DISCUSSION

5. Discussion

Continuous use of wastewater for irrigation leads to heavy metals accumulation in the plants that can be observed as growth retardation as a result of alterations in biochemical process (Mishra and Singh 2000; Arun *et al.*, 2005; Singh and Kumar, 2006; Sharma *et al.*, 2006 and 2007 and Gupta *et al.*, 2009). Heavy metals are known as growth inhibitors and exerting various adverse effects on the plants, and this can lead to wider phytotoxic responses and decrease the yield and quality of agricultural crops (Kopyra and Gwozdz, 2003; Atici *et al.*, 2005 and Faheed, 2005). In addition to the toxicity effects of heavy metals even at low concentrations, heavy metals can accumulate throughout the food chain and cause a serious ecological and health hazards (Rana and Masood 2002, b ; Kumar *et al.*, 2006, b; Kilic *et al.*, 2009 and Yang Yingli *et al.*, 2010). It has provoked the emergence of biological technologies for cleaning aquatic environment from heavy metals, because it has been suggested as a cheaper and more effective alternative to the traditional physicochemical methods for removing toxic heavy metal from wastewaters (Volesky and Holan 1995 and McKay *et al.*, 1999).

Microorganisms, including algae, bacteria, yeast, fungi are known to be capable of concentrating metal species from dilute aqueous solution and accumulating them within their structure (Aksu *et al.*, 1990) because the cell wall of most of these biosorbents consists of lipid, polysaccharides and proteins which provide different functional groups such as carboxyl, hydroxyl, carbonyl, etc., groups that can form coordination complexes with metals (Wong *et al.*, 2000 and Abu Al- Rub *et al.*, 2004). Recent studies have been focused on the efficiency of marine algae to remove heavy metals (Chen and Mulchand, 2005; Romera *et al.*, 2006 and Ahluwalia and Goyal, 2007).

Regarding the adsorption experiments, algal biomass have been used as an adsorption agent for heavy metals in non- living form because of their capability to accumulate heavy metals to the same or greater extent than living cells (Aksu *et al.*, 1991).

In general, the heavy metals uptake capacities have varied significantly for different types of biomass (Malkoc and Nuhoglu, 2003). Red algae also contain cellulose and sulfated polysaccharides made of galactanes (agar and carragenates). Green algae are mainly cellulose and protein bonded to polysaccharides to form glycoproteins.These compounds contain several functional groups (carboxyl, sulfate groups, amino, hydroxyl, ...) that have identified as the main metal- sequestering sites (Romera *et al.*, 2007). The binding of metal to active sites of the cell wall is closely related to some intrinsic metal property, such as ionic radii and electronegativity of atoms (Chong and Volesky, 1996).

The solution pH was the most important parameter affecting the adsorption of metal ions (Sheng *et al.*, 2004; Kumar *et al.*, 2006, a; Sari and Tuzen, 2008, b and Iftekhar *et al.*, 2009) influencing the algae surface charge as well as the metal speciation in solution (Brinza *et al.,* 2009).

It was observed that in most cases, the removal efficiency was markedly low under highly acidic conditions (pH less than 4). Metal adsorption increased with increase in pH reaching to a maximum around pH 5 and then showed a rabid decline under highly alkaline conditions (more than pH 7) [Tables (2-6) and Figures (1-5)]. These results were in conformity with those of Kumar *et al.* (2006, a); Pavasant *et al.* (2006); Romera *et al.* (2007) and Sari and Tuzen (2008, a and b).

Little biosorption occurred at low pH <4 because there was a high concentration of proton in the solution and this proton competed with metal ions in forming a bond with the active sites (the functional groups) on the surface of algae. This bonded active sites thereafter became saturated and was inaccessible to other cations. In contrast, when the pH increases, the concentration of protons decrease and negatively charged biomass surface can interact with the positively charged metal ions and the biosorption was reached maximum. The highest removal of heavy metals by almost algal biomass was occurred at pH 5- 7depending on the electronegativity and ionic radius of the atoms (Vijayaraghavan *et al.*, 2008) and the type of biomass (Romera *et al.*, 2007).Whereas, the number of negatively charged active sites increased, faciliting a higher electrical attraction to positively charged metal ions and the biosorption was reached maximum. The decrease in biosorption at higher pH is might be attributed to the precipitation as metal hydroxides due to the increasing concentration of OH^- ions (Kilic *et al.*, 2009) and lower polarity of heavy metals ions (Kumar *et al.*, 2006, b).

Our results disagree with those of Amany *et al.* (2007) concerning their work on adsorption of toxic chromium using *Ulva lactuca* which decreased with the increase in pH from 1.0 to 11.0. The maximum removal was occurred at initial pH 1.0, suggests that bind of the negatively charged chromium species occurred through electrostatic attraction to the positively charged functional groups on the surface of sorbent cell wall due to the presence of more functional groups carrying positive charges at pH< 3.

The dependence of heavy metals sorption on time was studied by varying sorption time from 5 to 120 min. It is obvious that the percent of removal increases with rise in contact time up to 60 min when the sorption equilibrium were attained and after that there was no significant changes in adsorption with further increase in contact time [Tables (7-11) and Figures (6-10)]. This change in percentage removal may be due to the fact that, initially all adsorbent sites are vacant and the solute concentration gradient is high (Malkoc and Nuhoglu, 2003).

Our data recorded were in good agreement with the finding of Sari and Tuzen (2008, a) who stated that the biosorption efficiency of Pb^{+2} and Cd^{+2} ions by *Ulva lactuca* as a function of contact time and temperature.

On the other hand, our results were in disagreement with those found by Pavasant *et al.* (2006) who found that the sorption of Cu^{+2}, Cd^{+2}, Pb^{+2} and Zn^{+2} by *Caulerpa lentillifera* rapidly reached equilibrium within 20 min, since the algae was dried and biological functions were no longer active, the sorption could only take place on the surface of the cell. Therefore the sorption equilibrium took place quickly within 20 min and no further sorption was observed thereafter. Also, Suzuki *et al.* (2005) reported that the sorption of Cd by *Ulva* biomass was saturated after 12 h.

Biosorbent dose is a significant factor to be considered for effective metal removal as it determines sorbent- sorbate equilibrium of the system (Ahaly *et al.*, 2005 and Hanif *et al.*, 2007).

It could be concluded that the heavy metals removal percentage increased with increasing concentration of the algal biomass and the maximum biosorption of the metal ions was attained at about biomass dosage 40 mg/L [Tables (12-16) and Figures (11-15)]. This trend could be explained as a consequence of the increase in overall surface area and more pore volume for the biosorption (Malkoc and Nuhoglu, 2003), which in turn increase in binding sites with increasing biomass concentration.

These results are in harmony with those reported by Amany *et al.* (2007) who investigated the removal of chromium from wastewater using of *Ulva lactuca* and found that the extent of biosorpion is proportional to specific area which can be defined as the portion of the total area that is available for biosorption.

Our results disagree with Sari and Tuzen (2008, a), who found that the maximum biosorption of Pb(II) and Cd(II) using *Ulva lactuca* was attained at about biomass dosage, 20 mg/ L and it was almost same at higher dosage as a result of a partial aggregation of biomass at higher biomass concentration, which results in a decrease in effective surface area for the biosorption (Karthikeyan *et al.*, 2007), and also with Romera *et al.* (2007) who reported that the best sorption of cadmium, nickel, zinc, copper and lead using six different algal species (green, red and brown) were obtained with the lowest biomass concentration used (0.5 g/L), because high biomass concentration can exert a shell effect, protecting the active sites from being occupied by metal and lower specific metal uptake, that is a smaller amount of metal uptake per biomass unit.

It became clear that adsorption of highly toxic metals (Cd^{+2}, Pb^{+2}, Ni^{+2}, Zn^{+2}, Cu^{+2}) using algal biomass is effective as a technology for processing wastewater containing heavy metals. So the highly efficient marine algae species in removal of different heavy metals (*Ulva lactuca*, *Jania rubens* and *Pterocladia capillacea*) were chosen for treatment of low quality water (El-Batts drainage water and synthetic solution) before irrigation of wheat and faba bean crop plants.

The efficiency of algal treated water to improve the final percentage of germination, seedling enzymes, some vegetative growth parameters, photosynthetic pigments and chemical components of wheat grains and faba bean seeds were investigated.

Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxicity effect of heavy metals on plants (Wang and Zhou, 2005 and Ahsan *et al.*, 2007). Consequences of stress caused by heavy metals are a number of important disorders of metabolic processes, including disturbances in seed germination (Pal *et al.*, 2006; Ahsan *et al.*, 2007; Bybordi and Tabatabaei, 2009 and Szollosi *et al.*, 2009) .

Increasing heavy metal concentration, caused inhibition in the final percentage of germination in wheat grains and faba bean seeds when compared with control plants [Table (17) and Figure (16)], as general responses associated with the toxicity, and this stress lead to the decrease in activity of different enzymes and the breakdown of growth proteins. The promotion of final percentage germination of wheat grains and faba bean seeds was noticed under irrigation from algal treated water which may be due to the sorption of toxic heavy metals by algae, as compared to those irrigated from non- treated water, but remained less than the control. This increase indicates that the role of algae treated water in reducing the concentration of heavy metals and enhancement the final percentage germination of wheat grains and faba bean seeds. These results are in a kind of agreement with (Koprya and Gwozdz, 2003 and Radic *et al.*, 2010).

In connection, Barcelo *et al.* (1986) stated that the reason for the decrease in the germination percentage of cowpea seeds may be related to the negative effect of cadmium on water absorpion, transport and reducing the availability of water in the embryo axis. Rascio *et al.* (1993) stated that increasing amount of cadmium concentration limits the nutrient transport to shoot, therefore inhibits germination processes.

Similar observations concerning the inhibitory effects of heavy metals on germination percentage of wheat grains were reported by other authors, Munzuroglu and Geckil (2002); Song *et al.* (2002); Upadhayay *et al.* (2008); Yang Yingli *et al.* (2010) and Asgharipour *et al.* (2011), whereas for faba bean seeds, Rabie *et al.* (1992); Jyoti *et al.* (1994) and Madhulike Gupta (2011) and on the other plant species Hsu and Chou (1992); Weiqiang *et al.* (2005) and Cavusoglu *et al.* (2009).

Free radical generation is one of the initial responses which stimulated in the presence of heavy metals, which can seriously disrupt normal metabolism (Halliwell and Gutteridge, 1993). The exposure of plants to heavy metals provoke pronounced responses of antioxidative systems which play an important role in the cellular defense strategy and protect the plants to some extent against oxidative damage like antioxidant enzymes (Tawfik, 2008) but the direction of response was dependent on the plant species, metal used, the intensity of stress (Odjegba and Fasidi 2007), the organs and the growth stage (Shaddad *et al.*, 2007). Bonnet *et al.*

(2000) and Khudsar *et al.* (2004) reported that a key role of reactive oxygen species-scavenging enzyme in the protection against harmful oxidative reaction resulting from heavy metal stress.

A variation in activity of both peroxidase and catalase enzymes has been noticed with respect to heavy metals concentration (Shah *et al.*, 2001 and Solanki *et al.*, 2011). Activities of these enzymes might increase in order to cope with the oxidative stress imposed by heavy metals on plants, as was repeatedly found in other experiments (Ianelli *et al.*, 2002). Alternatively, they might be diminished if the toxic effects of higher concentrations of heavy metals were greater than can be tolerated and combated by the antioxidant enzymes (Kasim, 2005).

The role of peroxidase as stress enzymes in plants has been widely accepted and it used as potential biomarker for sub lethal metal toxicity in examined plant species (Bonnet *et al.*, 2000; Radotic *et al.*, 2000 and Gao *et al.*, 2008).

The activity of peroxidase enzyme in wheat seedlings decrease as compared to the control after irrigation with all non-treated water (El-Batts drainage water and synthetic solution). On the other hand, the algal treated water specially at synthetic solution of 60 and 100 ppm heavy metal concentrations induced a highly significant increasing in peroxidase activity of wheat seedlings [Table (17) and Figure (17)]. Our studies coincide with Sharma and Sharma (1996) who showed that the same gradual decline in activity of peroxidase at 0.05, 0.5 Mm Cr in wheat plant. Knorzer *et* *al.* (1996) indicated that high concentration of heavy metals can inhibit action of peroxidase.

On contrast, in fabe bean seedling, the peroxidase activity increased over the control in case of both all non- treated water and algal treated water except in case of 20 ppm synthetic solution which induced significant reduction in peroxidase activity [Table (17) and Figure (17)]. The same results was obtained by Shaddad *et al.* (2007) who reported that sewage and algal processed sewage induced an increasing in peroxidase activity in broad bean.

Numerous studies reported that the peroxidase activity response to excess copper can vary among plant species and among different tissue (Passardi *et al.*, 2005). Often, the increase in antioxidant enzymes activity is identified as the key in the prevent of sewage damage, while sensitive species typically exhibit either no change or decrease in activity (Rahmana and Ebrahimzedeh, 2005).

Catalase is one of the most important plant enzymes catalyzing the dismutation of hydrogen peroxide, and is known as a mediator of oxidative damage into oxygen and water (Lin and Kao, 2000; Mittler, 2002 and Miller *et al.*, 2008). Also catalase plays an important role in plant defense, aging and senescence (Mittler *et al.*, 2004).

The obtained results [Table (17) and Figure (18)] showed that in wheat and faba bean seedlings, all algal treated and non-treated water induced a marked reduction in catalase activity when compared with control plants except at non-treated 100 ppm and algal treated 60 ppm in faba bean seedling which induce a slight increase in catalase activity. In this concern, a decrease in catalase activity has been reported in other plant species exposed to different heavy metals (Prasad *et al.*,1999; McGeer *et al.*, 2000; Verma and Dubey, 2003; Draobzkiewicz *et al.*, 2004; Gao *et al.*, 2008; Sreedevi *et al.*, 2008 and Choudhary *et al.*, 2010). The decrement in catalase activity may be associated with degradation caused by induced peroxisomal proteases or may be due to photo inactivation of enzyme (Sandalio *et al.*, 2001). Under highly stressed condition, decrease antioxidant enzyme activity may be the result of imposition of oxidative stress i.e. imbalance in generation and metabolism of reactive oxygen species (Cakmak, 2000; Shah *et al.*, 2001 and Rai *et al.*, 2004).

The inhibitory effect of heavy metals stress induced by irrigation with low quality water and synthetic heavy metals before and after treating with algae on the vegetative growth parameters of wheat [Table (18- 19) and Figure (19- 24)] and faba bean [Table (20- 21) and Figure (25- 30)] is in agreement with the results obtained by some authors using different plants (Geuns *et al.*, 1997; Vassilev *et al.*, 1998; Grifferty and Barrington, 2000; Fecht-Christoffers *et al.*, 2003; Shukla *et al.*, 2003; Kasim, 2005; Roy *et al.*, 2005; Wang and Zhou, 2005; Jamal *et al.*, 2006; Agrawal and Mishra, 2007; Ghani and Wahid, 2007; Upadhyay *et al.*, 2008;

Faizan *et al.*, 2011; Perveen *et al.*, 2011; Souguir *et al.*, 2011 anc Fatma *et al.*, 2012).

The stimulatory effect of heavy metals removal on roo length were confirmed by other investigators who found that the root length of faba bean increased when irrigated from treated polluted water (Abd El- Kader and Mahmoud, 2007).

The change in root growth characteristics is probably due to the consequences of the direct exposure of the radical to metal toxicity and preferential accumulation of metals in the emerging roots followed by slow mobility to the plant shoots (Godzik, 1993 and Fargasova, 1994).

As far as the fresh and dry weight of shoot is concerned, it followed the same trend of reduction as in the case of shoot and root length after irrigation with non-treated water, and are in accordance with the results obtained by Mazen (1995); Shukla *et al.* (2003) and Mane *et al.* (2010).

The inhibitory effect of heavy metals on pigments biosynthesis in wheat [Table (22- 23) and Figure (31- 32)] and faba bean [Table (24- 25) and Figure (33- 34)] is in accordance with the result obtained by some authors Stobart *et al.* (1985); Oncle *et al.* (2000); Patra *et al.* (2004); Zengin and Munzuroglu (2005); Gajewska *et al.* (2006); Maksymiec *et al.* (2007); Sarita and Abha (2007); Yang Yingli *et al.* (2011) and Kaur *et al.* (2012).

The photosynthetic apparatus appears to be very sensitive to the toxicity of heavy metals. Heavy metals affect the photosynthetic functions either directly or indirectly, inhibit enzyme activities of the Calvin cycle and cause CO_2 deficiency due to stomatal closure (Seregin and Ivanov, 2001; Bertran and Poirier, 2005 and Linger *et al.*, 2005).

Bruzynski (1987) and Drazkiewicz (1994) assumed that the decrease in photosynthesis might be due to Pb which is known to inhibit chlorophyll synthesis either to impaired uptake of Mg and Fe by plants and also, due to increased chlorophyllase activity.

In connection Sandalio *et al.* (2001) and Kasim (2005) assumed that the inhibitory effect of heavy metals on the pigment biosynthesis due to the accumulation of reactive oxygen species (which can cause severe damage to thylakoid membranes). Carotenoids is a non anzymatic antioxidant, playing vital role in the protection of plants by scavenging the free radicals generated under heavy metal stress (Sinha *et al.*, 2007), but it was not able to ameliorate the negative effect caused by excess heavy metal absorption in plants (Singh and Agrawal, 2011). It is argued that the fact that cadmium has an effect on chlorophyll biosynthesis is due to prevention of photoactive protochlorophyll reductase complex formation and aminolevulinic acid (ALA) synthesis. Sulphdryl groups of this enzyme complex providing chlorophyll synthesis are blocked by cadmium, but ALA synthetase enzyme is blocked by complexation of active thiol groups with cadmium (Stobart *et al.*, 1985).

Siedlecka *et al.* (1997) and Pence *et al.* (2000) reviewed that cadmium can be accumulated to higher levels in the aerial organs, prefentially in the chloroplasts and disturbs the chloroplast function by inhibiting the activities of enzymes of chlorophyll biosynthesis and CO_2 fixation.

The stimulatory effect of heavy metals removal by other means on photosynthetic pigments biosynthesis were confirmed by other investigators who found that the decrease of harmful effect of cadmium improve the concentration of photosynthetic pigments in leaves of soybean (Fouda and Arafa, 2002 and Fatma *et al.*, 2012) and of tomato plants (El- Gamal *et al.*, 2003).

The depressive or promotive action of low quality water or synthetic heavy metals before and after treating with algae on the biosynthesis of total carbohydrate in wheat grains and faba bean seeds [Table (26- 27) and Figure (35)] is in accordance with the results obtained by other investigators Stiborova *et al.* (1987); Rabie *et al.* (1992); Narwal and Sing (1993); Eleiwa (2004); Mansour and Kamel (2005); Rai *et al.* (2005); Nitika *et al.* (2007); Bhardwaj *et al.* (2009); Hamid *et al.* (2010) and Aldesuquy *et al.* (2011).

In contrast, sugar contents increased significantly at low dose of copper, but were adversely affected by the increase in its concentration (Dharam *et al.*, 2006).

The depressive action of heavy metals on total protein content of plants irrigated with non- treated water when compared with control plants is in accordance with results obtained by Rana and Masood (2002, a); Tandon and Gupta (2002) and Sanjoy *et al.* (2010) in case of *Triticum eastivum*, and with Costa and Spitz (1997); Mohan and Hosetti (1997); Samantary (2000); Dharam *et al.* (2007) and Latif (2010) in case of various plants because heavy metals caused blocking of essential functional groups in biomolecules as proteins by inactivation of SH- groups in enzymes active centers (Baranowska, 2003 and Mithofer *et al.*, 2004) . It thought that decrease in total soluble protein content may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et al.*, 2002), various structural and functional modification by the denaturation and fragmentation of proteins (John *et al.*, 2009 and Monteiro *et al.*, 2009).

According to Pal *et al.* (2006) the reduction in protein content was due to the interaction with thiol residues of proteins and replacement them with heavy metals in metallo proteins. However, Ge Cailin *et al.* (2009) stated that most of the induced proteins played important role in biochemical reactions involved in tolerance of wheat to heavy metals stress.

The bioaccumulation of single metal is known to be influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture (Peralta-Videa *et al.*, 2002 and An *et al.*, 2004).

The results indicated that the accumulation of heavy metals (Cd^{+2}, Ni^{+2}, Cu^{+2}, Pb^{+2} and Zn^{+2}) in polluted wheat grains and faba bean seeds was increased by irrigation with El- Batts drainage water when compared with control plant seeds, and increased progressively with increasing heavy metals concentrations of synthetic solution 20, 60, and 100 ppm. It is clear that algal treatment induced decrease in heavy metals concentrations in seeds compared with the non- treated water [Table (26- 27) and Figures (37-38)].

Similar observations concerning an increase of heavy metals content in grains when poor quality water was used for irrigation were reported by Feizi (2001); Abd El- Hady (2002); Rana and Masood (2002, a); Zein *et al.* (2002) and Jouzdan *et al.* (2007).

In this concern, there are several reports of high accumulation of cadmium in grains of rice, wheat and barley grown on soils irrigated with cadmium- rich effluents (Pandey, 2006). The copper concentrations in wheat grains increased with an increase in the level of applied Cu and was maximum at 2.5 mg Kg^{-1} Cu (Kumar *et al.*, 1990 and Kumar *et al.*, 2009).

On the other hand, our results are disagree with Shaddad *et al.* (2007) who reported that the analysis of Ni and Cd content showed that their accumulation seemed to be increased in wheat and broad bean plants irrigated with algal treated water when compared with wastewater and control plants tested.

Thus, algal treatment may be an effective for reduction of heavy metals in the drainage polluted water and in this case the drainage water can be used for irrigation.