INTRODUCTION

Heavy metal is defined as metal with a specific weight higher than 5gm/cm^3 or an atomic number more than twenty. All the heavy metals are toxic at relatively high concentration. The heavy metals are heterogeneous group of elements which greatly differ in their chemical properties and biological function. According to Tiller (1989) heavy metals are considered as environmental pollutants. Heavy metals are ubiquitous and persistent pollutants present in a myriad of industrial effluents. Copper, Mercury, Lead, Zinc and Cadmium are considered as heavy metals.

According to Davis (1980) heavy metals are metallic elements of a density greater than 6gm/cm^3. Although the term heavy metal is well established in literature, its definition is based on a rather arbitrary chosen parameter (e.g. Jarvis 1983) sets the density threshold for heavy metals with widely different chemical properties.

Among the various toxic substances heavy metals are particularly severe in their action. They contaminate soil, plants, and enter food chain posing health problems to human beings. Toxicity of these metals have been shown to be dependent upon their concentration. Chaphekar and Shetye (1988) reviewed the work done on effects of various toxic metals on community structure, production and physiological functions of plants and significance of bioindicators of metal pollution especially with reference to Indian work.

Copper is known to be an essential micro nutrient for plants. Since the pioneer work of Sommer (1931), later supported by the more conclusive experiment of Arnon and Stout (1939), but excess copper is a common environmental pollutant arising from metal mining processes as well as from numerous other industrial, urban and agricultural activities (Foy et al.,1978). Fresh water plants and fishes are killed by less than 1ppm of copper. Fish absorb it through their gills.

The strong binding of copper by organic matter also determines its behavior in aqueous media: The natural legends present in the water can be responsible for decreasing bio available copper with respect to total dissolved matter (Baccini, 1985).

Beside the direct toxic effect of Cu in plants that are likely to arise in the situation referred to above, it must be taken into an account that Cu can also indirectly decreases primary production, though its inhibitory effect on the processes responsible for nutrient cycling in the soil and water and its utilization by plants.

Lead is one of the heavy metal. Lead is natural compound and also a contaminant on our environment. It is most abundant of the natural heavy elements and the accepted average value for the lead content of the earth’s crust is 15ppm (Wampler 1972).

Lead is introduced into the environment from both natural and anthropogenic sources and is circulated throughout the environmental compartments by means of chemical and physical processes as well as it enters the food chain and gets incorporated into the plants and animal tissue. Lead is found mainly in upper horizon of soil (Swaine & Mitchell 1960., John 1971). Lead is a non essential element is taken by plant species primarily via their roots. Pb deposited on the leaves of trees most to the soil system with annual leaffall or may be washed.

Mercury is one of the few pollutant and the organic pollutant known to have caused serious ill – health nervous disorders and numerous death in recent times. Mercury pollution has now become a matter of serious concern in several parts of the world. Factories especially those which produced chlorine and caustic soda add much mercury in our environment (Nath and Nath, 1990). Mercury is discharged into the atmosphere from the burning of fossil fuel, the release of industrial waste and the use of agrochemicals (Norton 1971).

Mercury does not have nutritional value and it is extremely toxic even in very dilute concentration. Among all the heavy metals mercury compounds act as the effective pollutants. Toxic effects of mercury on plants include abscission of older leaves, growth reduction and general decreases vigorous (Heck and Brandt 1971) inhibition of root and leaf development (Siegel et al., 1973), leaf necrosis (Waldron and Terry 1987a, 1987b).

Onion (Allium cepa L.) is a dry weather and cold crop. Onion is a most important vegetable for all people – poor or the rich. It increases special taste in all types of food. It has a black seeds. The inflorescence is umbel and the flower colour is white. Onion is used as a fresh food and as a dry food. The fresh green leaves are also used as a vegetable.

In Gujarat onion variety -------------- is used as large scale for cultivation. This variety was selected for the study. Copper being essential element but phytotoxic at higher concentration, lead and mercury are toxic, mercury is most toxic for plants were selected as a heavy metals.

Following were the objectives :

(1) To study the effect of increasing dose of heavy metals on seed germination and growth of onion.
(2) To study the effect of heavy metals as a soil pollutant on growth of onion seedlings.
(3) To differentiate the effect of copper, lead and mercury on germinated onion seeds.
(4) To study the effects of heavy metals on metabolite level in germinating onion seeds.

REVIEW OF LITERATURE

Copper, lead and mercury are the heavy metals. Copper is essential but it is toxic at higher concentration while lead and mercury is phototoxic and induces toxic symptoms even at low concentration. Heavy metal toxicity in plant is studied and reviewed by number of workers (Foy et al., 1978, Larcher 1980, Levitt 1980, Pag et al., 1981, Sandman & Boger 1983, Woolhouse 1983, Khan & Khan 1994, Shaw et al., 1986, Verkleij and Schut 1989, Show 1989, Baker & Walker 1989). An attempt has made here to review the only related topics.

HEAVY METAL & SEEDLING GROWTH

In the life cycle of higher plants seed germination and seedling growth are important events. Seed germination and seedling growth are described by (Khan 1977, Bewley and Black 1982, 1985). The seed germination and seedling growth are controlled by number of factors. Soil is one of the important factors for germination of seed and growth of seedlings. The soil contaminated with toxic metals is considered as stress for germination as well as further growth of the seedlings.

The excessive quantities of copper become poisonous for many plant species (Waiwright and Woolhouse 1975, Marshner 1986). Higher plants are more resistant than algae. But they are never - theless injured by relatively low concentrations of heavy metals and develop necrosis in the presence of copper (Repp 1963).

The toxic effect of copper on morphology and physiology on *Thlaspi ochroleucum* seedlings were studied. Seedlings created with 16 µM of copper or more, showed lower root and shoot yield, than seedlings in the controlled solution.

Wong and Bradshaw (1982) reported that 0.02ppm copper in culture medium inhibited root growth of rye grass. When plant species like poplar, oat, maize, rye grass, lettuce, spinatch and beans were grown on metal embedded sludge, growth was inhibited.

Root was effected more than shoot by copper toxicity (Gupta and Mukherji 1977, Baszyski et al., 1982, Stiborova et al., 1986a). Copper was accumulated in the root so effect was more on root than on shoot.

For the whole plant level copper inhibits vegetative growth and induces the symptoms or senescence. Lead contamination can bring about crop failure at any stage of plant development. During seed germination the inhibitory effect of lead contamination are readily seen in terms of a significant reduction in root hair development on the emerging radicle. Lead contamination higher than 1000ppm inhibits radish seed germination (Lane & Martin 1980).

Numerous studies have shown a significant reduction of growth with increasing lead concentrations. This includes studies with oats (Fiussello and Molinari 1973), Soybeans (Huang et al., 1974), Corn (Carlson et al., 1975), and Rice (Fiussello & Molinari 1973). Lead (300 µM) inhibited pod fresh weight in soyabeans by 35% (Huang et al., 1974).

There is no evidence that Pb is essential for the growth of plant species, conflicting reports of growth stimulations and reduction are plentiful. Growth stimulations at low concentrations have been reported for various plants. During seed germination the inhibitory effect of lead contaminations are generally seen in terms of significant reduction in root hair development and on the emerging redicle.

Only very limited information is available about lead toxicity during seed germination and early seedling growth. The effect of toxic concentration of natural lead acetate on the germination and growth of rice (*Oryza sativa* L.) seeds are reported. Elongation, inhibition was first detected at lead acetate concentration of 10^{-3} M and become more pronounced with increasing concentrations. Root growth was more strongly inhibited than shoot growth and no germination occurred at 10^{-1} M (Mukherji and Maitra 1976).

During the last two decades it has been well documented that compared to other metals mercury and its compounds are more toxic for seed germination. Mercury inhibit seed germination and seedling growth of rice (Mukherji and Ganguli, 1974). Mercury compounds used as fungicides in agriculture also increase the levels in the environment. The total global release of mercury in the atmosphere due to human activity has been estimated to be of 2000 to 3000 tons per year (Wito, 1991).

The percentage germination was decreased with increasing concentrations of mercury. Similarly length of root and shoot was also affected by heavy metal concentration. The seedling growth was inhibited by the mercuric chloride. With concentration of mercuric chloride there was progressive decline in the respiration rate of seedlings. Kalimuthu and SivaSubramanian (1990) reported the decrease in percent germination of maize with increasing concentrations of lead and mercury.

Inhibition of seed germination due to lead, mercury and arsenic in bajra, mustard, jowar, methi, alfalfa, clusterbean, peas, sunhemp, mung, radish and bhindi is reported (Mhatre and Chaphekar, 1982). Metal toxicity not only affects the length of primary root but changes the architecture of the entire root system (Breckle, 1989). Although the length of root is widely used to assess the tolerance of plant to metal (Wilking, 1978). The cause of decreased root elongation and their consequences for whole plant performance are poorly understood. The different metal may inhibit root growth by different mechanisms.

HEAVY METALS AND METABOLISM :

Metabolism, the sum of all enzymatic reactions occurring in the cell can be disturbed by a great number of toxic agents. Heavy metals may interfere with the enzymes, essential for various processes. Hormones and other regulatory processes may be affected, ultimately metabolic rate is uncontrolled, there may be accumulation or accession of the normal substrate of the defective enzyme (Jernelov et al., 1978).

Heavy metals bring a considerable decrease in the rate of all the metabolic processes of the cell including nucleic acid synthesis, cell division and protein contents (Siegle, 1974, Vaulina et al., 1978, Maitra and Mukherji, 1979, Wickliff et al., 1980, Nag et al., 1981, Rani et al., 1987, 1988, Kalib 1994).

The relatively strong affinities of heavy metals ions for side chain ligands of protein (Vallee and Ulmer, 1972) indicate that enzyme activities and other functional proteins are one of the primary targets of metal toxicity.

PROTEIN METABOLISM :

Protein synthesis can be disturbed at many levels at a variety of mechanisms either by affecting the nucleic acid metabolism of structure, or in the protein forming system itself. Toxic agents acting directly on ribosomes, RNA enzymes of Co-enzymes may also have a drastic influence on protein synthesis. All biosynthetic processes can be disturbed in one way or another.

Ions and ionic flexus have been found to play important role in controlling protein synthesis of plant leaves (Ralph et al., 1980). Lower dose of Hg stimulated protease activity in Pisum sativum (Sharma 1985). Protease and RNAase were depressed in germinating pea seedlings in the presence of lead (Mittal and Sawhney, 1990).

The protein content was decreased while free aminoacid contains was increased. The decline in protein with corresponding rise in protease activity due to heavy metal stress suggests the promotion of catabolic activities. Thus imposition of heavy metal stress may include early senescence (Bhattacharyya and Chaudhuri , 1994).

MATERIALS AND METHODS :

Copper,lead and mercury were selected as a heavy metals and their salts namely copper sulphate – $CuSo_{4,}$ lead acetate $(CH_3COO)_2Pb$ and mercury chloride $HgCl_2$ were used in the study.In the present study seed germination and seedling growth were noted in relation to individual heavy metal may to lead to better understanding the ways in which plants are affected by those individual metal.

Expt.1 Effect of increasing concentrations of heavy metals on growth of Onion seedlings.(petriplate expt.)

Uniform graded seeds of Onion(Allium cepa L.) were germinated in priplates (9cm D 50 seeds per dish) under laboratory conditions.the media for germination are as follows:-

1; Distilled water – DW

2; $CuSo_4$ 10^{-5} M

3; $CuSo_4$ 10^{-4} M

4; $CuSo_4$ 10^{-3} M

5; $(CH_3COO)_2Pb$ 10^{-5} M

6; $(CH_3COO)_2Pb$ 10^{-4} M

7; $(CH_3COO)_2Pb$ 10^{-3} M

8; $HgCl_2$ 10^{-5} M

9; $HgCl_2$ 10^{-4} M

10; $HgCl_2$ 10^{3} M

The experiment was continued upto 15 days.The effect ofheavy metals on onion seedlings were studiedas follows;

(A) STUDY ON PERCENT GIRMINATION

Radical emergence was noted from15 seeds of each treatment on completion of 6 day, 8 day,10 day and 15 day and it was expressed as percent germination.

(B)STUDY OF SEEDLING GROWTH

15 day old seedlings at random from each treatment were selected. The elongation of root and shoot was measured; mean was calculated and expressed as cm/seedling. The embryo was dissected out from each seedling and fresh weight in two lots i.e. 10 embryo was weighted , fresh weight was expressed as g/seedling. The seedlings were dried in oven maintain at 80^{o} c.for 48h, dry weight was noted and expressed as g/seedling.The percent moisture was calculated using the data of fress weight and dry weight and expressed as percent moisture.Following formula was used for calculation.

Fress weight – Dryweight

Frefh weight

Expt-II STUDY ON GROWTH OF GERMINATING ONION SEEDS AS INFLUNCED BY TOXIC CONCENTRATION OF HEAVY METALS.

The uniform graded seed of Onion were germinated in sterilized petriplate(9cm D, %) seeds per dish) lined with Whatmann filter paper No.1. 10^{-4} M was selected as toxic concentration of heavy metal. The media for germination were DW (control). 10^{-4} M $CuSO_4$, 10^{-4}M $(CH_3COO)_2Pb$, 10^{-4} $HgCl_2$.The experiment was carried out at 20+ 2^{0} c under laboratory conditions. The effect of heavy metals on growth of germinating seedlings was studied as follows:

STUDY ON SEEDLING GROWTH:-

10 seedlings from each treatment were studied at the interval of 2 day that up to 15 day. The method was already mentioned in experiment 1.

Expt-lll STUDY ON METABOLITES LIKE PROTEIN AND TOTAL AMINO ACID IN GERMINATING ONION SEEDS AS INFLUNCED BY GRADED CONCENTRATIONS OF HEAVY METALS.

STUDY ON METABOLITES;-

Uniform graded seed of Onion were germinated in petriplate (9 cm D, 50 seeds per dish) lined with sterilized filter paper under laboratory condition . Seeds were germinated in DW , 10-3M,10-4M,10-5 M of CuSO4,(CH3COO)2Pb and HgCl2 upto 15 days and seedling were analysed for the following metabolites.

1; PROTEIN CONTENT

2; TOTAL AMINO ACID

The method for the above mentioned biochemical parameters are follows:-

1, Protein content:-

Prptein was determined using the method of Lwry et al (1951). Weighted amount of dried plant material was ground in 80% ethanol and extracted twice. The residue was first washed with cold 5% perchloric acid (to remove sugars and soluble protein fractions) centrifuge and secondly it was washed with mixture of ethanol :ether : chloroform in the ratio of 2: 2: 2 (to remove acid soluble fractions and lipids) and centrifuged. The protein fraction was dissolved in 1N Sodium hydroxide and kept for one hour and centrifuged. The protein fraction was dissolved in 1N Sodium hydroxide and kept for one hour and centrifuged. The supernatant was made up to 5m.l. with sodium hydroxide and used as aliquot. 2m.l. of the above aliquot was added 5m.l. of Lowry reagent (prepared by mixing 50ml reagent A which is 2% sodium carbonate in 0.1N NaOH and 1ml of reagent B which is 0.5% Copper sulphate in 1% sodium potassium tartarate) and incubated at room temperature for 30 minutes. The colour was developed by adding 0.5ml Folin phenol reagent. After 10 minutes O.D. was read at 660nm on systronic 106 spectrophotometer. The following regression equation was prepared by using non concentration of casin.

$X = 236.6 \quad Y – 35.22$

The protein content was expressed as mg/g dry weight.

2. Total amino acid content:-

The content of amino acid was determined following the method Harding and Maclean (1916). The reaction system contained 0.5ml of ethanol extracted of the material 1 ml 10%pyridine and 1 ml of 2% ninhydrin reagent was stoppered and heated in water bath at 100^{O}C for 30 minutes . Violet blue colour was developed, later it was cooled and diluted with distilled water to a final volume of 10 ml. O.D. of the violet blue colour was read at 570nm. The following regression formula was prepared using isolucine as standard.

$X = 413.42 \quad Y – 19.23$

The amino acid contain was expressed as mg/g dry weight.

RESULTS AND DISCUSSION :-

Expt.1 EFFECT ON INCREASING CONCENTRATION OF HEAVY METALS ON GROWTH OF ONION SEEDLINGS.

A: Study on percent germination :

Table :1 represents the data on effects of heavy metals on percent germination of onion seedlings. Onion seed germination was 90% during first 6 days slowly it was increased and on completion of 10 days it was 100%, provided that seeds were germinated in DW. Percent germination of onion seed was not affected when seeds were germinated in graded doses of Cu. 90% germination was found with Cu 10^{-5}M. Cu is micro nutrient, higher concentration was considered as phytotoxic. From the present data it seems that 10^{-5}M Cu may not be considered as toxic concentration for strouting the seeds of onion.

When the seeds were germinated under increasing concentration of lead acetate, it was found that seed germination was not much affected upto 10^{-4}M. 10^{-3}M lead delayed the seed germination. 10^{-3}M lead remarkably reduced the seed germination. Lane and Martine (1980) reported that the reddish seed germination was inhibited by lead concentration higher than 1000ppm. With increasing concentration of $HgCl_2$ percent germination was inhibited, the concentration was 10^{-5} M and above, the effect was severe. Seed germination test was considered as one of the parameter for assessment of metal toxicity (Wang and Keturi 1990).

From the present data it is found that some concentration of different metals gave different effects. The toxic effect was maximum with mercury than with lead. It is already noted that mercury is most toxic element to the plants (Kamp Nelson 1971, Mukherji and Ganguli 1974, Chaphekar and Mhatre 1982, Ranjine et al., 1989, Vyas et al., 1997). Thus seed germination of onion was strongly inhibited by higher concentration of mercury.

B. STUDY ON SEEDLING GROWTH :-

Table: 2 represents root elongation was decreased by all the heavy metals. Adverse effect on root elongation was progressively increased with increase in concentration. The seedling growth was not possible under 10^{-5} M Hg. The toxicity was in the range of Hg > Pb > Cu. Higher concentration of copper inhibited the root growth (Hoga and Rauser, 1981, Smilde 1981, Wong and Bradshaw 1982, Vyas et al., 1997). Root growth was inhibited by lead supporting the finding of Baumhartf and Welch, 1972, Rolfe 1973, Mukherji and Maitra 1976, Wilkins 1978, Lane and Martin 1980, Martin 1981.

Mercury is highly toxic. Number of workers have reported that mercury decreased root elongation. From the present experiment it was found that root elongation was maximum with mercury which supports the finding of Chaphekar and Mhatre (1982), Mhatre and Chaphekar (1982), Nag et al., 1989. Asthan and Salgare (1991) reported that shoot elongation was decreased by copper, lead and mercury. All the metals at the higher concentration inhibited the root elongation but different metals may inhibit root growth by different mechanism (Wilkins 1978).

The shoot growth was also inhibited by heavy metals but effective concentration differed with metals 10^{-5} M of Cu and Pb did not cause adverse effect not only that at this concentration copper stimulated the growth while the mercury drastically reduced it, 10^{-4} M concentration of Cu and Hg inhibited the shoot length. 10^{-3} M concentration of both the metals remarkably reduced the shoot elongation while shoot growth was not possible in 10^{-3} M mercury. It seems mercury should be considered as highly toxic element for onion seedlings.

Root elongation was affected more than shoot elongation with all the metals. Root is the first organ to contact metal concentration, it accumulates significantly higher metal amount than the upper plant parts (Brackle 1989). Present data suggests that root elongation study may be selected as physiological parameter for differentiating metal toxicity in onion seedlings.

Table :3 represents the effect of heavy metals on % moisture of 15 day old onion seedlings. The lower concentration of copper and mercury enhanced the percent moisture in embryo . Such effect of Pb was also noted in the seedlings germinated with higher concentration of heavy metal. The above data suggests that heavy metals increased the water uptakes by the seedlings. The effect of heavy metals on plant water relation was discussed by Barcelo and Poschenrieder 1990. Heavy metal disturbed the water balance. Vyas et al., 1997 reported that heavy metal irrespective of its nature stimulated the water uptakes in fenugreek, thus plants may survive under heavy metals.

From the above data it is suggested that Cu, Pb and Hg having 10^{-5} M and higher concentration inhibited the seedling growth. Inhibitory effect was correlated with concentration. Hg was most effective while Pb was less effective. Root growth was affected more than shoot growth by all the heavy metals.