Assessment of pollution at the former uranium waste dumpsite near kaji-Say Village/Kyrgyzstan: a genetic and physiological investigation

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1. Introduction

Nuclear energy has become one of the most important alternative energy sources especially after the mid-20th century (Rezaei et al., 2017). As is known, the most important raw materials of this energy are uranium and thorium (Li et al., 2019; Nisbet et al., 2019). In conjunction with this, nuclear accidents (Baillifa et al., 2016) (exposure to radioactive dose due to radioactive contamination by presence of radioactive materials affecting people and/or the environment, or an event involving radioactive contamination) (Ahearne, 2011; Goodfellow et al., 2011), testing of nuclear weapons (Pravalie, 2014), the spread of radioactive substances to the environment because of waste from nuclear industry cause excessive exposure of all living organisms to radiation in the environment (Kobashi et al., 2020; Sevbitov et al., 2020).

The Central Asian region that hosts the republics of the former Soviet Union, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan is still suffering from past practices and exploitations of the Soviet era, and these practices and exploitations have had significant impacts on human health and the fragile ecosystems of the region (Amineh & Rakel, 2012; Yuxuan, 2021). In the past, the radioactive materials needed by the Soviet Union were provided from Kyrgyzstan; thereby, there are 36 uranium tailings sites and 25 uranium-mining dumpsites in the country (Svck, 2003), one of which is located on the southern shore of Issyk-Kul Lake near Kaji-Say Village in the Ton region (Lind et al., 2013).

Issyk-Kul, a slightly saline nonfreezing closed mountain lake, is situated on the northern slopes of the Tian Shan mountain belt with having an arid climate (Romanovsky, 2002; Vermeesch et al., 2004). The Kungey Ala-Too in the north (reaching 4770 m) and the Teskey Ala-Too in the south (exceeding 5200 m)
are the mountain ranges surrounds the lake. The both mountain ranges have mainly of crystalline basement rocks from Archean and Middle Paleozoic ages, covered by volcano-sedimentary and sedimentary strata of Devonian-Carboniferous age (De De Batist et al., 2002). The rocks being on the eastern, central, and western parts of the Teskey Range are originated from the Paleozoic granites and granitoids, Late Ordovician granitoids and Paleozoic granitoids, respectively, while the rocks being on the eastern and western parts of the Kungoy Range are originated from Paleozoic granitoids and Late Ordovician granitoids, respectively (Academy of Sciences of the Kyrgyz SSR, 1987). Hence, the lithology around the glacial areas is mainly the same in the two mountain ranges. The 834 glaciers having a total of 650 km² with a volume of 48 km³ feed the lake (Salamat et al., 2015; Shabunin & Shabunin, 2002). Although prevailing climate is continental in the area, due to the topography, it shows spatial variability (Narama et al., 2010). The temperature around the lake basin reaches up to 19–20°C in July and shows no downfall below 2–3°C in January. The recorded annual precipitation rates in the area between the years of 1951 and 2012 are ranged from 136.7 mm (Balykchy) to 412.2 mm (Kyzyl-Suu), with an overall average of 276.5 mm (Alifijiang et al., 2020).

The population in the area surrounding Lake Issyk-Kul is dense comparing with the other areas of Kyrgyzstan (Alifijiang et al., 2017). According to the statistical data from Kyrgyz Republic, the death rate percentage from malignant tumors in the Kyrgyz Republic is about 61.7 in the entire population, whereas in the Issyk-Kul region, the rate is about more than 1.5 times higher among the general population of the Republic. The death rates of the malignant diseases recorded in the Issyk-Kul region are as follows: stomach cancer (10%), lung cancer (8.6%), and malignant tumors of the lymphatic system (3.9%), respectively (Tynymbekov, 1999; Liu et al., 2020).

The Kaji-Say region is approximately 2.5 km away from the southern coast and approximately 180 m above the lake level (1680 m above sea level) (Stegnar et al., 2013). The coal-bed containing uranium was discovered in 1948 (Lind et al., 2013). Uranium was recovered as acid leak from the ash formed after burning of coal containing uranium as in the form of uranium oxide in coalmine (Kaldybaev & Chinar, 2018). A thermal power plant ran by coal burning was built near the coal mine. The plant, which worked until 1967 for the processing of uranium from coal ashes, left about (150–400) x 10⁷ m³ of radioactive ash in the Kaji-Say region (Gavshin et al., 2005). A 6-m-thick layer of existant ash as a result of uranium mining was buried with industrial equipment in an area used as dumping site (about 10,800 m²) (Djebraev et al., 2012, 2008; Uralbekov et al., 2011). In nature, uranium naturally is found as a mixture of the three isotopes that are ²³⁸U, ²³⁵U, and ²³⁴U. As known having a half-life of 7.038 x 10⁸ years, ²³⁵U exists in nature to the extent of 0.72%. This isotope is of having special importance because of being undergoing fission with slow neutrons (Grethe et al., 2006).

At present, due to the pressure of natural and anthropogenic reasons, the threat of radioactive pollution in the vicinity of Kaji-Say increases significantly. Since the radioactive waste buried in the former mine dump near Kaji-Say is exposed to floods, erosion, and mud streams; hence, the radiation and/or radioactive material leakage from the area is a possibility (Kulenbekov & Merkel, 2012). For example, according to the data covering the period of 1973–1999, the average monthly temperature in the lake basin arisen, resulting in an acceleration of glaciers melting and following increased river runoffs during the summer periods (Romanovsky, 2002). Furthermore, in the research area where a river flows near the waste dumpsite, possibly polluting this river, reaching into Issyk-Kul Lake (Tynymbekov & Hamby, 1999). Therefore, the main objective of this study is to determine the extent of radioactive pollution and to determine the effect of this pollution on living organisms in the region.

2. Materials and methods

2.1. Study area

Soviet nuclear industry left imprints in the several regions of Kyrgyzstan through exploitations of mining of radioactive ores and minerals as strategic raw materials with the development of nuclear energy. These exploitations from the Soviet era had significant impacts on human health and the fragile ecosystems of these regions. Radioactive wastes from nuclear facilities were casually buried in uranium mining dumpsites (Sevcik, 2003), one of which is located on the southern shore of Issyk-Kul Lake near Kaji-Say Village in the Ton region, 270 km from Bishkek. Kaji-Say region is approximately 2.5 km away from the southern coast and approximately 180 m above the lake level (1680 m above sea level) (Stegnar et al., 2013).

Lake Issyk-Kul is the second largest high altitude lake in the world and one of the most important biological and economic resources of Kyrgyzstan. Among the lakes, which are 1607 meters above sea level (Batist et al., 2002), Lake Issyk-Kul comes second in the region after Lake Titicaca. The basin of Lake Issyk-Kul is one of the most important natural areas of the Kyrgyz Republic with its size of 22,080 km², which constitutes half of the Lake Issyk-Kul oblast covering 43,144 km² area. Lake Issyk-Kul oblast lies between 41°08’ and 42°59’ N latitudes and 75°38’ and 80°18’ E longitudes (Baetov, 2006). The longest and widest distances of the lake are 178 km and 60 km, respectively. The deepest point of the lake is 668 m, having an average
depth of 278 m and its total area is 6236 km$^2$. The overall circumference of Lake Issyk-Kul is 688 km (Amanaliev, 2008). There are almost 118 rivers and streams feeding the lake (Kulenbekov & Merkel, 2012).

The sampling stations (Figure 1) in the research area near Kaji-Say Village were indicated on map with bolded-red numbers. The numbers stand for: 1, where the former uranium processing plant is located; 2, where uranium waste dump site is located; 3 and 4, where the sites after the former uranium waste dump site in the direction of the lake are located; and 5, where the station 2 is located 5.3 km away from.

![Figure 1](image_url). The satellite images showing Lake Issyk-Kul in Kyrgyzstan, Kaji-Say region in southern section of Lake Issyk-Kul, and the former uranium waste dump site and surrounding area located at Kaji-Say region in southern section of Lake Issyk-Kul. The sampling sites are indicated on map with red numbers, where number 1 stands for the location of the former uranium processing plant, number 2 stands for the former waste dump site, numbers 3 and 4 stand for the sites after the former uranium waste dump site in the direction of the lake, and number 5 stands for as control located 5.3 km away from the station 2 (The images taken from Google Earth and modified using Google Earth Program, 2021).
2.2. Plant material employed in the study (P. abrotanoides Kar.)

P. abrotanoides was chosen as the study material because it grows naturally in the vicinity where the former uranium waste dumpsite located. Perovskia consisting of seven species from Lamiaceae family is distributed in southwestern and central Asia (Mamadalieva et al., 2017; Mohammadhosseini et al., 2019; Pourhosseini et al., 2018). P. abrotanoides also known as Caspian Perovskia is a medicinally important perennial (diploid $2n = 2x = 20$) with an outcrossing mating system herb containing different class of compounds including essential oils, phenolics, flavonones, irregular triterpenes, steroids, and a large amount of abietane-type norditerpenoidquinones (Armitage, 2008; Oreizi et al., 2015; Perveen et al., 2006, 2009; Zaker et al., 2015). The plant is utilized as medicine by local people for the treatments of several diseases. These of are including leishmaniasis, typhoid fever, atherosclerosis, cardiovascular diseases, and liver fibrosis. For the evaluation of the antileishmanicidal effect, the plant root extracts at different concentrations were used on the promastigotes of Leishmania major and a dose-dependent antileishmanial activity was detected (Jaafari et al., 2007). The isolated tanshinones (Cryptotanshinone, 1β-hydroxycryptotanshinone, 1-oxocryptotanshinone and 1-oxomiltirione) from the roots of Perovskia abrotanoides inhibited the growth of cultured malaria parasites (3D7 strain of Plasmodium falciparum) causing typhoid fever (Sairafianpour et al., 2001). Also, due to possessing a large number of phytochemicals and phytoconstituents, the plant in the traditional Chinese medicine was used for the treatments of atherosclerosis, cardiovascular problems, and liver fibrosis (Beikmohammadi, 2012; Moallem & Niapour, 2008).

2.3. Determination of radiation rates in the study area

This study was carried out to evaluate the radioactive contamination at the former uranium waste dump site in the Kaji-Say village in Ton District/Kyrgyzstan. The portable equipment used for the determination of radioactivity in this study was Dosimeter SRP 68–01. The coordinates and elevations of all stations where the research carried out were determined using a GPS device (Garmin, eTrex, 12 Channel Handheld). Radioactivity screening in the study area was carried out according to the instructions of the equipment manufacturer. Measurements were done in mR/h (microrontgen/hour). Radioactivity screenings were performed in five stations shown in Table 1. Readings were taken as the values between maximum and minimum oscillation points of the analog pointer.

2.4. Collection, preparation, and analysis of samples

The soil profile of Lake Issyk-Kul is of having arid soil in the west and semi-arid soil in the middle and the east, and it is mainly composed of loam, clayey loam, and sandy clay loam (Kulenbekov & Merkel, 2012). Largely wooded tundra as the territorial characteristic is seen in the upstream region while grassland, irrigated land, shrub land, dry land, and settlements are the surface covers seen in the downstream region (Kulenbekov &

Table 1. The information and radiation readings recorded at work stations in the former Soviet uranium processing plant and waste dump area in the Kaji-Say Village in Ton Region-Lake Issyk Kul/Kyrgyzstan.

| Stations | Numbers of Readings | GPS Coordinates | Altitude (Meter) | Radiation Rates (in mR/h) |
|----------|---------------------|-----------------|-----------------|--------------------------|
| Station 1 | 1.                   | 42.152630 N 77.219471 E | 1745           | 25                       |
|          | 2.                   | 42.152032 N 77.219475 E | 1755           | 16–17                    |
|          | 3.                   | 42.153347 N 77.219629 E | 1733           | 18–21                    |
| Station 2 | 1.                   | 42.153995 N 77.217800 E | 1716           | 36–38                    |
| (The former uranium waste dump site) | 2.                   | 42.154186 N 77.217722 E | 1710           | 40–42                    |
|          | 3.                   | 42.153632 N 77.217969 E | 1720           | 60–100                    |
|          |                      |                 |                 | (200–300 where cracks are seen in the soil) |
| Station 3 | 1.                   | 42.156696 N 77.216006 E | 1682           | 20–21                    |
|          | 2.                   | 42.157282 N 77.216018 E | 1677           | 18–19                    |
|          | 3.                   | 42.156398 N 77.216226 E | 1684           | 19–21                    |
| Station 4 | 1.                   | 42.164050 N 77.213854 E | 1646           | 17–19                    |
|          | 2.                   | 42.164558 N 77.214250 E | 1648           | 17–18                    |
|          | 3.                   | 42.163846 N 77.213546 E | 1648           | 18–20                    |
| Station 5 | 1.                   | 42.158614 N 77.153580 E | 1618           | 18–19                    |
| (As control) | 2.                   | 42.158314 N 77.153589 E | 1621           | 17–19                    |
|          | 3.                   | 42.158012 N 77.153588 E | 1623           | 16–18                    |
Merkel, 2012). At the dumpsite near Kaji-Say,uraniferous brown coal outcrops and piles of radioactive waste with fine-grained, gray ash and compacted slag were found along with 1- to 6-m-thick non-radioactive, alluvial, and deluvial material used as cover (Gavshin et al., 2004).

Plants take various mineral nutrients as well as metals together with their roots from the soil and accumulate them in their own body (in root, stem and leaf parts). In a sense, the pollution occurred in the soil can be estimated for this reason (Osma et al., 2014; Eskin et al., 2019). In this respect, depending on the degree of contamination, they are also affected by this situation as in other living things. In connection with this, the degree of contamination can be estimated in this way by means of various measurements in order to make estimation on the degree of radiation leakage by detection of physiological status of the plant and induced genotoxic damage occurred in the plant.

In this study, the leaf, stem, and root parts of P. abrotanoides and their co-located soils as study materials were collected from uranium waste dumpsite area and surrounding area located at Kaji-Say region in southern section of Lake Issyk-Kul/Kyrgyzstan. The plant and soil samples were taken from the uranium waste dump site and from three sites close to the waste dump site, relatively away from the waste dump site in the direction of lake and the control site, 5.3 km away from the uranium waste dump site. The soil samples were collected from an average depth of 15 cm (for keeping the deviation to a minimum because the top of the soil being contaminated through air) and from the distance not more than 90 m in one site. A minimum of five random samples (for each plant part and co-located soil) were taken at each station for once for a statistically valid evaluation.

For the determination of the contents of some elements (Ca, Fe, K, Mg, Mn, Na, Ni, and Zn) and uranium in the collected plant part and soil samples, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Agilent Technologies, 7700 Series) was employed. The plant part (leaf, stem, and root) samples were washed with deionized distilled water and placed in the envelopes. After drying process done at 80°C for 48 h, the plant part and soil samples (500 g each) were grounded in mortar and then passed through a 1.5 mm sieve and each 0.2 g weighed sample was placed in Teflon vessel (heat and pressure resistant). Then, 8 mL 65% HNO3 (Merck) was added to each plant sample. Also, 6 mL HNO3 (65%), 3 mL HCl (37%) and 2 mL HF (48%) (Merck) were added to each soil sample taken from a depth of about 10 cm using a shovel. Following solving using microwave oven (Berghof-MWS2) at 145°C for 5 min, 165°C for 5 min, and 175°C for 20 min and cooling processes, the samples were passed through 1–2 μm filter paper and filled up to 50-mL volume with ultrapure water in falcon tubes. Using multi-element stock solutions 1000 ppm (Merck), standard solutions were prepared. Finally, the reproducible concentrations of Ca, Fe, K, Mg, Mn, Na, Ni, U, and Zn were determined using ICP-MS.

All of the statistical evaluations were done through utilizing the element concentrations of plant parts (root, stem, and leaf – dry weights) and related concentrations of co-located soil samples using MANOVA (multivariate analysis of variance) with Tukey’s post-hoc HSD and Pearson Correlation Analysis were done using IBM SPSS Statistics version 20 software. A statistically significant difference was assessed at two levels as ** p < 0.01 and * p < 0.05. Sampling stations and plant parts were chosen as factors for Manova tests (Table 8).

2.5. Analysis of genotoxicity

Inter-simple sequence repeat (ISSR) analysis was performed for analyzing the genotoxic effects of radiation leaking from the former waste dump site near Kaji-Say Village using genomic DNA from the leaf part of P. abrotanoides. P. abrotanoides is a perennial herb (Jaafari et al., 2007). As known, all organisms including plant species are susceptible to the radiation to some extends and has lethal effects effects at both the cell and the organism levels causing genetic mutations, cancer formation, and finally cell death (Tominaga et al., 2004; Wright & Coates, 2006). To justify the extend of radiation, P. abrotanoides as study material was chosen after confirmation of observation of individuals grown in the area. Frozen leaf tissue (0.5 g) was ground using a mortar. Following modified CTAB, DNA extraction protocol (Aboul-Ftooh Aboul-Maaty & Abdel-Sadek Oraby, 2019; Fu et al., 2017; Hanjalic et al., 2018) was employed for genomic DNA extraction using the grounded leaf tissue. At final step, the genomic DNA obtained was air-dried for 4 h for removing ethanol and 100 μL RNase-free water was added for dissolving the genomic DNA in it. The concentrations of the obtained genomic DNA were determined using a Nano Volume Spectrophotometer (Optizen NanoQ). PCR products were obtained by using 15 different primers for ISSR analysis and specific and stable band profiles were produced in our study. PCR mix was prepared for 25 μL, containing 2.5 μL PCR buffer (1X), 1 μL from each dNTP (10 mM dNTP mix), 1 μL of primer (Santegen), 50 ng of genomic DNA, and 5 units of Taq DNA polymerase, and filled up with sterile de-ionized water to final volume. An AERIS-BG096 Gradient Thermal Cycler was used for the amplifications. The PCR programme was as follows: initial denaturation at 94°C for 5 min; followed by 45 cycles of denaturation at 94°C for 1 min, primer annealing at 53°C for 45 s, elongation at 72°C for 1 min and a final extension step at 72°C for 10 min. Amplification
products were run on a 1.6% agarose gel was used (in 1X TBE) for separation at 90–100 V (70 mA) for 90 min. Following ethidium bromide staining, DNA bands were visualized under UV light. A molecular size marker (GeneRuler 100 bp Plus DNA Ladder, ready-to-use, Thermo Fischer Scientific) was utilized for analyzing and estimating of sizes of amplification products. The genetic profiles of the samples were created by scoring clearly observed bands. Gel Analyzer 2010 Software program was employed for determination of DNA variations in ISSR profiles via band counting and size and band intensity assignments.

## 3. Results

### 3.1. Radiation readings in the study area

The information and radiation readings recorded at work stations in the former Soviet uranium processing plant and waste dump area located near the Kaji-Say Village were presented in Table 1. According to our data from Table 1, the highest radiation readings were recorded in station 2 where the former waste dump site is located. The levels of readings were found to be in ranges of 36–100 mR/h (200–300 mR/h in the areas where cracks are seen in the soil) in this site. The levels of readings at the station 5 (as control), which is approximately 5.3 km from the station 2, were recorded in ranges of 16–19 mR/h. The levels of readings at station 1, where the former uranium processing plant located, varied between 16 and 25 mR/h. And the levels of readings at the stations 3 and 4, where the sites after the former uranium waste dump site in the direction of the lake are located, were found to be in ranges of 18–21 mR/h and 17–20 mR/h, respectively. Monitoring of exposure and dose rates of radiation, and approximate exposure rate for radiation from space (cosmic radiation), is performed by EPA’s RadNet system, and the data show that it varies in the ranges of 0.0023 and 0.1107 mR/h (EPA, 2021).

On an average, Americans are exposed to a radiation dose of about 620 mR each year. Half of this dose comes from natural background radiation. As well, the dose limit from NRC-licensed activity is about 5000 mR for a year (USNRC, 2021).

### 3.2. The mineral nutrient elements and uranium contents in plant part and soil samples used as study materials

The average contents of Ca, Fe, K, Mg, Mn, Na, Ni, U, and Zn (in mg kg⁻¹ DW) in plant part and soil samples were shown in Table 2. According to our results, the average

Table 2. The concentrations of Ca, Fe, K, Mg, Mn, Na, Ni, U, and Zn (in mg kg⁻¹ DW) in the parts (leaf, stem and root) of *P. abrotanoides* and in the co-located soil samples determined in the stations where the study was carried out.

| Element | Station 1 | Station 2 | Station 3 | Station 4 | Station 5 |
|---------|-----------|-----------|-----------|-----------|-----------|
| Ca      | 2190.811 ± 54.392<sup>b</sup> | 2166.475 ± 45.621<sup>b</sup> | 4077.068 ± 93.209<sup>a</sup> | 4064.558 ± 82.304<sup>c</sup> | 2715.156 ± 39.586<sup>c</sup> |
| Mg      | 2749.987 ± 57.004<sup>c</sup> | 820.760 ± 13.264<sup>c</sup> | 769.155 ± 11.445<sup>c</sup> | 1383.346 ± 16.322<sup>c</sup> | 1259.808 ± 20.193<sup>c</sup> |
| Fe      | 39.966 ± 6.097<sup>c</sup> | 189.188 ± 3.071<sup>c</sup> | 253.043 ± 1.100<sup>c</sup> | 505.145 ± 6.698<sup>c</sup> | 391.681 ± 8.225<sup>c</sup> |
| Mn      | 1.287 ± 0.001<sup>c</sup> | 0.198 ± 0.001<sup>c</sup> | 0.018 ± 0.001<sup>c</sup> | 0.018 ± 0.001<sup>c</sup> | 0.018 ± 0.001<sup>c</sup> |
| Na      | 1.525 ± 0.001<sup>c</sup> | 0.480 ± 0.001<sup>c</sup> | 0.792 ± 0.001<sup>c</sup> | 1.051 ± 0.001<sup>c</sup> | 0.962 ± 0.001<sup>c</sup> |

**<sup>a</sup> p < 0.01 and <sup>b</sup> p < 0.05.**
lowest and highest element accumulations (in mg kg\(^{-1}\) DW) in the parts of plant were found to be: 769.1 and 4551.9 in stems and roots recorded in station 3 for Ca; 6796.8 and 55,964.3 in roots and leaves recorded in station 1 for K; 411.9 and 3506.3 in roots and leaves recorded in station 1 and the control station for Mn; 79.4 and 1987.7 in stems and roots recorded in station 4 and the control station for Fe; 13.2 and 71.6 in stems and leaves recorded in station 3 and the control station for Mg; 286 and 134.4 in stems and leaves recorded in stations 2 and 3 for Ni; 0.4 and 1.7 in stems and roots recorded in stations 3 and 2 for U; and 8.4 and 50.4 in roots and leaves recorded in station 2 and 3 for Zn, respectively. And, the average lowest and highest element accumulations (in mg kg\(^{-1}\) DW) in the soil samples were found to be: 2790.2 and 10,865.2 recorded in the control station and station 1 for Ca; 3302.7 and 10,595.2 recorded in stations 3 and 1 for K; 2710.5 and 13,221.9 recorded in stations 2 and 1 for Mg; 13,305.4 and 35,091.0 recorded in control station and station 2 for Fe; 305.0 and 726.6 recorded in stations 1 and 2 for Mn; 141.7 and 329.6 recorded in control station and station 1 for Na; 8.6 and 33.7 recorded in control station and station 2 for Ni; 4.2 and 7.1 recorded in control station and station 2 for U; and 29.8 and 73.5 recorded in control station and station 2 for Zn, respectively.

The results from the Pearson Correlation analysis performed using the element concentrations from the plant parts and the soil samples were given in Table 8. According to this table, it is seen that U had a high positive correlation with Ca, Fe, Mg, Mn, and Ni (>0.58, >0.89). On the other hand, there was a high negative correlation with K (> −0.58). As seen in Table 8, this shows that K values are adversely affected depending on the volume of U, in other words, U significantly inhibits K uptake. K also showed a high negative correlation with Fe as with U (> −0.50). This situation is thought to be related with the significant reverse downfall and rises in the amount of Fe and K in the soil (Table 8). Among other elements, there were high positive correlations: between Ca and Fe-Mg-Mn-Ni (> 0.52, > 0.84); between Fe and Mn-Ni-Zn (> 0.65, > 0.99); and Zn with Mn-Ni (> 0.68, > 0.70).

3.3. **Assessment of radiation-induced genotoxic damage in *P. abrotanoides* grown in the study area using ISSR-PCR analysis**

Here, ISSR-PCR analysis was used for identification of possible mutations. By ISSR technique, using primers generated by binary, triple, quadruple, and quaternary nucleotides can be used for amplifying a region between two microsatellite sites and the resulting PCR products can be determined after staining with ethidium bromide on agarose gels (Boopathi, 2020; Kougioumoutzis et al., 2021).

For evaluation of genotoxicity on the genomic DNA of *P. abrotanoides* caused by radiation leakage from the former waste dump site located near Kaj-Say Village, the comparative ISSR-PCR analyzes were carried out using ISSR-PCR profiles obtained from our samples. For PCR amplifications, 15 different primers were utilized but specific and stable band profiles were produced using 2 of them in our study. Distinguishable banding patterns including increase or decrease in band intensities and loss or gain of bands were produced using primers designated as (GA)\(^8\) C and (CA)\(^8\) G between exposed and unexposed groups. Representative ISSR-PCR profiles were shown in Figure 2 as well as in Tables 3–7. The comparisons between the ISSR-PCR profiles of plant samples were done on the basis of the changes mentioned above in the specific ISSR bands. The molecular sizes of the bands (in bp) obtained with (GA)\(^8\) C and (CA)\(^8\) G ranged from 189 to 833 and from 225 to 569, respectively. Molecular sizes of extra bands (in bp): approximately 189, appeared at station 1 using primer (GA)\(^8\) C; and approximately 430, appeared at stations 1–4 using primer (CA)\(^8\) G whereas there were disappearing of some normal bands (in bp): approximately 443 at station 1; 379, 665 and 833 at station 2; 379, 443, 655, 833 at station 3; 443 and 655 at station 4 using primer (GA)\(^8\) C and approximately 476 at station 1; 392 and 476 at station 2; 392 and 476 at station 3; 476 at station 4 using primer (CA)\(^8\) G (Figure 3 and Tables 3–7). Decreases and increases in band intensities were found to be existed at all stations in comparison with control station (Figure 3 and Tables 3 and 5).

4. Discussion

4.1. The levels of radioactivity readings

According to Table 1, compared to the control (station 5), very high radioactivity readings were recorded at station 2 (between 36 and 100 mR/h and even between 200 and 300 mR/h in the disturbed parts of the soil) where the radioactive materials buried and the radiation level was relatively higher at station 1 (between 16 and 25 mR/h) where the uranium processing plant located compared to the other stations. Since causing genetic mutations, cancer formation, and finally cell death, radiation has lethal effects at both the cell and the organism levels (Tominaga et al., 2004; Wright & Coates, 2006), and certain organisms show resistance to radiation to some extent (Moller & Mousseau, 2016). The reason for the mutation formations detected on the genomic DNA of the plant species used in this research could be the high radiation levels seen in stations, especially in station 2 (Moller & Mousseau, 2015).
Occurrence of uranium in the plant parts and in co-located soils

The normal worldwide distributions of uranium (in mg kg$^{-1}$ DW) in uncontaminated soils and plants are in ranges of 1.9–4.4 (Kabata-Pendias, 2001) with an average 2.7 (Reimann & Caritat, 1998). U levels (in mg kg$^{-1}$ DW) determined as between 1–10 and <1 were defined as moderate and low accumulator plants by...
Table 5. Representative results of ISSR profiles using primer (CA)8 G. Alterations were indicated as appearing and disappearing bands, and changes in band intensities.

| Molecular sizes of bands (in bp) obtained from control station | Station 1 | Station 2 | Station 3 | Station 4 |
|---------------------------------------------------------------|-----------|-----------|-----------|-----------|
| 569                                                           | %12.5(−)  | %28.6(−)  | %38.1(−)  | %14.7(−)  |
| 476                                                           | Disappearing of normal band | Disappearing of normal band | Disappearing of normal band | Disappearing of normal band |
| 430                                                           | %14.3(−)  | %35.0(−)  | %38.7(−)  | %20.7(−)  |
| 392                                                           | Appearing of new band | Disappearing of normal band | Appearing of new band | Disappearing of normal band |
| 310                                                           | %11.0(−)  | %35.0(−)  | %38.7(−)  | %20.7(−)  |
| 225                                                           | %47.6(−)  | %38.7(−)  | %35.0(−)  | %38.7(−)  |

Increase and decrease in band intensities were shown in % with plus (+) and minus (−), respectively, compared to bands obtained from control station.

Table 6. Variations identified as numbers and molecular sizes (in bp) of appearing and disappearing bands in ISSR profile of P. abrotanoides using primer (CA)8 G.

| Stations | Numbers of bands obtained using primer (CA)8 G | Band sizes (in bp) |
|----------|-----------------------------------------------|-------------------|
|          | Numbers of bands                              | A (appearing)     | B (disappearing) |
|          | (n) a (appearing) b (disappearing)            |                   |                  |
| Station 1| 6 1                                           | 430 476           |
| Station 2| 6 1                                           | 430 476           |
| Station 3| 6 1                                           | 430 476           |
| Station 4| 6 1                                           | 430 476           |

a: numbers for appearing of new bands, b: numbers for disappearing of normal bands;
A: sizes for appearing of new bands, B: sizes for disappearing of normal bands.

Table 7. Number and intensity changes in ISSR profiles of P. abrotanoides obtained using primer (GA)B C and primer (CA)8 G in comparison with radiation dose at stations where the experiments were carried out where the experiments were carried out.

| Station | Radiation dose (in mR/h) | Numbers of bands using primer (GA) B C | Numbers of bands using primer (CA) B G |
|---------|--------------------------|----------------------------------------|----------------------------------------|
|         | Total (n) a d           | Total (n) c d                          |                                        |
| Station 1 | 36–100                 | 7 5 -                                   | 6 3 1                                  |
| Station 2 | 16–25                   | 6 3 1                                  | 6 - 3                                  |
| Station 3 | 18–21                   | 6 1 1                                  | 6 - 3                                  |
| Station 4 | 17–20                   | 6 4 -                                   | 6 - 4                                  |

The normal lower and upper levels (in mg kg−1 DW) of element contents found in plant species and in soil are given in Table 9 given below along with related literature.

4.3. Assessments on the element contents found in the plant parts and in co-located soils

The normal lower and upper levels (in mg kg−1 DW) of element contents found in plant species and in soil are given in Table 9 given below along with related literature.

The concentrations of Fe, Mg (except station 1, found to be higher than normal limits), Mn, Ni, and Zn were found to be within normal limits in soils at all stations, whereas the concentrations of Ca (except station 1), K (except station 1), and Na were found to be lower than normal lower limits (Table 2). And even if Fe, Mg, Mn, Ni, and Zn concentrations were within normal limits, the relative higher concentrations of

Markert (1992). The U level in soil samples was detected as higher than upper normal limit at all stations and the U level had a tendency in getting gradual increase toward to station 2. U concentrations were found to be in ranges of <0.01–0.06 mg kg−1 FW (Kabata-Pendias & Pendias, 2001). U levels in the leaf and root of the plant samples were found to be higher than normal limits at all stations (except control station for leaf part, the U level was within the normal limits) and although U levels were detected within normal limits in the stem of the plant samples at stations 3, 4, and 5 (control), U levels were higher than normal limits at stations 1 and 2. Once again, the U levels in the plant samples had a tendency in getting gradual increase toward to station 2 (Table 2). It should be noted here that the effects of U are expected to be much higher because of burial of the enriched radioactive uranium.

Table 8. Correlation coefficients between the elements determined in the plant parts of P. abrotanoides and in the co-located soil samples used in this study.

| Correlation Matrix (r) | Fe | K | Mg | Mn | Na | Ni | U | Zn |
|------------------------|----|---|----|----|----|----|---|----|
| Ca                     | .524** | −.267 | .844** | −.539* | −.240 | .551* | .729** | .380 |
| Fe                     | −.504* | .341 | .992** | −.214 | .981** | .889** | .648** | .223 |
| K                      | −.135 | −.448* | −194 | −.447* | −.579** | .289 | .700** | .700** |
| Mg                     | .364 | −.174 | .368 | .575** | .289 | .700** | .700** | .700** |
| Mn                     | −.240 | .977** | .879** | .700** | .700** | .700** | .700** | .700** |
| Na                     | −.179 | −.181 | −.304 | .861** | .684** | .476* | .476* | .476* |

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
them (except Mg, found to be higher at station 1) were noticed at station 2 (Table 2). In addition, the concentrations of Ca and K were found to be lower than the normal lower limits at all stations, except station 1 for both elements. The Fe, Mg (except stations 4 and control, found to be higher than the normal upper limits), Na, and Ni concentrations were recorded within normal limits in the stem parts of the plant at all stations whereas Ca, Mn (except station 2, was within normal limits) and Zn concentrations were found to be lower than normal lower limit in the stem parts of the plant at all stations (Table 2). A relative higher concentration was recorded at station 1 for Ca in comparison with the other stations although it was lower than the normal lower limit. The concentrations of Ca (except stations 1, 2, and control, were lower than normal limits), K (except station 1, was higher than the upper limit), Mn, Na, Ni, Pb, and Zn (except station 4, was lower than normal limits) concentrations were determined as within normal limits in the leaf parts of the plant at all stations whereas Mg concentration was in ranges of higher than acceptable limits in the leaf parts of the plant at all stations (Table 2). But it was noticed that although the Mg concentration was higher than normal upper limit at stations 1 and 2, the relative low concentrations for Mg were detected in comparison with the other stations. And the element concentrations in the root parts of the plant at all stations: were within ranges of normal limits for Ca (except station 1 and control, were lower than the normal lower limit), Mg, Mn, Na, and Ni; and were lower than the normal limits for Zn (Table 2). Once again, although the Mg concentration was within normal limits at stations 1 and 2, the relative considerable lower concentrations for Mg were detected in comparison with the other stations.

The data proved that uptake and accumulation of certain elements were altered extensively in *P. abrotanoides* grown at the sites having radioactive leakage causing reductions on the uptake pattern of certain elements and increments on that of others in comparison with control. The data imply that the high
level of radiation in the area causes alterations on mineral nutrition uptake and metabolism of the plant used as the study material.

4.4. The results of Pearson correlation analysis

According to our results from the Pearson correlation analysis given in Table 8, U influenced the mineral nutrient uptake by the plant and had alterations on the mineral nutrient metabolism. For K, it can be said that U directly reduced its absorption from the soil and the plant tried to correct this situation as by Fe uptake. The relationships between other elements appeared to be significant results of the plant's response to maintain its biological balance. The element contents of plant parts and soil samples from the stations were given in Table 2, also consisting MANOVA with Tukey test results.

According to the Tukey test results, it was determined that there were significant differences between localities for the concentrations of Ca, Fe, Mg, U, and Zn elements. Although the samplings were conducted at five different locations for these five elements, the results showed that these five elements were divided into three classes and significant differences were occurred between them. For U, stations 1 and 4 were formed a group while stations 3 and 5 were formed another group, and station 2 was separated from them. For Ca and Mg, stations 1 and 2 were constituted as one group while stations 3 and 4 were placed together as another group, and station 5 as control was separated from them. For Fe, stations 1, 2, and 3 were placed together as a group while stations 4 and 5 were separated independently from the others. For Zn, stations 2 and 4 were constituted as a group while stations 3 and 5 were placed together as another group, and station 1 was separated from them.

4.5. Evaluation of genotoxicity in P. abrotanoides

For evaluating genotoxic effects of radioactive leakage caused by the former uranium waste dump on the genomic DNA of the leaf cells of P. abrotanoides, a comparative analysis was performed using profiles obtained from the ISSR-PCR experiments. For the analysis, 2 out of the 15 ISSR primers produced distinguishable banding patterns while the others failed for yielding banding profiles produced using the samples collected from the sites research done. The comparisons between the ISSR profiles were carried out according to the alterations (i.e. increase or decrease in band intensities and the loss or the gain of bands) occurred on the specific ISSR banding patterns. The band profiles of the amplification products obtained with: primer (GA)8 C produced 1 new band appeared in the samples collected from station 1; and primer (CA)8 G produced 4 new bands (430 bp) appeared in the samples collected from stations 1, 2, 3, and 4 (one at each station) (Figure 3 and Tables 3–7) whereas 10 normal bands (379, 443, 655, 833 bp) were disappeared in the samples collected from stations 1, 2, 3, and 4 employing primer (GA)8 C: one at station 1; 3 at station 2; 4 at station 3; and 2 at station 2 and 6 normal bands (392 and 476 bp) were disappeared in the samples collected from stations 1, 2, 3, and 4 employing primer (CA)8 G: one at station 1; 2 at station 2; 2 at station 3; and 1 at station 2 (Figure 3 and Tables 3–7). Decreases and increases in band intensities were noticed at all ISSR-PCR profiles representing the samples collected from all stations research carried out using primer (GA)8 C and primer (CA)8 G (Figure 3 and Tables 3–7).

Similar reports previously done by other authors have consistency with ours showing DNA alterations induced by genotoxins using ISSR markers. Examples are: Gamma-radiation exposure resulted in emerging of different ISSR-PCR patterns in Egyptian Soybean Varieties (Gaafar et al., 2017); Difenoconazole- and tebuconazole-induced changes in the amplification profiles of the simple sequence repeats and intersimple sequence repeats in the root tips of Allium cepa (Bernardes et al., 2015); Lead-induced alterations in the ISSR profiles were observed following ascending lead exposure in Pistia stratiotes (Neeratanaphan et al., 2014); Ethylmethane sulfonate exposure caused substantial alterations on genetic materials of Artemia franciscana and Artemia parthenogenetica detected by in the ISSR patterns (Sukumaran & Grant, 2013); Genotoxic effects of Cd, Pb, and Zn using ISSR markers on Sphagnum palustre (Sorrentino et al., 2017) and Eruca sativa (L) (Al-Quraiin, 2010) were shown. This may be due to damage existed on genomic DNA. Excessive exposure on the samples due to radioactive leakage may cause in generating different types of damage occurred on the genomic DNA, including single- and double-strand breaks, modified bases, abasic sites, DNA-protein cross-links and oxidized bases (Alhumaydhi et al., 2020; Gonzalez-Hunt et al., 2018; Hsiao & Stapleton, 2004). Alterations on DNA structure resulting from the cases involving great variety of lesions and mutations can influence the kinetics of PCR (Kuriakose & Prasad, 2008; Mo et al., 2020; Ozijigt et al., 2016). Thus, appearances/disappearances as new or losses of normal bands or increases/decreases in band intensities in ISSR-PCR banding profiles could have been arisen from the availability or absence of priming site/s in conjunction with these structural modifications in DNA sequences (Kamal et al., 2020; Rai et al., 2018).

4.6. Final remarks

The data from representing radiation-induced ISSR-PCR profiles along with data from radioactivity readings and
ICP-MS data for determination of uptake patterns of certain elements were used for revealing current state and impacts of radioactive contamination on living organisms caused by uranium waste dump. A phenotypic measurements were not performed between the exposed and unexposed (from station 5) intact plant samples. So, no data are available for pheno- typic comparison. The extent of the DNA damage, done by radioactive contamination, was serious on the leaf cells of P. abrotanoides in comparison with control, especially at station 2 recorded. And, in conjunction with this, mutations on the genetic material as existing of new bands and disappearing of normal bands and increases and decreases in band intensities compared with control were noticed at all stations. Therefore, there are two possibilities for explanation of this: 1) mutations on genomic DNA of the plant used in this study due to extensive radioactive leakage occurred at the station 2 were transferred from generation to generation and spread out to the other areas or 2) the existing radioactive leakage was not limited to the station 2 and the occurrences of mutations were detected in all stations (except control) due to the fact that there is leakage toward to other stations. The second possibility is more real because of radiation readings recorded at other stations were higher than control station although the reading levels were not extensive. The radiation readings (in mR/h) revealed that the levels of radiation leakage were extensive, especially at station 2 where the radioactive material buried in comparison with control.

5. Conclusion

Approximately 600,000 tons of radioactive waste were buried in an area near Kaji-Say Village located very close to Lake Issyk-Kul. The radiation leakage readings detected at the waste dump site reach up to 300 mR/h. At present, under inadequate maintenance conditions, the former radioactive waste dumpsite is left unprotected. At present, due to the pressure of natural and anthropogenic reasons, the structural foundations of mine waste dumps are gradually losing their integrities. If radioactive leakage occurs in this area, Lake Issyk-Kul, one of the most beautiful places in the world will be contaminated. Considering that more than 400,000 people live in the towns and villages surrounding the lake (United Nations, General Assembly, 2001), the consequences of contamination can be severe and our data suggest that the level of radioactive leakage detected in the area could cause problems in people living around.

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Informed consent

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