IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Impact Of Lead And Chromium On Morphological Parameters, Chlorophyll And Protein Content Of Spinacia Oleracea

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Abstract :

A study was conducted to evaluate the impact of metals like lead and chromium on morphological parameters, amount of chlorophyll and protein content of Spinacia oleracea. The absorbance and accumulation of these two metals in the edible part (leaves) of the plant was also studied. Pot experiments were carried out to treat the plants with various concentrations of both the metals and their impact on different parameters were investigated and compared with the control. It was found that all the morphological parameters, the three types of chlorophyll (a, b and total) as well as the total proteins get reduced in the plants treated with lead and chromium in comparison to control. The effect of chromium in increasing concentrations on the morphological parameters was found to be more adverse than lead, while the adverse impact of increasing lead concentrations were more on the chlorophyll and protein contents. However the metals (Pb and Cr) did not accumulate in the leaves in significant amounts.

Key words : Absorbance, Accumulation, Morphological parameters, Chlorophyll, Protein.

1. INTRODUCTION

Practices such as urbanisation, industrialization, development in agriculture, technologies are some of the evidence for the growth of a country but these activities also contribute to the increase in the environmental pollution. These practices influence the environment directly or indirectly in adverse ways causing addition of undesirable substances ; in the air and making its quality poor, in water making it unsafe for drinking and sometimes even for irrigation purposes, in soil leading to deterioration of its quality. These undesirable substances polluting the environment may be gases like sulphur dioxide, carbon dioxide, nitrogen oxide etc, soluble and insoluble matters or metals like mercury, arsenic, lead, copper, manganese, chromium, cadmium etc. The activities such as use of fertilisers, insecticides and pesticides in the agricultural fields, disposal of industrial wastes especially from smelting, pipe manufacturing etc and mining add up the metals to the air, water and soil in the environment which can affect the living beings in various ways, especially the plants. Although the use of different types of plants in absorbing the undesirable substances from the environment, especially soil and hence reducing the pollutants is frequently studied nowadays.

Numerous studies have revealed that various types of the plants have the ability to absorb and accumulate metals which affect the plant growth, metabolism and may lead to plant stress. According to [1], the barley plant bears the ability to absorb various types of heavy metals from soil irrigated with metal treated water and these adsorbed metals have the effect on growth of the plant. [2] [3], in their study have reported that the chromium exhibits toxic effects on the wheat plant and Mung Bean respectively, at its various growth phases and also on the photosynthetic potential of the plant. [2] also explained the toxic effects of chromium on the morphological characteristics and yield of two varieties of chickpea. [4] studied the potential of the okra plant to extract lead from the soil and its accumulation in roots, stem and leaves of okra plant.

In the present study we are primarily focused on the impact of metals such as lead (Pb) and chromium (Cr) on certain parameters of Spinacia oleracea (spinach plant) including morphological (growth) parameters, its chlorophyll content and the amount of protein in the spinach plant leaves. The study also aimed to check the ability of Spinacia oleracea to absorb metals from soil, to check the accumulation of metals (Pb & Cr) in the edible parts of the plants (leaves) and lastly to evaluate the extent of influence of two metals i.e Pb and Cr on various parameters of spinach plant as mentioned above.

Spinacia oleracea is an angiospermic plant belonging to the family Amaranthaceae. It is an annual plant with dark green leaves which are the edible part. The larger leaves are present more towards the base and the smaller ones at the apex of the plant. It increases to a height of about 30 cm. The flowers are yellow-green in colour and not very clearly visible. They contain numerous seeds. The plant bears a tap root system. The stem is green and slender like. The spinach leaves are widely used as vegetables and are known to have a good nutritional profile. It is rich in protein, carbohydrates, vitamins (A, C, K) and minerals, especially calcium, potassium and iron [5]. Various researchers, over the past years have studied the potential of Spinacia oleracea to absorb and accumulate metals from the soil or water used for irrigation purposes [6] [7] [8] [9] [10]. Spinacia oleracea is known to absorb various metals, most commonly lead and chromium due to its strong roots [8] but the matter of concern is the amount of metals accumulated in the leaves which are the edible part of the plant.

The lead is a widely used metal for different purposes like manufacturing of paints, corrosion resistant pipelines, batteries, electrical devices, in gasoline and as electrodes. [11]. Lead exists in the environment naturally as well as entering the environment through fertilisers and pesticides, industries, automobiles, smelters, Sewage and Urban wastes, either in the metallic forms or in the form of its oxides, chlorides and bromides [11]. These salts of lead drop on the surface of soil, water or may be present in the air hence causing entry of lead in the environment.

Chromium is a trace element required by organisms to aid in metabolism of major biomolecules (carbohydrates, proteins and lipids) but higher its concentration may be hazardous [11]. Chromium enters the environment through industries mainly involved in processing and manufacturing of various types of chemicals and minerals. It is also produced as waste from industries such as steel making, plating of metals, tanning of leather, textile dyeing, cement factories etc. The chromium is mostly added to the environment either through waste water or solid wastes from these industries and subsequently reaches to the soil, water and air [11].

Both the metals, lead and chromium are hazardous to living beings in some or the other way and may undergo biomagnification in the food chains when they are absorbed by plants from the soil, or when the plants are irrigated by metal contaminated water [12].

Lead in plants affects the morphological characteristics and the photosynthetic efficiency. It also causes inhibition of ATP formation and DNA damage by formation of free radicals in large amounts [13]. Lead mainly affects the processes like seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content [13]. According to [14] [15], chromium may cause stunted plant growth, chlorosis in new leaves, diminished photosynthesis, damage of roots and shoots and may even may finally lead to death of the plant.

2. MATERIALS AND METHODOLOGY

Experimental design :

Plastic pots filled with 1 kg of agricultural soil were used to grow the plants. The soil was obtained from agricultural land collected from the depth of around 20 cm and pretested for pH, electrical conductivity and the presence of lead and chromium. The pH was found to be 8.77, electrical conductivity 187.37 μmho/cm, lead (Pb) was not detected while chromium (Cr) was present in traces. Temperature was 20 degree celsius.

In each pot, eight seeds of almost equal size, of Spinacia oleracea were sown and allowed to germinate and grow in normal climatic conditions during the December 2022. The seeds were bought from the local market of Indore, Madhya Pradesh, India.

The whole experiment consisting of experimental and control pots was performed in triplicates. All the plants were watered twice a day with 200 ml of tap water each time initially and then 100 ml each time subsequently. Germination began after a week of sowing. Here the plants were thinned down to four per pot.

After 15 days of growth, when the plantlets were visible, the experimental plants were treated with different concentrations of lead and chromium. While no treatment was given to the control plants.

2.1 Treatment of plants with lead -

To contaminate the soil with lead (in the form of lead nitrate), the solution was prepared by dissolving 1.599 gm of lead nitrate [Pb $(N03)_2$] in 1000mL of distilled water (The solution contains 1 gm Pb/L). The stock solutions were diluted as required to prepare 50, 100, 150 mg /100 ml.

2.2 Treatment of plants with chromium -

Chromium was added in the form of potassium dichromate (K_2CrO_7) which consists of hexavalent chromium [Cr(VI)]. The stock solution was prepared by adding 2.745 gm potassium dichromate in 1000 ml distilled water to get a solution of concentration 1gm Cr/L.

The stock solution was diluted accordingly to make solutions with concentration of 20 mg/100 ml, 40 mg/100 ml and 60 mg/100 ml.

The lead was added to the experimental pots in such a way that the concentration of lead becomes 50 mg/kg, 100 mg/kg and 150 mg/kg of soil in respective pots. These pots were considered as Pb1, Pb2 and Pb3 respectively. Similarly the chromium solution was added to the experimental pots in such a way that the concentration of chromium becomes 20 mg/kg, 40 mg/kg and 60 mg/kg of soil in respective pots. These pots were considered as Cr1, Cr2 and Cr3 respectively.

Once the treatment was done, all the plants were allowed to grow till complete growth, that is, for a duration of 45 days (approximately seven weeks and then the final investigations such as determination of growth parameters, chlorophyll and protein contents, metal concentrations in treated soil and edible part (leaf) of the plant and the bioconcentration factor were done.

2.3 Measurement of soil parameters -

After 45 days of plant sowing, the pH was calculated using pH metre (Metler Toledo MA 235) and electrical conductivity (EC) using electrical conductivity analyser (Analabs concal 5). The initial value of pH was 8.77, EC was 187. 37 µmho/cm and temperature was 20 degree celsius.

2.4 Measurement of growth parameters -

Various growth parameters such as root length, shoot length,, leaf length, leaf diameter of the plant were measured using a centimetre scale according to [16]. The fresh weights of root, shoot and leaf were measured using an electronic digital balance (Wensar Arigo 994). To measure the dry weights of roots, shoots and leaves, firstly the fresh parts were dried in an oven for 12 hours at 80 degree celsius [17] and then weighed using digital balance. In addition, the whole length of all the experimental and control plants were measured. These parameters were determined after 45 days (approx. 7 weeks) of sowing the plants.

2.5 Estimation of Chlorophyll -

The extraction and estimation of chlorophyll a, chlorophyll b and the total chlorophyll content of all the plants was completed using 80% acetone v/v according to the method given by [18] using the following equations -

$$\text{Total Chlorophyll: } 20.2(A\ 645) + 8.02\ (A663)$$

$$\text{Chlorophyll a: } 12.7(A663) - 2.69\ (A645)$$

$$\text{Chlorophyll b: } 22.9(A645) - 4.68\ (A663)$$

2.6 Estimation of Protein -

The extraction and determination of the amount of total proteins in all the experimental and control plants was determined using the method of Folin and Lowry [19].

2.7 Determination of metals (Pb and Cr) -

To determine the metals (Pb and Cr) in the treated soil and the leaves of all the experimental and control plants, the samples were first digested.

2.7.1 Digestion of soil :

The soil was digested by adding 1 gm of metal treated soil with 5 ml of nitric acid (HNO_3). The mixture was heated on a hot plate till the soil got completely dissolved. The solution obtained was cooled and 50 ml of distilled water was added. After 10 minutes, the mixture was filtered and filtrate was used for detection of metals (Pb and Cr).

2.7.2 Digestion of plant leaves :

The cleaned washed spinach leaves were air dried for a day and then oven dried at 80 degree celsius for about 48 hours and converted into uniform, fine powder with the help of a mortar and pestle. 1 gm of leaf powder was added to 10 ml conc. sulphuric acid (H_2SO_4) and boiled on a hot plate till the powder was completely dissolved followed by addition of 10 ml distilled water. With occasional stirring the boiling continued till the evolving fumes became black to yellowish in colour. The material was cooled and filtered. The filtrate was used for metal (Pb and Cr) detection.

The concentration of lead and chromium in digested soil and leaf samples was done using Atomic Absorption spectrophotometer (Perkin - Elmer, PinAAle 900H). The wavelength used for lead was 283.31nm and that for chromium was 357.87. Slit size used for Pb and chromium was 0.7 nm. Fuel/Gas used in the cylinder was acetylene/air.
All the reagents used were of analytical grade and throughout the experiment distilled water was used wherever required.

2.8 Measurement of BCF -
After determining the metal (Pb and Cr) concentrations in leaf (edible part) samples of control and treated plants and the respective soil samples, their ratio was calculated to assess the amount of metals absorbed and accumulated in the leaves. This ratio of metals concentration in the leaf to the metal present in the soil is known as the bioconcentration factor (BCF) which is calculated by the formula as given by [4] [20] and [21]

$$\text{BCF} = \frac{\text{Conc. of metal (Pb / Cr) in leaf (edible part) (in mg/Kg)}}{\text{Conc. of metal (Pb /Cr) in soil (in mg/Kg)}}$$

According to [22] if BCF is found to be less than 1, it indicates less absorption of metals from soil to plant. On the contrary, if BCF is found to be more than 1, it indicates more absorption of metal by the plant.

2.9 Statistical Analysis
The results were expressed as mean and standard deviation of determined values of all the parameters. These mean values and standard deviations of all the obtained values for bioconcentration factors, growth parameters, soil parameters and metals (Pb and Cr) in soil and leaf samples were calculated using excel sheet (windows 10).

3. RESULT AND DISCUSSION

3.1 **Soil parameters** - The presence of lead and chromium in soil affected the pH which decreased to 7.23 from the initial pH (8.77). However, electrical conductivity was found to be almost the same. There was no change observed in the temperature also.

3.2 **Morphological (growth) parameters** - Table 1 below shows the effect of increasing concentrations of lead and chromium on morphological parameters in the terms of mean and standard deviation of morphological parameters (all lengths in cm ; weights in gms).

Table 1 : Mean and standard deviation of Morphological parameters (all lengths in cm ; weights in gms

S. No.	Growth parameters	Control	Pb1	Pb2	Pb3	Cr1	Cr2	Cr3
1.	Root length (cm)	6.8 ± 0.26	6.3 ± 0.43	6.0 ± 0.17	5.8 ± 0.1	6 2 ± 0.26	6.1 ± 0.2	5.9 ± 0.17
2.	Root fresh weight (gm)	0.352 ± 006	0.322 ± 0.012	0.308 ± 0.008	0.288 ± 0.006	0.316 ± 0.005	0.291 ± 0.004	0.276 ± 0.009
3.	Root dry weight (gm)	0.038 ± 0.009	0.034 ± 0.009	0.031 ± 0.005	0.028 ± 0.007	0.032 ± 0.005	0.031 ± 0.003	0.026 ± 0.014
4.	Shoot length (cm)	27.4 ± 0.55	24.1 ± 0.4	23. 8 ± 0.49	19.2 ± 0.15	23.4 ± 0.26	22.5 ± 0.55	19.0 ± 0.17
5.	Shoot fresh weight (gm)	2.793 ± 0.045	2.109 ± 0.25	1.886 ± 0.026	1.710 ± 0.009	2.068 ± 0.15	1.832 ± 0.04	1.744 ± 0.007
6.	Shoot dry weight (gm)	0.363 ± 0. 18	0.354 ± 0.005	0.338 ± 0.49	0.332 ± 0.018	0.331 ± 0.017	0.337 ± 0.02	0.276 ± 0.017
7.	Leaf length	9.2 ±	8.8 ±	8.2 ±	7.6 ±	8.4 ±	7.9 ±	7.5 ±

	(cm)	0.2	0.55	0.17	0.48	0.4	0.26	0.36
8.	Leaf diameter (cm)	7.8 ± 0.26	7.6 ± 0.26	7.4 ± 0.17	6.9 ± 0.2	7.3 ± 0.36	7.1 ± 0.55	6.9 ± 0.1
9.	Leaf fresh weight (gm)	0.889 ± 0.08	0.846 ± 0.07	0.813 ± 0.05	0.772 ± 0.04	0.832 ± 0.07	0.802 ± 0.06	0.754 ± 0.04
10.	Leaf dry weight (gm)	0.207 ± 0.008	0.198 ± 0.002	0.184 ± 0.003	0.178 ± 0.002	0.195 ± 0.005	0.175 ± 0.002	0.166 ± 0.003
11.	Whole length of plant (cm)	34.2 ± 0.3	32.8 ± 0.05	32.1 ± 0.3	31.8 ± 0.17	31.8 ± 0.7	31.4 ± 0.4	31.0 ± 1.2

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Various parameters associated with roots, shoot and leaves of Spinacia oleracea were studies in the control plants as well the plants treated with increasing concentrations of lead and chromium and it was discovered that due to the presence of the two metals i.e Pb and Cr almost all the parameters exhibited a decrease by various percentages (Figure 1). Maximum effect was observed on roots then shoot and then on leaves. Moreover, the effect of chromium was more deleterious than lead. As depicted in the figure 1, the root length (Rt. lng.) was decreased by 7.35%, 11.76%, 14.70% in Pb1, Pb2 and Pb3 while by 8.82%, 10.29% and 13.23% in Cr1, Cr2 and Cr3 respectively. The reduction in percentage of root fresh weight (Rt. Fr. Wt.) was 8.52, 12.5, 18.18, 10.22, 17.32 and 21.59 and root dry weight (Rt. dry wt.) was 10.1, 16.3, 26.0, 13.2, 17.6, 30.9 in all the pots with increasing concentrations of lead and chromium. The reduction % in shoot length (st. lng.) as 13.04, 13.13, 29.92, 14.59, 17.88 and 30.65 respectively. Shoot fresh weight (st. fr. wt.) reduction percentages were 24.43, 32.47, 38.77, 25.95, 34.40 and 37.55. Shoot dry weight (st. Dr. wt.) reduction percentages were found to be 2.3, 6.8, 8.3, 2.7, 7.1 and 9.7. Similarly the different parameters of leaves were also reduced as leaf length (leaf lng.) reduction percentages were 4.34, 10.86, 17.39, 8.69, 14.13 and 31.0 while leaf diameter (leaf diam.) were decreased by 2.56%, 5.12%, 11.53%, 6.41%, 8.97%, 11.53% respectively in all the lead and chromium treated plants. Fresh leaf weights leaf fr. wt.) were reduced by 4.83%, 8.54%, 13.16%, 6.41%, 9.78% and 15.18%. A decrease in the percentage of dry weight of leaves (leaf dr. wt.) were 4.34, 11.11, 14.0, 5.79, 15.45 and 19.80 respectively. Whole length (Whl. lng) of the Spinacia oleracea plants were decreased by 4.09%, 6.14%, 7.01%, 7.01%, 8.18% and 9.35% in all lead and chromium treated plants as compared to control.

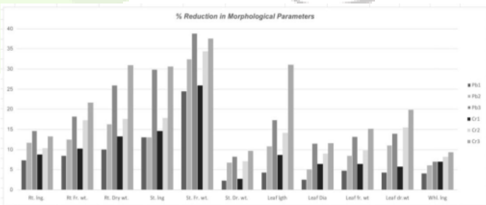

Figure 1 : Reduction % of morphological parameters due to lead and chromium in experimental plants.

3.3 Chlorophyll Content - Table 2 below shows the results (effect of increasing concentration of lead and chromium on chlorophyll content of Spinacia oleracea) of chlorophyll content in terms of mean and standard deviation of obtained chlorophyll values. Chla = chlorophyll a ; Chlb = chlorophyll b ;Total chl. = Total chlorophyll, values in mg/gm) -

Table 2 : Mean **and standard deviation of chlorophylls (mg/gm)**

S. No	Chloro-phyll content	Control	Pb1	Pb2	Pb3	Cr1	Cr2	Cr3
1.	Chla (mg/gm)	17.98 ± 0.15	12.98 ± 0.58	12.51 ± 0.27	10.62 ± 0.61	14.11 ± 0.11	14.03 ± 0.11	10.13 ± 0.22
2.	Chlb (mg/gm)	6.35 ± 0.29	5.65 ± 0.56	4.20 ± 0.14	2.88 ± 0.35	5.34 ± 0.17	4.58 ± 0.45	4.34 ± 0.23
3.	Total chl. (mg/gm)	24.34 ± 0.32	18.64 ± 0.26	16.79 ± 0.32	13.86 ± 0.59	19.45 ± 0.57	18.61 ± 0.33	14. 47 ± 0.23

The mean value of chlorophyll content (in mg/gm) in control plants were found to be 17.98, 6.35 and 24.34 for chlorophyll a, chlorophyll b and total chlorophyll respectively (Table 2).
The plants treated with different concentrations of lead showed reduction in their chlorophyll content (Figure 2). The Pb1 showed 27.18 % reduction in its chlorophyll a, 11.02 % in chlorophyll b and 23.41 % in total chlorophyll content. In Pb2 the reduction in chlorophyll a, chlorophyll b and total chlorophyll was 30.03 %, 33.85 % and 31.01 % respectively. The reduction percentage of the three types of chlorophyll in Pb3 was 40.93, 54.64 and 43.05, indicating that as the lead concentration in the soil was increased, the chlorophyll content (all the three types) showed a significant decrease. Similarly, the chromium treatment also affected the amount of all three types of chlorophylls (Figure 2). In Cr1 the decrease in % of chlorophyll a was found to be 21.52, chlorophyll b was 15.90 and total chlorophyll was 20.09. In Cr2 the reduction percentage was 21.96 (quite similar to Cr1) for chlorophyll a, 27.87 for chlorophyll b and 23.54 for total chlorophyll. If we compare the effect of lead and chromium on chlorophyll content of spinach plants, the effect of lead is found to be more adverse as compared to chromium.

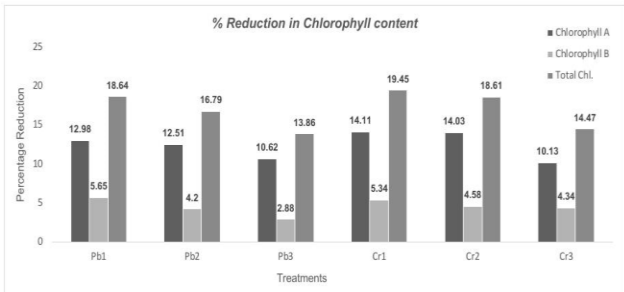

Figure 2 : Reduction % of chlorophyll content in experimental plants.

3.4 Protein Content - Table 3 indicates the results (effect of increasing concentration of lead and chromium on protein content of Spinacia oleracea) as mean and standard deviation for protein content **(values were obtained in mg/100 gm of leaf).**

Table 3 : Mean and standard deviation of Proteins (mg/100 gm)

S. No	Protein content (mg/100 gm)	Control	Pb1	Pb2	Pb3	Cr1	Cr2	Cr3
1.	–	2.94 ± 0.35	2.81 ± 0.2	2.48 ± 0.08	2.06 ± 0.42	2.74 ± 0.18	2.53 ± 0.26	1.89 ± 0.17

The effect of lead and chromium treatment was also observed on the total protein of spinach leaves. The percentage of total proteins in the lead treated spinach plants as well as chromium treated spinach plants was found to be reduced (Figure 3). The total amount of proteins in the control plants was calculated as 2.94 mg /100 gm (Table 3). In Pb1 it was reduced by 4.42 %, by 5.64 % in Pb2 and by 29.93 % in Pb3. The reduction percentage in Cr1 was 6.80, in Cr2 it was 13.94 ,while in Cr3 total reduction percentage was 35.71. This aspect indicated that although the treatment of spinach plants with lead and chromium decreased the protein content but the higher levels as in Pb3 and Cr3 were significant.

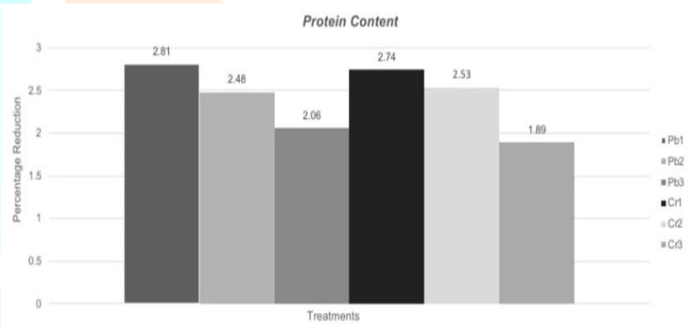

Figure 3 : Reduction % of protein content in experimental plants.

Finally it can be stated that the reduction in root length, shoot length and protein content due to increasing concentrations of lead was in accordance with the results of [23].

3.5 Metals and bioconcentration factors (BCF) - Table 4 below shows the mean values and standard deviation of the amount of lead and chromium determined in leaves and soil and the derived BCF (Bioconcentration factor).

Table 4 : Mean and standard deviation of Lead & Chromium and BCF

Heavy metal concentration				
S. No.	**Treatments**	**In leaf (mg/Kg)**	**In soil (mg/Kg)**	**BCF**
1.	Pb1 (50 mg/kg)	0.396 ± 0.004	3.76 ± 0.12	0.105
2.	Pb2 (100 mg/kg)	0.419 ± 0.013	4.96 ± 0.27	0.826
3.	Pb3 (150 mg/kg)	0.528 ± 0.009	6.13 ± 0.17	0.086
4.	Cr1 (20 mg/kg)	0.612 ± 0. 024	3.37 ± 0.23	0.181
5.	Cr2	0.689 ± 0.009	3.58 ± 0.16	0.192

	(40 mg/kg)			
6.	Cr3 (60 mg/kg)	0.701 $\pm$ 0.009	4.82 $\pm$ 0.30	0.145

In the above table, all the calculated BCF are less than one specifying its insignificance [22].

5. Conclusion

Different types of metals when present in the agricultural soil may affect the plants adversely if their occurrence is in elevated levels. Various parameters of such plants might be affected due to the presence of metals. The present study revealed that the metals such as lead and chromium when present in the soil, definitely affect the parameters such as morphology including root length, root fresh and dry weight, shoot length, shoot fresh and dry weight, leaf length, leaf diameter, fresh and dry weight of leaves and the whole length of the plant. The effect on leaf parameters is of more concern as they are the edible parts of the plant Spinacia oleracea. Due to the presence of both the metals (Pb and Cr) a decrease in all the morphological parameters was seen. If we compare the effect of lead with chromium, the chromium has more adversely affected the morphological parameters as compared to lead.

The stress build up due to presence of heavy metals (Pb and Cr) in the plants also found to be affecting the chlorophyll content (a , b and total) and the protein contents. The increasing concentration of these heavy metals in the soil also keep increasing the reduction percentage of growth parameters, chlorophyll and the protein content. The reduction in Chlorophyll content of Spinacia oleracea might influence the photosynthetic efficiency of the plant.

In contrast to these findings, it was found that although the lead and chromium was absorbed by the Spinacia oleracea plant, its translocation towards the leaves is insignificant. This is indicated by the bioconcentration factor being below 1 (< 1) in the present work. However, the absorption and bioconcentration might be higher in other parts of the plants (roots and shoots). Further studies on the role of Spinacia oleracea in absorption and accumulation of heavy metals is required which may show significant results in this concern.

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