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It is our great pleasure to present this Supplement Issue on “*Macedonian Pharmaceutical Bulletin*” to the scientific and professional community. This supplement includes the short communications from the *Sixth Congress of Pharmacy in Macedonia with International participation*, as the largest gathering for the pharmacy profession held in the Republic of Macedonia. The main theme of the Congress was “Modern pharmacist - bridging science with practice”.

A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. The contributions submitted to the Congress included 6 plenary lectures, 84 section lectures, and more than 240 posters. This Congress, followed the excellent international tradition, was attended by close to 1000 domestic and foreign participants. We received 326 short paper submissions from more than 25 countries. These numbers show that our Congress is aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

We would like to thank all internationally prominent researchers for their contribution to reinforcing the overall quality of the Congress. They give the state of the art of the recent advances in the field of pharmacy research.

Sincere thanks to the hosts of the Sixth Congress of Pharmacy in Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

We acknowledge the sponsoring companies: the platinum sponsor AD ALKALOID, Skopje, the golden sponsor PLIVA, the silver sponsor EUROFARM and the bronze sponsor SEPTIMA, for the permanent support to our efforts during the organization.

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process and in the organization of the program. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank the authors, whose work was the essential part of the congress and contributed to a very successful event. Besides the many academic staff and professionals who contributed to the success of the Congress, we are grateful to the students who participated with oral presentations and posters.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

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The present issue of *Macedonian Pharmaceutical Bulletin* is a special issue of the 6<sup>th</sup> Congress of Pharmacy in Macedonia with international participation.

This issue of *Macedonian Pharmaceutical Bulletin* contains short papers accepted by the scientific committee for the presentation at the Congress.

The authors are fully responsible for the contents of their short papers.

All reviewers that were involved in the short papers revision process are sincerely acknowledged.



## Approach to detect possible genotoxic effects of metals in plants

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### Introduction

During the intensive research in past decades, the scientific community and regulatory agencies also, focused their interest on measuring contaminant levels in tissues and environmental samples and on understanding the mechanisms of toxicity of different and pervasive contaminants. Among them, metals were always in focus because of possible detrimental and long lasting effects on living organisms (Gjorgieva et al., 2011; Stafilov et al., 2010). DNA damage and genotoxic stress are important parameters that are followed in a case of chemicals exposition as an indication of carcinogenicity (Aydin et al., 2015; Kecec et al., 2010; Villatoro-Pulido et al., 2013). Plants are unique systems in their ability to serve as in situ monitors for environmental genotoxins. Molecular assays related to DNA-based techniques, like Random Amplified Polymorphic DNA (RAPD), are very sensitive method of screening for nucleotide sequence polymorphisms that are randomly distributed throughout the investigated genome (Gjorgieva et al., 2012).

The current study was designed to assess the effects of long term, high metal exposition (cadmium, lead, copper, nickel and zinc) on DNA damage.

### Materials and methods

Four different plants [*Taraxacum officinale* (Asteraceae), *Matricaria recutita* L. (Asteraceae), *Robinia pseudoacacia* L. (Fabaceae), and *Urtica dioica* (Urticaceae)]

were chosen as model systems for genotoxicity assessment in a case of metal stressors. Plant samples were collected from two different areas, metal polluted (area around city of Veles, known for its lead and zinc industrial activity in the nearest past) and referent area (without metal exposition, Plačkovica Mountain). Element analysis (analyzed by Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES)), DNA extraction (frozen plant samples were used for DNA isolation by using REExtract-N-Amp Plant PCR Kit (Sigma-Aldrich)) and RAPD-PCR analysis were performed with samples included in this study. Amplifications were performed in a DNA thermo cycler according to protocol of Enan, 2006. Seven different primers (with 60-70% GC content) were used. Sequences (5'→3') from Primer 1 to 7 were: Primer 1 - GGTGCGGGAA; Primer 2 - GTTTCGCTCC; Primer 3 - GTAGACCCGT; Primer 4 - AAGAGCCCGT; Primer 5 - AACGCGCAAC; Primer 6 - CCCGTCAGCA; Primer 7 - GGCCTGAGG. All amplifications were repeated twice in order to confirm the reproducible amplification of scored fragments. Only reproducible and clear bands were scored for the construction of the data matrix.

### Results and discussion

RAPD technique was used to quantify DNA sequence changes. Agarose-gel electrophoresis of extracted DNA from all samples, revealed total of 37 bands with different molecular weights ranging from 1250 to 5000 bp. It generated distinctive polymorphism value of 72.97% (27 bands) total in four plant species investigated. Polymorphism in the RAPD profiles is scored as the presence or absence

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of DNA bands in comparison to the control. The numerical analysis based on the banding pattern obtained from the samples exposed to metals was compared with that of the control sample via hierarchical cluster analysis. RAPD profiles generated by samples exposed to metals were different from those obtained using control DNA. Samples which were close to the pollution source (high metal-exposition area) showed considerable polymorphism. The diagnostic analysis (considering band intensity differences or the disappearance and/or appearance of RAPD bands) and the phenetic numerical analysis are important parameters in the RAPD method (Bhaduri and Fulekar, 2015; Gjorgieva et al., 2012; Zhiyi and Haowen, 2004). Amplification with primer 1 and primer 2 yielded not enough significant polymorphism, so we find that this primer sequences are not suitable for fingerprinting genome of this four plant species. When amplification is performed with primer 3 and 5, the highest number of new bands is obtained (9 and 8 bands, respectively), with exception of primer 5 for *T. officinale*, where probably there is no suitable sequence in the genome of this plant for annealing of above mentioned primer. The dendrogram constructed using NT-SYSpc programme (Rohlf, 1994) is statistical presentation of the obtained data and showed that there is grouping in separate clusters of the same plant species collected from two different areas, according to modifications in the RAPD "fingerprints" following metal exposure. In accordance with literature data that considered concentration of 40 mg L<sup>-1</sup> of lead and 30 mg L<sup>-1</sup> of cadmium (Aydin et al., 2013; Aydin et al., 2015) as the point of maximum appearance and disappearance of new bands in RAPD assay, we assume that high metal content in all samples from Veles area, determined in present study, was directly involved in DNA damage detected which can lead to initiation of mutations.

## Conclusion

RAPD assay is a valuable tool to evaluate the effects of toxicants on organisms under optimized conditions and for genotoxicity studies. RAPD markers can be used as diagnostic markers. In this regard, induction of DNA band changes with one kind of a stressor (found in areas with metal pollution) might provide evidence of its genotoxic potential.

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