Concentration- and time-dependent effects of strontium on *Lens culinaris* Medik.

Hakan Sepet¹* and Murat Çanlı²

1 Kırşehir Ahi Evran University, Engineering and Architecture Faculty, Department of Environmental Engineering, TR-40100 Kırşehir, Turkey
2 Ahi Evran University, Mucur Vocational School, Department of Chemistry and Chemical Processing Technologies, TR-40500 Mucur, Kırşehir, Turkey
* correspondence: hakansepet@gmail.com

ABSTRACT:
This study investigates how strontium (Sr) ions act on meristematic root tip cells of lentil (*Lens culinaris*) with changing parameters (time and concentration). Plant seeds were exposed to both a standard solution of Sr for different lengths of time (1/4, 1/2, 1, 2, 4, 8, 12, 16, 20 and 24 hours) at a fixed concentration of 1.0 molL⁻¹ (M) and Sr ions at various concentrations (0.05, 0.1, 0.25, 0.5 and 1.0 M) for a certain length of time (12 hours). The seeds treated with Sr were made to sprout and microscopic examination focused on the root tips. The aim of microscopic examination was to clarify chromosomal abnormalities of cell division. Microscopic examination showed that various abnormalities occurred in cells of the seedlings, abnormalities such as chromosome adherence, chromosome breakings, bridge chromosomes, chromosome dispersion, chromosome shrinking, fish bones and ring chromosomes. Those abnormalities were detected several times for each treatment depending on the different periods and concentrations. Adsorption and absorption of Sr inside lentil seeds were detected by the spectroscopic method. Removed and excess amounts of Sr ions were found by spectroscopic determination. Statistical evaluation of the results was used in order to reveal the differences and similarities. The results showed that while there is a positive correlation with the concentration parameter, there is a negative correlation with the time parameter. Over 90% of Sr was removed from the solution during 12-hour exposure. Lentil seeds can be accepted as good bioaccumulators of Sr ions only for an exposure period shorter than 12 hours at an Sr concentration of 1 M.

INTRODUCTION
Cytogenetic studies are vital to an understanding of plant responses to outside influences (Saxena et al. 2009; Özdemir et al. 2015; Garlich et al. 2016). Andronic (2012) examined the effect of viruses on tomato plants and DNA. Cytogenetic effects of metals, especially heavy metals, are in the focus of research efforts because of the huge amount of environmental pollution caused by metal-based industries (Aksoy & Deveci 2012; Pesnya 2013; Sepet et al. 2014; Özdemir et al. 2015). Those pollutants can have a direct effect on plant cytogenetics by causing small or large chromosomal changes (Gupta 2006). Plants uptake metals from soils and accumulate them in different parts, from roots to leaves (Baranowska-Morek & Wierzbicka 2004; Maestri et al. 2010; Sepet et al. 2014).

Metals have a positive effect on plant growth until their concentration reaches a certain point (Tangahu et al. 2011; Bhargava et al. 2012; Çanlı 2018; Sabovljević 2013; Sepet et al. 2014; Özdemir et al. 2015). Those pollutants can have a direct effect on plant cytogenetics by causing small or large chromosomal changes (Gupta 2006). Plants uptake metals from soils and accumulate them in different parts, from roots to leaves (Baranowska-Morek & Wierzbicka 2004; Maestri et al. 2010; Sepet et al. 2014).
et al. 2018), and they also change the structure of chromosomes inside the plant (Özdemir et al. 2015; Çanlı 2018). In several investigations, six types of alteration in chromosomes were named, viz., bridges, C-mitosis, fragments, laggards, multipolarity and stickiness (Kuczyński et al. 2016; Özkul et al. 2016). Moreover, Utsunomiya et al. (2002) described abnormalities such as chromosome segregation, absence of cytokinesis, cytomiexitis, cell fusion, irregular cell shape, chromosome bridges and genomic separation. According to Özdemir et al. (2012), chromosome abnormalities are classified into three categories: chromosome breaking, chromosome adherence and chromosome dispersion. There is another more detailed classification that includes fish bone in addition to chromosome adherence (Özdemir et al. 2015). When chromosomes do not detach at the end of the metaphase and remain adherent as indicated by Pekol et al. (2016), they look sticky (Wang et al. 2014). This appearance is referred to as “fish bone” in the latter study. The cytogenetic effects of metals have remained in the focus of recent research efforts (Kiran & Şahin 2005; Janas et al. 2010; Sepet et al. 2014; Özdemir et al. 2015).

Strontium (Sr) is a metal element with a bright white colour commonly found in the Earth’s crust (about 0.04% of the Earth’s crust) (MacMillan et al. 2000). Previous investigations treating the mechanism of metal pollution in plants demonstrated that Sr can be easily absorbed by them (Çelik et al. 2005; Manzatu et al. 2015; Lemtiri et al. 2016). Strontium can replace calcium in some foods and be accumulated in human bones and teeth (Burk & Allen 2019; Katzenberg & Grauer 2019). Serégine & Kozhevnikova (2005) and Kudo et al. (2015) found that metals inhibit maize seed germination in the following order: Mg > Cd > Ni > Pb > Sr. Wójcick-Kosiór et al. (2019) studied the effect of Sr on soybean sprouts in low concentrations (between 1 and 10 mM) for 72 hours. They found the best germination at 5 and 10 mM concentrations of Sr.

Accumulation of metals in plants has been of great interest to researchers (Jakovljević et al. 2019; Prica et al. 2019) because there is no need to spend more money on other removing techniques. Jakovljević et al. (2019) focused on the heavy metal tolerance of Pontederia maculata in order to gauge its usefulness as an accumulator. In addition, Prica et al. (2019) investigated bioaccumulation of heavy metals in Phragmites australis. Sevik et al. (2020) were interested in determination of lead (Pb) and magnesium (Mg) in some landscape plants. Bürger & Lichtscheidl (2019) investigated the reactions of plants to stable and radioactive Sr isotopes.

Lentil is interesting because it is able to accumulate metals and show effects of metal toxicity on its growth processes in every part (Talukdar 2013). Talukdar (2013) used lentil to understand the effects of arsenic (As) accumulation on the plant, but his work only involved exposure of the plant to solid sodium arsenate. He found that as is accumulated in different amounts in eight genotypes of lentil. Janas et al. (2010) pointed out that accumulation of copper ions occurs in vacuoles of lentil and its root cell walls. Çanlı (2018) focused on determining aberrations in Lens culinaris Medik. caused by barium (Ba) and vanadium (V) ions. Aksoy Dalgiç & Dane (2007) and Aksoy Dalgiç et al. (2007) studied the effect of fusilade at four different concentrations on seed germination, mitotic frequency, α-amylase activity and shoot and root growth in L. culinaris. Even though there are several studies treating L. culinaris (Talukdar 2013; Sepet et al. 2014; Hossain et al. 2019; Laskar et al. 2019; Mitra & Paul 2020; Pandey & Sengar 2020), no effort has been made to understand the effect of Sr on chromosomal change in L. culinaris, especially at such a high Sr concentration.

In this study, we investigated how Sr treatment of Lens culinaris for different lengths of time and at different concentrations of Sr affected root tip cells of the plant. The main objectives of the study were to clarify the influence of Sr on cell division, ascertain chromosomal abnormalities in the treated plants and establish the nature of Sr bioaccumulation.

**MATERIAL AND METHODS**

All experiments were carried out in laboratory conditions. Lentil seeds, which have 2n = 14 chromosomes, were used to clarify the effects of Sr in the study. Meristematic cells located in the root tips of lentil seedlings were prepared for microscopic examination of chromosomes as explained in Singh (2003).

Ten seeds were used for each treatment and as a control group. The seeds were chosen for being sound, plump and equal-sized. They were left in 10% sodium hypochlorite for 10 minutes in order to prevent contamination (Sepet et al. 2014). To remove excessive hypochlorite, the seeds were washed five times with distilled water, after which they were dried on filter paper at 25°C for 1 hour. A 1.0 M standard solution of Sr, which was prepared from strontium nitrate (Sigma Aldrich), was applied to the seeds for different lengths of time. Using an overdose of Sr helped us to shorten the exposure time needed to see abnormalities occurring in the cells.

Seeds were kept in the Sr standard solution at fixed concentration of 1.0 mol L⁻¹ (M) for 1/4, 1/2, 1, 2, 4, 8, 12, 16, 20 and 24 hours, a batch being left in purified water for 24 hours as a control. To establish influence of the concentration parameter, seeds were kept at different concentrations of Sr (0.05, 0.1, 0.25, 0.5 and 1.0 M) for 12 hours, the control group being left in water for the same period of time. Distilled water was then used to remove the metal solution remaining on the seeds, which were allowed to germinate in petri dishes at 20–25°C. For fixation, root tips 1.5-2 cm long were cut and left in a fixative consisting of a solution of ethyl alcohol and glacial acetic acid in a ratio of 3:1. Stock root tips were
stained by the Feulgen method (Murray et al. 1992) to get them ready for more detailed examination. In microscopic determination of cell abnormalities, homologous areas were preferred. The number of mitotic cells was determined. The mitotic index was calculated based on the frequency of division in cell images obtained under a microscope. The occurrence and frequency of chromosomal abnormalities in the cells were established by hand counting from the microscope images. A Leica DM 3000 microscope with motorised screening was used for photographing the preparations.

Spectroscopic determination of Sr ions was accomplished by flame atomic absorption spectroscopy (AAS) using a PG-990 atomic absorption spectrometer (PG Instruments, United Kingdom). After calibration of the atomic absorption spectrometer with reference material, five standard solutions of SrNO₃ were prepared to determine the calibration equation between absorbance and concentration, after which all the exposed solutions were administered in flames to the machine. With reference to the standard solutions, all calculations were converted automatically by the atomic absorption spectrometer in light of the concentration-absorbance graph. The remaining statistical procedures such as calculation of the Pearson correlation between variables were carried out using the SPSS 22 program package. The percentage of adsorbed Sr was calculated using the following equation:

\[
\% \text{ adsorbed Sr} = \frac{(\text{amount of Sr to which seeds were exposed} - \text{amount of Sr left in solution}) \times 100}{\text{amount of Sr to which seeds were exposed}}
\]

**RESULTS**

The relationship between the concentration of Sr to which seeds were exposed and adsorbed amount of it is presented in Fig. 1. Absorption of Sr ions from the seeds increased for up to 4 hours, in parallel with decrease in the mitotic index (Table 1). At 8 hours of exposure, while absorption decreased, the mitotic index increased again. Germination was not observed after 8 hours of application.

### Table 1. Mitotic index of root tip cells of *Lens culinaris* at different times of exposure to Sr in a concentration of 1M.

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>Mitotic Index ± <em>S.D.</em></th>
<th>Time (Hours)</th>
<th>Mitotic Index ± <em>S.D.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4</td>
<td>18.80±4.75</td>
<td>8</td>
<td>8.42±1.94</td>
</tr>
<tr>
<td>1/2</td>
<td>16.09±5.10</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>11.22±3.82</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>7.88±4.14</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>6.72±3.43</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td><strong>Control Group</strong></td>
<td><strong>19.12±6.35</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
with Sr (in almost 35% of the counted cells). There were also fish bone abnormalities after 1 hour of treatment (in over 35% of the counted cells). For the other abnormalities, the percentages of counted cells exhibiting them varied between 5 and 30%. The fish bone type abnormality was recorded in between 19 and 37% of the counted cells.

**Concentration parameter.** At the end of 12 hours of application at different concentrations, the amounts of the solutions which were absorbed by the seeds and the amounts remaining in solution are given in Table 3. The maximum absorption was recorded at a concentration of 1.00 M (93.8%), while the lowest absorption was at 0.05 M (6.67%). There was a direct relationship between the concentrations. The correlation between Sr concentration and the adsorbed amount of Sr was $r = 0.9964$.

**DISCUSSION**

The results indicate that Sr stress caused certain abnormalities such as fish bones, chromosome adherence, chromosome dispersions, bridge chromosomes, chromosome breaking, chromosome shrinking and ring chromosomes (Fig. 3). SEPET et al. (2014) found chromosome dispersions to be the abnormality with the highest frequency in lentil seeds (occurring in 52% of the counted seeds) after exposure to titanium for different lengths of time. Also, ÇANLI (2018) found them to be the abnormality with the highest frequency in lentil seeds after exposure to barium and vanadium, occurring in 36.4 and 26.7% of the counted seeds, respectively. In a similar study, chromosome adherence and bridge chromosomes were the most frequently observed abnormalities in cells of lettuce after exposure to an extract of *Macroptilium lathyroides* (SILVA et al. 2018). In another study, KIRAN & ŞAHIN (2005) obtained similar results in testing the effects of lead exposure on *L. culinaris*: they found that concentration increase caused a decrease in cell division and several mitotic anomalies such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges. In our study, those abnormalities showed no regular distribution. After exposures to Sr for more than 8 hours, there was no seed production in the plant. This result showed that after 8 hours, disorder in mitotic cell division prevented the plant from producing any seeds.

**Fig. 1.** Relationship between concentration of Sr to which seeds were exposed and amount of Sr adsorbed.

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Concentration of Sr to which seeds were exposed (M)</th>
<th>Amount of Sr left in the solution (M)</th>
<th>Adsorption of Sr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>½</td>
<td>1</td>
<td>0.05</td>
<td>95</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.06</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.06</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.06</td>
<td>94</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0.09</td>
<td>91</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0.19</td>
<td>81</td>
</tr>
</tbody>
</table>

Table 2. Adsorption of Sr by seeds of *L. culinaris* with changing exposure time as detected by flame-AAS (1.00 M).

<table>
<thead>
<tr>
<th>Concentration of Sr to which seeds were exposed (mg Sr/L)</th>
<th>Adsorption of Sr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>6.67</td>
</tr>
<tr>
<td>9.0</td>
<td>52.2</td>
</tr>
<tr>
<td>22.5</td>
<td>49.6</td>
</tr>
<tr>
<td>45</td>
<td>88.5</td>
</tr>
<tr>
<td>90</td>
<td>93.8</td>
</tr>
</tbody>
</table>

Table 3. Adsorption of Sr (%) by seeds of *L. culinaris* with changing concentration (at 12 h).
then let the excess back into the solution. The amount of the chemical absorbed by the seeds is returned into the solution as time progresses. The fact is that some metal ions were transferred to the plants, while others were sent out from them, precisely as claimed by Yin-Xiao et al. (1986). The correlation between Sr concentration and the adsorbed amount of Sr exists because the chemical retention rate of seeds increased with increasing concentration.

CONCLUSION

Highlights of the study can be listed as below:
- There is no simple correlation between concentration, exposure time and the type of abnormalities.
- Every concentration of Sr to which seeds are exposed unquestionably creates at least one type of abnormality in the cells.
- Up to a certain time of exposure, lentil seeds can be used as a bioaccumulator for removal of Sr.

In the course of further study, lentil seeds can be applied for purposes of bioaccumulation to fields containing a high Sr concentration. Also, the impact of strontium on uptake of macroelements and macronutrients by lentil or other plants can be investigated in the future.

REFERENCES


Ova studija istražuje kako joni stroncijuma (Sr) deluju na meristematske ćelije korena sočiva (*Lens culinaris*) sa promenljivim parametrima (vreme i koncentracija). Semena biljaka su bila izložena standardnim rastvorima stroncijuma u različitim vremenskim periodima (1/4, 1/2, 1, 2, 4, 8, 12, 16, 20, i 24 sati) u fiksnoj koncentraciji od 1.0 molL\(^{-1}\) (M) i jonima Sr u različitim koncentracijama (0.05, 0.1, 0.25, 0.5, i 1.0 M) u određenom vremenskom periodu (12 sati). Semena tretirana stroncijumom su proklijala i vrhovi korenčića su istraživani mikroskopom, sa ciljem razjašnjavanja hromozomskih abnormalnosti pri deobi ćelija. Istraživanja mikroskopom su pokaza alo anormalnosti u ćelijama klijanaca u smislu srastanja hromozoma, pucanja, hromozomskih mostova, disperzije hromozoma, njihovog skupljanja, riblje kosti i prstenastih hromozoma. Ove abnormalnosti su uočene nekoliko puta u svakom tretmanu zavisnom od različitih peroda i koncentracija. Adsorpcija i absorpcija Sr unutar semena sočiva su uočene spektroskopski. Spektroskopski su uočene i otklonjene suvišne količine Sr. Zatim, statistička evaluacija rezultata je primenjena kako bi se uočila značajnost sličnosti i razlika. Rezultati ovih analiza su sa jedne strane ukazali na pozitivnu korelaciju sa koncentracijom kao parametrom, a sa druge, na negativnu korelaciju sa vremenom. Više od 90% Sr je uklonjeno iz rastvora tokom 12-časovne ekspozicije. Semena sočiva se mogu prihvatiti kao dobri bioakumulatori jona Sr jedino pri ekspoziciji u vremenskom periodu kraćem od 12 sati pri koncentraciji od 1 M Sr.

**Ključne reči:** abnormalnosti hromozoma, bioakumulator, sočivo, citogenetički efekti