

Influence of various concentrations of uranium mining waste on certain growth and biochemical parameters in gram

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SUMMARY

The importance of plants is continuously increasing for clean up of contaminated and polluted ecosystems with radioactive metals. Importance of gram for the purpose is very limited in the literature. In the present investigation effects of different uranium tailing concentrations (25, 50, 75 and 100%), conditioned with soil were studied on *Cicer arietinum* L. by analysis of various growth (shoot-root length, shoot-root fresh weight, shoot-root dry weight, seed number plant⁻¹ and seed weight plant⁻¹) and biochemical parameters (chlorophyll contents and soluble leaf proteins). All the growth parameters and chlorophyll contents showed gradual but significant decrease with an increase in tailing concentration. Soluble protein (leaf) level showed maximum increase at 75 per cent tailing concentration. Plant material was not found sufficient at 100 per cent tailing concentration for analysis of any growth and biochemical parameter. Survival of gram plants over 80 days on higher tailing concentrations (up to 75%) shows that it may be helpful for revitalization of uranium mining waste.

Key Words : Uranium, Tailing, *Cicer arietinum* L., Growth parameters, Biochemical parameters, Phytoremediation

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Uranium is naturally occurring radioactive element. Comparatively little information is available with respect to uranium in soils. Its average concentration in earth's crust is 4 mg kg⁻¹ (Hursh and Spoor, 1973). The world wide data summarized by Kabata-Pendias and Pendias (1984) give uranium values in a narrow range of 0.79 to 11 mg kg⁻¹. Though the level of uranium in soils is generally low, in LOCAL areas it may exceed due to fission by products of nuclear testing and reactor operations (Entry *et al.*, 1996; Fuhrmann *et al.*, 2004), improper waste storage practices (Jones and Serne, 1995; Liator, 1995), agricultural practices (Rossler *et al.*, 1979;

Aery and Jain, 1998) and uranium tailings (Sheppard and Thiabault, 1984; Jagetiya and Purohit, 2006; 2007).

The tailings are large quantities of waste material, result of mining and milling. These consist of overburden from strip and open pit mines as well as by products from the ore proceed in milling facilities (Hossner *et al.*, 1998). The tailings are disposed at a place known as tailings dam or tailings impoundments (Jain, 1996).

Even if the tailing site is physically secure against wind or water erosion, intrusion by flora and fauna, earthquakes and chemically secure against contamination of surrounding soils, surface waters or ground waters, the retention emanating from the tailings may still give rise to an unacceptable risk to the LOCAL environment and nearby communities compared to pre-mining conditions because uranium ores and therefore, the tailings are often associated with elevated concentrations of heavy metals, giving rise to the potential for chemical toxicity from surface water or ground water contamination.

Umra mine (Udaipur, India) was in operation between years 1957 to 1962 in the form of exploratory mine (Jain, 1996).

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Table A : Various physical and chemical properties of uranium tailings amended with garden soil. (Values in the parenthesis indicate percentage increase/decrease over the control)

Tailing concentration	pH	Conductivity (m mho/cm)	Biological carbon (%)	Phosphate (Kg/hac)	Potash (Kg/hac)
0%	7.25 ± 0.07	0.74 ± 0.09	0.52 ± 0.31	26.0 ± 5.65	High
25%	7.55 ± 0.07 (4.13%)	0.73 ± 0.12 (- 1.35%)	0.52 ± 0.10 (0.00%)	24.0 ± 5.65 (- 7.69%)	High
50%	7.80 ± 0.14 (7.58%)	0.56 ± 0.14 (- 24.32%)	0.52 ± 0.14 (0.00%)	26.0 ± 5.65 (0.00%)	High
75%	8.05 ± 0.07 (11.03%)	0.37 ± 0.09 (- 50.0%)	0.45 ± 0.00 (- 13.46%)	16.0 ± 5.65 (- 38.46%)	High
100%	8.60 ± 0.28 (18.62%)	0.13 ± 0.00 (- 82.43%)	0.15 ± 0.00 (- 71.15%)	18.0 ± 0.00 (- 30.76%)	High

where High* > 337 kilogram/hectare

The benefaction of the ore resulted in the production of large amount of waste, which is lying in the form of large black heaps on the western flanks of Umra. The waste is quite inhospitable as only a few plant species are growing on their outskirts.

The conventional clean up technologies are either inadequate or too expensive and unsafe. Now a day bioremediation (the use of living organisms to control and destroy contaminants) is of increasing interest to minimize some of these problems. When green plants are used to remove heavy metals from the soil, bioremediation is known as phytoremediation. It is the most rapidly developing environment friendly and cost effective technology (Chen *et al.*, 2000; 2005).

In the present investigation growth performance of certain plants was studied in different uranium tailings to know:

- The optimum concentration of the garden soil for conditioning the tailing, which can be used for revitalization.
- The effect of different tailing concentrations on various growth and biochemical parameters of gram.
- To assess the phytoremediation potential of gram.

MATERIALS AND METHODS

Uranium tailings were collected from Umra region in plastic bags and later air-dried. Tailing was conditioned with garden soil in the ratio of 0 : 100, 25 : 75, 50 : 50, 75 : 25 and 100 : 0. The physical and chemical properties of garden soil as well conditioned tailings at various levels are described in Table A. Two kilogram of each mixture was filled in earthen pots. Ten seeds of uniform size of gram (*Cicer arietinum* L.) were sown equidistantly at the depth of 2.5 cm. All the treatments were replicated three times to record the observation. Watering was done everyday as recommended by agricultural practices and pots were placed on the greenhouse bench in completely randomized design. Seed germination and visual toxicity symptoms were noticed. Contents of chlorophyll and soluble proteins (leaf) were

estimated according to the method described by Arnon (1949) and Bradford (1976), respectively.

Plants were uprooted for the observation of shoot-root length; shoot-root fresh weight and shoot-root dry weight. Plants were dried in an oven at 80°C for 48 hours and weighed for dry weight.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Growth parameters :

The results are shown in Tables 1 to 5. Early visible symptoms of the toxicity were the disturbance of germination and yellowing of leaves. Germination percentage showed negative correlation with an increase in tailing concentration. Seeds were either failed to germinate or died in seedling stage at 100 per cent tailing concentration (Table 1).

Shoot and root length as well as shoot and root fresh and dry weight remarkably decreased at all the tailing concentrations (negative correlation was observed). Maximum decrease in above mentioned parameters were 82.2, 42.4, 95.8, 70.3; 95.3 and 83.33 per cent, respectively over the controls was observed at 100 per cent tailing concentration (Table 2 and 3).

All the tailing concentration resulted in significant decrease in seed number plant⁻¹ as well as seed weight plant⁻¹ over the controls 54.6 and 63.5 per cent decrease was observed in seed number plant⁻¹ and seed weight plant⁻¹, respectively at 50 per cent tailing concentration. Plants did not show any reproductive growth on 75 and 100 per cent tailing concentrations (Table 4).

Biochemical parameters :

The results are shown in Table 5. A gradual but significant reduction in the content of chlorophyll a, b and total chlorophyll was observed with increasing concentration of uranium tailing. Maximum decrease was found at 75 per cent tailing concentration which was 32.37, 38.46 and 32.82 per cent, respectively for chl a, chl b and total chlorophyll (Table

Table 1 : Showing effect of various uranium tailing concentrations on percentage seed germination and visual toxicity symptoms of gram (values in the parenthesis indicate percentage increase/decrease over the control)

Tailing concentrations	Seed germination percentage	Visual toxicity symptoms
0%	100.00 ± 0.00	-
25%	100.00 ± 0.00 (0.00%)	Yellowing of leaves
50%	93.33 ± 1.53 (-6.70%)	Yellowing of leaves
75%	73.33 ± 1.53 (-26.67%)	Yellowing of leaves
100%	50.00 ± 2.00 (-50.00%)	Yellowing of leaves
S.E.	0.76	
C.D. (P=0.05)	2.395	
C.D. (P=0.01)	3.407	
r	-0.9271**	
r ²	0.8595	
Y	191.369 ± (-) 1.6964	

*and ** indicate significance of values at P=0.05 and 0.01, respectively

Table 2 : Showing effect of various uranium tailing concentrations on shoot-root length (cm) of gram (values in the parenthesis indicate percentage increase/decrease over the control)

Tailing concentrations	Shoot length	Root length
0%	27.35 ± 2.75	7.64 ± 1.68
25%	25.81 ± 2.14 (-5.63%)	7.26 ± 0.40 (-4.97%)
50%	17.35 ± 2.41 (-36.56%)	7.09 ± 0.97 (-7.20%)
75%	5.30 ± 2.16 (-80.62%)	5.61 ± 1.69 (-26.57%)
100%	4.88 ± 3.59 (-82.16%)	4.40 ± 0.08 (-42.41%)
S.E.±	1.54	0.673
C.D. (P=0.05)	4.848	2.119
C.D. (P=0.01)	6.896	3.014
r	-0.9599**	-0.9465**
r ²	0.9214	0.8959
Y	106.795 ± (-) 3.5193	226.322 ± (-) 27.5504

*and ** indicate significance of values at P=0.05 and 0.01, respectively

Table 3 : Showing effect of various uranium tailing concentrations on fresh and dry mass (g plant⁻¹) of gram (values in the parenthesis indicate percentage increase/decrease over the control)

Tailing concentrations	Fresh mass		Dry mass	
	Shoot	Root	Shoot	Root
0%	3.70 ± 0.02	0.65 ± 0.01	0.38 ± 0.09	0.06 ± 0.00
25%	3.42 ± 0.01 (-7.57%)	0.58 ± 0.04 (-10.77%)	0.36 ± 0.11 (-5.26%)	0.05 ± 0.01 (-16.67%)
50%	1.91 ± 0.02 (-48.38%)	0.41 ± 0.01 (-36.92%)	0.15 ± 0.03 (-60.53%)	0.04 ± 0.01 (-33.33%)
75%	0.71 ± 0.15 (-80.81%)	0.29 ± 0.00 (-55.38%)	0.04 ± 0.02 (-89.47%)	0.03 ± 0.00 (-50.00%)
100%	0.16 ± 0.04 (-95.67%)	0.18 ± 0.02 (-72.31%)	0.02 ± 0.01 (-94.74%)	0.01 ± 0.01 (-80.00%)
S.E.±	0.0408	0.0121	0.0379	0.00447
C.D. (P=0.05)	0.129	0.038	0.120	0.014
C.D. (P=0.01)	0.183	0.054	0.170	0.020
r	-0.9809**	-0.9941**	-0.9574**	-0.9864**
r ²	0.9621	0.9883	0.9166	0.9730
Y	98.644 ± (-) 24.5679	134.769 ± (-) 200.8754	91.864 ± (-) 220.3390	127.027 ± (-) 2027.0271

* and ** indicate significance of values at P=0.05 and 0.01, respectively

5).

Soluble protein contents were increased significantly with increasing tailing concentration. The increase was maximum at 75 per cent tailing concentration, which was 40.38 per cent over the controls.

Uranium is a naturally occurring element. Its average concentration in earth's crust is 4 mg kg⁻¹ (Hursh and Poor, 1973). Naturally it exists in the form of 14 isotopes, mainly ²³⁴U, ²³⁵U and ²³⁸U with a relative abundance of 0.0055, 0.720 and 99.27 per cent, respectively (Purohit, 2006).

There is contradictory information on the toxicity of soil uranium to plants. Stoklasa and Penkava (1928); Becquerel and Rousseau (1947); Fevilli (1948); Drobkov (1951); Canon (1952); Sultanbeav (1971); Morishima *et al.* (1976); Sheppard *et al.* (1992) reported that low levels of uranium concentration stimulated plant growth while Jain (1996); Aery and Jain (1998);

Hafez and Ramadan (2002) and Jagetiya and Purohit (2006; 2007) showed detrimental effects of uranium.

Results of this study show that the lower as well as the higher concentrations of uranium tailings were toxic to all the crops with respect to all the parameters studied.

Uranium as well as other radionuclides and their decay products present in the tailings exhibit two different health hazards. The first is the long time influence, because of short distance a radiation of the uranium, staying in the biomass, which could cause the development of cancer and genetic defects by deformation of chromosomes. The particles emitted during disintegration of uranium seem to produce continuous ionisation in the medium, which interfere with the metabolic processes (Jagetiya and Purohit, 2006).

The second hazard of uranium tailings is the short time chemical toxicity of soluble compounds UO₂²⁺, by influencing

Table 4 : Showing effect of various uranium tailing concentrations on reproductive growth of gram (values in the parenthesis indicate percentage increase/decrease over the control)

Tailing concentrations	Number of seeds plant ⁻¹	Weight of seeds plant ⁻¹
0%	5.50 ± 0.50	0.52 ± 0.10
25%	4.50 ± 0.50 (18.18%)	0.36 ± 0.04 (-30.77%)
50%	3.00 ± 0.5 (-45.45%)	0.19 ± 0.01 (-63.46%)
75%	- (-100%)	- (-100%)
100%	- (-100%)	- (-100%)
S.E. ±	0.289	0.0361
C.D. (P=0.05)	0.999	0.125
C.D. (P=0.01)	1.513	0.189
r	-0.9934**	-0.9998**
r ²	0.9868	0.9997
Y	110.526 ± (-) 19.7368	79.024 ± (-) 151.4688

* and ** indicate significance of values at P=0.05 and 0.01, respectively

Table 5 : Showing effect of various uranium tailing concentrations on chlorophyll contents and soluble protein (leaf) contents [mg g⁻¹ (fresh weight)] of Gram (values in the parenthesis indicate per centage increase/decrease over the control)

Tailing concentrations	Chlorophyll			Soluble protein (leaf) contents
	Chl a	Chl b	Total	
0%	0.83 ± 0.02	0.39 ± 0.04	1.31 ± 0.24	11.41 ± 2.42
25%	0.77 ± 0.22 (-7.23%)	0.35 ± 0.02 (-10.26%)	1.18 ± 0.00 (-9.92%)	12.37 ± 0.48 (8.41%)
50%	0.63 ± 0.07 (24.10%)	0.30 ± 0.00 (-23.08%)	1.00 ± 0.16 (-23.66%)	14.13 ± 0.23 (23.84%)
75%	0.56 ± 0.02 (32.53%)	0.24 ± 0.02 (-38.46%)	0.88 ± 0.02 (-32.82%)	16.01 ± 1.24 (40.32%)
100%	- (-100%)	-(-100%)	- (-100%)	- (-100%)
S.E.±	0.0671	0.0141	0.0835	0.8
C.D. (P=0.05)	0.219	0.046	0.272	2.609
C.D. (P=0.01)	0.337	0.071	0.419	4.016
r	-0.9875**	-0.9960**	-0.9971**	0.9905**
r ²	0.9751	0.9921	0.9942	0.9811
Y	216.491 ± (-) 256.6180	196.230 ± (-) 496.0317	222.222 ± (-) 169.0821	(-) 174.847 ± (-) 15.7537

* and ** indicate significance of values at P=0.05 and 0.01, respectively

directly the function of internal organs. Chemical toxic effects on plants would be caused by ^{99}Tc and ^{129}I before radiological effects are predicted to occur (Amiro and Sheppard, 1994). For most of these radionuclides, radiological dose is the source of potential impact, but for uranium itself, chemical toxicity can supersede radiological dose. Finally, the issue of chemical toxicity of uranium has become complicated by the presence in the literature of reports of exceptional toxicity, as low as 0.5 mg uranium kg^{-1} dry soil (Sheppard *et al.*, 2005).

Phytoremediation is emerging as an attractive alternative to energy-intensive high cost traditional cleaning methods. This new technology employs the use of higher plants capable of accumulating high levels of contamination in different plant parts. After harvesting plant biomass can be disposed of in a final repository after volume reduction (ashing) (Fuhramnn *et al.*, 2004; Jagetiya and Sharma, 2009). Present investigation demonstrates that gram can tolerate, uptake and accumulate uranium hence, can be used to filter contaminated soil and in revitalization of hazardous radioactive waste sites. Present study also indicates the necessity of addition of garden soil (properties described in Table A) before revegetation. The conditioning can improve the quality of tailings and provide better environment by alteration in nutritional status.

The available information for phytoremediation utility of gram in the public literature is limited. Our estimates could consequently have inadequacies. Our estimates, therefore, have to be considered as a preliminary one. However, the methodology that we have followed has been described in sufficient detail so that it could be used to modify estimates, when new data is available.

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