

## ENDANGERED BIODIVERSITY IN THE REGIONS OF “STOMANA” AND “KREMIKOV TZI” HEAVY METAL PLANTS IN BULGARIA

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### ABSTRACT

The decreased biodiversity disturbs ecological balance and leads to long-term changes in the ecosystems. In the efforts to solve the problem we need to know cause and effect in order to study the mechanisms of species extinction.

Reduction of biodiversity leads to decrease in genetic diversity, which is of critical importance for the survival of populations since it forms the potential for adaptation. Any kind of stress selects for the most tolerant genotypes and leads to reduced genetic variability. Low genetic diversity lowers the chance for adaptation to future changes in the environment and acts as a factor for the extinction of species.

The aim of the current research is to study the genetic diversity alterations in polluted with heavy metals regions. Subject of the study are populations of the biomonitoring plant *Taraxacum officinale* (common dandelion). Samples were collected from two contaminated and two control sites. Polluted areas are located around two metal plants: “Stomana”, which is still working, and “Kremikovtzi” which was closed five years ago. For studying the genetic diversity two regions from chloroplast DNA of *T. officinale* were amplified and restricted. Restriction fragment length polymorphism (RFLP) analysis showed decreased genetic diversity in the populations from heavy metal polluted regions compared to the plants from the control region. No recovery in the genetic variability was detected in the population of “Kremikovtzi”, despite five years since its closure.

Our results are consistent with the hypothesis of lowering genetic diversity in populations exposed to heavy metal stress and pose the problem about the time required for their recovering.

**Key words:** heavy metal pollution, adaptation, *Taraxacum officinale*, genetic variation, cpDNA markers

### Introduction

Heavy metal pollution in Bulgaria is a consequence of fifty years extensive developing of heavy industry. Plants growing on polluted soils face strong selective pressure. As a result only highly adapted individuals survive and give descendants which decreases genetic diversity within populations. Genetic variation is the “playground” for processes like natural selection and adaptation. Reduced diversity lowers the chance for adaptation to future environmental changes and acts as a factor for species extinction.

The wide geographical distribution and the broad ecological amplitude make *Taraxacum officinale* a common biomonitoring plant for studying the effect of heavy metal pollution [2, 3, 4, 7]. Moreover dandelions reproduce asexually via apomixis which excludes genetic variation as a result of meiotic recombination. This makes this species a suitable model for the examination of population genetic diversity [1, 9].

Sampling sites include two control clean areas and two polluted with heavy metals regions around “Stomana” and “Kremikovtzi” metal works. While “Stomana” metal works is still working, “Kremikovtzi” was closed five years ago. This provides a good opportunity to compare the two threatened with heavy metal populations of dandelion populations.

Two questions were to be answered: First, is there a difference in the genetic structure between plant populations from clean and polluted with heavy metals regions. Second, is there a difference in the plant genetic variability between the populations from the still working “Stomana” factory works and the population from the closed “Kremikovtzi” metal factory.

To examine the genetic diversity two regions from chloroplast DNA of *T. officinale* were chosen for amplification and further restriction. RFLP analysis possesses some advantages compared to other genetic methods used in the genetic diversity studies. On one hand it is more sensitive in the detection of genetic differences than the broadly used allozyme variation. On the other hand it is technically easier to perform than the analysis of highly polymorphic VNTR (variable-number-tandem-repeat) loci.

**Materials and methods**

*Taraxacum officinale* plants were collected during the years 2010-2012. Sampling sites include two polluted areas around “Stomana” and “Kremikovtzi” metal works and two control rural areas – next to the villages Bosnek and Lokorsko (sites locations and soil heavy metal content described in [6]). Genomic DNA was obtained from leaf samples (as described in [5]) and was used to perform polymerase chain reaction (PCR). Two regions from the chloroplast DNA (cpDNA) were amplified. The first pair of primers was *psbA-trnH* for the amplification of *psbA-trnH* intergenic spacer of cpDNA and the second one - *aB48557-fA50272* for obtaining the region between *trnT* and *trnF* genes of cpDNA [8].

The PCR products were restricted as follows: the *psbA-trnH* intergenic spacer was digested with *DraI*, and the *trnT-trnH* region – with *EcoRI* enzyme. Obtained restriction fragments were separated on 1,2 or 1,5 % agarose gel electrophoresis and analyzed with Gel-Pro Analyzer™3.1. Every unique restriction profile was considered a genotype. The percentage of every single genotype in a population was used to determine the dominant genotype and to evaluate the genetic diversity within the studied populations.

**Results and discussion**

RFLP analysis of cpDNA region *psbA-trnH* of *T. officinale* revealed four different genotypes, presented on fig. 1A. The distribution of genotypes among the populations from the clean and polluted environments is shown as percentages on fig.1B and C. It is hard to determine a dominant genotype within the populations from the clean regions. On the contrary, it is obvious that there is a dominant genotype in the populations from the heavy metal polluted areas. More than a half of them possess genotype “0” – a fact that speaks for increased genetic similarity.

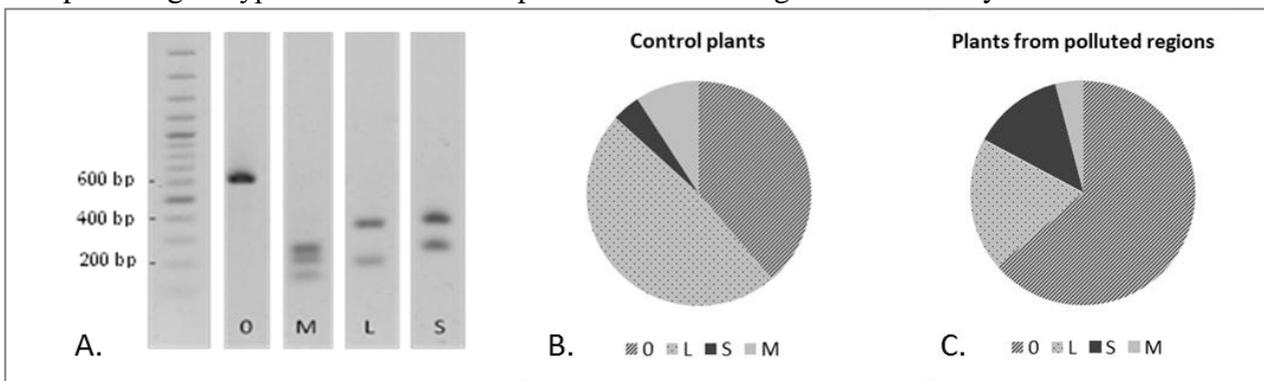


Fig.1. A. Four genotypes (named 0-M) obtained after restriction of *psbA-trnH* intergenic spacer of cpDNA. First lane shows 100 bp molecular marker. B, C. Distribution of genotypes in the populations from clean and polluted regions.

Similar pattern of genetic diversity was shown by the second molecular marker - *trnT-trnF*. Six restriction profiles were obtained and their distribution among populations was evaluated (fig. 2). Extremely low genetic diversity was estimated among plants collected from contaminated sites with more than 80% of them possessing the same dominant genotype "a" (fig. 2C).

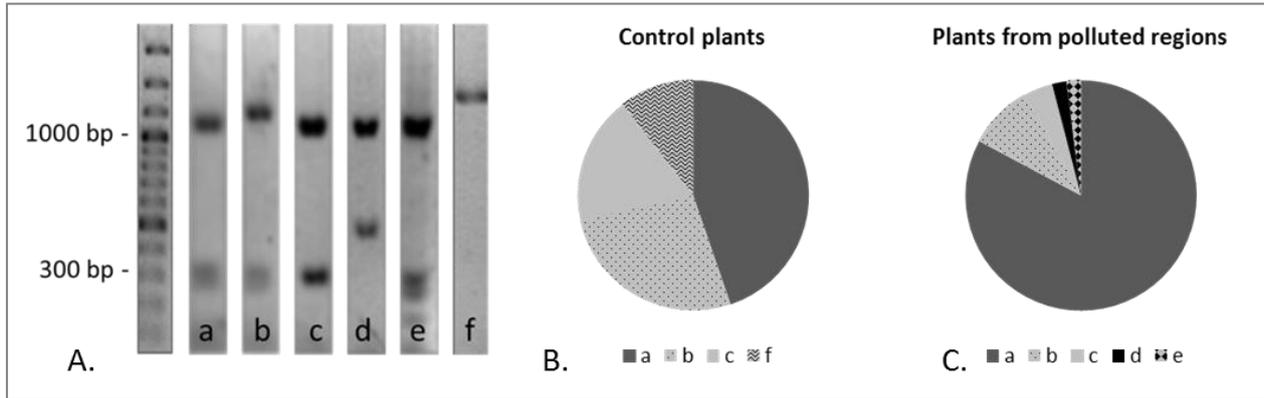


Fig.2. A. Six genotypes (named a-f) obtained after restriction of *trnT-trnF* region of cpDNA. First lane shows 100 bp molecular marker. B, C. Distribution of genotypes in the populations from clean and polluted regions.

Having in mind that “Kremikovtzi” metal works closed down five years ago it was of interest to check if the equilibrium of presented genotypes has started to recover in comparison with the population from the still working “Stomana”. Surprisingly, no such trend was found (fig. 3). Not only there wasn’t higher diversity, but the similarity in “Kremikovtzi” turned out to be higher than” this in “Stomana”. At one of the markers used (*psbA-trnH*) the genetic identical individuals from “Kremikovtzi” were twice as much as these from “Stomana” (fig.3A). At the other used marker - *trnT-trnF*, the genetic similarity in the population from “Kremikovtzi” reached almost 90% (fig. 3B).

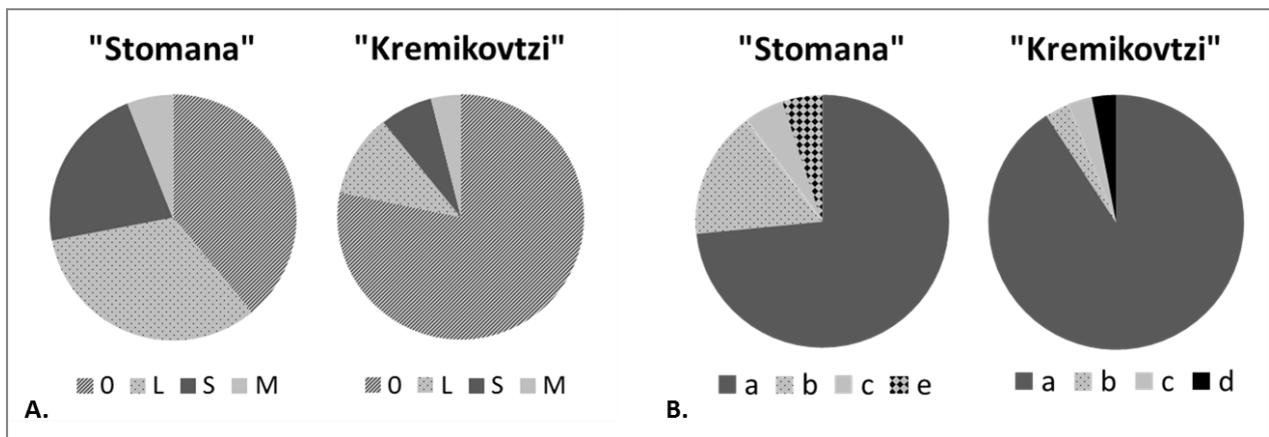


Fig.3. Distribution of genotypes in the populations from “Stomana”and “Kremikovtzi”.  
A. *psbA-trnH* genetic marker. B. *trnT-trnF* genetic marker.

Our research demonstrated lower genetic diversity in *T. officinale* populations from contaminated with heavy metals regions compared with rural ones. The results are consistent with the hypothesis for the pollution-induced selection pressure. According to it only few genotypes survive which is a thread for the adaptive capacity of the population. There is a lack of recovery of genetic variability in the population from the closed “Kremikovtzi” works. This poses the problem about the long-term consequences of pollution-induced stress over ecosystems and requires measures to be taken against current and future contamination in industrial areas.

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