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Genetic Changes In Common Dandelion (*Taraxacum officinale*) Induced by High Concentrations of Heavy Metals in Soil

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ABSTRACT

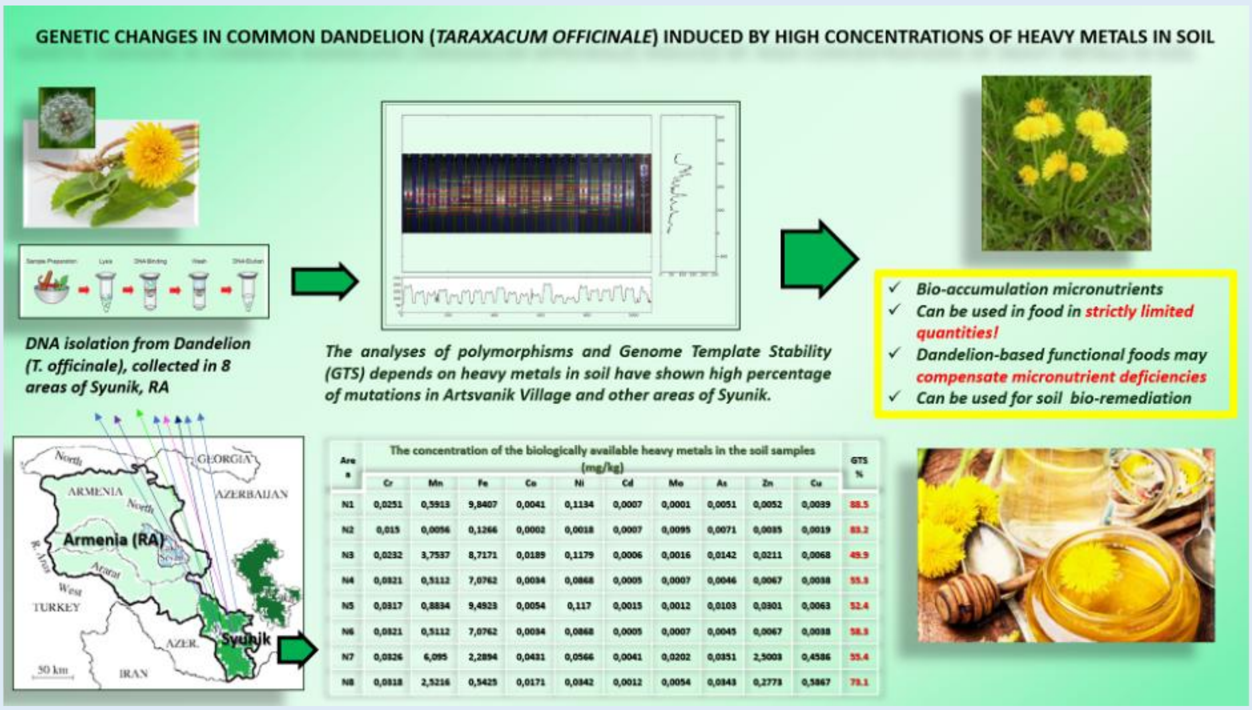
Background: Heavy metal contamination poses a significant environmental threat in developing regions such as the Syunik Province of Armenia, where agricultural lands coexist with metal mining operations. Heavy metals are genotoxic, therefore, the study of genetic changes in wild and cultivated edible plants here is essential for evaluating the safety of traditional plant-based foods (“zhengyal,” honey, herbal teas, etc.). *Taraxacum officinale* (dandelion) is notable for its use in traditional diets and the accumulation of micronutrients. Thus, it’s prospective for both environmental monitoring and functional food development.

Context and purpose of this study: The relation between heavy metal contamination of environment and DNA polymorphisms of *T. officinale* from Syunik, Armenia was studied.

Results: Significant genetic variation was observed among the samples, with the highest polymorphism (95.46%) in Artsvanik Village and the lowest (73.24%) in Ttujur District. Increased soil concentrations of Fe and Mn were correlated with greater DNA polymorphism, suggesting a higher mutation rate in contaminated areas. These results point to a direct relationship between heavy metal exposure and genome instability in dandelion plants.

Conclusions: Significant genetic changes were observed in samples from all surveyed locations in Syunik. *T. officinale* is able to accumulate heavy metals and may be used in ecotoxicological and remediation research as bio-indicator. Also, it could be recommended for further research as potential source for bioactive supplements and functional foods elaboration with diuretic, antioxidant, anti-inflammatory, anti-aging, and immunomodulatory properties.

Keywords: *Taraxacum officinale,* food safety, heavy metals, soil contamination, PCR, polymorphism



INTRODUCTION

Among wild-growing plants, common dandelion (*Taraxacum officinale*) is widespread in Central and Southern Europe, including almost all climatic zones of the Republic of Armenia (RA), and has been extensively studied as a bio-indicator plant for environmental impact assessment [1,2]. Ethnobotanically, it is traditionally used in RA as both an edible and medicinal plant. In recent years, this plant has also been widely applied in the bioremediation of soils, because the confirmed properties of resistance to heavy metals increased concentrations [3-5].

Heavy metals are dangerous for human and animals in overdose high, low and even in trace concentrations, depends to a particular element. They are toxic and pose the risks of: genotoxicity, infertility, carcinogenicity, neurodegradations, etc. Anyhow, some of them are extremely necessary for human metabolism (Zn, Fe, Mn, Mg, etc.) in very low concentrations, and in case of deficiencies also could become a cause of the various disorders. They are naturally occurring in the Earth's crust, but human activities have significantly increased their concentration in the environment. Number of investigations have shown the direct correlation between the level and type of heavy metal contamination in soil of areas near the mines and heavy metals content in plants [6,7]. Syunik region is a risk-zone of heavy metal contamination, because of the variety of metal mines here for over then 65 years and up to now. Simultaneously, it's one of the agrarian regions of Armenia. Therefore, here the traditional consumption of *T. officinale* as a food or medication, becoming unsafe. And thus, the assessment of biological risks of that practice is very important [8,9].

In this research genetic changes in *T. officinale* samples collected, from Syunik's various points, potentially contaminated by heavy metals, were studied.

MATERIALS AND METHODS:

Sample collection: The sample collection took place in different zones of Syunik province of RA: N1 – Trilateral crossroad of Chapni Village (39°16'11.80"N, 46°27'39.73"E); N2 – Sevaqar Village (39°16'17.55"N, 46°26'43.27"E); N3 – Artsvanik Village (39°15'55.68"N, 46°28'21.68"E); N4 – The Syunik village, the area near the river (39°11'59.66"N, 46°28'11.13"E); N5 – the surroadings of Atchanan Village (39°14'14.35"N, 46°25'50.82"E); N6 – Atchanan Village (39°14'2.16"N, 46°25'52.25"E); N7 – The upper part of Kapan city (39°13'22.88"N, 46°20'55.95"E); N8 – Ttujur District (39° 9'27.69"N, 46° 6'49.29"E). Soil sample collection was carried out from the same points for the assessment of heavy metals consistence, using the generally accepted CP-MS (ELAN 9000, PerkinElme) analytical techniques [10-12]. Totally triplicate samples from the 8 sampling points were screened for the genetic stability [13].

DNA isolation and polymorphism analyses: The plant DNA was extracted using DNeasy Plant Pro Kit. The quantity (purity) and quality of the isolated DNA was evaluated using Thermo Scientific MultiscanGO µDrop™ plate [14]. For qualitative assessment 2% agarose horizontal gel-electrophoresis with ethidium bromide was applied [15,16]. PCR (Polymerase Chain Reaction) was performed with 20 random 10 nucleotides RAPD (Random Amplified Polymorphic DNA) OPA (Oligonucleotide Primer Arbitrary) primers: *OPA-01:*CAGGCCCTTC; *OPA-02:* TGCCGAGCTG; *OPA-03:* AGTCAGCCAC; *OPA-04:* AATCGGGCTG; *OPA-05:* AGGGGTCTTG; *OPA-11:* CAATCGCCGT; *OPA-12:* TCGGCGATAG; *OPA-13:* CAGCACCCAC; *OPA-14:* TCTGTGCTGG; *OPA-15*: TTCCGAACCC; *OPA-06:* GGTCCCTGAC; *OPA-07:* GAAACGGGTG; *OPA-08:* GTGACGTAGG; *OPA-09:* GGGTAACGCC; *OPA-10:* GTGATCGCAG; *OPA-16*: AGCCAGCGAA; *OPA-17:* GACCGCTTGT; *OPA-18:* AGGTGACCGT; *OPA-19:* CAAACGTCGG; *OPA-20:* GTTGCGATCC ("Operon Technologies"), using HS-Taq PCR-Color kit ("Dia.M"), using Qiagen genetic analyzer, Qiaxcel DNA screening kit and PyElph 1.4 and GenAlEx 6.502 software packages [17,18]. The level of polymorphism of RAPD fragments and the genome stability was calculated for both contaminated and control samples. The changes in RAPD fragments were marked as +1. The Genomic template stability (GTS, %) was calculated for each sample, using the formula (1):

$$(1)\ GTS\ (\%) = 1 - a/n \times 100,$$

where *a* – the average where a was RAPD polymorphic profiles detected in each sample treated and *n* the total number of bands in the control. Polymorphism was observed by the disappearance of normal bands and/or appearance of a new bands in comparison to control samples: RAPD profiles of genomic DNA of lab-grown *T. officinale*. The integrity of genomic DNA was assessed by agarose gel-electrophoresis. [19,20]. All the experiments were performed in triplicate series and repeated three times independently under similar conditions. For the statistical assessment MS Excel program package was used [21].

RESULTS

Concentrations of DNA in all the samples were measured. In N1, the DNA concentration was 891.24 mcg/mL; in N2 – 647.32 mcg/mL; in N3 – 458.15 mcg/mL; in N4 – 880.10 mcg/mL; in N5 – 785.28 mcg/mL; in N6 – 746.78 mcg/mL; in N7 – 763.35 mcg/mL; and in N8 – 568.18 mcg/mL. According to spectrophotometric analysis, the absorbance ratio at 260 nm/280 nm for DNA isolated from genomic samples ranged from 1.85 to 1.90, indicating DNA suitable quantity and high degree of its purity. According to the assessment of genomic DNA integrity, the results indicate a high level of stability in these plants under high concentrations of heavy metals. It correlates with literature data, about the ability of *T. officinale* to exhibit a significantly high resistance to increased concentrations of heavy metals in environment [22]. Genotoxicological analysis of genomic DNA RAPD profiles of plant from Sevaqar, Artsvanik, Atchanan, Chapni, Kapan city, and Ttujjur district had a high level of genetic polymorphism (Table 1, Fig. 1)

Table 1. Polymorphism (Pol.) analysis of *T. officinale* from the different areas of Syunik.

OPA Primer	Samples of *T. officinale*							
	N1	N2	N3	N4	N5	N6	N7	N8
OPA-01	6	5	8	9	7	6	7	6
OPA-02	5	8	7	9	5	6	5	6
OPA-03	6	6	6	7	6	5	4	10
OPA-04	7	6	9	8	4	5	6	9
OPA-05	6	8	8	8	10	5	5	7
OPA-06	7	5	7	7	9	7	7	8
OPA-07	8	5	9	9	9	6	5	8
OPA-08	4	7	9	9	6	6	3	10
OPA-09	3	6	8	8	9	5	5	7
OPA-10	5	8	9	9	6	7	6	3
OPA-11	5	8	9	6	7	5	7	5
OPA-12	8	5	8	6	6	4	6	6
OPA-13	4	6	9	5	6	2	6	7
OPA-14	5	4	6	9	10	3	5	6
OPA-15	5	8	9	9	9	3	8	8
OPA-16	7	5	9	6	8	6	6	6
OPA-17	7	6	9	5	6	5	6	6
OPA-18	6	7	8	7	6	5	7	5
OPA-19	6	7	9	9	4	5	4	5
OPA-20	9	8	11	8	7	2	8	4
Pol., %	86.35	89.32	95.46	98.23	88.25	82.37	86.15	73.24

A comparably high levels of polymorphisms in dandelion were registered in all the studied territories of Syunik, what correlates with literature data about the contaminations increased risks in this area [23,24].

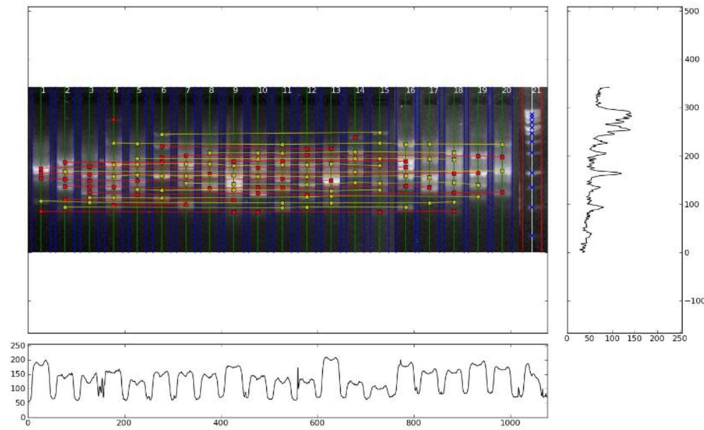


Fig. 1. Genomic DNA RAPD profiles of *T. officinale* (the sample N8).

Polymorphism maximal level was identified in Artsvanik village, while in Ttujjur District it was the minimal. Then, the Genome Template Stability (GTS) was assessed by RAPD profiles and the data about heavy metals consistence in soil (Table 2).

Table 2. The concentration of some heavy metals in the soil and the plant genome stability.

N	The concentration of the biologically available heavy metals in the soil samples (mg/kg)										GTS%
	Cr	Mn	Fe	Co	Ni	Cd	Mo	As	Zn	Cu	
N1	0,0251	0,5913	9,8407	0,0041	0,1134	0,0007	0,0001	0,0051	0,0052	0,0039	88.5
N2	0,015	0,0056	0,1266	0,0002	0,0018	0,0007	0,0095	0,0071	0,0035	0,0019	83.2
N3	0,0232	3,7537	8,7171	0,0189	0,1179	0,0006	0,0016	0,0142	0,0211	0,0068	49.9
N4	0,0321	0,5112	7,0762	0,0034	0,0868	0,0005	0,0007	0,0046	0,0067	0,0038	55.3
N5	0,0317	0,8834	9,4923	0,0054	0,117	0,0015	0,0012	0,0103	0,0301	0,0063	52.4
N6	0,0321	0,5112	7,0762	0,0034	0,0868	0,0005	0,0007	0,0045	0,0067	0,0038	58.3
N7	0,0326	6,095	2,2894	0,0431	0,0566	0,0041	0,0202	0,0351	2,5003	0,4586	55.4
N8	0,0318	2,5216	0,5425	0,0171	0,0342	0,0012	0,0054	0,0343	0,2773	0,5867	73.1

DISCUSSION

The main scientific novelty and actuality of this research is the primary evaluation the genotoxic effects of heavy metal exposure on *T. officinale* in the Syunik, using RAPD-PCR analysis. The findings demonstrate that it can serve as a bio-indicator for both eco-monitoring of soil contamination and ecotoxicological risks evaluation. This experience could be also prospective for the safety evaluation of other traditionally edible and medicinal wild plants of region: aveluk or horse sorrel (*Rumex confertus*), urtica (*Urtica dioica*), etc. [25]. Moreover, the study supports the potential of *T. officinale* as a source of novel bioactive compounds for functional food formulations. Also, the main advantage of the applied approach is the use of local populations that have adapted and evolved under the influence of local conditions as a system for assessing the impact on genetic material. During the research, significant changes in the observed RAPD profiles, such as the disappearance and/or appearance of bands compared to the control samples—were evaluated. The presence or absence of these bands, in comparison with the control, indicated strong genetic differences among plants growing under different conditions. And PCR with RAPD markers enables the identification of DNA changes caused by heavy metals [26-28]. The 10-mer oligonucleotides used, with a GC content of 50–70%, resulted in stable amplification, producing approximately 180 bands. The molecular weight of them ranged from 105 bp (primer 2) to 1150 bp (primer 16). The assessment of genetic profiles along with the heavy metal content and the analysis of their potential mobility in soil, revealed a direct correlation between the contamination level and polymorphisms. In studied samples, an increase in the concentrations of biologically mobile elements led to an increased number of polymorphic bands in the RAPD profiles. A strong positive correlation (correlation coefficient = 0.95) was observed between Fe and Mn concentrations and RAPD bands number. The higher the concentration of Fe and Mn (or Fe alone) in the soil, the greater the genetic differences observed in the DNA profiles, as evidenced by the appearance and disappearance of DNA bands [29,30]. The analyses have shown that the higher the concentration of heavy metals in plants and/or soil, the lower the GTS level, indicating a higher mutation rate in genome and, consequently, the greater ecotoxicological risks.

Potential application of *T. officinale* for bioactive food supplement elaboration: Dandelion is commonly used in traditional honey and other meals in Syunik. It's more used among the other plants for the specific honey. Anyhow, it suggests significant risks to human health in case of its uncontrolled consumption. On the other hand, the strictly controlled application of it may hold great potential. It is directly related to the presence of diverse bioactive compounds (vitamins A, C, K, E, B_1, B_2, B_6, B_9, antioxidants, etc.) in it [31-33]. Dandelion has anti-inflammatory, diuretic, cardio-protector, anti-diabetic properties, etc. because of some compounds which regulate lipids and carbohydrates metabolism [34,35]. Also, this plant has a potential for the elaboration of new bioactive food supplements and additives both for human and animals. It's based on the fact that micronutrients, absorbed by *T. officinale* from the soil are converted into a highly bioavailable form and may have beneficial therapeutic effects for patients with corresponding deficiencies. Thus, the dandelion honey and jam produced in Syunik might be used as a functional food aimed at addressing the aforementioned micronutrient deficiencies, which are dangerous for both adults and children and have led to serious consequences [36-38]. Moreover, the production of such bio-supplements would not demand the significant economic costs and could be potentially implemented in context of circular economy principles development in RA and the mitigation of mining-related risks in Syunik [39-41].

CONCLUSION

The different genotypes of dandelion *T. officinale*, exposed by heavy metals allowed us to come to the conclusion it's bio-accumulation abilities. Thus, its consumption in Syunik should be strictly limited. Anyhow, this property can be potentially used for the elaboration of specific food supplements and functional foods, based on *T. officinale*, prescribed against some deficiencies of micronutrients. Also, this plant might be used for bioremediation of soils.

Abbreviations: dNTP, deoxyribonucleoside triphosphate; GTS, Genomic template stability; OPA, Oligonucleotide Primer Arbitrary; PCR, Polymerase Chain Reaction; RAPD, Random Amplified Polymorphic DNA.

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