DISCUSSION

A. EFFECT OF DIFFERENT CONCENTRATIONS OF DYE MONASTRAL FAST BLUE ON RHIZOBIUM SPECIES.

In relation to Rhizobium the effect of dye Monastral Fast Blue BS indicate a gradual decrease in all the parameters with different concentrations but the lower concentration showed similar effects in comparison to control. The explanation for these result which are resembled with the work of different workers (Ahmed et al. 2012, Stan et al. 2011, Kruzatz et al. 2011) with different plants. There are a no. of views for this. In regards of this a wide range of soil properties such as pH, organic matter content, clay content, iron oxide content etc. They alter the effects of various metal levels in soil microbes (Babich H., & Stotzky G. 1980). Of these, soil pH is often found to have the largest effect owing to its strong effect on solubility and specification of metal both in soil as a whole and particularly in the soil solution. Thus, each unit decrease in pH results in approximately two fold increase in the concentrations of metals (Christensan TH, 1984 and Sanders JR, McGrath SP and Adams TM 1986). CEC Directive also points to
the fact that the limit values may be reduced in soil with pH values lower than six.

DHA is widely used in evaluating the metabolic activity of soil microorganism. Because dehydrogenises are not active independently of the parent microbial cell as extra-cellular enzymes in soil. The measurement of the DHA was a good overall indicator of microbial activity. DHA has been used as an indicator of microbiological activity in semi-arid Mediterranean soil (Garcia C, Hernandez T and Costa F, 1994). A significant correlation between soil DHA and heavy metal contents has been reported (Leiros MC et al. 2000). Some researchers (Casida LE et al. 1964) suggested that dehydrogenase assay is a useful indicator for evaluating the effect of toxic metals on the soil microbial activity. Other investigations have suggested that dehydrogenase assay is a more sensitive indicator of heavy metal effects on soil microbiological properties than other soil parameters (Lenhard G 1963). Although no investigation have been carried out during present work. But it might be the DHA activity which was responsible for dose dependent effects.

Our results suggested a good relationship between DHA inhibition and heavy metal contamination. Additional microbiological properties, such as microbial counts (heterotrophic aerobic bacteria, actinomycetes, fungi and symbiotic nitrogen fixers) and ATP content, as indicators of soil microbial biomass
were also responsible. As a biological indicator process, symbiotic nitrogen fixers were chosen because these bacteria are sensitive to small amounts of heavy metals (Lorenz SE et al. 1966). Nitrogen fixation is performed by phylogenetically and physiologically diverse groups of prokaryotic organisms. Because this phenomenon is quickly activated positively or negatively when environmental conditions are changed (Martensson AM, 1993). Results also support the idea that this group of soil bacteria plays an important role in monitoring the possible impact of heavy metal contamination. This study also demonstrated that changes in soil conditions due to heavy metal contamination have a large negative effect on soil microbes, as evidenced by the results of the microbial counting and ATP content measurements.

As stated earlier, the principal contaminants of the contaminated soil samples under study were Hg and As. Arsenic may have a direct effect on the microbial populations present in soil (Speir TW et al. 1999, Maliskewska-Kordybachi B). Worldwide, soil arsenic concentrations average 6.0 mg/kg and commonly range from 0.1 to 40 mg/kg (Tamaki S et al. 1992). Arsenic in soil or other metals are present in various forms because of their interaction with various soil components; therefore, the total concentration of a particular metal in soil cannot provide a precise index for evaluating its influence on soil microorganisms and enzyme activities. (Maclaren RG et al. 1998). Temperature
changes, particularly during dry spells, may help potentiate metal toxicity (Duker AA et al. 2005).

The effect of heavy metals on the number of culturable bacteria remains unclarified, as finding differ between studies (Baath E 1989). In this investigation the total number (in CFU) of bacteria, fungi an actionomyces was reduced in the contaminated site. However, fungi and actinomycetes seem to be less sensitive than culturable hetrorophic bacteria or even symbiotic nitrogen fixers. Various studies have found that fungi are more resistant than bacteria to long-term heavy metal contamination (Baath E 1989; Fliessbach A et al. 1994; Frostegrad A et al. 1996). (Hayat S et al. 2002). However, studies of Hayat's et al. (2002) were performed under in vitro conditions, and the ability of microbes to tolerate a definite level of heavy metals under natural conditions might be different owing to the complex nature of the soil environment.

Most studies of heavy metals have been carried out with soil samples incubated under laboratory conditions. As reported in recent review (Speir TW et al. 2002), there was relatively little data from long term studies on microbial responses to heavy metal contamination caused by chronic industrial pollution. In our study, which was performed under field conditions, important long term changes in morphological characteristics have been observed. It was also apparent from this investigation that certain
groups of soil microbes are more sensitive to long term heavy metal contamination. With various parameters various workers had observed dose dependent effect of heavy metal, viz., Ignatov OV et al. (2001), Kamnev AA et al. (2005), Voigt A, Hendershot WH (2006), Stazkovic et al. (2011), Ahmed and Khan (2010), Khan (2012). Our work were also inconsistence of results of these workers i.e. higher the dose greater the inhibition and lower dose had less or no significant effect.

B. EFFECT OF DIFFERENT CONCENTRATIONS OF DYE MONASTRAL FAST BLUE BS AND RIZOBIUM INOCULANT ON TEST CROP : GREEN GRAM (Vigna radiata L. Wilczek):

The present piece of work is undertaken to study the effect or different concentrations of the dye and Rhizobium inoculant on various physiological aspects such as seed germination and its physiology, plant growth, metabolism and productivity of Green gram (Vigna radiata L. Wilczek).

1. Seed Germination and Germination Physiology:

Seed germination and seedling growth are primary events in the life cycle of a crop plant. Their success ensure healthy growth and better yield. Seed germination unfolds the hidden embryo to develop into a healthy plant. To ensure the effect of the dye and Rhizobium inoculant during germination and the seedling growth,
seed were treated with different concentrations of dye and *Rhizobium* inoculant. The treated seeds, which were allowed to germinate in petri-plates between moist filter papers, showed different effects. Lower doses were found parallel to control. In Green gram, seed germination in control plants gets completed within 7. The embryo emerges out of the seedcoat within 48 hrs.

The promotion in seed germination observed in the present study with treatments of lower concentration of the dye and *Rhizobium* inoculant indicates that treatments improved the permeability of the testa, thus allowing faster diffusion of water into the cotyledons and then to the embryos. Higher concentrations of the dye and *Rhizobium* inoculant, which inhibited seed germination seems to be due to an alteration in the permeability of the testa allowing poor diffusion of water molecules into the endosperm of the seeds.

The emerging seedling requires nutrients for its proper growth they are provided by the hydrolytic activity of enzymes such as amylase and protease present in cotyledons/endosperm. For proper understanding of the seedling growth, the activity of these two key hydrolytic enzymes was considered essential. The activity has been determined in terms of their hydrolytic products i.e. reducing sugars and the amino acid formed by the enzymes respectively. The lower concentration of the dye and *Rhizobium* inoculants which stimulated amylase and protease activity, resulted in the availability of more nutrients for the developing embryos. The treatments of higher concentrations of the dye and
Rhizobium inoculant inhibited the activities of amylase and protease consequently and may probably reduced the supply of the reducing sugars and the amino acid, the two most important raw materials required for cell growth. Treatment of higher concentrations may also inhibit seedling growth by checking translocation of metabolites from cotyledons/endosperm to the developing seedling. Studies on seed germination and physiology of germination as affected by pollutants and metals have been also carried out by Vallee and Ulmer (1972), Chandra and Mathur (1985), Beg et al. (1993), Gautam and Srivastava (1998), Nirangana et al. (1999) and Anuradha and Rao (2002), Gupta and Shukla (2003), Lal and Mishra (2003), Malla and Mohanty (2005) and Singh et al. (2006), Mumthas et al. (2010 Solanki et al. (2011).

These studies carried out with the various pollutants and metals used by these workers indicate that all of them had a dose dependent effect on seed germination of various crops and also dose dependent effect on the activities of amylase and protease, the key hydrolyzing enzymes which regulate the supply of metabolites such as reducing sugars and amino acids to developing embryos. GA₃ is a growth regulator i.e. known to stimulate the synthesis of amylase during seed germination (Chrispeel and Varner, 1967; Tanaka and Akazawa, 1970). The enzyme catalyzed hydrolysis and solubilization of starch in germinating seeds have also been reported by Ching (1972). The protease activity make amino acid available for the developing
seedlings (Beever and Splittstoesser 1968). The proteolytic enzymes showed great diversity with respect to their specificity for peptide bond linkage and pH optima (Mayer and Mayber, 1978). The non-supply of metabolites to the developing embryo, thus resulted inhibition of seedling growth. The seedling dry weight is another parameter, which gives indication of the effect of pollutants on seedling growth. In the present work, treatment of dye and Rhizobium inoculant had similar effects on seedling growth. The treatments of lower concentration was parallel to control, while those of higher concentration inhibited growth. Different workers on several plants have made similar observations.

Saravanan et al. (1996) reported that the higher concentration of copper sulphate affects seed germination and growth severely. Arora and Chauhan (1996) observed that concentrated effluents from tannery showed inhibitory effect on Hordeum vulagre. Pillai and Pugazhendi (1996) reported that diluted effluents from dairy were found effective in promoting seed germination, growth, chlorophyll content and protein content. Prasannakumar et al. (1997) reported a gradual decrease in germination, seedling growth and pigment content on Vigna mungo with increase in effluent concentration from chemical industry.
2. Effects on Nitrogen Utilizing Enzymes:

Effect of the dye and *Rhizobium* inoculum have been studied on key nitrogen utilizing enzyme i.e. nitrate reductase (NR). Treatments of the dye and *Rhizobium* inoculant in the present investigation in the primary leaves had similar effects. The lower concentration had no significant effect, while higher concentration had inhibitory effect. Such effects are not different with those observed by various workers with different plants. Kiermeir *et al.* (1963), Wallach (1973), Prasad *et al.* (1980), Mathur *et al.* (1988), Singh *et al.* (1987), Sinha *et al.* (1988), Assche and Cligster (1990), Uma and Sasikala (1998) and Saxena and Saxena (2002), Quadri *et al.* (2003), Zutshi (2003) and Xiong (2006), Solanki *et al.* (2011).

3 Effects on Nitrogen Metabolism:

In the present investigation treatments of the dye and *Rhizobium* inoculant when used at low concentration had no significant effect on the total free amino acids in the primary leaves, but higher concentrations of the dye and *Rhizobium* inoculant showed inhibitory effect. The general trend in the various fractions of nitrogen, viz. soluble and insoluble nitrogen in the primary leaves with treatments of the dye and *Rhizobium* inoculant, were found to be similar i.e. treatments of lower concentration had no significant effect on this fractions over control, but treatments of higher concentrations reduced the nitrogen content.
The changes in the various fractions of nitrogen in the primary leaves are reflection of the changes induced in the activities of the enzymes of nitrogen metabolism. Generally, in beginning, a young leaf has a high amino acid pool formed due to high respiratory activity combined with high GDH or GOGAT pathways. With growing age of the leaf and also of their demand for reproductive organs, the amino acid pool decreases with simultaneous increase in other fractions particularly the insoluble and soluble nitrogen. Variation in this general pattern are brought about by exogenous application of growth regulators, pesticides, pollutants etc. The data of present investigation showed that treatment of dye and Rhizobium inoculant affected the general pattern of various nitrogen fractions. The treatment of lower concentration had no significant effect on these fractions over control, where as the higher concentrations decreased them. Such observations are similar to those observed by various workers with different plants. Valle and Ulmer (1972), Guneyman et al. (1991), Khan and Jain (1995), Giller (1998) and Neogy et al. (2002), Pandey and Agarwal (2002), Zulthi (2003), Yao et al. (2003), Chandra (2004), Chaudhry S.P. et al. (2006), Sridevi et al. (2008), Kuriakosa and Prasad (2008).

4. Effect on Carbon Metabolism:

Changes in the chlorophyll and the total reducing sugar in the leaves are two indicators of the changes induced by the
pollutants in the carbon metabolism of a plant. The result of the present investigation shows that treatment of dye and *Rhizobium* inoculant on various chlorophyll fractions and the total reducing sugars were similar. Treatments of lower concentration of the dye and *Rhizobium* inoculant had no significant effect on Chl-a, Chl.-b and the total chlorophyll over control, whereas the higher concentration had inhibitory effects. From the present data, it may also concluded that treatments of lower concentration of dye and *Rhizobium* inoculant had no significant effect on photosynthesis over control, but higher concentration inhibited it because of the production of reduced amount of sugars. Similar observation were reported by various workers with different plants. Carlson *et al.* (1975), BAszynski *et al.* (1980), Nag *et al.* (1981), Brookes and McGrath (1984), Burzynski (1985), Upadhya and Pandey (1991), Prasad *et al.* (1991), Bacillo (1997), Saxena and Saxena (2002), and Pratibha and Rathore (2002), Zulthi (2003), Yao *et al.* (2003), Jha and Dubey (2005), Chtterjee (2006), Zeng LS *et al.* (2006), Ahmed *et al.* (2012).

5. Effect on Plant Growth:

In the present study the growth as affected by treatment of dye and *Rhizobium* inoculant has also been investigated in term of plant height, number of Branches/plant, number of nodes/plant, length of internodes/plant, number of pods per plant and length of pods/plant etc. The dye and *Rhizobium* inoculant had similar effects on plant growth. Lower concentration of the dye and
*Rhizobium* inoculant had no significant effect on plant growth, while higher concentrations inhibited it. Such effects of the dye and *Rhizobium* inoculant are not different from those observed by other workers with different plants. Holden (1961), Kashin (1971), Roth et al. (1971), Dutta and Boissaya (1977), Vesper and Weiden Soul (1978), Austenfield (1979a), Hela and Ormrod (1982), Manker and Martin 91984), Farooqi and Singh (1993), Singh and Singh (1994), Khan and Jain (1995), Singh and Singh (1995), Saxena and Saxena (2002), Gehlot et al. (2003), Bashan and Ramchandran (2004), Nath et al. (2005), Heikens A et al. (2005), Alexander A & Kamnev A (2005), Alvarez et al. (2006), Zeng Q et al. (2006), Sridevi et al. (2008), John et al. (2009), Kauchout et al. (2009), Mumthas et al. (2012).

6. Effect on Productivity:

The effects of the dye and *Rhizobium* inoculant on crop productivity measured in term of average dry weight of seeds number of seeds/pod dry weight of plant, total reducing sugar content in seeds, total nitrogen and total protein content in seeds showed that each parameter was affected in similar way. Low concentration of the dye and *Rhizobium* inoculant had no significant effect over control, while higher concentration had inhibitory effects.

The productivity of a crop plant depends upon two most important plant metabolism, viz. photosynthesis which provides
photosynthetase to the developing fruits and the seeds and the nitrogen metabolism that provides the various nitrogenous compounds required for growth. The effects of dye and Rhizobium inoculant on carbon and nitrogen metabolism as discussed earlier are in consistent with their effects on yield. Similar observation were reported by various workers with many plants. Chucka et al. (1937), Kusaka et al. (1971), Kim et al. (1986), Singh (1984), Bharatveer (1986), Pundeer and Singh (1994), Singh and Singh (1995), Kwun et al. (1999), Neogy et al. (2001) and Shetty and Magu (2001), Kumar (2002), Zutshi et al. (2003), Appleton JD (2005), Chatterjee et al. (2006), Sridevi et al. (2008), Solanki et al. (2011).

7. Effect on Nitrogen Metabolism of the Root Nodules:

Root nodules are special sites of atmospheric nitrogen fixation in the roots of pulse crops. Their effectiveness is determined by the presence of a pink pigment called the leghaemoglobin, which regulates the supply of oxygen to the nitrogen fixing anaerobic enzyme, the nitrogenase presence in the bacteroids inside the root nodules.

In the present investigation, treatment of the different concentrations of the dye and Rhizobium inoculant had similar effect on leghaemoglobin. The lower concentrations had no effect on leghaemoglobin concentration, but higher concentrations decreased it. It is very clear from the data, the lower concentrations were found almost similar to thrash control concentration where
as the higher were inhibitory. Such effect have been observed by other workers in different legumes (Smith et al. 1978, Mathur 1986, Hamidi et al. 1974, Ghosh 1995, Dube et al. 2003, Chaterjee et al. 2006). Changes in the various nitrogen fractions in root nodules reflect the effect of exogenous treatments on activities of nitrogenous glutamine synthetase, etc and can be correlated with their activities (Sridevi et al. 2008, Ahmed and Khan 2010, Kruzatz et al. 2011, Ahmed et al. 2012).

All of these reports are in agreement with the present investigation i.e. all of the different test materials with different cultivars showed similar result i.e. dose dependant effect. Based on the data reported in the thesis, it may be concluded that application of the metal-based dye in Green gram may be done after establishing their proper dose, which should not be harmful to the crop plant. Their frequency of application through irrigation channel on the crop plant is also to be determined. When all such parameters are kept in view, their application for irrigation to the crop would be more beneficial to the farmers.

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