

REVIEW

CHROMIUM CHEMISTRY AND UPTAKE BY PLANTS

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INTRODUCTION

Chromium (Cr) is considered as a soil and water pollutant mainly introduced by the various industrial wastes using chrome salts for processing. Although Cr is recognised as an essential microelement for human and animal nutrition, conflicting reports are available on the stimulatory and inhibitory effects of Cr on plants. The differential adsorption, uptake and distribution of Cr^{3+} and CrO_4^{2-} in different plant species were reported recently and efforts have also been made to grow tolerant crops in these polluted zones as a phytoremediative measure. In this paper, the uptake of Cr in plants, its accumulation, translocation and factors governing the uptake has been reviewed in detail.

Occurrence

Chromium is found in all phases of the environment including air, water, soil and virtually all biota. It ranks 21st among elements in crustal abundance and the average concentration in the continental crust has been reported to be 125 mg/kg with a commonly observed range of 80 - 200 mg/kg. The Cr content in soil is largely dependent on parent material with an average of 40 mg/kg. However, in soils derived from serpentine parent materials, Cr levels can reach 125000 mg/kg or more. Fresh water Cr concentration generally range from 0.1 - 6.0 $\mu\text{g/l}$ with an average of 0.1 $\mu\text{g/l}$, while sea water averages 0.3 $\mu\text{g/l}$ and range from 0.2 to 50 $\mu\text{g/l}$ (Bowen, 1979). Fresh water Cr concentrations can actually be higher, depending on the soil Cr levels in the surrounding watershed (Pacyna and Nriagu, 1988). The measured background atmospheric concentration varied from 5.0×10^{-6} to 1.2×10^{-3} $\mu\text{g/m}^3$ in remote areas and

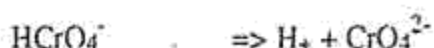
in urban areas it averages from 0.015 to 0.03 $\mu\text{g/m}^3$.

Anthropogenic sources of Cr are mainly from the industrial processes like metallurgical, chemical and refractory brick industries, pigment manufacture, plating / metal finishing, corrosion inhibition, organic synthesis, leather tanning and as wood preservative (Losi, Amrhein and Frankenberger, 1994).

Chemistry and speciation

Chromium can exist in oxidation states ranging from 2⁺ and 6⁺ and are normally found within the pH and potentials common in environmental systems and of these, Cr^{3+} is generally considered to be the more stable form. Its form and chemistry is more complex than the hexavalent form. Because of its less affinity to O_2 it is known to form numerous complexes with both organic and inorganic ligands. Among the OH^- , SO_4^{2-} , NO_3^- and CO_3^{2-} , OH^- is found to be effective in complexing. Major Cr (III) hydroxy species include $\text{Cr}(\text{OH})^{2+}$, $\text{Cr}(\text{OH})_3^0$ and $\text{Cr}(\text{OH})_4$ (Rai and Zachara, 1988).

The hexavalent Cr is a strong oxidiser and, as a result exists only in oxygenated species that are very soluble and pH dependent (Fig 1) according to the following equilibria (Nieboer and Jusys, 1988).



Under highly acidic conditions, H_2CrO_4 is the dominant species, while between the values of 0 and 5.9, HCrO_4^- dominates and, at or above pH 6.0, CrO_4^{2-} prevails.

The Cr VI chemistry in environmental systems is largely confined to that of the chromate ion. The speciation of Cr III and Cr VI will generally depend on a variety of environmental factors, including pH, concentration and the available ligands. In most natural systems, hexavalent Cr will be present as CrO_4^{2-} and major trivalent species include hydroxides and various organic complexes. Chromium is known to undergo various chemical and biological reactions in natural systems that govern speciation and, in turn, environmental behaviour. Figure 2 illustrates the possible fates of Cr in soil/water systems. The important reactions include oxidation/reduction, precipitation/dissolution, and adsorption / desorption.

Nutrition and toxicity

The Cr is an essential component of animal and human nutrition and found notably associated with glucose metabolism. The trivalent form is nutritionally useful, while the hexavalent form is toxic and mutagenic. The biotoxicity of chromate is largely a function of its ability to cross biological membranes and its powerful oxidising potential. The toxic and mutagenic effects of chromates on microbes are also well documented. Ross, Sjogren and Bartlett (1981) found that 10 - 12 mg/l Cr VI was inhibitory to soil bacteria in liquid media, whilst, at this Cr III had no effect. Ajmal, Nomani and Ahmad (1984) reported that chrome electroplating waste was toxic to saprophytic and nitrifying bacteria, with toxicity increasing directly with the Cr VI content of the waste. Since, heavy metals occur naturally at high concentration in various environmental systems, resistance/tolerance mechanisms have been evolving within microbial communities since the advent of life.

Heavy metals and plants

The pivotal role of trace metals in plant nutrition at lesser concentration is documented long before. Cr as one of the trace element is reported to produce stimulatory effects at lesser concentrations (Lisk, 1972) but contradictory views are also documented (Huffman and Allaway, 1973). A technical review was summarised by Borel and Anderson (1984) on the various chemical and nutritional aspects of Cr including physical and chemical properties, its biologically active form, *in*

vivo uptake and transport, its metabolic functions, deficiency states and factors influencing its metabolism. Chromium toxicity is rare in natural systems, probably because the majority is in trivalent form.

Mechanisms of metal uptake

The heavy metals were taken up by two different processes i.e., passive and facilitated/active.

Passive uptake is effected upon concentration differences and based on the diffusion processes, requiring no specific processes / carrier. The ion diffuses through the bulk phase of the solution to the solution membrane interface. The passive uptake can only continue if the membrane is freely permeable to the ion, which implies that there is a continuous extension from the free space into and through the cells which form the boundary of root or leaf. Otherwise some specific mechanisms must exist for the transfer of the ion across the selectively permeable membrane.

Facilitated uptake requires metabolic activity but is not directly linked to a specific energetic process. Typically, uptake is facilitated by the secretions from the cell either of H^+ or a meal binding agent, so that the chemical form of the metal may be altered, possibly by a ligand replacement reaction, thus giving a more favourable structure for uptake. It is interesting to note that the rhizosphere is now recognised to be substantially different from the bulk soil phase and root secretion is known to be quite extensive. Facilitated uptake will be affected by metabolic changes also. Additionally, active uptake can sometimes be recognised by the ability to accumulate the element in question. The organism may continue to take up the nutrient even when the internal concentration exceeds the external concentration (Peterson and Girling, 1981).

Chromium uptake by plants

Native Cr in non serpentine soils tend to exist in very insoluble oxides and trace inclusion in various soil minerals and is relatively unavailable to plants (Allaway, 1968). Soluble Cr if added to soils is likely to revert to insoluble oxides and hence less available and also the Cr reaching *via*

sewage sludge is generally low in bioavailability to plants.

The Cr uptake occur either in chromate or chromic form by the plant. The uptake of chromate is an active process and follows Michelis Menten kinetics *via* sulphate carrier and mostly accumulated by the shoot. In contrast, the chromic ion uptake is a passive process occurring mostly in roots (Peterson and Girling, 1981). The chemical form of Cr inside the plants vary among the plant species. The predominant form of soluble Cr in serpentine plant extracts was found as trioxalatochromate III ion which is not present in the xylem sap. Hence, the transport of Cr as chromate in xylem sap might be analogous to that of SO_4 and PO_4 , principally as inorganic anions.

Unknown low molecular weight complexes of Cr, not associated with subcellular organelles have also been reported in leaves of barley (Skeffington, Shewry and Peterson, 1976), lucerne and bean. The chemical form of Cr in plants is undoubtedly of considerable importance in human and animal nutrition. The unknown Cr compounds in wheat grains although present at low levels have been reported to be biologically active and the oxalato complex identified in cauliflower leaves is found readily available to animals.

Accumulation and localisation in tissues

Cr is distributed in all the cellwall constituents but the protopectins, lignin and residue - cellulose are the fractions which contain most of Cr. CrO_4^- was also fixed on walls which prove that chromate are partly reduced before entering the cell. The reduction phenomenon is not known but as for SO_4 the plasmalemma could be the reduction site (Myttenaere and Mousny, 1974).

Autoradiography of roots showed no preferential areas of location of ^{51}Cr and it is mostly present in a soluble non particulate forms in the case of barley (Shewry and Peterson, 1974). One third of root radioactive Cr was soluble in 80 per cent ethanol in the form of three ^{51}Cr complexes, the predominant being identified as trioxalatochromate III ion which was also present in stem and leaf extracts (Lyon, Peterson and Brooks, 1969). In shoots ^{51}Cr transported to the leaves in 24 h growth in $^{51}\text{CrO}_4^{2-}$. Autoradiograph

of leaves showed that it was concentrated in the apices of the young leaves.

Factors affecting the uptake

Generally the heavy metal absorption by plants is influenced by the characteristics of the soil support system (organic matter content, CEC and above all, pH) and by the characteristics of the plants themselves. When a soil is amended with products such as sewage sludge, the heavy metals which these brought may hold will be contained in the organic matrix. These may lead to the increasing solubility of some of the metal (due to the formation of soluble organic complexes) or to their immobilisation and subsequent reduced possibility of being assimilated by the plant depending on the molecular weight of the metal humus complexes formed and the state of the organic matter itself.

Total availability of Cr

Total uptake of Cr by the plants was independent of the amount of Cr applied, the plant took up a similar fraction even over eight orders of magnitude (Sheppard, Sheppard and Thibault, 1984). But in the hydroponis studies, the ^{51}Cr concentration increased proportionally with increasing levels of application in kale, soybean and bushbeans. Exposure period and level of ^{51}Cr affected the nuclide concentration significantly, but, not the distribution. The concentration was higher when the plants were exposed throughout the growth cycle than for shorter periods of time, but the greater efficiency of incorporation into soybean seeds occurred when plants were exposed during the reproductive growth phase (Schmitt and Weaver, 1984). Although crop absorption of heavy metals has increased with the sludge application rates, the total uptake amounted to less than 1 per cent of the metal introduced through sludge application (Chang *et al.*, 1984; Dowdy and Ham, 1977; Keefer, Singh and Horvath, 1986).

Type of vegetal species

The species differed in their ability to accumulate Cr in either shoot or root. Species differences in about 10 fold in the tops and 5 fold in the roots. Cauliflower seedlings followed by beetroot seedlings accumulated most of the Cr

while, mung bean and barley accumulated the least. In general, beetroot and maize plants were found to be good accumulators of Cr (Hernandez, Moreno and Costa, 1991). Comparative uptake of the Cr content by some of the crops is listed in Table 1 and 2. The toxicity effect of Cr on the yield and quality of radish (Yiyong, Tongchou and Boer, 1989a), pepper (Yiyong, Tongchou and Boer, 1989b), rye grass and red clover (Nishimura and Takayashi, 1982) was also studied.

Lysimeters studies on the uptake of Cr in alfalfa and chard in loam and sandy soils revealed that the tissue concentration of ^{52}Cr was relatively high (Table 3). The means averaged for both plants were 2.6 and 6.8 $\text{mg}^{52}\text{Cr}/\text{kg}$ plant for loamy soil, and 18 and 57 $\text{mg}^{52}\text{Cr}/\text{kg}$ plant for the sandy soil, for the 20 and 50 $\text{mg}^{52}\text{Cr}/\text{kg}$ soil treatment, respectively. These are above the toxic thresholds observed for beans and corn but, as noted, no toxicity in terms of reduced shoot growth occurred. The toxicity threshold for chard and alfalfa may be as high as 57 $\text{mg Cr}/\text{kg}$ (Sheppard *et al.*, 1984).

The data on the comparative uptake of $^{51}\text{Cr}^{3+}$ and $^{51}\text{CrO}_4^{2-}$ by different plant species indicate that massive accumulation of $^{51}\text{Cr}^{3+}$ in roots of all the plant species (Table 4). In the shoots the concentrations ratios of both the forms of Cr were the highest for peas followed by beans, tomatoes and the cereals. The concentration factor (C.R) for $^{51}\text{Cr}^{3+}$ in the roots was the highest for barley and

significantly lower concentration in the other plants. In the case of $^{51}\text{CrO}_4^{2-}$, there are no significant differences in the roots of barley, peas and beans (Ramachandran, D'Souza and Mistry, 1980). In the case of trees of maples, spruces and beeches, the Cr was found accumulated in fine roots and the distribution in soybean and cucumber using X ray microanalyser was also studied (Yamaguchi *et al.*, 1982).

Transport of Cr up the root is very low accounting for the low levels of Cr in the tops of plants. In laboratory experiments, less than 1% of the accumulated Cr was transported to the shoots. Evidently, the element enters the vascular tissue with difficulty but once there it can be rapidly transported. However, significantly more of chromate form was transported to the shoot than chromic form.

Type of soil

The uptake was higher in acid soils than calcareous soils. Prolonged incubation doubled the amount of Cr extracted by barley seedlings in both soils. The alkaline soil had an amazing capacity to buffer against Cr and where upto 142 kg/h of Cr was applied there was no increase in uptake (Dowdy and Larson, 1975; Moreno *et al.*, 1996). For even at pH 5.8 Cr exists in hydrated oxide form of such low solubility that it should not be available for plant uptake. Though the type of soil affected the uptake, the depth of soil did not have any significant effect. Results suggest that the availability did not change over the aeration

Table 1. Cr content in some crops

Crops	Cr concentration (ppm)	Reference
Serpentine plants	20000	Lyon <i>et al.</i> (1969)
Shrubs and trees	0.2 - 0.6	Hanna and Grant (1962)
Forest and Pastures	0.4	Schroeder, Balass and Tipton (1962)
Orchard leaves	2.6	Bowen (1979)
Pine needles	2.6	Peterson and Girling (1981)
Tomato plants	4.5	Peterson and Girling (1981)
Lichens and mosses	>5.0	Peterson and Girling (1981)
Maize	4.6	Renan <i>et al.</i> (1979)
Spinach	10	Peterson and Girling (1981)
		Misra and Jaiswal (1982)
Fruits	20*	Mertz (1969)
Cereals	40*	Peterson and Girling (1981)
Peas, Beans, sweet corn	10 - 40*	Cary, Allaway and Ison (1977)
White clover	0.35	Czekala (1990)

* in ppb

Table 2. Mean Cr concentration of some of the plant species grown on sandy soils

Plant	Cr conc. (ppm)		References
	Leaf	Stem	
Horseweed	7.6 (4.2)	6.4 (3.0)	Martin <i>et al.</i> (1996)
Dogfennel	9.4 (12.4)	8.1 (2.4)	Martin <i>et al.</i> (1996)
Chicory	9.2 (7.1)	3.4 (1.6)	Martin <i>et al.</i> (1996)
Alfalfa	0.24* (1.5 - 1.9)*		Sheppard <i>et al.</i> (1984)
Chard	0.20* (0.21 - 0.24)*		Sheppard <i>et al.</i> (1984)

* in per cent : Figures in parentheses indicate the uptake on loamy soils

Table 3. Total ^{51}Cr and the proportion of chromium taken up by the plants (Sheppard *et al.*, 1984)

Soil	Plant sp.	^{51}Cr treatment	% uptake
Loam	Alfalfa	0	1.49
		20	1.40
		50	1.94
	Chard	0	0.57
		20	0.76
Sand	Alfalfa	0	0.24
		20	0.20
		50	0.21
	Chard	0	0.22
		20	0.18
		50	0.22

gradient imposed by the depth of soil column (Sheppard *et al.*, 1984).

pH and salt concentration

The ^{51}Cr accumulated in the root was significantly lower at pH levels greater than 7.0. Similar effects were observed with $^{51}\text{CrO}_4^{2-}$. However the shoot uptake of Cr^{3+} was more than CrO_4^{2-} at pH 4.0 and above this pH the uptake of both forms was similar. This could be due to biological reduction of CrO_4 to Cr^{3+} at surface of the root cells as described.

A significant reduction in uptake of both ^{51}Cr and $^{51}\text{CrO}_4^{2-}$ by plant roots and shoots was observed with an increasing ionic strength of the external solution. When treated with dinitrophenol, significant reduction in $^{51}\text{Cr}^{3+}$ uptake by shoots were observed at 10⁻³ and 10⁻⁴ M. In $^{51}\text{CrO}_4^{2-}$, the significant reduction was observed only at 10⁻⁴ M. Maize plants with increasing amounts of CrO_4^{2-} from 10⁻⁷ to 10⁻⁴ M showed growth inhibition and reduced uptake in turn, changing the plastid

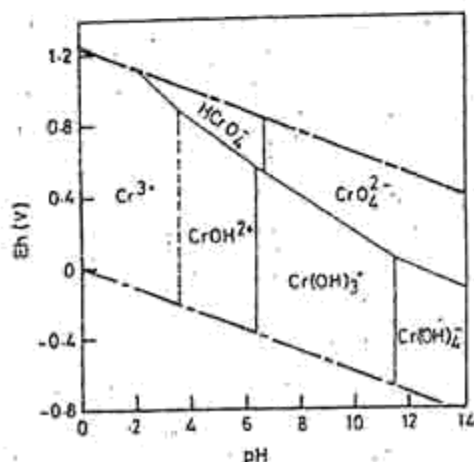


Fig.1. Aqueous speciation of chromium at various Eh and pH values (Losi *et al.*, 1994).

Table 4. Uptake of ^{51}Cr by different plant species (Ramachandran *et al.*, 1980)

Plant	$^{51}\text{Cr}^{3+}$			$^{51}\text{CrO}_4^{2-}$		
	C.R.		T.I.	C.R.		T.I.
	Shoot	Root		Shoot	Root	
Beans	0.84	928	0.48	0.54	749	0.38
Peas	1.49	969	0.34	1.24	855	0.29
Tomato	0.19	984	0.13	0.16	671	0.14
Maize	0.15	1202	0.03	0.11	621	0.05
Barley	0.17	2071	0.02	0.10	995	0.03

C.R. - Concentration Ratio : T.I. - Transport Index

pigments and lipoquinones (Krupa, Ruzkowski and Gilowska, 1982).

Forms of Cr

Cr III uptake was more than Cr VI form. In maize, Mishra *et al.* (1995) reported that for the root, the observed order of uptake was Cr III sand > Cr VI sand > Cr III soil > Cr VI soil. Significantly high uptake of Cr by roots in the sand culture (Cr III) is attributed to the effect of root exudates and degradation products on the mobilisation of the Cr III. In aerial parts of the plants a higher uptake was observed when the initial supply was Cr VI. The trends observed are explained on the basis of the redox behaviour of Cr.

However, bound and dialisable Cr is quite the same when Cr^{3+} or CrO_4 was used and the addition of carrier increase very little the proportion of fixed Cr. This indicated that the

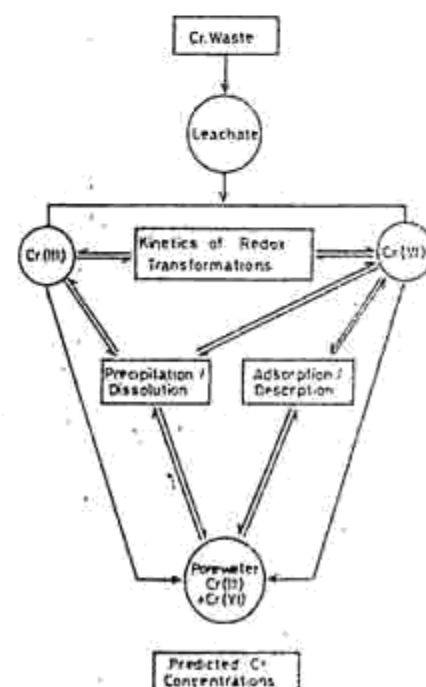


Fig.2. Fate of chromium applied to soil/water system (Losi *et al.*, 1994)

chemical form in which Cr is added to the nutrient solution does not influence the Cr distribution and fixation inside the protoplasm. Further, ^{51}Cr is bound to different isolated proteins confirming that the process is non specific. The chelated Cr forms are much less sorbed but its translocation rate is higher (30 per cent) than those observed for non chelated forms (Myttanaere and Mousny, 1974).

The phytotoxic effects of chromate was more compared to chromic ions. Perhaps, this is related to the higher concentration of Cr found in the tops of plants grown in chromate rather than chromic treated soils and solutions. Nevertheless, plants showing visual toxicity symptoms may not contain more Cr in their tops than normal plants.

Concentration of other anions and cations

The added anions and cations play a major role in effecting/inhibiting the Cr uptake. The transport processes of Fe and Cr to plant is found similar. The Cr significantly enhanced the growth of bushbeans if grown along with Fe. This beneficial effect of low Cr concentration was neither correlated to the changes of Mn, P or Fe tissue concentration nor to Cr induced alterations of the Fe/Mn and P/Fe ratios. Also the effect of Cr on the subcellular iron distribution or on the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio cannot be excluded (Bonet, Poschenrieder and Barcelo, 1991). Fe and Cr are found associated in small electron dense granules deposited on cellwall. When grown in chromic solutions only low concentration of Fe was found associated with Cr (Peerson and Girling, 1981).

The addition of lime increases the uptake significantly in plants by 3 ppm (Goodroad and Caldwell, 1979), but it depended on the source and the quantity applied. But phosphorus did not have any effect. However, KH_2PO_4 application was found to detoxify the small rate of CrO_3 on acid soils and decreased the toxicity in neutral soils (Otabbong, 1989). Ca stimulated the uptake of chromate but the increase was non linear. Se and SO_4 inhibit the uptake (Shewry and Peterson, 1974).

Soil amendments

The soil amendments with chelated fertilisers prepared from fish extract increased the concentration of Cr, Co, Pd and Ni in corn plants. The results of regression analyses indicated that the interactions between Cr, Co, Ni, Pb, Ti, Mb, V and

Zn were significantly associated (Sadiq and Hussain, 1993).

Soils amended with cocomposted sewage sludge (CCSS) reduced the dry wheat dry matter production in coastal soils which may be probably due to the decreased N availability caused by the C/N ratio (52:1) of the compost and slightly increase the soybean growth (6-12%). The CCSS had little effect on the concentration of Cr, Cd or Pb in wheat and soybean but consistently increased Cu, Ni and Zn in vegetative tissues (Sims and Kline, 1991). The others amendments like chicken manure and sewage sludge (aerobically and anaerobically composted) did not have any deleterious effect on the crops.

SUMMARY

Chromium occurs naturally in various crustal materials and is discharged into the environment as industrial waste. The environmental behaviour of Cr is largely a function of its oxidation states and there are a number of possible fates through which it reaches the soil/ water and take up by the plants, animals and microbes. Native Cr as such occur mostly in the trivalent form, unavailable to the plants. The amount of Cr present in the soil doesn't have any significant role in uptake however, a direct correlation was observed in hydroponics and nuclear tracer studies. It was taken up by the plants in the chromate or chromic forms which gets oxidised before entering into the root. Most of the Cr was accumulated in the roots than shoots but its localisation was not definite presumably, the vacuoles. The alkaline soils have a good buffering capacity for Cr than acid soils. The addition of Fe enhanced the uptake but not the phosphates, sulphates and the lime. The addition of Cr as sewage sludge is found safe compared to the other heavy metals but it dependent on the species and other environmental factors.

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EFFECTS OF GA₃ AND THIOUREA ON PROTEIN, PEROXIDASE ACTIVITY AND CHLOROPHYLL STATUS IN SEEDLINGS OF ANOLA

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ABSTRACT

The experiment was conducted in the laboratories of the Department of Horticulture, G.A.U., Anand during 1993. Three concentration each of GA₃ and thiourea, distilled water and control (total 8 treatments) were given to the seeds of Anola with 36 h. of subsequent soaking and drying thrice. Observations were recorded 6, 8 and 10 week intervals on protein peroxidase specific activity and chlorophyll content. GA₃ 500 ppm was effective in increasing the protein content of leaves and stem whereas no treatment was effective in enhancing the peroxidase specific activity in leaves, stems and roots. GA₃ 250 ppm was again helpful in increasing the total chlorophyll content of the leaves at all the three intervals.

KEY WORDS : Anola, seedling, protein, peroxidase activity, chlorophyll

Anola (*Phyllanthus emblica* Linn.) is one of most important fruit crops of the tropics and subtropics and broadly cultivated for its fruits rich in vitamin c and commercially exploited for hair dyes, medicine, ink etc. (Ram, 1983). Till recently, the main emphasis has been given on the yield and fruit preservation and very little on improvement in seed germination in anola raised for rootstocks. The phenomenon of seed germination is associated with changes in several protein constituents. Proteins are broken down during germination with a concomitant rise in amino acids and amides followed by de novo synthesis of protein and enzymes in the germinating parts of embryo. Peroxidases widely distributed in plants and peroxidase activity is related to cell growth and differentiation. The aim of the present investigation was to find out the possible role of protein and peroxidase enzyme for altering seed germination.

MATERIALS AND METHODS

Present investigations were carried out at the Department of Horticulture, Gujarat Agricultural University, Anand Campus during 1993 in completely randomised design with four replications and eight treatment viz., three concentrations each of GA₃ and Thiourea (250, 500 and 750 ppm), distilled water and control (untreated). Pre-sowing treatments of seed soaking and subsequent drying processes were carried out for 36 hr followed by a surface sterilisation with 0.2 percent mercuric chloride. Treated seeds were allowed to germinate in pots and for chemical analysis samples were taken at 6 weeks (Ist stage), 8 weeks (IInd stage) and 10 weeks (IInd stage) interval. Protein percentage peroxidase specific activity (Change in O.D/hr./mg. protein) in leaves, stem, leaves and roots and chlorophyll content (mg/g fresh weight) were determined by employing normal methods.