

CHAPTER 4
DISCUSSION

DISCUSSION

The present investigation was undertaken with an objective to assess the impact of fertilizer factory effluent on the growth and metabolism of four selected *Capsicum* plants. The tolerance limit of the effluent with respect to *Capsicum* plants was considered. An attempt was also made to tap the potential use of industrial effluents for beneficial purposes. Many reports are available regarding the utilization of the effluent for irrigation purpose. (Day, 1973; Pound and Crites, 1973; Bauwer and Chaney, 1974; De Anil, 1980; Dixit *et al.*, 1986; Sahai and Srivastava, 1988; Gautam and Bishnoi, 1990; Devi, 1991; Swaminathan and Vaidheeswaran, 1991; Gautam *et al.*, 1992; Kannabiran and Pragasam, 1993; Pandey and Sony, 1994; Pathak *et al.*, 1998; Ramana *et al.*, 2001; Dhanker and Joginder, 2002; Narwal, 2006; Tharakeshwari and Shoba, 2006; Kamlesh Nath *et al.*, 2007; Sheela and Deepa, 2007).

4.1 Physico-chemical examination of effluent

The physico-chemical analysis of fertilizer industry effluent showed a pH 5.2-7.3 which was within the permissible limit and slightly acidic (pH 5.5 to 9; IS: 2490-1981). The suspended solids in the effluent were 19-26 mg/l which was very lower than permissible limit (100mg/l; IS; 2490-1981). Dissolved solids were found to be 700-952 mg/l which was also below the permissible limit (2100 mg/l; IS: 2490-1981) free ammonia and nitrates content revealed very low values as 0- 0.88 and 0-1.06 mg/l respectively.

Ammoniacal nitrogen present in the effluent (9-45 mg/l) was near to the standard value of 50mg/l (IS: 2490-1981).

Dissolved oxygen in the effluent was 5-6.8 mg/l that indicates the better quality of the effluent. BOD of the effluent was recorded as 6-8.1 mg/l which was lower than the permissible limit (30mg/l; IS: 2490-1981). COD of the effluent was 15.8-19.8 mg/l which was much lower than that of the standard limit (250mg/l, IS: 2490-1981). Oil and grease 4.8-8.0 mg/l were found in the effluent. Chloride content present in the effluent was found to be 246-348.45 mg/l which was below the tolerance limit (1000 mg/l; IS: 2490-1981).

Phosphates were recorded as 1.24-10.2 mg/l that was higher than the tolerance limit (5mg/l; IS: 2490-1981). Sulphate 194.38mg/l, residual chlorine 0-0.22 mg/l, fluoride 0.64-0.90 mg/l, sodium 10-13.12 mg/l were below the tolerance limit (1000, 50, 2, 60 mg/l, IS: 2490-1981). Copper 0.2-0.04 mg/l, lead 0-0.02 mg/l, zinc 0-1.05 mg/l which was also recorded to be below the tolerance limit (3, 0.1 and 5 mg/l; IS: 2490-1981).

Phenolic compounds, cyanides, sulphides, boron, arsenic, chromium, nickel, hexavalent chromium and total chromium were not detected in the effluent.

4.2 Germination and seedling morphology

4.2.1 Germination

In the present study maximum seed germination was recorded at 25% and 50% concentration of effluent. At higher concentration above 50% of effluent the seed germination percentage was retarded as compared to control. Similar results were reported by Shukla and Moitra (1995), Augusthy and Mani (2001) in *Vigna radiatus* L., Singh and Misra (1987) in crop plants, Neelam and Sahai (1988) in *Sesamum indicum* L., Ashok and Pankaj (2000) in *Cicer arietinum* L., Verma and Mather (1974), Ravi and Srivastava (1988); Sahai and Srivastava (1988), Kannabiran and Pragasam (1993), Vinod Sharma *et al.* (2002). Indira *et al.* (2001) and Palanivel *et al.* (2004) observed a complete inhibition of germination at 100% effluent concentration on *Capsicum annum* L. in dairy effluent. In 75% concentration although radicle pierced the seed coat in a few seeds subsequent growth was inhibited. Only one plant survived at this concentration.

Subramani *et al.* (1998) reported a progressive decrease in seed germination and seedling growth with the increasing concentration of fertilizer factory effluent. Similar findings have been documented by Mishra and Bera (1996). Tharakeshwari and Shobha (2006) also supported the findings of Mishra and Bera (1996) by using distillary effluent in *Eleusine coracana* L..

The effect of fertilizer factory effluent on seed germination and seedling growth on *Vigna radiata* L., *Arachis hypogea* L., *Glycine max* L., *Oryza sativa* L. and *Sorghum bicolor* Moench was investigated by Sundaramoorthy *et al.* (2000) and found that the percentage of germination and seedling growth was maximum in the 10% diluted effluent than the control. While undiluted effluent elicited an inhibitory effect due to excess amount of solid present in effluent. Similar results were reported by Neelam and Sahai (1988), Vijayakumari and Kumudha (1990), Goel and Kulkarni (1994), Ghosh and Kumar (1998, 2000), Prabhakar *et al.* (2006), Nagda *et al.* (2006) and Sheela and Deepa (2007).

Ghousebasha and Adhiyaman (2004) reported 100% germination of green gram seeds in control as well as undiluted (100%) effluent of Paper factory. Here the germination was not adversely affected in the experimental plants. Therefore it is obvious that the effluent did not influence on the physiological activity of germinating seeds, but it stimulates the germination. A similar trend was reported in black gram, green gram and cow pea seeds (Suresh Babu and Vivekanandan, 1999) treated with different concentration of distillery spent wash. The ability of seeds to germinate under high osmotic pressure differs with variety as well as species (Ungar, 1987)

In the present study reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and

water, thus reduction in the amount of water absorption takes place, which results into retardation of seed germination. The salt concentration outside of the seed is known to act as limiting factor and it might be responsible for delay in germination. Similar findings have been reported by Bhumbra *et al.* (1968), Adraino *et al.* (1973), Gupta (1979), Lakshmann (1987), Rajaram *et al.* (1988), Heikal (1989) and Gautam *et al.* (1992).

It is observed in the present study that the higher concentration of the effluent deprived the germinating seeds of the necessary dissolved oxygen which is so necessary for the growth and development of young seedling. Similar observations were made by Maguire (1973) and Mhatre and Chaphekar (1982).

During germination, seeds imbibe considerable amount of water to attain the critical level. The embryo thus gets activated and the respiration sets on at a faster pace which requires a suitable supply of dissolved oxygen for energy liberation. A part of the energy liberated is utilised in building new protoplasm which results in the growth of the new embryo (Chiny, 1972; Bowley and Black, 1978; Salisbury and Ross, 1986; Jabeen and Abraham, 1997).

According to Handas (1976) and Saxena *et al.* (1986), the low amount of oxygen in dissolved form due to the presence of higher concentration of solids in the effluent reduces the energy supply through anaerobic respiration resulting in restriction of the growth and development of the seedling.

Similar observations have been made by Ramesh Kumar *et al.* (1990), Swaminathan and Vaidheeswaran (1991) and Malla and Mohanty (2005).

In the present study the promotion of seed germination upto 50% effluent concentration might be attributed to the presence of optimum level of plant nutrients such as nitrates and phosphates and the ability of seeds to absorb them. Similar findings have been reported by Dolar *et al.* (1972), Rajannan and Oblisami (1979), Sahai *et al.* (1988), Augusthy and Mani (2001) and Lakshmi and Sundaramoorthy (2004).

Higher concentration of the nutritious solution such as nitrogen and sulphates negatively affected germination level and rate (Perez Fernandez *et al.*, 2006; Sheela and Deepa, 2007).

In the present study the inhibitory effect of higher concentrations of fertilizer factory effluent on seed germination can also be attributed to the presence of oil and grease which might have obstructed water absorption by the seeds and resulted in inhibition of germination at higher concentration of effluent. *Capsicum* seeds being dry, thin and membranous should be properly hydrated for the initiation of germination. Similar inhibitory effect on seed germination was noticed by Indira *et al.* (2001) and Crowe (2002).

There is also a chance that certain physiological factors of seeds were altered by the effluent and which in turn affected the seed germination and seedling growth. This is in consonance with the reports of Bahadur and

Sharma (1988). The paper and pulp mill effluent has a high sulphate content and this inhibits the uptake of potassium and magnesium and in turn will affect the seed germination and seedling growth. In the present study also the sulphate content in the effluent may be acting as an inhibiting factor. Parallel results had been reported by Handas and Stribbe (1973) and Saxena *et al.* (1986).

Sheoran and Gary (1983) and Vijaya Kumari *et al.* (1990) reported that excess accumulation of chloride ions (Cl^-) under salt stress, reduces the turgor pressure inside the cells of treated seedlings which in turn caused reduction in growth of *Cicer arietinum* L. and in some millet and pulse crops respectively. The inhibition of growth at higher concentration of effluent due to toxic effect of chloride was reported by Kott *et al.* (1966) and Saxena and Jabeen (1990).

In the present study also the chloride ions (Cl^-) in the higher concentration of effluent may be acting as one of the inhibiting factors. Misra and Misra (1984) also reported a decrease in the seedling vigour due to the high concentration of sodium and chloride in the effluent.

The enhancement effect at lower concentrations of effluent may be due to the presence of essential nutrients like Zn^{2+} and Cu^{2+} in the effluent. The lower concentration of which serve as a micronutrient and it may stimulate germination at higher diluted level.

An initial stimulating effect of essential nutrients like Zn^{2+} and Cu^{2+} on seed germination at lower concentration was observed, which has earlier been explained in the work of Liza (1997) and Anitha (2002).

The concentration dependent effect of heavy metals on germination and seedling growth was also described earlier by Sharma (1982, 1983), Pratima *et al.* (1996), Maury and Verma (1997), Mazon (1995) and Chandra and Kulshreshtha (2004).

Ghosh *et al.* (1999) and Ashok and Pankaj (2000) explained a negative correlation between the effluent concentrations and seedling growth on the basis of inter relation between pH and phosphate content of effluent. At higher pH monovalent phosphates are converted into divalent form and trivalent form. Monovalent form of phosphate is only soluble in water. Other forms of phosphate precipitates in an insoluble form making it unavailable to the plant.

Salisbury and Ross (1993) reported that different concentrations of effluent with different pH values give different forms of phosphate to the growing seedlings. At pH up to 7.1 monovalent forms of phosphate was available which slowly gets converted into trivalent due to increase in pH. Due to non-availability of phosphate the germination and seedling growth were severely affected.

In the present study the inhibition of germination beyond 50% effluent concentration might be due to an interrelation between pH and phosphate content of effluent. pH plays an important role in breaking dormancy and enhancing seed germination (Keeley and Fotheringham, 1998). Killi (2004) proved germination enhancement of *Gossypium* sps. at increasing pH. However Goubitz *et al.* (2003) reported that high pH negatively affects seed germination in *Pinus halepensis*. Perez-Fernandez *et al.* (2006) reported that high pH negatively affected the germination rate of seeds but had no effect on the germination percentage.

4.2.2 Length of the radicle and plumule

In the present study root and shoot length were slightly increased at lower concentration (upto 50%) of the effluent. Reduction in the root and shoot length were evident with 75% and 100% effluent treated plants. Inhibition of root and shoot length at higher concentration of the effluent is due to the high level of total dissolved solids, suspended solids, volatile solids and high level of chlorides present in the effluent which interfered and inhibited the uptake of other elements like K, Ca, P and Mg by the plants. Similar results were reported by Tabraj *et al.* (1964), Augusty and Mani, (2001) and Nath and Sharma (2002).

In higher concentrations of the effluent the germinating seeds would get low amount of oxygen which restricts the energy supply and retards the growth and development of seedling. Parallel results had been reported by

Handas (1976) and Bahadur and Sharma (1989). Subramani *et al.* (1998) reported a progressive decrease in seedling growth with the increasing concentration of fertilizer factory effluent. Similar inhibitory effect in growth induced by higher concentration of factory effluent was noticed in several other crops also (Sahai and Srivastava, 1986; Neelam and Sahai, 1988; Sahai and Srivastava, 1988; Swaminathan and Vaidheeswaran, 1991; Kannabiran and Pragasam, 1993; Ghosh *et al.*, 1999; Vinod Sharma *et al.*, 2002; Singh *et al.*, 2004). Murukumar and Chavan (1987) had reported that high concentration of effluent decreases dehydrogenase activity that is considered as one of the biochemical changes which may have disrupted germination and seedling growth. This is in consonance with the studies of Vijayakumari and Kumudha (1990), Rajaram and Janardhan (1988), Anil and Bishwas (2005) and Tharakeshwari and Shobha (2006).

Singh *et al.* (2004) reported that at 20% paper mill effluent concentration the mean length of seedlings of the *Trigonella foenumgraecum* L. and *Brassica nigra* L. were promotory when they were compared with control conditions of the seedlings. At 20% to 100% effluent concentration the mean of the seedlings of all the species was inhibitory. Similar toxic effects were observed by Nag *et al.* (1981).

Jerome and Ferguson (1972) reported that different types of ions which are present in the effluent inhibit the activity of hydrolytic enzymes, required at the time of germination and early growth. Reduction in photosynthetic rate

may also be considered as a reason for reduced growth of treated seedlings (Mukhiya *et al.*, 1983).

Mala Neogy (2002) observed a major toxic effect of $Al_2(SO_4)_3$ appeared to be manifested in the root system. Length of roots as compared to shoots of the seedlings was more affected due to increase in concentration of Aluminium. Toxic effects of aluminium on root tips are considered to be mediated through decreased export of cytokinins to shoot and deficiency of cytokinins has been held responsible for Al-induced inhibition of shoot growth (Massot *et al.*, 1994).

Inhibition of the primary root growth led to an increase in the number of lateral roots as the apical root meristem was damaged (Bulevins, 1993). In the present study inhibition of root elongation is considered to be the evident effect of metal toxicity in plants. Cell division at the root tip and cell elongation in the extension zone is two different mechanism in root growth, both of which are affected by the presence of metals. It was observed that the fertilizer factory effluent contains chloride, sulphate, phosphates and the heavy metals such as Cu, Pb and Zn. These were in consonance with the reports of Adrini *et al.* (1994) and Manisha Mall *et al.* (2004).

The deterioration in root growth and the severity of inhibition noted in the root concurred with the general observation that root accumulates more pollutants and translocates little to the above ground shoot. Similar findings were reported by Van Bruwerne *et al.* (1984) and Davies (1991). In the

present study nutrients such as nitrates and phosphates present in the diluted effluent might have played a role in promoting plant growth in lower concentration and at the 25% and 50% effluent may be in a beneficial level. This dilution might be the optimum concentration where the nutrients are richer and the factors inhibiting the uptake of elements and some physiological process are ineffective. This leads to the better growth of radicle and plumule. Similar suggestions have been made by many researchers (Dolar *et al.*, 1972; Rajannan and Obilsami, 1979; Sahai *et al.*, 1983; Neelam and Sahai, 1988; Rajaram and Janardhanan, 1988; Jabeen and Saxena, 1990; Vijayakumari and Kumudha 1990; Kannabiran and Pragasam, 1993 and Om *et al.*,1994). The stimulated seedling growth at lower concentrations of Cl^{2+} and Zn^{2+} followed by a decreased result in higher levels are in agreement with the earlier related work of Maury and Verma (1997), Nagoor and Vyas (1999) and Behera *et al.* (1999).

4.2.3 Vigour Index

Vigour index was used as a parameter to screen the tolerance and toxicity of heavy metals in the work of Sharma and Saran (1992) and Asha and Ketewa (1999). In the present study plants irrigated with 50% effluent found to show maximum enhancement in vigour index in all the treatments. The vigour index was found to be reduced than control in higher concentration in treated population. Excess amount of ions in plants imposes stress causing physiological constrains leading to decreased vigour and plant

growth in higher concentration of effluent. This is supported by the result of Ouzounidou (1993). 1% distillery effluent and 5% effluent irrigation were recommended to increase the vigour index of the *Phaseolus aureus* Roxb. and *Pennisetum typhoides* Rich (Kannan, 2001).

4.2.4 Phytotoxicity

Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity (Kapanen and Itavaara, 2001). In the present study phytotoxicity was found to increase with higher concentrations. This is in keeping with the findings of Chaney *et al.* (1978), Somashekar *et al.* (1984), Wang and Williams (1989), Kumar *et al.* (2003) and Saayman-du-Toit (2006).

The negative values recorded in the present study revealed the tolerance characters of the plants. The treated plants of Ujwala and *Capsicum* Wild were found to be more tolerant than Jwalamukhi and Jwalasakhi. The negative values unveiled the tolerant character of Ujwala and *Capsicum* Wild. The reports of Jetty and Srivastava (1995), Harrington *et al.* (1996), Asha Arora and Katewa (1999) and Anitha (2002) are also in tune with this.

Regarding the phytotoxicity of water pollutants, all species did not respond equally to the effluent. Some were relatively tolerant while others were sensitive. This has been reported earlier by Kumar and Mukesh (2001).

4.2.5 Growth, Phytomass, Productivity and Yield

The various growth parameters like height of the plant, length of the root, number of branches, number of leaves, days taken to flower, number of fruits per plant, weight of fruits, length of fruits, number of seeds per fruit, phytomass and net primary productivity were studied both in control as well as in the various concentrations of effluent treated plants.

The fertilizer factory effluent had a dual effect on the growth of the plants. Lower concentration of effluent treated plants showed a considerable enhancement in the various morphological parameters studied. The overall growth of 25% and 50% effluent treated plants exhibited more vigour over the control. However the inhibition increased with the increase in the concentration of the effluent. 100% concentration of effluent produced the most severe reduction in all the morphological parameters studied except number of days taken to flower.

In the study of Banerjee *et al.* (2004) on *Casuarina equisetifolia* Forst. a significant increase was observed in all the morphological parameters upto the concentration of 60% of distillery effluent and beyond this there was a decline. A drastic decline in growth and biomass was observed in 100% effluent treated plants.

Similar inhibitory effect in growth, induced by higher concentrations of factory effluent was noticed in several other crops also (Sahai and

Srivastava, 1986; Neelam and Sahai, 1988; Sahai and Srivastava, 1988; Bahadur and Sharma, 1989; Patel, 1991; Swaminathan and Vaidheeswaran, 1991; Kannabiran and Pragasam, 1993 and Iqbal and Mehta, 1998).

In the present study the decrease in growth has been variously attributed to the presence of high amount of dissolved, suspended and volatile solids, sodium sulphate, richness of chlorides *etc.* may be negatively affecting the overall growth of the treated population. Similar findings have been reported by Mishra and Sunandashoo (1989), Ashraf *et al.* (1992), Bansal *et al.* (1992), Rema Subramanian *et al.* (1993), Dutta and Boissya (1996) in Paddy, Dutta and Boissya (1998), Anoop Singh *et al.* (2002) in wheat, Sheela and Deepa (2007) in *Capsicum frutescence* L. and Kamlesh *et al.* (2007) in crop plants like wheat, pea, black gram and mustard.

Bahadur and Sharma (1989) pointed out the possible role of oil and grease on the soil surface in reducing the availability of soil oxygen and there by reducing respiration and other metabolic processes. They have also suggested that adhering of oil and grease on the root hair zone can obstruct the entry of water and decrease plant growth. Gradual accumulation of oil and grease in the soil might have led to the depletion of soil air and soil water, necessary for plant growth, which in turn might have caused reduction in the productivity and biomass was reported by many workers (Kich and Braun, 1978; Rao and Nandakumar, 1983; Ramesh Kumar *et al.*, 1990; Swaminathan and Vaidheeswaran, 1991).

Swaminathan *et al.* (1992) have also reported that when viscose factory effluent was added to the soil for irrigation purpose the effluent decreased the hygroscopic moisture, water holding capacity *etc.* making the soil unfit for plant growth.

In the present study reduction in growth at late vegetative phase can be attributed to the presence of fairly high amount of oil and grease present in the higher concentration of effluent .

Lower concentration of effluent treated plants showed a considerable enhancement over the control in the various morphological parameters like height of the plants, length of the roots, number of branches and leaves, number of fruits per plant, length and weight of the fruits and number of seeds per plant, where as number of days taken for flowering showed an opposite effect. 100% and 75% effluent treated plants produced flowers very much earlier than control and other concentration.

Since the fertilizer factory effluent contains optimum level of plant nutrients such as nitrates, phosphates, and small amount of micronutrients like Cu^{2+} and Zn^{2+} might be attributed to the favourable effect of growth parameters at the lower concentration of effluent.

The reports of Rajannan and Oblisamy (1979), Bhosale (1985), Misra (1987), Swaminathan and Ravi (1987), Neelam and Sahai (1988), Augusthy and Mani (2001) are in agreement with the present findings.

Pandey (2006) reported in plants of *Spinacia oleracea* L. and *Raphanus sativa* L. raised in pot culture method and irrigated with effluent from electroplating industry showed visual toxic symptoms like curled leaf tips, burned leaves, stunted growth, necrosis followed by chlorosis in leaves and finally death of the plants. Severity of toxicity was less in plants treated with diluted effluents. Similar findings were also reported on *Capsicum frutescence* L. treated with distillery factory effluent (Sheela and Deepa, 2007).

In the present study also treated population exhibited some toxic symptom on the leaves like chlorosis and rough leaf surface as the concentration of the effluent increased. The leaf chlorosis caused by effluent toxicity may be an after effect of the inhibition of Fe translocation from root to shoot. This will lead to a decrease in the leaf chlorophyll content as observed in the present study. Agarwala *et al.* (1977) also share the same view.

Narasimharao and Narasimharao (1992) observed that irrigation with the effluent of *Bhadrachalam* Paper Board Industry, Andhrapradesh did not show any deficiency or toxicity symptoms in rice and cotton crops.

In the present work root inhibition was more pronounced in treated population. Number of branches and leaves were also adversely affected as a result of these the phytomass and productivity of the plants also showed considerable decrease over the control. All these effects were concentration

dependent. Flowering and fruiting too had increased over the control. In all the experimental plants an increased yield was noticed upto 50% effluent concentration.

Yield reduction as a result of higher effluent concentration is reported by many (Sahai *et al.*, 1983; Arora and Azad, 1985; Ramesh Kumar *et al.*, 1990; Bansal *et al.*, 1992 ; Khan and Jain, 1995 and Kaushik *et al.*, 2005).

Shilpa and Pal (2005) studied the effect of phosphorus and potassium on grain yield and quality. P_2O_5 and K_2O increased the grain yield and protein content. Ramana *et al.* (2006) studied the effect of sewage water on the yield of groundnut. At 25% concentration of sewage considerable increase in yield, number of pods and seed weight were observed, but the higher concentration of sewage showed decreased yield. Similar results were reported by researchers (Adishesha *et al.*, 1997; Kannabiran and Harilal, 1998; Vijayakumari, 2003; Raina and Raina, 2005; Jaikwad *et al.*, 2005 and Girisha *et al.*,2006).

In the present study 50% concentration of effluent showed maximum yield followed by 25% concentration. Presence of nutrients might be responsible for the increase in the growth and yield of *Capsicum* plants.

Dutta and Boissya (1999) reported reduction in yield and test weight of the plant *Oryza sativa* L. treated with high concentration of paper mill

effluent. Similar results were observed by Sahai *et al.* (1983), Bansal *et al.* (1992), Agrawal *et al.* (1994) and Pradhan *et al.* (2001).

In the present study more or less same results were observed in *Capsicum* plants treated with higher concentration of effluent. The inhibitory effect of growth and yield may be due to higher concentration of effluent, high level of dissolved solids like Na, Cl and sulphate *etc.* which possibly inhibit the uptake of other elements like K, Ca, P and Mg by the plants. Similar result was reported by Ramesh Kumar *et al.* (1990). Absence of either noticeable pollen sterility or excess flower drop might be the reason for reduced number of seeds and fruits. It may be due to some physiological disturbance as in agreement with findings of Srihari Reddy and Rao (1981) in *Capsicum annuum* L.

As reported by Aery and Sarkar (1991) the stressed plants may be utilising their energy for the survival in the hostile environment, which otherwise could be used for their growth process. At such a situation the overall growth of the plants will be reduced, which in turn will reflect on the productivity and yield of the plants.

4.2.6 The stomatal index

In the present study the stomatal index of the mature leaves, treated with different concentration of effluent -25%, 50%, 75% and 100%- indicated low values compared to that of control. This may be due to the decreased

turgor as well as cell division and expansion. Bindu and Bera (2001) reported the effect of Cadmium on the stomatal indices of *Mungbean*, with an increase in Cadmium concentration the stomatal indices of both upper and lower epidermis decreased.

The reduction in stomatal index in polluted leaves was reported by Sharma and Butler (1973) and Inamdar and Chaudhari (1984). Almost the same result was observed by Wadkar *et al.* (1984) in the stomatal behaviour of *Cynodon dactylon* Pers.

The stomatal frequency in the ventral surface of leaf of seven days old seedling, increased gradually with increase in concentration of Aluminium sulphate in ground nut (Swaminathan *et al.*, 1992).

4.2.7 Biochemical parameters

a) Chlorophyll content

The chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of the plants treated with different concentrations of effluent showed various degrees of variation over the control. Higher dilution of effluent had caused considerable increase in the chlorophyll content. In all the treated population chlorophyll *a* showed more increase than chlorophyll *b*. Where as in lower dilution of effluent, the pigment contents were highly reduced.

The enhancement observed in the pigment content of the plants under study may be taken as a favourable effect of the optimum level of nitrogen,

phosphate and other micronutrients. Earlier reports confirm the present observation (Sahai *et al.*,1979; Rao and Nandakumar, 1983; Sahai and Srivastava, 1985; Nanda *et al.*, 1986; Jabeen and Saxena, 1990; Balasouri and Prameela Devi, 1994; and Taghavi and Vora, 1994).

But at higher concentrations of the effluent, the mineral contents will increase considerably and this will interact adversely with the physiological conditions necessary for the synthesis of chlorophyll. The higher concentration of the elements in the effluent must have interfered with the photosynthetic machinery which resulted in the significant decline of the chlorophyll pigment.

According to Shiva (1941) the toxic doses of a particular element may cause the deficiency of other elements. The toxicity of effluent at higher levels may be a result of its interference with the uptake of other elements or it may be inhibiting certain enzyme system causing changes in metabolites.

The leaf chlorosis caused by effluent toxicity may be an after effect of the inhibition of Fe translocation from root to shoot. This will lead to a decrease in the leaf chlorophyll content as observed in the present study. Agarwala *et al.* (1977) share the same view.

Determination of the chlorophyll content of plants is often carried out to assess the impact of environmental stress, as changes in pigment content

are linked to visual symptoms of plant illness and photosynthetic plant productivity.

Parekh *et al.* (1990) used chlorophyll *a/b* ratio as a stress indicator in *Empetrum nigrum* leaves. The pigment content which showed a marked reduction at higher concentrations of effluent have been documented by a number of workers (Rao and Nandakumar, 1983; Sahai and Neelam, 1987; Rajendran, 1990; Balasouri and Prameela Devi, 1994; Reshmi and Panigrahi, 1994; Gadallah, 1996; Barik *et al.*, 1999; Ghosh *et al.*, 1999; Prabhakaran *et al.*, 1999; Srinivasa and D'costa, 1999; Verma *et al.*, 1999; Monni *et al.*, 2001; Muthusamy and Jayabalan, 2001; Ramana *et al.*, 2002).

Vijaywargiya and Pandey (1991) reported that sodium fluoride caused reduction in chlorophyll *a* and chlorophyll *b* and total chlorophyll of *Sorghum* seedlings.

Vijayawargiya and Pandey (1991) also reported that increase in exposure time of *Sorghum* seedlings decreased the amount of protein. Katz and Shore (1955) suggested that fluoride compounds may cause degradation of chlorophyll. Constantinidou and Kozlowski (1979) concluded that decrease of protein contents was due to decrease in photosynthesis.

Taghavi and Vora (1994) reported that the level of protein contents was directly propotional to the chlorophyll content. Mishra and Behera (1991) and Chaturwedi *et al.* (1995) have also reported that leaf chlorophyll

and leaf area were reduced upon irrigation with the effluent of higher concentration, while these parameters were enhanced at lower concentration of effluent.

Dutta and Boissya (1998) reported that chlorophyll and protein contents in wheat leaves were more at 50% irrigation over control. While 100% effluent irrigation resulted in reduction of the same.

b) Carotenoid

The carotenoid is a very important pigment. The light energy absorbed by the carotenoids is transferred to chlorophyll *a* for photosynthesis. So its destruction causes various growth abnormalities.

As a result of 25% effluent treatment the carotenoid content of all the four plants showed an increase over the control. 50% effluent too had caused the carotenoid content to increase over the control, but to a lesser degree. At the same time 75% and 100% effluent had highly reduced the carotenoid content of the treated population.

The minerals and metals of the effluent affect the membrane structure and their permeability may account for the reduction in the carotenoid content at higher concentration of the effluent. Epstein (1972) also share the same findings.

c) Total protein content

Protein is the most sensitive macromolecule affected by effluent. Measurement of protein and protein enzymes might therefore provide a useful criterion for the evaluation of the phytotoxicity of effluent. Mishra and Behera (1991) investigated effects of pulp and paper industry effluent in relation to both concentration and time of exposure to the effluent on Rice seedlings, protein content showed a decreasing trend with increase in effluent concentration and time. The cotton yarn dye effluent showed increase in proteins at 25% and 50% and were decreased with increase in the higher concentration (Saravanamoorthy *et al.*, 2005).

In the present work the effluent showed a dual effect on protein content. Lower concentration caused considerable increase in the protein content of the treated plants. But 75% and 100% effluent caused an inhibition of the protein content.

Higher concentration and longer periods of exposure to industrial waste water indicate tissue injury and lowering of protein content reported by Fikriye (2006) in bean. Decrease in protein content was recorded in Cadmium exposed *Lemna minor* plants by Singh *et al.* (1994) and Vivek and Jaswant (2006).

Increasing doses of chromium caused reduction in sugar and protein in paddy leaves. At increasing doses of chromium, catalase and peroxidase

activity was found to be reduced (Singh *et al.*,2006). A depressive effect in protein content was observed in bean grown in Zn nutrient solutions (Chaoui *et al.*,1995).

d) Total carbohydrate

Reduction in photosynthetic rate will lead to reduced accumulation of the assimilatory products (Harris *et al.*, 1970). When the photosynthesis and photosynthetic pigment contents are adversely affected naturally the photosynthetic process will be reduced and this may account for the reduced content of total carbohydrate in the effluent treated plants.

The dual nature of effluent was very clear in its effect on the total carbohydrate content. In all the treated plants 25% and 50% effluent concentration produced a considerable increase in the total carbohydrate content. As the concentration of the effluent increased the impact also increased. The 100% concentration proved to be the most unfavourable one, followed by 75%.

As described earlier, when the overall growth of the plants was promoted as a result of the optimum levels of nutrients in the 25% and 50% effluent concentration, the photosynthetic activity also was enhanced. This caused an increase in the carbohydrate content. The increase in protein content may be due to the absorption and transportation of the nitrate in the effluent. This has been also reported by Van Dobban (1961, 1962).

The various negative characters in the higher concentration of the effluent like low dissolved oxygen, high amount of suspended solids, dissolved solids, volatile solids, ammoniacal nitrogen, chlorides, sulphates *etc.* will be behaving as barriers for the normal growth of the treated plants. The various toxic effects of the effluent might prevent the normal growth of the plant as they move through the phenological phases. When the photosynthetic machinery is affected, naturally the carbohydrate content also will be affected.

These findings are in agreement with the views of Chapman *et al.*(1939) and Defedar (1983), Wadkar *et al.* (1984), Muthuchelian *et al.* (1988) and Swaminathan *et al.* (1992). Irrigation of plants with industrial effluents produces a marked change in growth criteria depending on plant and/or the stage of growth. On the other hand combination of industrial waste water with VAM caused an increase in the total carbohydrates and total nitrogen.

Swaminathan and Vaidheeswaran, (1991) observed that the pure dyeing factory effluent decreased the amount of physiological parameters while diluted effluents increased the carbohydrate, chlorophyll and protein in *Arachis hypogea* L.. Mishra and Behera (1991) reported that carbohydrate content showed a decreased trend with increase in pulp and paper industrial effluent concentration and time. Mahmoud *et al.* (2005) reported that

different concentrations of Cd and Pb induced marked inhibition in the contents of soluble carbohydrate and proteins.

4.2.8 Cytological studies

Analysis of the genotoxic potential of a substance through the investigation of the induction of chromosome alterations represents an effective method for biomonitoring studies and for the analysis of the extent of pollution (Fielder *et al.*, 1991 and Harden, 2001).

In the present study, the cytological damage caused by the effluent is observed only in minor frequencies, but the frequency of aberrant cell was more than control in treated population. The clastogenic aberrations in the root tip cells are attributed mainly to the abnormal activity of the spindle organization. Stickiness of chromosomes has earlier been reported to be produced in root tip cells by many chemicals. Adam and Rashad (1984) assigned the induction of stickiness of chromosomes to liquification of chemical material which hinder the normal separation.

The chromosomal aberrations induced by the fertilizer factory effluent include deeply stained cells, stickiness, fragments, unorientation, bridges and laggards. Such aberrations have been reported to be caused by large number of chemical agents besides plant extracts (Sharma and Paneerselvam, 1990).

According to Mann (1977) the chromosomal aberrations constitute a significant portion of the genetic damage. Stickiness normally occurs due to

breakage and reunion of sticky ends of chromosomes, fragments are formed due to deletion caused by chemical treatment (Lea, 1955; Kaur and Grover, 1985 and 1985a). Stickiness of chromosomes observed in the treated cells reveal the depolymerisation effect of effluent on the nucleic acid of the chromosomes. Soheir *et al.*(1989) reported a high level of chromosomal stickiness in metaphase stage after herbicide treatment.

Patil and Bhat (1992) reported that stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. The formation of small fragments can be attributed to the chromosomal breakage due to effect of the effluent.

Non-orientation of chromosomes at metaphase and anaphase were possibly due to disturbances in the formation of spindle (Bhamburkar and Bhalla, 1980; Soheir *et al.*, 1980; Banerjee and Sharma, 1981; Shehab and Adam, 1983; Soheir *et al.*, 1983; Zeerak, 1991). Laggards reported in the present investigation may be due to chromosome breakage or malfunction of spindle apparatus.

Anaphasic bridges may be formed either due to unequal exchange or dicentric chromosomes which are pulled equally to both the poles (Sax, 1940; Najjar and Soliman, 1980; Badar, 1983; Shehab and Adam, 1983).

The formation of bridges could be attributed to chromosomal stickiness (EL-Khodary *et al.*, 1990). Induction of bridges and breaks may lead to loss

of genetic material (Salam *et al.*, 1993). The effect of silk dyeing industry effluent is a clear indication of the mitoclastic and clastogenic property of the effluent.

The abnormalities observed included stickiness of chromosomes, fragments, bridges, laggards and vacuolated nuclei. Here the silk dyeing industry effluent acted as potential mutagens (Sudhakar, 2001). Naser *et al.*(2005) screen the cytological impact of silk industry discharge on *Vicia faba* L. It was noticed that the mitotic index had fallen corresponding to the increasing dosage of effluent used. Similar abnormalities were also reported many (Ravindran and Ravindran, 1978; Shanthamurthy *et al.*, 1979; Venegas *et al.*, 1994; Thangapandian *et al.*, 1995; Kaushik *et al.*, 1996 and Kumar and Prasad, 1998).

Dane *et al.* (2006) reported that low concentration of minerals in waters of effluent channels of textile thread factory and Olmuksa Paper Mill has some positive effects on root growth and mitotic division in onion root tip cells.

Aybeke *et al.* (2000) observed that total protein amounts were fallen and mitotic abnormalities and mitotic cell division frequency were increased depending on the concentration of olive oil mill effluent and time of the treatment.

The action of carbonaceous sugar mill effluent in *Hordeum* was mitodepressive which may be eliminated after dilution of effluent as opined by Arindom (1999). A 2% dilution of distillery effluent not only inhibited mitosis but also induced a 10-fold increase in the frequency of chromosome abnormalities in onion root tip cells (Chaurasia, 1994).

Rajrendiran (1990) reported an impact of distillery effluent on *Helianthus annuus* L. var. Ec 68414. A decreased seed germination, seedling growth, chlorophyll content and chromosomal abnormalities were observed with increase of effluent concentration.

Similar chromosomal aberrations were also reported after treatment with industrial effluent of Durgapur barrage water (Shekhar Banerjee and Ray, 1984) and effluents of Tisco factory (Ganesh Thakur and Roy, 1986).

The impact of Tamla Nalah river water contaminated with industrial effluent, on *Allium cepa* L. was conducted by Malabika and Banerjee, 1986. Effects of Ganga water contaminated with industrial effluents on root tip mitosis of *Allium cepa* L. were indicated (Azam and Biswas, 1987). Effects of Sapucaizinho river, Brazil contaminated with tannery effluent on root tip mitosis of *Allium cepa* L. were reported by Silvia and Maria (2004).

Abnormalities in cell division were observed in root tip cells of *Vicia faba* L. treated with Titanium factory effluents (Abraham, 1988). Effluents from the Titanium factory produced morphological abnormalities in flowers

and somatic mutations in the staminal hairs of *Tradescantia* clone O₂ (Abraham, 1989). Toxic effects of pollutants in the environment were manifested in the plant *Crotalaria laburnifolia* L. growing near the Titanium factory by the presence of growing retardation, inhibition of cell divisions, chromosome clumping and a variety of chromosomal aberrations (Abraham and Abraham, 1991).

All chromosomal aberrations that could be easily detected microscopically in the form of fragments, extreme clumping and stickiness, laggards, early and delayed movements of chromosomes could be attributed to the effect of the agents used for treatment. Reports are available regarding cytogenetic damage induced by industrial effluents (Clowes, 1951; Lilly and Thoday, 1966; Ohno, 1960; Chang, 1966; Ray and Banerjee, 1983; Adam and Rashad, 1984; Pandita, 1986; Ray *et al.*, 1988; Chaurasia, 1992; Ray and Saha, 1992; Barman and Lal, 1994; Barman *et al.*, 1999; Barman *et al.*, 2000; Kishu *et al.*, 2000).

Ayşe (2005) observed reduction in the mitotic index and increased frequency of abnormal mitosis on root tip cells of *Allium cepa* L. when treated with food preservatives like potassium metabisulphate and potassium nitrate. Richa and Gopal (2004) on *Helianthus annuus* L. showed mitotic abnormalities after EMS treatment. There has been quite a number of investigations in the past which highlighted the effect of a variety of chemicals on cytological system (Shanthanamurthy and Rangaswamy, 1979;

Misra, 1982; Bandyopadhyaya and Bose, 1983; Gohil and Asha, 1983; Anjana Roy *et al.*, 1984; Archana Sharma, 1984; Chaudhary and Sajid, 1984; Mazrooei and Kabritu, 1984; Ahmad and Sinha, 1986; Chaurasia and Sinha, 1986; Dixit and Dubey, 1986; Mukherjee and Sharma, 1986, 1987; Younis *et al.*, 1988; Mukerjee *et al.*, 1990; De Campos *et al.*, 2003; Kaymak and Muranli, 2005). The presence of chlorides and sulphates forms causal factor or chemicals for the enhanced cytotoxicity in effluent in the present case also.

Jayaprakash (1994) reported the effect of chromium on the mitotic activity in the root meristems of *Allium cepa* L. with increase in concentration. Cr inhibited both root growth and mitotic index due to the action of heavy metal as a prophase poison. Cytogenetic effects of zinc sulphate were tested on somatic chromosomes of *Vicia faba* L. The treatment of 100 ppm of any duration and 2 hrs treatment of 250 ppm enhanced the mitotic index. But a fall in mitotic index has been noticed when concentration and duration of the treatment were increased (Hitendra Kumar *et al.*, 2007). The possible adverse effects of zinc pollution had been reported by several workers like Bonnet *et al.* (2000); El-Ghamery *et al.* (2003) and Tandi *et al.* (2004). Mahmoud *et al.* (2005) reported different types of chromosomal aberrations in response to heavy metals like cadmium and lead. The above mentioned abnormalities have also been reported by using Cadmium and Selenium by Mukherjee and Sharma (1988) and Cadmium and lead (Mukherjee *et al.*, 1990).

4.2.9 Pollen characteristics

The pollen, which is the highly reduced, haploid male gametophyte in higher plants, is a unique and vital functional unit represents the sole medium through which the entire male genetic attributes are transmitted to the next generation; and thereby it ensures the perpetuation of species. Pollen characteristics play a crucial role in the biology of sexual reproduction of plants as well as breeding programme and seed setting. Successful fruit set and high crop yield generally depend up on viable pollen grains (Vasil, 1974). As pointed out by Rana *et al.* (1996) pollen fertility is very sensitive to temperature changes. Pollen sterility and seed viability here showed a slight link.

The recent facilities of Scanning Electron Microscopy (SEM) and Interference Microscopy have become very useful tools for palynological studies with increased precision and accuracy (Le Thomas, 1980, 1981) and this has opened up profound avenues for better understanding of the exine ornamentation patterns, which are especially useful for its detailed studies.

The aperture characters are based on their form, number and distribution, and they show variation in different plants at various taxonomic levels so as to be of use in the identification of genera, species and varieties. Several palynologists have focused their attention on the aperture in a phylogenetic consideration (Walker, 1976).

Sterility of pollen is due to many factors such as chromosomal aberrations, gene action or cytoplasmic influences that cause abortion or modification of entire flowers, stamens or pistils or that upset the development of pollen, embryo sac, embryo or endosperm. Male sterility is of two types, genetic and cytoplasmic (Duvick, 1965; Edwardson, 1970).

The morphological characters of pollen grains are those relating to the germinal aperture, exine ornamentation, exine strata, size and shape, of which the aperture character is considered to be of primary importance, exine surface pattern secondary and the others as tertiary. In the present study the light as well as scanning electron microscopic studies revealed that the pollen characteristics were not affected by the various concentrations of the effluent. Pollen from both control and plants received effluent treatment showed similar characteristics.

4.2.10 Leaf and seed surface characteristics

The available literature reveals that not much work has been done on the epidermal characters of the plants worked out in the present study. In the present study the SEM characteristics of both leaf and seed surface showed additional deposition of wax and coatings that increases the hard texture as the concentration of the effluent increased. This may be due to the results of stress induced changes for protection. Neogy *et al.* (2002) reported a general trend of increased stomatal index with simultaneous rise in the number of trichomes around the midrib on the ventral surface of leaf of seven days old

seedling were noticed in the leaves through scanning electron microscopy. This is due to the effect of increasing concentration of heavy metals adhered to the leaf surface. The seed coat layers did not restrict water uptake (imbibition) to any extent, despite the presence of wax filled cells.

Organic compounds and toxic heavy metals absorbed and stored in plant may make them unsafe for consumption with biomagnification of pollutants in plants, the environmental health problem acquires dangerous dimensions (Walsh, 1976). Many toxicants may enter human system initially through herbivores as reported by Buchi and Lisk (1990) and Pandey *et al.* (2007).

Use of effluent as irrigation water provides avenue for reducing the disposal problem of effluent and enhancing the productivity of crops owing to the use of chemicals adhered in effluent as nutrient (Sims and Riddell, 1998; Roygard *et al.*, 1999; Anoop Singh *et al.*, 2002).

The high concentration of the effluent may be acting as a barrier for the normal functioning of the plants metabolic system. As a result the overall growth of the plant will be affected. This in turn will influence the fruit and seed development. The variation in seed coat texture and roughness of leaf surface may be a result of the disturbances experienced by the treated plants during the various developmental stages.

Besides inhibiting the metabolic processes effluent even causes a disturbance in cell division (Abraham, 1989). In the present study, it was observed that effluent had a dual effect on almost all the parameters studied. Lower concentrations of effluent produced an enhancing effect on the various plant characters, whereas 75% and 100% effluent proved to be unfavourable for the overall growth.

All the plants treated with 25% and 50% effluent exhibited better results compared to the control in almost all the parameters studied. Though this points to the nutritive value of the effluent in lower concentrations, its detrimental effect to plant growth when used as such, should always be given prime concern.