STUDY OF THE EFFECT OF HEAVY METAL POLLUTION ON THE GENOME OF PLANT SPECIES *TARAXACUM OFFICINALE* WEB. (ASTERACEAE) - A PRELIMINARY REPORT

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ABSTRACT

Pollution of the environment with heavy metals leads to damages in the genome of the living organisms. Both, the element entity and the genome profile of an organism are called ionome. Assessment and detailed understanding of the changes which heavy metal pollution causes in the ionome of the common dandelion *Taraxacum officinale* are subject of this research. The ionome of plants collected from the regions around two metallurgic plants in Bulgaria was studied. The results showed serious changes in the ionome of this indicator organism. Interestingly, the gross changes in the elemental composition of this plant species do not lead to the expected multiple DNA damages. This, however, does not rule out the possibility heavy metal pollution to lead to major genome rearrangements which will be assessed in future research.

***Key words**: environmental pollution, ionome, DNA, Taraxacum officinale Web.*

I. INTRODUCTION:

Metals in low concentrations as microelements are vitally important for living organisms, however, they are highly toxic as environmental contaminants. The accumulation of heavy metals in the environment is one of the main causes of pollution in the industrialized countries. The devastating effect of high concentrations of metal elements on the living organisms is well documented. The primary sources of pollutants are the burning of fossil fuels, the mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and sewage (Kabata-Pendias and Pendias, 1989). Above certain levels metal elements indigested by animals and humans or accumulated by plants could be highly toxic, but even more deleterious are their genotoxic and teratogenic effect. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. In addition the negative effect of copper, manganese, zinc, has also been documented [Stojanov, 1999]. Therefore investigating the regions with high levels of metal pollution is vitally important for the human health.

Often, the monitoring of the environment is performed by studying of certain plant species grown in areas suspected for pollution. Such plant species can accumulate polluting substances. From soil and water, all plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo and Ni (Langille and MacLean, 1976). Certain plants also have the ability to accumulate heavy metals which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg (Baker and Brooks, 1989). However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved in a number of different plant species (Ernst *et al.*, 1992). The combination of the element composition and the genome entity of a living organism is called ionome. The ionome can change depending on the environment in which the organism lives.

Accumulation of heavy metals in the plants leads to toxicity or at least to severe changes in the metabolic status of the plant. Due to chemical properties of these elements serious damages in the cellular DNA have to be expected. One of the most popular methods for assessing of DNA damages is the Single Cell Gel Electrophoresis (SCGE) or also called - Comet assay. Growing in popularity this method is increasingly used in genotoxic ecology for monitoring the environment.

In this research we used common dandelion *Taraxacum officinale* as an indicator species to produce damages in its ionome as a result of pollution with heavy metals.

II. MATERIALS AND METHODS:

Sites of the collection of probes

The probes were collected from two clean regions officially declared as clean - south skirts of Vitosha mountain and south skirts of Stara planina mountain and two polluted regions – waste depot of ore flotation of the metallurgic plants “Kremikovci’- Sofia and “Stomana”- Pernik.

Samples of 40 plants *Taraxacum officinale* Web. (Asteraceae) together with samples of the soil in which they grow were collected for the purpose of this research.

Chemical analysis of soil and plant probes

The analysis of the soil and plant probes was performed according to methodology published elswhere [Djingova et al., 2003].

Comet assay

The method of Comet assay was performed with the modifications for plant tissues developed in our laboratory [Peycheva *et al*., 2004; Peycheva *et al*. in press].

Briefly, nuclei from plant leaves were isolated after lysis of plant cells and centrifugation in sucrose gradients. Nuclei in appropriate concentration (usually 1×10^5 nuclei/ml) were mixed with PBS (2.68 mM KCl, 1.47 mM KH_2PO_4, 137 mM NaCl, 8.06 mM Na_2HPO_4; pH 7) and with equal volume of 1.4% low gelling agarose (Sigma) and were spread as micro-gels on microscopic slides. After solidifying of the agarose, the slides were submerged in denaturing solution (30 mM NaOH, 10 mM EDTA, pH 12.6) and were incubated for 30 min at 4ºC. After that the slides were placed in an electrophoresis tank and electrophoresis was run in the denaturing solution at 0.45 V/cm for 10 min. Neutralization of the gels in 0.5 M Tris-HCl, pH 7 for 5 min and 5 min in distilled water followed the electrophoresis. The obtained comets were observed under an epi-fluorescent microscope.

FACS (Fluorescent Activated Cell Sorter) analysis

Young intact leaf tissues from plants were chopped in isolation buffer (15 mM Tris, 2 mM EDTA, 0.5 mM spermin tetrahydrochloride, 80 mM KCl, 20 mM NaCl, 0.1 % triton, 0.3 mM β-mercaptoethanol). For an internal standard leaves from *Pisum sativum* “Kleine Rheinländerin, 2C=8.84 pg were co-chopped together with the leaf tissue for each probe [Greilhuber and Ebert, 1994]. The probes were incubated with 50 µg/ml RNase and 50 µg/ml propidium iodide for one hour.

The analysis was performed on a FACS machine (BD FACS Calibur™ Flow Cytometer) equipped with argon laser operating at λ = 488 nm. We used CellQuest and WinMDI 2.8 software to analyze the results.

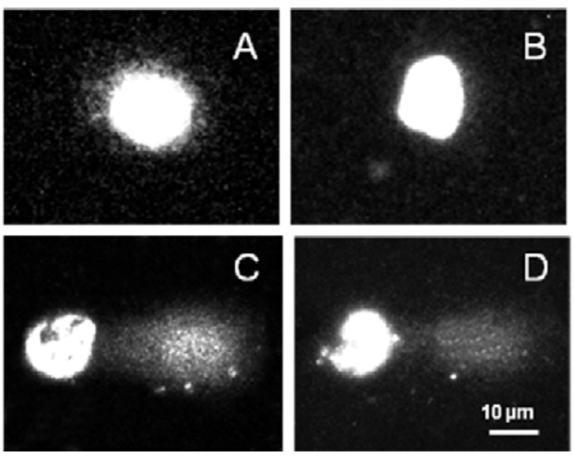

Figure 1. Comet assay of *Taraxacum officinale.* A and B – examples of nuclei with undamaged DNA. C and D – examples of comets from nuclei in which DNA is damaged.

III. RESULTS AND DISCUSSION:

The constituency of the environment is

important for the assessment of its influence on the organism. As a first step in our research we performed chemical analysis of the soil probes taken from two polluted and two clean regions in Bulgaria (see Materials and methods). The results showed higher concentration of the metal elements in some of the soil probes taken from polluted regions than that taken from clean places. The concentrations of Ni, Cu, Mn, Cd, Pb, Zn, As and Fe.

Surprisingly, the concentrations of the same heavy metals in the plant samples of *Taraxacum officinale* collected from the same places showed lower values of the heavy metals than that found in the respective soil. Therefore, the suggestion by some authors [Husain and Khan, 2010] that the plant species *Taraxacum officinale* is from the group of so-called accumulator plants is questionable.

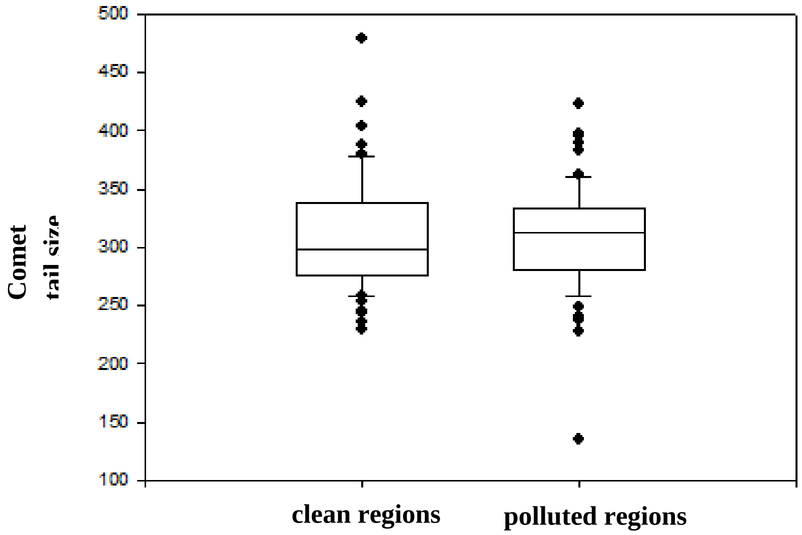

Figure 2. Comet assay analysis of *Taraxacum* plants collected from clean and polluted regions.

It was of interest to proceed the genomic answer of the plant to the found high levels of heavy metals in soil. The method of Comet assay is capable to detect not only cuttings in DNA but also chemical modifications in its molecule. Some pictures of comets from *Taraxacum* plants obtained by this method are shown in Figure 1.

It can be seen in the figure that the comets obtained from this plant are normal in appearance. The quantification of the Comet assay by measuring more than 100 objects per sample revealed unexpected results. There was no difference in the size of the comets tails of the comets obtained from plants living in clean and polluted areas (Figure 2). This result signifies that there is no more damaged DNA in plants growing in the polluted areas in comparison with the plants, which grew in clean areas. This unexpected result could be explained by a higher activity of DNA repair systems in the plants from polluted regions. Broader and more detailed study is needed in order to clarify this question.

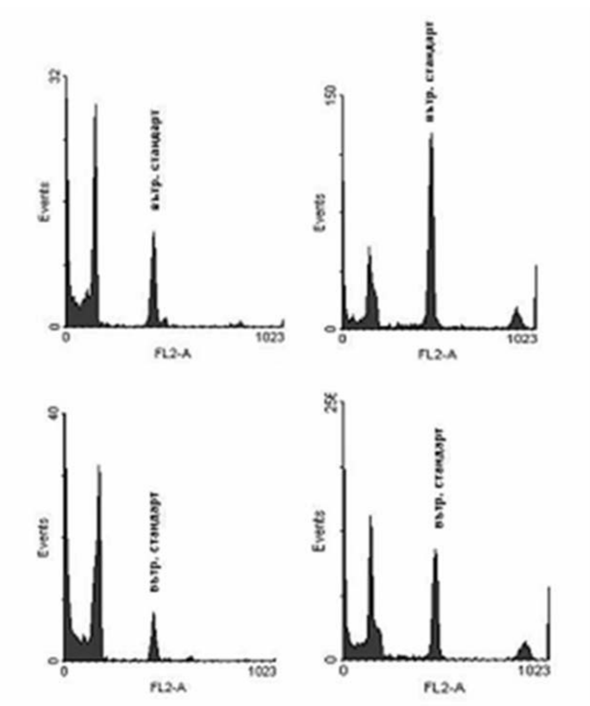

Figure 3. FACS analysis. Examples of FACS analysis of 4 probes (two clean and two polluted) *Taraxacum.*

Another method, which can reveal gross genome rearrangements by measuring the genome size, is the FACS analysis [Palomino et al., 2008]. By performing FACS analysis on plant samples taken from both, clean and polluted regions we were not able to detect significant differences between the two types of probes [Figure 3]. Obviously, years of growing on the polluted with heavy metal soil does not lead to alteration of the plant genome size in a large scale.

CONCLUSIONS:

Chemical analysis of the collected soil probes taken from regions around metallurgic plants does not confirm the expected high concentrations of most of the metal elements. We have detected high concentrations for As, Fe and only for one probe of Pb. Altogether these results are not unexpected since both metallurgic plants use iron-ore in their production and therefore high Fe concentrations in soils around them have to be expected. Unexpected was the results of not finding of DNA damages in the plants growing on these soils. However, big genomic rearrangements can be expected and such will be searched using other genetic methods.

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