1.367 ± 1.072	-	-	-
1	0.547 ± 0.012*****	500	22	47.8	0.167 ± 0.017******	460	27	43.3
2	0.413 ± 0.085*****	450	26	42.4	0.139 ± 0.001******	375	30	34.5
3	0.167 ± 0.033*****	400	29	37.1	0.1 ± 9.813******	300	35	26.5

5000 cells (5 slides) per concentration of each water samples. *: ($P<0.05$) level of significance of root growth inhibition compared with the control and Formalin. **: non-significant.

The obtained results from the collected water samples show non-statistically significant (Table 2). It was observed that the 25% tested concentration found the highest root growth for Sorex (3.083 ± 1.583) and Rosario (3.261 ± 0.570) both with the first highest number of dividing cells and fewer number of aberrant cells. It was followed by 50% tested concentration for Sorex (2.896 ± 1.199) and Rosario (2.278 ± 0.222) were second highest root growth and recorded the second highest number of dividing cells with a fewer number of aberrant cells. Lastly, at 75% tested concentration has the least root growth (2.817 ± 0.570) from Sorex and (2.597 ± 1.119) in Rosario also recorded the fewer number of dividing cells with the highest number of aberrant cells. Hence, root growth primarily the identification in which there were toxic substances that could be found in the aquatic environment. In this relation, MI or mitotic index also represents the cytotoxicity and

genotoxicity levels in which there is a decreasing and increasing MI (Fiskesjo, 1985; Fernandez et al., 2007). Moreover, the alterations during cell cycle may be attributed to the genotoxic potential of a substance (Nefic et al., 2013).

Table 2 *Allium cepa* L. root length and cytological effects from collected water samples.

	Sorex Water Samples				Rosario Water Samples			
Concentration (%)	**Mean Root Length ± S.E (cm)**	**No. of dividing cells**	**No. of aberrant cells**	**Mitotic Index (%)**	**Mean Root Length ± S.E (cm)**	**No. of dividing cells**	**No. of aberrant cells**	**Mitotic Index (%)**
25	3.083 ± 1.583**	660	10	66	3.261 ± 0.570**	550	11	53.9
50	2.896 ± 1.199**	640	13	62.7	2.278 ± 0.222**	500	13	48.7
75	2.817 ± 0.570	600	16	58.4	2.597 ± 1.119**	475	15	46

*5000 cells (5 slides) per concentration of each water samples. **: ns (non-significant).

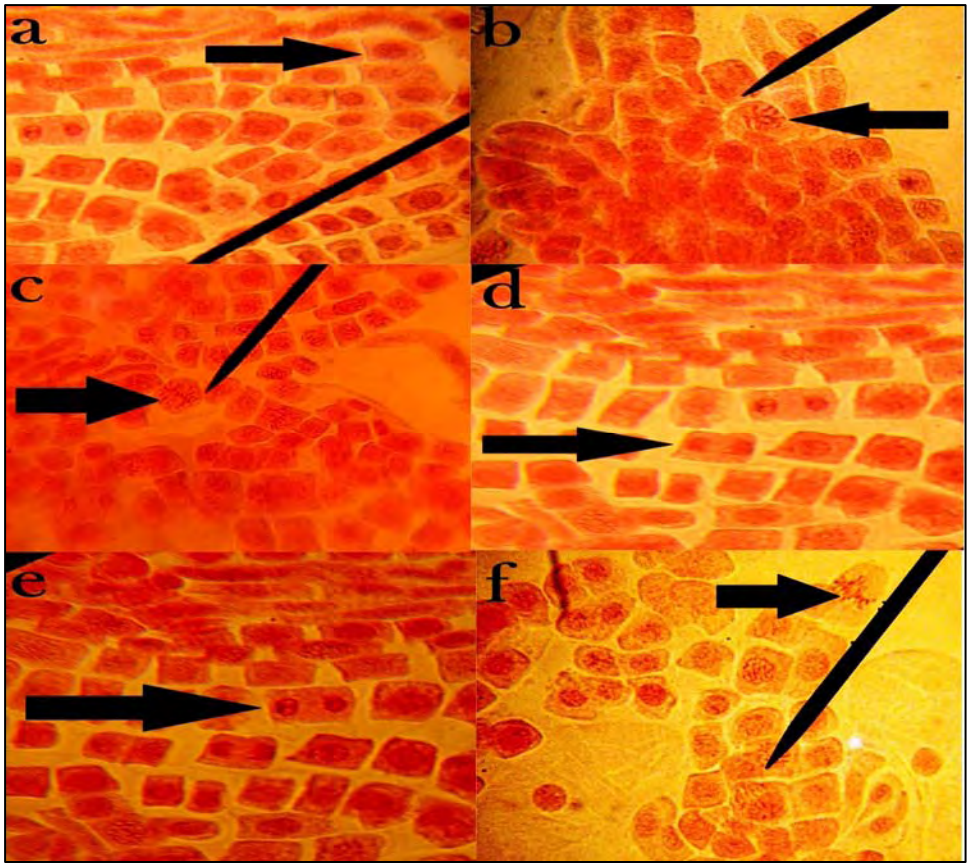

Fig. 2 Stages of mitotic division in root cells of *Allium cepa* L. (**a**) Interphase (**b**) Prophase (**c**) Metaphase (**d**) Anaphase (**e**) Telophase (**f**) spindle disturbance at prophase. Magnification 1000x.

The collected water samples from the mining areas shows a great number of aberrant cells and demonstrates fewer root growth. Morphologically the roots were smaller, bent in structure and crochet like in form. It might be due to the information that these areas were constantly experiencing pollutants coming from

the wastes of the mining activities. Microscopic analysis was shown in Fig. 2 were stages of mitotic division and chromosomal aberrations occur. From the study of Olorunfemi and Ehwre (2011), chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. As well as, it is considered as a result of genotoxic effects of various physical and chemical agents and is also estimates of exposure of various organisms to different physical and chemical agents (Pohren et al., 2013). On the same note, chromatin dysfunction is the immediate response that results to depolymerization of chromosomal DNA which is attributed from the environmental pollutants (Goujon et al., 2015).The chromosomal abnormalities such as stickiness, bridges, and laggards are some of the indications of genotoxicity (Dutta and Ahmad, 2016). Moreover, a concentration-dependent increased were observed among the dividing cells and aberrant cells of the treated and collected water samples. The observed abnormalities were seen into its roots morphology indicating genotoxicity. Furthermore, the advances of applying *Allium cepa* test contribute and enhance knowledge on detecting harmful substances in the water.

4 Conclusions

This study shows the importance of *Allium cepa* L. in testing genotoxic substances. The indication of chromosomal and mitotic aberration in the root tip cells suggests genotoxicity. Hence, mitotic index is a marker of increasing and decreasing of cells during mitosis. One-Way ANOVA shows that water samples treated with H2O2 were statistically significant ($P<0.05$) when compared to CH2O while the water sample collected from the mining areas shows statistically non-significant. The result indicates that H2O2 (Hydrogen Peroxide) and CH2O (Formalin) was a potential transporter of chromosomal aberration. The observed abnormalities were shown on the roots morphology. Likewise the response of *Allium cepa* genetic material helps to identify the effect of possible genotoxic substances that may pose genetic abnormality. Furthermore, this approach advances in monitoring an environmental condition, especially aquatic environs.

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References

Abusalama EA, Elhassan AM, Errami M, Salghi R. 2014. Moroccan Journal of Chemistry, 2(3): 125-135

Ashraf H, Husain Q. 2010. Studies on bitter gourd peroxidase catalyzed removal of p-bromophenol from wastewater. Desalination, 262(1-3): 267-272

Bagatini MD, Vasconcelos, TG, Laughinghouse, HD IV, et al. 2009. Biomonitoring hospital effluents by *Allium cepa* L. test. Bulletin of Environmental Toxicology and Contamination, 82: 590-592

Bakadir K, Kassale A, Barouni K, Lakhmiri R, Albourine AJ. 2016. Retention of a compound of herbicides, 2,4-dichlorophenoxy acetic acid, to a soil in the absence and in the presence of Cu(II) and Zn(II) cations. Journal of Materials and Environmental Science, 7(3): 1056-1063

Barberico A, Barros L, Voltolini JC, Mello MLS. 2009. Evaluation of the cytotoxic and genotoxic potential of water from the River Paraíba do Sul, in Brazil, with the *Allium cepa* L. test. Brazilian Journal of Biology, 69(3): 837-842

Bianchi J, Fernandes CCT, Marin-Morales MA. 2016. Induction of mitotic and chromosomal abnormalities on *Allium cepa* cells by pesticides imidacloprid and sulfentrazone and the mixture of them. Chemosphere.

144: 475-483

Dutta J, Ahmad A. 2016. Effect of azo dye corafix on root tip chromosomes of *Allium cepa* L. Journal of Chemical and Pharmaceutical Research, 8(5): 857-862

El-Shahaby AO, Abdel Migid HM, Soliman MI, Mashaly IA. 2003. Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. Pakistan Journal of Biological Sciences, 6(1): 23-28

Fernandes TCC, Mazzeo DEC. Marin-Morales MA. 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pesticide Biochemical and Physiology. 88: 252-259

Feretti D, Zerbini I, Zani C, Ceretti E, et al. 2007. *Allium cepa* chromosome aberration and micronucleus test applied to study genotoxicity of extracts from pesticide-treated vegetables and grades. Food Additives and Contaminants, 24: 561-572

Fiskesjo G. 1985. The *Allium* test as a standard in environmental monitoring. Hereditas, 102: 99-112

Fiskesjo G. 1988. The Allium test- an alternative in environmental studies: The relative toxicity of metal ions. Mutation Research, 197: 243-260

Fiskesjo G. 1993. The Allium test in wastewater Monitoring. Environmental Toxicology and Water Quality, 8(3): 291-298

Firbas P, Amon T. 2013. Allium chromosome aberration test for evaluation effect of cleaning municipal water with Constructed Wetland (CW) in Sveti Tomaž, Slovenia. Journal of Bioremediation and Biodegradation. 4(4): 189

Goujon E, Richard C, Goupil P, Ledoigt G. 2015. Cytotoxicity on Allium cepa of the two main sulcotrione photoproducts, xanthene-1,9-dione-3,4-dihydro-6-methylsulphonyl and 2-chloro-4-mesylbenzoic acid. Pesticide Biochemical and Physiology. 124: 37-42

Leme DM, Marin-Morales MA. 2009. *Allium cepa* test in environmental monitoring: A review on its Application. Mutation Research, 682: 71–81

Matsumoto ST, Mantovani MS, Malaguttii MIA, et al. 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. Genetic and Molecular Biology, 29: 148-158

Mesi A, Koplikua D. 2013. Food Environmental Proceedings Technology, 8: 19-26

Mittergger H, Da Silva J, Arenzon A, et al. 2007. Evaluation of genotoxicity and toxicity of water and sediment samples from a Brazilian stream influenced by tannery industries. Chemosphere, 67: 1211-1217

Mustafa Y, Arikan ES. 2008. Genotoxicity testing of quizalofop-P-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. Caryologia, 61(1): 45-52

Nefic H, Musanovic J, Metovic A, Kurteshi K. 2013. Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by Alprazolam. Medical Archives, 67(6): 388-392

Nunes E, De Lemos C, Gavronski L, et al. 2011. Genotoxic assessment on river wáter using different biological systems. Chemosphere, 84(1): 47-53

Olorunfemi DI, Ehwre EO. 2011. Chromosomal aberrations induced in root tips of *Allium cepa* by Squeezed *Garri* extracts. Report and Opinion, 3(1): 155-160

Pohren R, Thatiana C, Vargas VMF. 2013. Investigation of sensitivity of the *Allium cepa* test as and alert system to evaluate the genotoxic potential of soil contaminated by heavy metals. Water Air Soil Pollution, 224: 1460-1470

Rank J, Nielsen MH. 1993. A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. Hereditas, 118: 49-53

Rank J. 2003. The method of *Allium* anaphase-telophase chromosome aberration assay. Ekologija, 1: 38-42

Riffat A, Ahmad M. 2006. *Allium cepa* derived EROD as a potential biomarker for the presence of certain pesticides in water. Chemosphere, 62: 527-537

Rodriguez-Ruiz A, Asensio V, Zaldibar B, Soto M, Marigómez I. 2014. Toxicity assessment through multiple multiple endpoint bioassays in soils posing environmental risk according to regulatory screening values. Environment and Soil Pollution, 21(16): 9689-708

Siddiqui AH, Tabrez S, Ahmad M. 2011. Validation of plant based bioassays for the toxicity testing of Indian waters. Environmental Monitoring and Assessment, 179(1-4): 241-253

Tedesco SB, Laughinghouse HD IV 2012. Boindicator of Genotixicity. The *Allium cepa* Test. Journal of Environmental Contamination, 138-156

Turkoglu S. 2007. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutation Research, 626: 4-14

Webster PL, MacLeod RD. 1996. The root apical meristem and its magrin. In: Plant Roots- the Hidden Half (Waishel Y, Eshel A., Kafkafi U, eds) (2nd edition). 51-76, Marcel Dekker, New York, USA

Wierzbicka M. 1988. Mitotic disturbances induced by low doses of inorganic lead. Caryologia, 41: 143-163