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USE OF RAPD "FINGERPRINTING" TECHNIQUE TO DETECT GENOTOXIC EFFECTS OF HEAVY METALS IN PLANTS

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Metals constitute one of the major groups of genotoxic environmental pollutants possessing serious threat to human as well as environmental wellbeing. Heavy metals induce several cellular stress responses and damage different cellular components, proteins, membranes and DNA. "DNA alterations" include DNA damage (e.g. DNA adducts, breaks), mutations (e.g. point mutations and large rearrangements) and other possible changes (e.g. structural distortion) induced by chemical or physical agents. Random Amplified Polymorphic DNA (RAPD) 'fingerprinting' technique is based on the amplification of genomic DNA with 10 bp primers of arbitrary nucleotide sequence which anneals to multiple regions of the genomic DNA. The PCR reactions thus generate many amplicons of variable lengths (e.g. between 100 and 4000 bp) which can be separated by gel electrophoresis to obtain DNA fingerprint. RAPD assays were successfully used to detect genetic instability in DNA alterations in animals, bacteria and plants induced by low doses of pollutants, heavy metals among them also.

The objectives of this study were to detect DNA damage in plants induced by different metals using the RAPD technique. Plant samples of selected medicinal plants from the industrialized area were taken from different places between 10-100 m around the lead smelting plant "MHK Zletovo" in Veles, while for uncontaminated controls, plant samples were taken from Plačkovica Mountain, about 60 km from the city of Veles. Different plant organs (leaves, flowers, radix and stems) were analyzed by ICP-AES (Varian715-ES) for selected metals. Detection of genotoxic effect using RAPD involves the comparison of profiles generated from control (unexposed) and treated (exposed) DNA. Events observed following the metal exposure were a variation in the disappearance and appearance of new bands. These unique bands clearly differentiated the samples exposed to heavy metal stress, and could act as a marker for assessment of environmental exposition of these metals.

Key words: heavy metals, RAPD, DNA damage, plants