# LITERATURE REVIEW



### 2. Literature Review

The bioremediation of heavy metals from low quality water by marine algal powder and the effect of low quality water on wheat and faba bean plants will be reviewed as follows.

Biosorption is defined as the capacity of substrate to retain metallic species, ions forms and/ or ligands from fluids in the molecular structure of the cell wall. The biosorption mechanism include extracellular and intercellular bonds, as well as complex interactions that depended on the type of metal and the biosorbent structure. Also, bioremediation or biosorption is an innovative technology that employs inactive and dead biomass for the recovery of heavy metals from aqueous solution. As an alternative to traditional methods, its promising results are now being considered for application by the scientific community. In addition. wastewater, however, far from being simple or single-component, solutions are complex containing several metal ions simultaneously. In the biosorption of complex solutions, different metal ions may compete for the active sites present on the cell wall of the biomass. Consequently, the preference of the biomass for some metals is an important issue, which is affected by the type of biomass, its physiochemical characteristics, its preparation or previous conditioning and nature of metallic solution (Davis et al., 2003 a; Gavrilescu, 2004 and Rincon et al., 2005).

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Biosorption, which uses the ability of biological materials, is a relatively new technology to remove heavy metals from wastewater. The major advantages of the biosorption technology are the effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent material such as naturally abundant algae (Christian taty *et al.*, 2003 and Rakhshaee *et al.*, 2006). Many different biomasses materials such as, bacteria, fungi and yeasts can effectively uptake heavy metals and considered as good biosorbents for heavy metals recovery from wastewater, but recent researches have shown that algae are more effective than other biomasses (Chen and Mulchand, 2005; Romera *et al.*, 2006 and Ahluwalia and Goyal, 2007).

In this context, researches and development of new biosorbent materials has focused especially on algae, due to its high sorption capacity and its presentation in unlimited amounts in seas and oceans, especially brown and red varieties that grow exclusively in salt water. Macro algae also, are one of the promising types because they are stable and fast growing. Marine algae, otherwise known as a seaweeds have been reported to have high metal binding capacities due to the presence of polysaccharides, proteins or lipids on the cell wall surface containing functional groups such as, amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals (Ramelow *et al.*, 1992; Klimmek *et al.*, 2001; Davis *et al.*, 2003 a and b; Vijayaraghavan *et al.*, 2004; Brinza *et al.*, 2005; Suzuki *et al.*, 2005 and Romera *et al.*, 2007)

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Some green algae, are particularly useful in the respect of biosorption because of its wide distribution and relatively simple structure. It has a sheet like- thallus, which is two cells thick, resulting in a relatively high surface area of structurally uniform and physiological active cells (Turner *et al.*, 2007).

Earlier studies on heavy metals adsorption have shown that pH was the single most important parameter affecting the adsorption process. In all cases, metal adsorption by cells increases with increasing pH reaching to maximum and then showed rapid decline in adsorption.

Vijayaraghavan *et al.* (2005) revealed that the optimum pH values for the biomass, i.e. *Ulva reticulate* absorption for copper, cobalt and nikel were 5.5, 4.0 and 4.5, respectively. The biomass removed 72.6% of Cu at the first cycle of regeneration, 56.8% at the second cycle and 48.8% at the third one. Whereas, for Co the corresponding values were 40.7%, 39.8% and 36.4% for the three cycles, respectively. The metals uptake capacity for Cu, Co and Ni by the biomass were 56.3, 46.1 and 46.5 mg/g, respectively. High biosorption efficiency of this algae, low biomass costs, less dependency on the biomass (due to its reuse) and highly efficient elutant makes this process effective, cheep and alternative technique for metals bearing industrial effluents.

For artificial wastewater containing different metals, i.e. Cd<sup>+2</sup>, Zn<sup>+2</sup>, Cu<sup>+2</sup>, Cr<sup>+2</sup> and Ni<sup>+2</sup>, it was possible to remove each metal simultaneously using the *Ulva* biomass. At this case of several *Literature Review* 

types of heavy metal in the solution, their removal rate can determined by pH. The optimum pH for Cd+2 adsorption in such solutions was 8.0, when Ulva biomass used and the sorption capacity of Cd<sup>+2</sup> metal by this biomass was markedly low at pH less than 3.0 and more than 10.0, but high sorption potential was approximately at pH 6.0. The Cd<sup>+2</sup> concentration rapidly decreased during the first 2hrs and then gradually decreased and the sorption was saturated after 12hr (equilibrium sorption), for all initial concentrations. The removal rate of heavy metals, except for Ni<sup>+2</sup> were increased more than 80% at 20 gm/L of the biomass, when the pH was elevated to 5.0. In particular, the removal rate of Cu<sup>+2</sup> and Cr<sup>+2</sup> reached 95% by increasing the biosorbent dose of biomass to 50 gm/L. At pH 8.5, the removal rates of Cd and Ni improved, but the removal rates of Cu<sup>+2</sup> and Cr<sup>+2</sup> decreased to 80%. The alkali pretreated biomass gave the highest Cd<sup>+2</sup> adsorption (90.7% mg/g). whereas the value of non-treated biomass became 61.9 mg/g (Suzuki et al., 2005). At a given equilibrium concentration, the biomass uptake more metal ions at lower than at higher biomass cell densities (Mehta and Gaur, 2001). The optimum pH for maximum metal ions uptake per unit of biomass, i.e. Caulerpa lentillifera (a green macroalgae) in mol/kg were 0.14, 0.13 and 0.142 for  $Pb^{+2}$ ,  $Cu^{+2}$ ,  $Cd^{+2}$  at pH = 5.0. Total sorption capacities were approximately 0.09, 0.11 and 0.10 mol/kg for Pb- Cu, Pb- Cd and Cu- Cd binary mixtures, respectively which indicated that there was a limitation in maximum sorbent capacities at about 0.1 mol/ Literature Review

kg. On the other hand, the removal capacity of  $Cu^{+2}$  was 84% at pH 6.0,  $Cd^{+2}$  90% at pH 8,  $Pb^{+2}$  92% at pH 5.5 and  $Zn^{+2}$  80% at pH 6.5. In most cases, the removal efficiency increased steadily with pH and the sorption at low pH range usually took place with low removal capcity. The sorption of the four metal ions rapidly reached equilibrium within 20 min, and the sequence of maximum sorption capacities  $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$ , while the dose of 0.5 g dried algae/ 30 ml metal solution with a predetermined concentration (10 mg  $M^{+2}L^{-1}$ ) (Apiratikul and Pavasant, 2006 and Pavasant *et al.*, 2006).

The optimum pH value for high metals adsorption by six different types of algae (green, red and brown) for Cd<sup>+2</sup>, Ni<sup>+2</sup> and Zn<sup>+2</sup> was 6.0, whereas for Cu<sup>+2</sup> was 5.0 and for Pb<sup>+2</sup> was ranged between 3 and 5, according to biomass type. Green and red algae presented similar values of maximum capacity adsorption for all metals. In all cases, the values were much lower than those registered with brown algae, which besides being very high for all metals, reduced the metal equilibrium to very low levels and the best result was achieved with *fucus spiralis* (Romera *et al.*, 2007).

At a synthetic wastewater solution of Pb (II), Cu (II) and Cd(II), the sorption capacities by *Caulepra lentillifera* at initial concentration of 10 mg/L was rapid and reached the equilibrium within 20 min. An increase in algal mass resulted in a decrease in absorption capacity, but removal efficiency of metal increased steadily by increasing the sorbent dose, the optimal usage dose *Literature Review* 

were about 14.0, 17.5 and 10 gm/L for Pb, Cu, and Cd, respectively, and the order of maximum sorption capacity of biomass was Pb > Cu > Cd (Apiratikul and Pavasant, 2008).

The metal uptake capacity and removal efficiency are equally important in sorption experiments. Solution contained several heavy metals i.e. Pb<sup>+2</sup>, Cu<sup>+2</sup>, Mn<sup>+2</sup> and Zn<sup>+2</sup> when treated with *Sargassum* biomass (brown algae) gave maximum uptake of metals at pH above 4.0. For all the metal ions, biosorption rate was fast and most of the process was completed within 50 min. Increasing the metals concentration increased its uptake and among the four metals, *sargassum* recorded the highest uptake of 168.7 Pb mg/g, followed by Cu (45.2 mg/g), Zn (20.3 mg/g) and Mn (16.0 mg/g) (Vijayaraghavan *et al.*, 2008).

The pH dependence of metal uptake could related to the functional groups of the biomass and also the solution chemistry. At pH less than 5 metals are in their free ionic form and such as the sharp increase in metal uptake. A greater sorption of Cu<sup>+2</sup> and Zn<sup>+2</sup> by *Ulva fasciatd* sp. was observed at pH 5.0, as a result of lower numbers of competing H<sup>+</sup> and more legands were exposed with negative charges. But for pH values from 6 to 10, lower adsorption capacity was detected, due to the ions precipitation of Cu<sup>++</sup> and Zn<sup>++</sup>. Also, more than 75% of the metals ions adsorption was completed within 10 min and reached its equilibrium after 20 min and the adsorption did not change significantly with further

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increase in contact time by the biomass. The initial metal ions concentrations ranged from 20-100 mg/L, and adsorption maximum capacity (mg/g) for Cu and Zn were 26.88 and 13.5, respectively (Kumar *et al.*, 2006, a). The biosorption of Cu<sup>+2</sup> and Pb<sup>+2</sup> by *Fucus Spiralis* (marine brown algae) the initial pH was 4.5 and maximum sorption uptake of biomass remained particularly constant around 1mmol/gm. There was a significant natural decrease in Pb<sup>+2</sup> and Cu<sup>+2</sup> sorption level at high concentrations of Cd, Ni and Zn, sorption of Ni<sup>+2</sup> decreased in the presence of Cu<sup>+2</sup> and was less with Cd<sup>+2</sup>, but Ni<sup>++</sup> sorption was zero even at low Cu<sup>+2</sup> concentration. These results indicate that Cu<sup>+2</sup> controlled the sorption uptake of Cd<sup>+2</sup> and Ni<sup>+2</sup> by the biomass (Romera *et al.*, 2008).

Solution pH is an important parameter that affect biosorption capacity of heavy metals, also the biosorbent mass dose is a significant factor to be considered for effective metal removal. A little biosorption of Cu<sup>+2</sup> and Cr<sup>+2</sup> was occurred by rose waste biomass at pH < 4 but biosorpation capacity increased rapidly with pH increase to 5. The effective biomass dose for Cu<sup>+2</sup> and Cr<sup>+2</sup> maximum uptake was 1 gm/L, whereas sorption capacity increase with the increase in the initial ion concentration and reached 55.79 mg/gm for Cu<sup>+2</sup> and 67.34 mg/gm for Cr<sup>+2</sup> (Iftekhar *et al.*, 2009).

For Cd<sup>+2</sup> and Cu<sup>+2</sup> sorption by *sargassum* (seaweed), the higher pH values usually resulted in higher metal cation uptake due to lowered metal solubility. For Cd<sup>+2</sup>, it was the dominant species

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present at pH lower than 5.0, but at higher concentrations (4.0 Mm) the Cd<sup>+2</sup> predominance is shifted to lower pH values, however it remains the dominant species up to a maximum of pH 5.5. the optimal pH for high absorption was 4.5 and biosorption uptake was 0.90 mmol/gm. At final concentration of 10 mg/L metal uptake was 29.3 mg/gm, while at 200 mg/L the final metal uptake was 79 mg/gm. The maximum absorption for Cd<sup>+2</sup> and Cu<sup>+2</sup> were 0.79 and 0.93 mmol/gm (Davis *et al.*, 2000). On the other hand, Loutseti *et al.* (2009) showed that under micro algal/ bacterial treatments, the Cu<sup>+2</sup> removal from the solution was significantly higher to 60-80% at pH 4.0 and 5.5, but it was significantly lower than 50% at pH=2.0. No significant differences in Cd<sup>+2</sup> removal percentage were obtained between pH 4.0 and 5.5. Improved performance in metal removal, as pH increases from acid to more natural condition (optimum pH range 4.0-5.5).

Maximum uptake capacities of Pb<sup>+2</sup> and Cd<sup>+2</sup> from a solution contained the two metals by Protonated *Sargassum glaucescens* were 1.18 and 0.22 mmol/gm at pH 5.0 (Naddafi *et al.*, 2007). Also, Sari and Tuzen (2008, a) concluded that the maximum biosorption of Pb<sup>+2</sup> and Cd<sup>+2</sup> by *Ulva Lactuca* biomass was found to be 95% and 90%, respectively at pH=5.0. Whereas, at higher pH values, the biosorption yield of Cd<sup>+2</sup> was dramatically decreased, but at pH range 2-4, the biosorption yield was 20-40% for both pb<sup>+2</sup> and Cd<sup>+2</sup> ions. The maximum biosorption capacity was 34.7 mg/g for Pb<sup>2+</sup> ions and 29.2 mg/gm for Cd<sup>+2</sup> ions and the biosorption efficiency *Literature Review* 

increased with rise in contact time up to 60 min and after that it is almost constant. The biosorption efficiency is highly dependent on the biomass dosage in the solution, and the maximum biosorption of the metals ions was attained at 20 mg biomass/L, and it was almost same at higher dosages.

In respect of biosorption of copper from solutions, it was reported that the sharpest update of Cu+2 was observed between pH 3 and 4 and reached the plateau at pH 5.0. This result show the strong pH dependence of Cu (II) biosorption. The adsorption capacity increased with the increase in solution pH, and reached 0.80 mmol/g by using Padina sp. (a marine algae). This adsorption capacity was relatively high when compared with other bioadsorbents. Also, 90% of total soluble Cu (II) was removed from the solution within 15 min of agitation, afterwards, there were slower rates of update to about 30 min, (Kaewsarn, 2002). Another study indicated that the maximum biosorption capacity by Spirogura sp. was 133.3 Cu<sup>+2</sup> mg/gm dry weight of biomass at an optimum pH value of 5.0 in 120 min with 20 mg/L algal dose. The removal of 95.3% of Cu<sup>+2</sup> from solution was observed with HCl treatment at 15 min (Gupta et al., 2006). The adsorption capacity of Cu<sup>+2</sup> (initial concentration of 20 mg/L) by Ulva fasciata sp. biomass at 0.5 gm dose was increased from 7.52% to 96.43% with an increase in solution pH from 2 to 5. The maximum removal of Cu<sup>+2</sup> was observed at pH 5 in 20 min, whereas increasing pH from 6 to 10 decreased the adsorption capacity. Also, the maximum Literature Review

capacity of Cu<sup>+2</sup> removal was 26.88 mg/gm biomass, since the amount of Cu<sup>+2</sup> absorption increased from 94.88 to 95.86% with increasing algal dose from 0.1 to 0.5 gm (Kumar *et al.*, 2006, a). However, Ozer *et al.* (2009) found that the optimum pH for Cu<sup>+2</sup> biosorption by the green seaweed *Enteromorpha Prolifera* was equal 4.0 for initial Cu<sup>+2</sup> ion concentration of 200 mg/L and biosorbent dosage of 1.2 gm/L. The maximum biosoption capacity was 54.17 mg/gm at 25°c and equilibrium reached at 60 min, where the biosorbed Cu<sup>+2</sup> amounts did not change with further increase in contact time.

Biosorbents derived from three types of brown algae, namely

Sargassum hystrix, Sargassum natans and Padina povonia, have the highest lead removal capacity, respectively. Biosoption of Pb<sup>+2</sup> from aqueous solution was more efficient at higher pH values. The highest removal of Pb<sup>+2</sup> by Padina and Sorgassum biomass was occurred at pH 4-5 and significantly decreased by reducing pH values to 1.0. Biosoption of Pb<sup>+2</sup> was rapidly occurred onto algal biosorbents and most of the sorbed metal was bound in < 30 min of contact. The initial Pb<sup>+2</sup>ions concentration was 463.5 mg/L and biomass dose was 2 gm/L and contact time was 30 min., the maximum absorption capacity were 285, 238 and 217.4 mg/gm for S. hystrix, S. natans and padina biomasses, respectively (Jalali et al., 2002). The amount of Pb<sup>+2</sup> bisorbed by Sargasum sp biomass

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remains constant in the range of pH 2-7 for the two Pb+2

concentrations 43.5 and 65.3 mg/L. For the initial Pb<sup>2+</sup> concentration of 54.4 mg/L, the rate of Pb<sup>2+</sup> ions removal is extremely rapid in the first 10 min, but next it decreased significantly and approached equilibrium in about 60 min (Martins *et al.*, 2006).

Vijayaraghavan et al. (2006) revealed that Sargassum wightii biomass was found to be an effective biosorbent for nickel from two effluents. It was reported that, as the pH value increased, the amount of Ni<sup>+2</sup> uptake increased and the sharpest increase was attained between pH 4 and 4.5. However, in real effluents the influence of pH on Ni<sup>+2</sup> uptake was not well pronounced at higher pH values. At pH 4 and above, Ni<sup>+2</sup> uptake remained relatively constant at 18 and 14 mg/g for two effluents.

Regarding to Cd<sup>+2</sup> ions biosorption, Kaewsarn and Qiming (2001) found that at pH around 5 the Cd<sup>+2</sup> adsorption capacity by the marine algae *Padina* sp. leveled off at maximum value. The maximum Cd<sup>+2</sup> adsorption capacity of *Padina* sp. was 0.53 mmol/g and it considered high but less than the biosorbent of other marine algae. 90% of the total soluble Cd<sup>+2</sup> was removed from the solution within 35 min of agitation, then there was a small decrease in soluble Cd<sup>+2</sup>concentration during the test period of 5 hr. The algal adsorbent dose was 2 gm/L and initial Cd<sup>+2</sup> concentration was 2 mM. Likely using the marine algae *Fucus* sp. in Cd<sup>+2</sup> biosorption showed that the sorption of Cd<sup>+2</sup> was increased as pH increased and reached a plateau at pH 5.0 and the cadmium uptake was relatively *Literature Review* 

fast of the three algae and reached over 50% of the total biomass Cd<sup>+2</sup> uptake within 5 min of contact and over 90% of the total Cd<sup>+2</sup> was removed from solution in the first 25 min. The uptake capacity of Cd<sup>+2</sup> was as high as 80 mg Cd<sup>+2</sup>/gm raw seaweed (Herrero et al., 2006). The cadmium uptake decreased with decreasing the solution pH using the marine algae Gelidium (Vilar et al., 2008). At the concentration of 1 mmol/L of Cd+2, the marine algae Fucus vesiculosus obtained from the Bothnian sea absorbed more Cd (about 98%), compared the algae of the Irish sea. This result indicate that the algae from the Bothnian sea may be an efficient sorption substrate for Cd+2 removal for cadmium contaminated sea water and this algal species may also have applications for wastewater treatment. The cadmium absorption was higher during the first hour and reached maximum absorption after two hours. The algal dose was 5 gm fresh weigh and the pH value for maximum adsorption was 8.0-8.2, respectively (Brinza et al., 2009).

Excess of metal ions induces oxidative stress by increasing the formation of active oxygen species such as superoxide radicals (O<sub>2</sub>), single oxygen (<sup>1</sup>O<sub>2</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical (OH) in plant cell (Stohs and Bagchi, 1995 and Gallego *et al.*, 1999) or inhibition of antioxidant systems in plants (Ali, 2000)

Germination of seeds involves imbibitions of water, activation and formation of enzymes, mobilization of reserves from storage

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organs and growth establishment of seedlings. All these processes may be adversely affected by heavy metals (Jeliazkova and Craker, 2002; Verma and Dubey, 2003; Burhan *et al.*, 2001; Munzuroglu and Geckil, 2002; Mahmood *et al.*, 2007; Luksiene and Racaite, 2008; An Younjoo, 2004; Dharam *et al.*, 2006; Asgharipour *et al.*, 2011; Upadhayay *et al.*, 2008; Yang Yingli *et al.*, 2010; Madhulika Gupta, 2011).

On contrary, (Jamal *et al.*, 2006) reported that wheat showed enhanced seed germination at the level of mercury used from 25 to 100 ppm of HgCl<sub>2</sub> solution compared to the untreated control.

Different stress conditions inducing oxidative microenvironment, e.g. heavy metals contamination results in fundamental changes of both the activity and isoenzyme composition of the antioxidant enzyme system. One of the protective mechanisms is the enzymatic antioxidant system, operate with the sequential and simultaneous action of number of enzymes including catalase (CAT) and peroxidase (POD) (Hegedüs *et al.*, 2001).

Peroxidase have various physiological roles in plant cells and participate in many reactions including lignification, cross linking of cell wall polysaccharides, oxidation of indole -3- acetic acid, regulation of cell elongation and phenol oxidation all linked to growth reduction (Mocquot *et al.*, 1996). Peroxidase activity shows a close correlation with changes in physiological processes such as respiration, photosynthesis, CO<sub>2</sub> fixation, transpiration and gas
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exchange with obvious growth and fitness consequences and therefore has the potential to serve as a sensitivity indicator of compromised metabolic activity (Verklij and Schat, 1990). The increased production of peroxidase is considered to be due to the phytotoxic metal fraction or free metal not bound to cell walls or accumulated in vacuoles (Macfarlane and Burchett, 2001).

Peroxidase (POD) induction is a general response of higher plants to uptake of toxic amounts of metals, it has been observed in roots and leaves of various species after application of toxic doses of Zn, Cd, Cu, Ni and Pb (Van Assche and Clijsters, 1990; Gajewska et al., 2006; Abantika et al., 2008; Gajewska and Sklodowska, 2008). On the other hand, Gajewska and Sklodowska (2010) showed that the most pronounced increase in peroxidase activity in the shoots was found after Ni treatment in wheat plants.

Catalase is a heme – containing enzyme that catalysis the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidases involved in β- oxidation of fatty acid, the glyoxylate cycle and purine catabolism (Mckersie, 1996). On the other hand, faba bean plants treated with 10<sup>-5</sup> M of CuSo<sub>4</sub> or 10<sup>-6</sup> M of CdSO<sub>4</sub> solution or mixture of both metals showed significant reductions in the activities of antioxidant enzymes, i.e. CAT, SOD and POD (Kasim, 2005).

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Visible injuries and significant changes in growth and yield usually become apparent only after exposure to relatively high levels of pollutants, the effect of pollution on biochemical processes may be detected much earlier (Robe and Kreeb, 1979). The free metal fraction may produce secondary effects such as growth inhibition (Van Assche and Clijster, 1987). The reduction of growth could have been due to metal inhibition of root respiration (Billet *et al.*, 1974). Stunted growth, chorosis, necrosis, leaf epinasty and red-brownish discoloration are visible symptoms of severe metal phytotoxicity (Lepp, 1981; Woolhouse, 1993; Walnöfer and Engelhardt, 1984).

Biomass, root length and shoot height of wheat plant were decreased with increasing Cd and As concentrations. However, root length appeared to be the most sensitive parameter to Cd or As. Combined exposure to Cd and As produced greater toxicity to wheat than single exposure to each metal separately. Also, Cd and Hg inhibited root and shoot growth, and this stress caused water deficit and lipid phosphorylation in the seedlings (Liu and Zhang, 2007; Liu et al., 2007 and Ge Cailin et al., 2009). Therefore, metabolic changes in plant can serve as suitable indicators of pollution in the absence of visible symptoms (Castillo et al., 1987).

The heavy metals induced toxicity symptoms and growth arrest of *Vicia Faba* roots gradually to the Cd concentration and duration of the treatment (Soughir *et al.*, 2011).

Irrigating wheat plants with treated municipal wastewater from the beginning of rooting or at the tillering stage decreased the total biomass production significantly, compared to the control. However, the fresh and dry weights of plant were increased by increasing the sewages sludge levels in the soil. The biomass of wheat seedlings was increased by increasing rates of sludge to soil, and lime application enhanced the biomass and reduced heavy metal concentrations in seedlings (Ghanbari *et al.*, 2007; Mazen *et al.*, 2010 and Sudarshana *et al.*, 2010).

Fresh and dry weight biomass of faba bean plants decreased by applying dry sewage sludge to the soil. Contents of heavy metals were several times greater in sludge than in soil. Also, a significant reductions in fresh and dry weights of plants by exposing plants to  $10^{-5}$  M of CuSo<sub>4</sub> or  $10^{-6}$  M of CdSo<sub>4</sub> and a mixture of both solutions. Treatments caused reductions in fresh and dry weight were in the descending order: Cu+Cd > Cu > Cd. However, higher concentration of Pb (48 mM) caused a significant decrease in fresh weight, although it caused a significant increase in the dry weight of plants (Mazen, 1995; Kasim, 2005 and Kamel, 2008).

The photosynthetic pigments may have the potential to be employed as sensitive indicators of metal stress (Dietz et al., 1999). Inhibition of photosynthesis by heavy metal in higher plants is well documented (Clijsters and Van Assche, 1985; Prasad and Strzalka, 1999; Patra et al. (2004); Bertran and Poirier, 2005; Linger et al., 2005; Zengin and Munzuroglu; 2005; Sarita and Abha, 2007; Literature Review

Tawfik, 2008 and Kaur et al., 2012). Reduction in the levels of photosynthetic pigments including chlorophyll a and b and accessory pigments such as carotenoids, on exposure to heavy metals have been observed in many species for Cu (Van Assche and Clijsters, 1990), Pb (Wozny and Krzeslowska, 1993; Kastori et al., 1998; Oncel et al., 2000; Abantika et al., 2008; liu et al., 2010 and Yang Yingli et al., 2011), Zn (Krupa et al., 1996), Cd (Stobart et al., 1985; Oncel et al., 2000; Shukla et al., 2003; Abdel- Latif, 2008; Upadhyay et al., 2008; Ci Dun et al., 2010 and Duan et al., 2010) and Ni (Gajewska et al., 2006 and Szwarc et al., 2006).

Treatment with excess heavy metals decreased the chlorophyll and carotenoid content in wheat leaves, with increase in time of excision. A sharp increase in proline accumulation was observed with increasing concentration of heavy metals (Panda *et al.*, 2003).

Heavy metals were found to be induced a profound changes in carbohydrates content. In this respect, some authors have reported that the concentration of total, soluble sugars and starch decreased significantly with the toxic levels of heavy metals (Shukla *et al.*, 2003; Eleiwa, 2004; Nitika *et al.*, 2007 and Aldesuquy *et al.*, 2011). In the other hand, increasing heavy metals level significantly increased the carbohydrate accumulation (Miller *et al.*, 1969; Prokopiev, 1978 and Asgharipour *et al.*, 2011).

In plant species, the effect of heavy metals has resulted in a general decrease in the protein formation (Sharma et al.,1995;

Chaffei et al., 2004; Odjegba and Fasidi, 2006 and Sanjoy et al., 2010).

Odjegba and Fasidi (2006) have reported that the poor protein formation could be related to disruption of nitrogen metabolism in *Echhornia crassipes* exposed to high doses of heavy metals (Ag, Ni, Pb and Zn). In contrast to expectation, other investigators recorded an increase rather than decrease in protein of some plants (Freeman *et al.*, 2004; Liu *et al.*, 2007; Chandra *et al.*, 2009 and Ge Cailin *et al.*, 2009).

In addition to the previous results, Ni, Zn, Cu, Cd, Cr and Pb elements, accumulated from municipal solid waste amended soil in plants were at least concentration in grain, in comparison to other plants part (Mamata *et al.*, 2009). However, Zhangren *et al.* (2002) reported that increasing total soil content of selected metals could enhance grain Cd accumulation, and the increment of total Zn content in soil could lower the grain Pb accumulation.

Limed soil reduced Cd, Pb and Zn contents in wheat straw and grains, whereas Zn and Ni stimulated grain yield in other soils when they were in lower concentration (5 or 10 mg/kg soil), but with a further increase in Zn and Ni concentration, grain yield decreased, and Cd led to continous yield decrease with increasing its concentration (Tlustos *et al.*, 2006 and Sarita and Abha, 2007).

The contents of Fe and Mn were significantly greater in straw than in seeds of faba bean, whereas there was no significant differences between straw and seeds regarding to Cu, Zn and Ni Literature Review

contents, when plants were irrigated with raw wastewater (untreated) mixed with freshwater (1:2) or (1:6) and treated wastewater mixed with freshwater (1:6). The dry matter yield was greatest from raw wastewater mixed with fresh (1:6). Also, it was reported that the levels of most metals, i.e. Cd, Cu, Fe, Mn, Zn and Pb, decreased in bean fruits. (Eid and Shereif, 1996 and li feili et al., 2010).



# MATERIAL AND AND METHODS

### 3. Materials and methods

The present investigation was conducted at Fayoum Governorate, Egypt during the winter season 2010/2011 to study the effect of irrigation with low quality water (drainage contaminated effluent water) before and after the bioremediation of heavy metals by algae on germination percentage, seedling enzymes, some vegetative growth parameters, photosynthetic pigments and chemical components of wheat grains and faba bean seeds. To achieve these targets nine irrigation water treatments were carried out in a complete block design with three replications for each crop and the experiment was carried as follows:

### 3.1 Collection and preparation of marine algal biomass:

To achieve the bioremediation of heavy metals from low quality water, marine macroalgae *Ulva lactuca* (green), *Pterocladia capillacea*, *Jania rubens* and *Corallina mediterranea* (red) which used in this investigation were collected from the Mediterranean sea (Abu Quir beach), Alexandria, Egypt, during July 2010 (plate 1).

The collected algal species were delivered to the laboratory of Botany Department, Faculty of Science, Fayoum University, Egypt and rinsed thoroughly with distilled water in order to remove any adhering debris, the algae were oven dried at  $60^{\circ}$ C for 24 h, then samples were taken from each species, ground and sieved to a particle size of 500- 850  $\mu$ m. this particle size fraction was used for all algal experiments.

In order to study the biosorption of different algae species collected, a stock solution was prepared as follows:

### Preparation of heavy metal stock solutions:

The stock solutions (1000 ppm) were prepared by dissolving the following concentrations separately.

Materials and methods



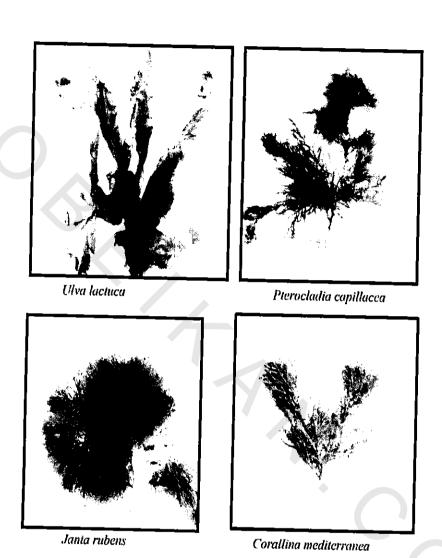


Plate 1: Collected marine macroalgal species.



- 2.2803 g of CdSO<sub>4</sub>. 8H<sub>2</sub>O in 1000 ml bidestilled water.
- 3.9294 g of CuSO<sub>4</sub>. 5H<sub>2</sub>O in 1000 ml bidestilled water.
- 1.8307 g of  $(CH_3 COO)_2$ Pb.  $3H_2O$  in 1000 ml bidestilled water.
- 4.0495 g of NiCl<sub>2</sub>. 6H<sub>2</sub>O in 1000 ml bidestilled water.
- 4.3981 g of ZnSO<sub>4</sub>. 7H<sub>2</sub>O in 1000 ml bidestilled water.

### Reagents and equipment:

Working solutions were prepared by diluting the stock solution with bidistilled water. The metal concentrations in solution were determined using PERKIN-ELMER A Analyst 700 flame atomic absorption spectrophotometer with deuterium background corrector. All measurements were carried out in an air/ acetylene flame.

A Perkin-Elmer model 2380 atomic absorption spectrometer (U.S.A.) was used with Pye Unicam (England) hollow cathode lamps for iron, manganese, zinc, cadmium, nickel and cobalt. Perkin Elmer hollow- cathode for lead and copper and a

conventional 10 cm silt burner head for air-acetylene flame were used.

### 3.2 Optimum pH determination:

In order to investigate the optimum pH for heavy metal biosorption, 10 mg/L metal solutions of various pH values (2-9) were prepared using 0.1 M NaOH or 0.1M HNO<sub>3</sub>. The final pH values were checked by pH – meter (Hanna Instrument, 8519, Italy). Approximately 1.0 g of algal powder was added to Flasks containing 100 ml of heavy metal solution at the required pH. Flasks were shaken for 2h at 200 rpm and room temperature (25±1°C). Samples were analyzed via AAS and the % of bioremoval was calculated according to the following equation.

Removal percentage =  $[(C_i - C_f)/C_i] \times 100$ 

Where  $C_i$  and  $C_f$  are the initial and final metal ion concentrations, respectively.

## 3.3 Optimum contact time determination:

A quantity of 1.0 g of biomass was added to 100 ml of a 10 mg/L metal solution adjusted to pH 5. Flasks were shaken for 2 hr at 200 rpm and room temperature ( $25\pm1^{\circ}$ C). Samples were taken at t=5, 10, 20, 60, 120 min. The metal concentrations were analyzed

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by AAS. The percentage of bioremoval was calculated as mentioned before

### 3.4 Optimum dosage determination:

In order to investigate the optimum biomass dosage for various heavy metal biosorption, different flasks containing100 ml of a 10 mg/L metal solution adjusted to pH 5 were prepared using various biomass in the range (1-40) g/L. Flasks were shaken for 2h at 200rpm and room temperature (25±1°C), the samples were analyzed via AAS and the percentage of the bioremoval was calculated.

According to the results detected from the experiments of pH, time of contact and algal dosage, the following 3 algal types *Ulva lactua, Jania rubens* and *Pterocladia capillacea* were chosen according to their high absorbant capacity to heavy metals and used in bioremdiation of heavy metals from low quality water.

### Water samples used in irrigation:

Fresh water samples were taken from the main irrigation water canal of Fayoum Governorate namely Bahr Youssef, as a control treatment for comparison with other low quality water treatments.

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The low quality water samples were collected from the main drains of Fayoum Governorate, which may used some times by farmers in irrigating their lands when the fresh Nile water become scarcer, and these drains namely:

- 1- El- Meiry drain, at Hawaret El- Maktaa village (Agric. drain water).
- 2- El- Batts drain, at Siyala village (Agric. drain contaminated with sewage effluents).
- 3- Fannous drain (Tanhalah) (Agric. drain water).
- 4- El- Wadi drain, Abu Genshou village (Agric. drain water).
- 5- Bahr Abu Denkash, (Agric. drainage mixed with Nile water).

Water of the five main drains mentioned before were

analyzed to determine their chemical composition such as: pH values (measured by pH meter), electrical conductivity (EC<sub>w</sub>) (measured by electrical conductivity bridge according to the method of Richards, 1954), total nitrogen (determined colorimetrically using Nessler solution, described by Sauter and Stoub, 1990). Soluble phosphorus (determined colorimetrically

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using ascorbic acid method described by Watanabe and Olsen,

1965), soluble Potassium (determined using inductively coupled plasma optical emission spectroscopy) and heavy metals (determined using Atomic absorption spectrometer).

The results of water analysis for the five drains are presented in Table (1), and it clearly show that El- Batts drain had the highest micronutrients and heavy metals concentrations when compared with the other four drainage water compositions. Therefore, El-Batts drainage water was chosen as the serious water quality used some times in irrigation and treated with the algal powder to estimate its efficiency in removing heavy metals from this low quality water type.

Table (1): Macro, micro-nutrients and heavy metal contents (mg/L) of the five drains.

Location	PH	ECw		Macro, micro nutrients and heavy metals (mg/l.)										
		Ds/m	N	P	K	Fe	Mn	Zn	Cu	Cd	Ni	Pb	Co	
Bahr Yossef	7.26	0.58	1.18	0.22	5.37	1.19	0.059	0.647	0.026	0.001	0.004	0.013	0.004	
(fresh Nile w.)														
El- Meiry D.	7.84	2.27	2.46	0.73	10.73	0.65	0.150	0.120	0.043	0.014	0.061	0.081	0.020	
El- Batts D.	7.16	2.97	5.38	1.29	14.21	0.86	1.489	0.244	0.172	1.440	0.214	0.207	0.027	
Fannous	7.81	1.66	1.99	0.58	10.12	0.57	0.142	0.111	0.032	0.013	0.049	0.066	0.016	
(Tanhalah D.)														
El- Wadi D.	7.85	3.20	 2.74	079	11.21	0.69	0.157	0.126	0.073	0.014	0.061	0.088	0.028	
Abou Denkash	7.74	1.67	2.00	0.51	10.50	0.54	0.143	0.109	0.076	0.011	0.036	0.082	0.019	

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# 3.5 Effect of irrigation with water treatments:

To study the effect of irrigation water before and after algal treatment on seedlings of wheat and faba bean plants.

The seeds of each crop plant were divided into nine groups. Each group was treated with different types of irrigated water as the following:

- 1- Seeds irrigated with fresh Nile water without additional heavy metals [Control].
- 2- Seeds irrigated with synthetic solution of 20 ppm heavy metals (4 ppm of Cd, 4 ppm of Cu, 4 ppm of Pb, 4 ppm of
- Ni and 4 ppm of Zn) [S.S 20].

  3- Seeds irrigated with synthetic solution of 20 ppm heavy metals (4 ppm of Cd, 4 ppm of Cu, 4 ppm of Pb, 4 ppm of
  - Ni and 4 ppm of Zn) treated with 10g mixture of algal powder (3.33g of *U. lactua* powder, 3.33g of *J. rubens* powder and 3.33g of *P. capillacea* powder) [Algal treated S.S 20].
  - 4- Seeds irrigated with synthetic solution of 60 ppm heavy metals (12 ppm of Cd, 12 ppm of Cu, 4 ppm of Pb, 12 ppm of Ni and 12 ppm of Zn) [S.S 60].

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- 5- Seeds irrigated with synthetic solution of 60 ppm heavy metals (12 ppm of Cd, 12 ppm of Cu, 4 ppm of Pb, 12 ppm of Ni and 12 ppm of Zn) treated with 10g mixture of algal powder (3.33g of *U. lactua* powder, 3.33g of *J. rubens* powder and 3.33g of *P. capillacea* powder) [Algal treated S.S 60].
- 6- Seeds irrigated with synthetic solution of 100 ppm heavy metals (20 ppm of Cd, 20 ppm of Cu, 20 ppm of Pb, 20 ppm of Ni and 20 ppm of Zn) [S.S 100].
- 7- Seeds irrigated with synthetic solution of 100 ppm heavy metals (20 ppm of Cd, 20 ppm of Cu, 20 ppm of Pb, 20 ppm of Ni and 20 ppm of Zn) treated with 10g mixture of algal powder (3.33g of *U. lactua* powder, 3.33g of *J. rubens* powder and 3.33g of *P. capillacea* powder) [Algal treated S.S 100].
- 8- Seeds irrigated with El- Batts drainage water without additional heavy metals [El- Batts drainage water].
- 9- Seeds irrigated with El- Batts drainage water treated with mixture of algal powder [El- Batts drainage water treated with algal powder].

The final percentage germination and enzyme activities were determine at the end of experimental periods.

# 3.5.1 The final percentage germination of seeds (%):

Seed germination experiments were performed as described by (Ali, 2000). A homogenous lot of healthy bean and wheat seeds were selected for uniformity of size and shape. Before germination, bean and wheat seeds were surface sterilized by soaking for 30 minutes in 2.5% sodium hypochlorite solution, rinsed several times with distilled water. The seeds were transferred to sterile petridishes (15 cm diameter) containing two sheets of Whitman No.1 filter paper, moisted with one type from the treatments. Each treatment were replicated 3 times. The seeds allowed to germinate in the darkness at 25 °C. the final percentage of germination was determined after 7 days as the following equation.

Germination (%) = 
$$\frac{Number\ of\ seeds\ at\ t\ time}{Number\ of\ total\ seeds} \times 100$$

# 3.5.2 Seedling enzyme activities:

To determine the enzyme activities, i.e. peroxidase and catalase in wheat and faba bean seedlings of 10- days old the following methods were adopted:

### 3.5.2.1 Seedling experiments:

Ten seeds were placed between folded paper towels, covered by plastic wrap, rolled up, and placed up right in 500 ml beakers. Eight ml of each irrigation treatments were used to saturated the towels in each treatments. Seedlings were left to grow in dark at  $25\pm1$  °C. Distilled water was added to compensate the evaporation loss. At the end of the experimental period (10 days) some enzyme activities were recorded.

#### 3.5.2.2 Extraction:

For assaying the activities of peroxidase, catalase the plant material was extracted following the method of Kar and Mishra (1976) with slight modifications as follows: 2 g fresh sample were homogenized in cold phosphate buffer (0.05 M at pH 6.5). The homogenate was centrifuged at 10,000 rpm for 10 minutes. The pigments were removed from supernatant by adsorbing with activated charcoal and filtered. The filtrate was completed to a known volume and used as an enzyme source.

# 3.5.2.3 Peroxidase (POD):

Peroxidase activity was assayed using a solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2.0 ml of 20 mM H<sub>2</sub>O<sub>2</sub> after addition of 20 mM pyrogallol, the color intensity was determined spectrophotometrically by PERKIN ELMER UV spectrophotometer within 60 seconds at 470 nm and 25 °C (Bergmeyer, 1974). One unit of enzyme conversion of one micromole of H<sub>2</sub>O<sub>2</sub> per minute at 25 °C (Kong *et al.*, 1999). The blank sample was made by using buffer instead of enzyme extract. The enzyme activities were assay.

# 3.5.2.4 Catalase (CAT):

Catalase activity was assayed following the method of Chen et al., (2000). The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H<sub>2</sub>O<sub>2</sub> phosphate buffer (0.16 ml of 30% H<sub>2</sub>O<sub>2</sub> to 100 ml of 50 Mm phosphate buffer pH 7.0). CAT activity was determined by measuring the rate of change of H<sub>2</sub>O<sub>2</sub> absorbance in 60 seconds with PERKIN ELMER UV spectrophotometer at 250 nm. The blank sample was made by using buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the

enzyme that reduced 50% of  $H_2O_2$  in 60 seconds at 25 °C (Kong *et al.*, 1999).

#### 3.6 Green house pot experiment:

To study the effect of irrigating wheat and faba bean plants with fresh and low quality water (contained heavy metals) before and after algal treatment on some growth parameters and some chemical components of the seeds, the following experiment was carried out as follows:

Fifty four pots of 30×30 cm area and 75 cm depth were filled with a mixture of clay and sand soil (2:1), and the pots were divided into two groups i.e. 27 pots for wheat and 27 pots for faba bean. Each 27 pots for each crop were subjected to the nine irrigation treatments, where each treatment was replicated for 3 times.

Wheat grains (Sakha-69 cv.) were planted as dressing in rows of 10 cm apart on November 15<sup>th</sup>, then irrigated with 2 liters of each irrigation water treatment to reach the maximum water holding capacity of the soil. After that water was added for each treatment every two weeks until harvesting.

For the faba bean experiment, 18 seeds of faba bean (Giza-843 cv.) were planted in hills of 5 cm between and rows of 10 cm apart on October 21<sup>st</sup>, then the pots were irrigated immediately with 2 liters of each water treatment for each pot and irrigated every 21 day. Twice dose of ammonium nitrate (33.5% N) each of 30 gm were added to wheat pots at 21 and 60 days from planting. The following measurements at 5, 9- weeks old plant and harvesting time were recorded for wheat and faba bean.

- 3.6.1 Shoot height (cm)
- 3.6.2 Root length (cm)
- 3.6.3 Shoot fresh weight (gm)
- 3.6.4 Soot dry weight (gm)

Plant samples were separately washed to remove the soil particles and the shoots were separated and weighed then, oven dried at 60-70 °C till constant weight was achieved.

- 3.6.5 Leaf number per plant for wheat and leaflets number per plant for faba bean.
- 3.6.6 Leaf area per plant for wheat and leaflets area per plant for faba bean was determined by using portable leaf area meter (Model LI 3100, LICOR, USA).

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3.6.7 Photosynthetic pigments i.e. chlorophyll a, b (mg/gm F.W.) were determined by using the carotenoids spectrophotometric method recommended by (Metzner et al., 1965) and applied for higher plants by (Ahmed et al., 1977, 1979 and 1980). A known fresh weight of the plant leaves was homogenized in 85% (v/v) aqueous acetone and then kept at least 6 hours in a refrigerator. After centrifugation for 5 minutes at 14,000 r.p.m. the supernatant, which contained the pigments was quantitatively taken and completed to known volume by 85% (v/v) aqueous acetone then the absorbance measured using PERKIN ELMER UV spectrophotometer. The extraction was measured against blank of pure 85% (v/v) aqueous aceton at three wave length of 452, 644 and 663 nm. Taking in consideration the dilution made, it is possible to determine the concentration of pigments fractions (chlorophyll a, b and carotenoids) as μg/ ml using the following equations.

chlorophyll a= 10.3 E663 -0.918 E644 µg.ml<sup>-1</sup>

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chlorophyll b= 19.7 E644 - 3.87 E663  $\mu g.ml^{-1}$  chlorophyll (a+ b)= 5.13 E663 + 20.41 E644  $\mu g.ml^{-1}$  carotenoids= 4.2 E452 -(0.0264 chl a+ 0.426 chl b)  $\mu g.ml^{-1}$ 

The results obtained from these equations were expressed as mg/g fresh matter of different treatments, and all extraction and measurements were carried out in dim light within maximum of six hours to avoid decomposition of pigments.

mg pigments/ g fresh matter = [Pigment content ( $\mu$ g/ml) × Extraction volume (ml) × Dilution factor]/ [Weight of extracted tissue (g) ×1000].

# 3.6.8 Chemical analysis of seeds:

At harvesting time some chemical components of wheat grains and faba bean seed, i.e. total carbohydrates, total protein and some heavy metals were determined as follows.

# 3.6.8.1 Total carbohydrates content:

For the determination of carbohydrates, the anthrone sulphuric acid method which was carried out by (Fales, 1951;

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Schlegel, 1956) and adopted by (Irigoyen *et al.*, 1992) was used. All carbohydrates were colorimetrically determined. A known weight of dried grounds seeds was heated in a water bath at  $100\,^{\circ}$ C for 2 h in 10 ml of 2 N HCl. The solution was cooled and transferred to a 100 ml measuring flask after filtration, followed by making up to a known volume with distilled water. The anthrone reagent freshly prepared consists of 150 mg anthrone and 100 ml of 72% (w/w)  $H_2SO_4$  (D =1.84). The procedure followed was to use 0.1 ml of extract with 3 ml anthrone reagent and placed in a boiling water bath for 10 minutes, after cooling, the absorbency of 625 nm was determined.

# 3.6.8.2 Total protein content:

Nitrogen contents of the seeds were determined by the micro-Kjeldahl technique (A.O.A.C., 1990). 1.0 g of dried grounds seeds was weighed into Kjeldahl digestion flask and a pinch of catalyst mixture (2.0 g of potassium sulphate, 1.0 g of copper sulphate and a pinch of selenium powder) were added followed by 10 ml concentrated H<sub>2</sub>So<sub>4</sub>. The flask was then heated cautiously Materials and methods

under fume hood until a greenish solution appears. After clearing, heating continued for more 30 min and allowed to cool. 10 ml distilled water was added into the digest and shaked thoroughly. Steam was passed through a Markham distillation. The digest was transferred into 100 ml measuring cylinder and diluted with distilled water until 100 ml mark. Under the condenser of the distillation apparatus, a receiver flask containing 10 ml of boric acid as indicator was placed, 10 ml of the diluted digest was introduced into the distillation unit and 10 ml of 40% NaOH solution was slowly added. The distillation was stopped by closing inlet stop cork first and then opened steam bypass. This was titrated with 0.01M HCl to give pink colour. The following equation can be used to determine the nitrogen concentration of a sample that weighs m grams using xM HCl acid solution for the titration:

$$\% N = \frac{x \text{ moles}}{1000 \text{ cm}^3} \times \frac{(v_s - v_b) \text{ cm}^3}{m \text{ g}} \times \frac{14 \text{ g}}{\text{moles}} \times 100$$

Where vs and vb are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. Protein content can be determined using the propriate conversion factor [6.25 for faba bean and 5.83 for wheat (A.O.A.C, 1965)].

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# % Protein = F} %N.

# 3.6.8.3 Determination of heavy metals content:

Seeds was analyzed according to Jones (1989), 0.5 gram oven dried seeds was placed in a 80 ml digestion pyrex tube, 4 ml concentrated nitric acid (HNO<sub>3</sub>) were added and let stand overnight. The digestion tube was placed into the digestion block and heated at 120 °C for one hour, then left to cool. Few drops of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added and digestion tube was placed back again in the digesion block where the temperature is 120°C. The addition of 30% hydrogen peroxide was repeated several times, and completed to 25 ml with 0.1 N HNO3. The solution was filtered through Whitman 42 filter paper. The filtrate was used for measuring the concentration of heavy metals by Atomic Absorpion Spectrophotometer (A.A. Spectrometry Thermo Elemental Type Solar 54/2001, Ser No.GE 710728)

# 3.7 Statistical analysis:

All the measurement collected for the two crops were subjected to statistical analysis described by Snedecor and Cochran, 1980. And the means were compared according the LSD test at 0.05 and 0.01 probability.

# RESULTS



#### 4. Results

Many recent researches concluded that the bioremoval of heavy metals can be affected significantly by physiochemical parameters of the aqueous solutions, such as pH, ion strength and concentration, contact time and by other characteristics of the biomass as biomass dosage.

# 4.1 Optimum pH for metal ions bioremoval:

The effect of different pH values on metal ions (Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup>, respectively) bioremoval efficiency of different algal biomass are given in Tables (2-6) and Figures (1-5).

It is obvious that the removal efficiency decreased at low acidic and high alkaline pH and the optimum pH value for high metals adsorption by algae ranged from pH 5 to pH 7.

The data presented in Table (2) and Figure (1) show that the maximum removal percent of  $Cd^{+2}$  ion by *C. mediterranea* and *U. lactuca* at pH 5.0 were 71.6 and 64.1%, respectively, 58.8% by *J. rubens* at pH 6, and 55.8% by *P. capillacea* at pH 7.0.

In respect of biosorption of Pb<sup>+2</sup>, the data in Table (3) and Figure (2) revealed that the biosorbtion of Pb<sup>+2</sup>was more efficient at pH 5.0 by *U. lactuca*, *C. mediterranea* and *J. rubens* (95.9, 79.5 and 79.2%, respectively). Also, *P. capillacea* reached a plateau at pH 6.0 and remove 83.6% of pb<sup>+2</sup> from the solution.

Data of Table (4) and Figure (3) indicated that at pH 5 the maximum Ni<sup>+2</sup> uptake by *U. lactuca* were 83.4% and *J. rubens* 

81.1% at pH 7. While the maximum removal of Ni<sup>+2</sup> by C. mediterranea and P. capillacea at pH 6 reached 75.98 and 74.9%,—respectively.

It is clear from Table (5) and Figure (4) that Zi<sup>+2</sup> absorption—percentage reached maximum by *P. capillace*, *U. lactuca* and *J. rubens* 98, 93 and 81.3%, respectively, at pH 6.0, and 76.7% by *C. mediterranea* at pH 5.0.

As can be seen from Table (6) and Figure (5) that the maximum removal efficiency of Cu<sup>+2</sup> at pH 6.0 was detected by *J. rubens* and *C. mediterranea*, 91.7 and 78.1%, respectively. Whereas, *P. capillace* remove 77.1% at pH 4, and *U. lactuca* remove 75% at pH 6.

Table (2): Effect of pH on Cd<sup>+2</sup> removal percentage by different

algal powder.

	gar ponter.		_							
Algal species	рН	2	3	4	5	6	7	8	9	10
Chlorophyta	U. lactuca	5.9	27.4	38.4	64.1	50	49.6	45.5	42.4	25.9
	C. mediterranea	5.9	18.6	55.3	71.6	54.9	65.2	45.13	21.3	2.5
Rhodophyta	J. rubens	37.8	42.8	43	57.33	58.8	50.7	46.9	44.6	12.7
	P. capillacea	6.7	20.9	34.9	42.9	46	55.8	27.9	18.4	13

Table (3): Effect of pH on Pb<sup>+2</sup> removal percentage by different

algal powders.

pH Algal species		2	3	4	5	6	7	8	9	10
Chlorophyta	U. lactuca			29.6			l	l .		41.5
	C. mediterranea	3.7	13.4	28.16	79.5	61.03	58.4	32.4	28.7	18.6
Rhodophyta	J. rubens	13.4	44.1	28.2	79.2	65.7	70.9	20.6	27.03	8.5
	P. capillacea	23.2	29.6	59.5	69.7	83.6	82.9	13.4	25.3	2.7

Table (4): Effect of pH on Ni<sup>+2</sup> removal percentage by different

algal powders.

Algal specie	рH	2	3	4	5	6	7	8	9	10
Chlorophyta	U. lactuca	13.4	23.5	48.5	83.4	66.6	60.8	73.2	61	66.5
Rhodophyta	C. mediterranea	9.1	25.6	49.28	64.08	75.98	52.05	29.3	15	6.3
1100 wopinytu	J. rubens	12.6	31.9	52.89	70.24	78.9	81.06	20.85	3.8	1.5
	P. capillacea	34	37.9	33. 9	64.4	74.9	29.38	31.7	17	6.5

Table (5): Effect of pH on Zn<sup>+2</sup> removal percentage by different algal powders.

_2	ugai powders	<u>.                                    </u>								
Al-al apagins	pH	2	3	4	5	6	7	8	9	10
Algal species  Chlorophyta	U. lactuca	20.5	39	59.4	85.2	93	66.7	50.4	52.7	41.5
	C. mediterranea	12	36.9	52.13	76.7	62	48.9	20.6	9.1	5.8
Rhodophyta	J. rubens	30.2	58.5	76.6	77.9	81.3	61.4	55.9	43.9	22.7
	P. capillacea	59	78.1	86.7	91.5	98	73.7	61.8	44.2	25.3

Table (6): Effect of pH on Cu<sup>+2</sup> removal percentage by different algal powders.

algai powders.									_	
Algal specie	pH	2	3	4	5	6	7	8	9	1⊏
Chlorophyt	U. lactuca	10.7	25.9	33.9	74.5	75	42.8	42.7	58.4	38
Rhodophyt	C. mediterranca	4.1	9.3	36.5	48.1	78.1	51.5	38.4	15.4	3.
a	J. rubens	19.8	33.2	43.9	87.1	91.7	50.5	31.4	11.8	6.
	P. capillacea	36.5	55.8	77.1	76	48.8	52.33	30.7	16	12
	<u> </u>									

Fig. (1): Effect of pH on Cd<sup>+2</sup> removal percentage by different algal powders.

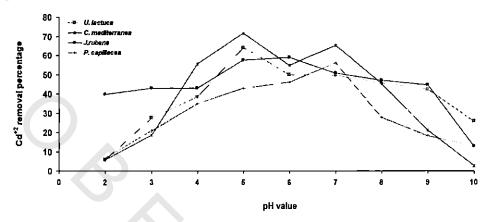


Fig. (2): Effect of pH on Pb<sup>+2</sup> removal percentage by different algal powders.

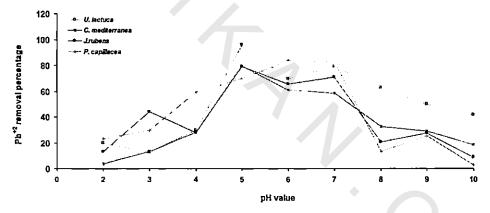


Fig. (3): Effect of pH on Ni<sup>+2</sup> removal percentage by different algal powders\_

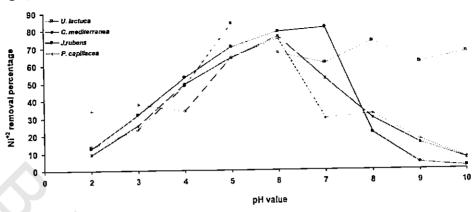


Fig. (4): Effect of pH on Zn<sup>+2</sup> removal percentage by different algal powders.

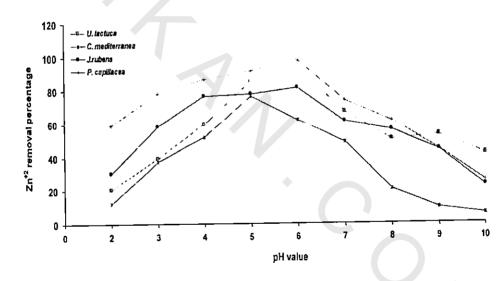
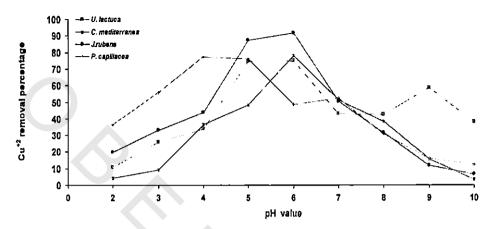


Fig. (5): Effect of pH on Cu<sup>+2</sup> removal percentage by different algal powders.



# 4.2 Optimum contact time for metal ions bioremoval:

The effect of different contact periods (5 min., 10 min., 20 min., 60 min. and 120 min.) on metal ion bioremoval efficiency of different algal biomass, are given in Tables (7-11) and Figures (6—10) for the metal ions (Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup> respectively).

It is clear from the obtained results that the efficiency of metal ion bioremoval increased as contact time increased. For almost all cases, the highest metal ion concentrations removed by different test algae was achieved at 60 minutes and above that it was more or less constant. The maximum efficiency of metal ion bioremoval at 60 min ranged between 38.2% and 90.3% for different algal sp. powders.

Table (7): Effect of contact time on Cd<sup>+2</sup> removal percentage by

different algal powders.

	Time	5	10mi	20	60	120mi
Algal specie		min	n	min	min	n
Chlorophyt a	U. lactuca	21.2	33	43.7	64.3	61.5
Rhodophyt	C. mediterranea	11.7	29.2	43.2	52.6	89.23
a	J. rubens	10	14.48	28.6	65.6	38.16
	P. capillacea	6.95	13.8	24.8	38.16	39.5

Table (8): Effect of contact time on Pb+2 removal percentage by

different algal powders.

Algal specie	Time	5 min	10mi n	20 min	60 min	120min
Chlorophyt a	U. lactuca	2.59	12.34	15.6	48.8	59.6
Rhodophyt	C. mediterranea	8.2	24.6	56	83.4	87.7
a	J. rubens	17.4	15.6	38.31	74.02	77.27
	P. capillacea	2.59	31.8	63.7	75.12	63.2

Table (9): Effect of contact time on Ni<sup>+2</sup> removal percentage by

	different algai F	JUW ue	<u> </u>			
	Time	5	10	20	60	120mi
Algal specie	s	min	lumin	20 min	min	n_
Chlorophyt	U. lactuca	10.8	25.5	35.4	43.5	47.39
a	_					
	<i>C</i> .	16.4	33.1	49.5	68.9	76
ny t	mediterranea			'		
Rhodophyta	J. rubens	12.8	20.5	45.6	61.1	56.45
	P. capillacea	4.12	6.2	11.4	59.5	61.2

Table (10): Effect of contact time on Zn<sup>+2</sup> removal percentage by different algal powders.

	Time	5	10mi	20	60	120mi
Algal species		min	n	min	min	n
Chlorophyt a	U. lactuca	15.36	48.26	79.80	80.4	81.6
Rhodophyt	C. mediterranea	13.2	42.2	62.5	74.9	93.5
а	J. rubens	25.8	45.5	54.68	69.9	67.5
	P. capillacea	12.5	35.9	77.47	90.26	88.2

Table (11): Effect of contact time on Cu<sup>+2</sup> removal percentage by different algal powders.

Algal speci	Time	5 min	10mi n	20 min	60 min	120mi n
Chlorophyt a	U. lactuca	19	42.8	72.3	79.95	80.43
Rhodophyt	C. mediterranea	21.5	35	48.13	52.6	89.2
a	J. rubens	24.8	50.6	68.5	89	95.4
	P. capillacea	17.75	34.8	62.6	77.8	74.01

Fig. (6): Effect of contact time on Cd<sup>+2</sup> removal percentage by different algal powders.

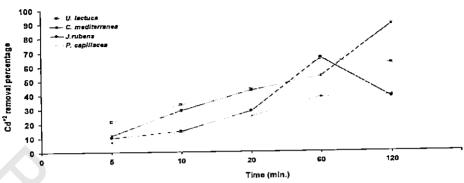


Fig. (7): Effect of contact time on Pb<sup>+2</sup> removal percentage by different algampowders.

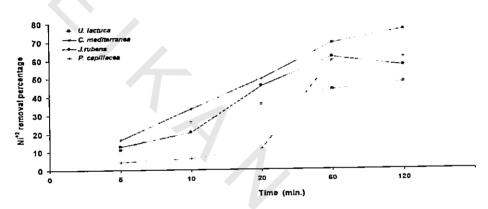


Fig. (8): Effect of contact time on Ni<sup>+2</sup> removal percentage by different algal powders.

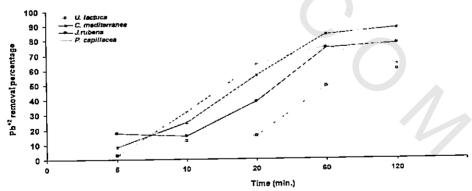


Fig. (9): Effect of contact time on Zn<sup>+2</sup> removal percentage by different algal powders.

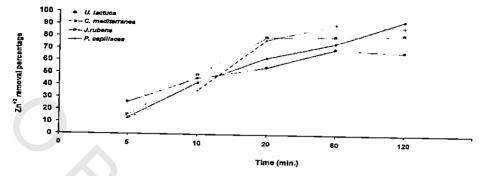
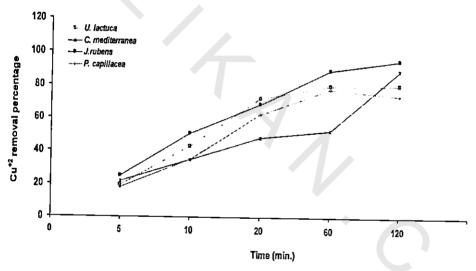


Fig. (10): Effect of contact time on Cu<sup>+2</sup> removal percentage by different algal powders.



#### 4.3 Optimum algal biomass for metal ions bioremoval:

Data in Tables (12-16) and Figures (11-15) show the effect of algal biomass on metal ion bioremoval efficiency. The results clearly reveal that the efficiency of metal ion bioremoval increases as algal biomasses increases from 1 g/L to 2 g/L to 5 g/L to 10 g/L to 20 g/L and 40 g/L. With all algae, the highest value of metal ions removal percentage was observed at 40 g/L and the maximum efficiency for removal of metal ions ranged between 86.7% by *U. lactuca* and 68.1% by *P. capillacea* for Cd, 92.7% by *C. mediterranea* and 79.7% by *P. capillacea* for Pb, 94.52% by *P. capillacea* and 65.34% by *J. rubens* for Ni, 92.7% by *J. rubens* and 89.3% by *C. mediterranea* for Zn, 92.6% by *J. rubens* and 71.2% *C. mediterranea* by for Cu.

Table (12): Effect of algal biomass on Cd<sup>+2</sup> removal percentage

by different algal powders.

B	iomass dosage		2	5	10	20	40
Algal specie	s	1g/L	g/L	g/L	g/L	g/L	g/L
Chlorophyta	U. lactuca	2.9	12.9	27.9	59.9	87.1	86.7
Rhodophyta	C. mediterranea	7.2	36.8	56.1	73	81.9	83.4
10.000 priytu	J. rubens	5.6	24.7	50.6	57.5	49.3	74.9
	P. capillacea	3.8	8.01	16.7	36.4	56.4	68.1

Table (13): Effect of algal biomass on Pb<sup>+2</sup> removal percentage by different algal nowders

	Biomass dosage		2	5	10	20	40
Algal speci	es	L	g/L	g/L	g/L	g/L	g/L
Chlorophyt a	U. lactuca	6.7	20.5	53.9 7	59.3	85. 93	80.12
Rhodophyt	C. mediterranea	9.4	23.7	63.5	84	95.2	92.7
а	J. rubens	2.48	32.8 6	43.6	76.1	83.5	89.3
	P. capillacea	7.9	18.2	45.7	69.35	47.98	79.7

Table (14): Effect of algal biomass on Ni<sup>+2</sup> removal percentage by different algal powders.

E	iomass dosage	10/1	2	5	10	20	40
Algal specie	s	1g/L	g/L	g/L	g/L	g/L	g/L
Chlorophyta	U. lactuca	3.7	8.5	28.9	47.7	97	91.78
Rhodophyta	C. mediterranea	17.3	45.3	59.2	61.8	73.7	79.9
Induophyta	J. rubens	1.5	5.8	36.2	66.5	67.2	65.34
	P. capillacea	1.2	7.3	22.4	62.32	93.5	94.52

Table (15): Effect of algal biomass on Zn<sup>+2</sup> removal percentage by different algal powders.

I	Biomass dosage	1 cr/T	2	E ~/T	10	20	40
Algal specie	s	1g/L	g/L	5 g/L	g/L	g/L	g/L
Chlorophyta	U. lactuca	4.55	17.4	35.62	78.62	67.6	90.9
Rhodophyta	C. mediterranea	20	35.7	57.4	78.1	85.7	89.3
Inouophytu	J. rubens	5.89	13.8	24.3	74.5	88.9	92.7
	P. capillacea	1.43	22.5	46.9	86.9	79.32	89.9

Table (16): Effect of algal biomass on Cu<sup>+2</sup> removal percentage by different algal powders.

	y arrier ent uiga	<u> </u>	uci s	•			
	Biomass dosage	lg/L	2	5 g/L	10	20	40
Algal species	5	1.8/1	g/L	J g/L	g/L	g/L	g/L
Chlorophyta	U. lactuca	10.4	35.9	64.24	71.3	79.81	80.3
District of	C. mediterranea	12.6	29.1	48.9	56.5	66.3	71.2
Rhodophyta	J. rubens	4.6	16.9	48.2	75.5	79.4	92.6
	P. capillacea	5.1	15.4	42.16	82.16	83.53	82.95

Fig. (11): Effect of algal biomass on Cd<sup>+2</sup> removal percentage by different algal powders.

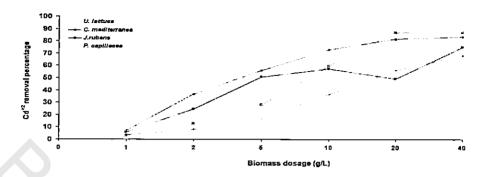


Fig. (12): Effect of algal biomass on Pb<sup>+2</sup> removal percentage by different algal powders.

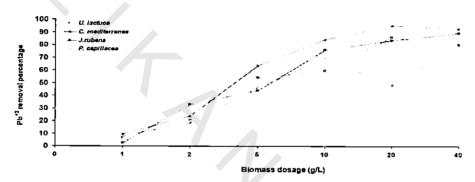


Fig. (13): Effect of algal biomass on Ni<sup>+2</sup> removal percentage by different algal powders.

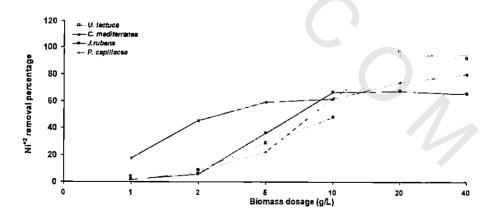


Fig. (14): Effect of algal biomass on Zn<sup>+2</sup> removal percentage by different algal power powers

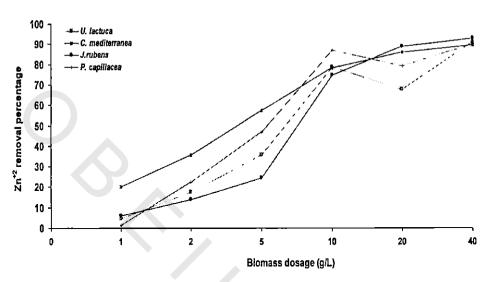
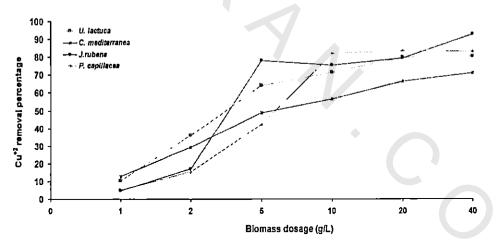


Fig. (15): Effect of algal biomass on Cu<sup>+2</sup> removal percentage by different algal Powders.



# 4.4 Effect of irrigation with low quality water before and after algal treatment.

This part of study show the effect of irrigation with low-quality water contained different concentrations of heavy metals-(Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Cu<sup>+2</sup> and Zn<sup>+2</sup>) before and after marine algal treatment on the final germination percentage, seedling enzymes, some vegetative growth parameters, photosynthetic pigments and some chemical components of wheat grains and faba bean seeds.

# 4.4.1 The final percentage germination of seeds:

The final percentage germination values of wheat grains and faba bean seeds at 7 days from planting as influenced by low quality water irrigation before and after algal treatment are presented in Table (17) and graphically in Figure (16).

#### 4.4.1.1 Wheat:

The data revealed that using El- Batts drainage water in irrigation significantly inhibited wheat grains germination by 23.33%, than irrigation with Nile fresh water (control). However, the reduction in the final percentage germination was only 13.33% than the control when El- Batts drainage water was treated by algae. On the other hand, it was clear that increasing heavy metal concentration in synthetic solutions from 20 to 60 or 100 ppm caused more and remarkable inhibition in wheat grains germination by 33.33, 36.67 and 43.34%, respectively, when compared with corresponding control. Irrigation with synthetic solutions contained 20, 60 and 100 heavy metals after algal treatment raised wheat germination percentage at 7- days when compared to non-algal treated ones by 15.0, 21.05 and 33.54%, respectively.

# 4.4.1.2 Faba bean:

The final percentage germination of faba bean seeds irrigated with El- Batts drainage water before algal treatment was significantly decreased by 16.67%, than irrigation with Nile fresh water. However, irrigation with algal treated water of El- Batts drainage gave an increase in seed germination percentage to reach about 96.66%. Irrigating seeds with synthetic solutions contained 20 or 60 ppm heavy metals before algal treatment inhibited the germination percentage significantly by 40%, as compared with the Nile fresh water (control) and without any differences between the two synthetic concentrations. However, a sharp inhibition in the germination percentage was occurred by using synthetic solution of 100 ppm concentration of heavy metals, and the germination percentage reached 46.66% only.

It is obvious that treating synthetic solutions contained 20, 60 and 100 ppm with algae and used it in irrigation, increased the germination percentage of faba bean seeds than non-treated solutions by 13.33% for these statements.

# 4.4.2 Seedling enzyme activities:

#### 4.4.2.1 Peroxidase (U/gm F.W.):

#### 4.4.2.1.1 Wheat:

Data in Table (17) and Figure (17) indicate that irrigation with El. Batts drainage water and synthetic solution contained 20, 60 and 100 ppm of heavy metals before algal treatment caused very highly significant reductions in seedling peroxidase activity by 13.76, 25.38, 58.46 and 64.32%, respectively, when compared with Nile fresh water irrigation.

Using algal treated water in irrigation gave fluctuated effect on seedling peroxidase activity. Irrigation with El- Batts drainage water or synthetic solution of 20 ppm heavy metals after algal treatment significantly reduced the seedling peroxidase enzyme by 11.92 and 28.9% when compared with corresponding untreated with algae. However, irrigation by synthetic solution contained 60 or 100 ppm heavy metals concentration decreased peroxidase activity. Treatment of these two synthetic solutions (60 and 100 ppm) by algae leads to increase in seedlings peroxidase activity by 48.96and 46.84%, respectively.

#### 4.4.2.1.2 Faba bean:

It is clear form Table (17) and Figure (17) that irrigation with El Batts drainage water or synthetic solutions contained 20, 60 and 100 ppm of heavy metals before algal treatment gave very highly-significant increases in seedling peroxidase activity by 24.60, 22.75, 26.11 and 23.63%, respectively, when compared with the Nile freshwater irrigation.

On the other hand, irrigation with algal treated water caused decrease in seedling peroxidase activity than the non- algal treated water by 24.57, 23.78, 2.07 and 11.34% for El- Batts drainage water and synthetic solutions 20, 60 and 100 ppm heavy metals respectively.

# 4.4.2.2 Catalase (U/gm F.W.):

#### 4.4.2.2.1 Wheat:

The results in Table (17) and Figure (18) demonstrated that the maximum catalase activity in wheat seedlings was detected from irrigation with Nile fresh water (control).

Irrigation with El- Batts drainage water or synthetic solutions contained 20, 60 and 100 ppm of heavy metal without algal treatment gave very highly significant decrease in catalase activity than the control by 60.6, 81.7, 81.82 and 75.75%, respectively.

The irrigation with algal treated El- Batts drainage water significantly reduce catalase activity in seedlings compared with non-treated drainage water by 57.7%.

Treatment synthetic solutions of 20, 60 and 100 ppm heavy metals with algae gave significant increases in catalase activity in seedlings than non-algal treated water.

#### 4.4.2.2.2 Faba bean:

As can be seen from Table (17) and Figure (18) that irrigation with El- Batts drainage water exhibit a very highly significant decrease in catalase activity of faba been seedlings by 53.33% than the control. However, in the case of non-treated synthetic solutions, the catalase activity of seedlings detected from irrigation with 20 or 60 ppm heavy metals solution significantly reduced by 70.91 and 33.33% than irrigation with the Nile fresh water, respectively.

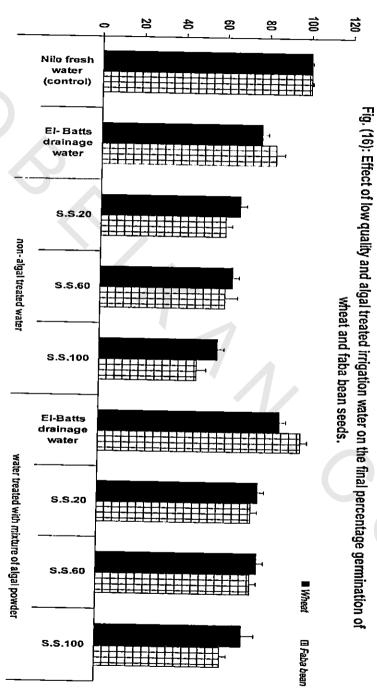
Regarding, irrigation with synthetic solution contained 100 ppm heavy metals, the catalase activity of seedlings significantly increased than the control (Nile fresh water) by 10.6%.

It can be noticed that irrigation with algal treated water caused very highly significant increases in the catalase activity, comparing with irrigation by non-algal treated water, except at 100 ppm heavy metals of algal treated, the catalase activity reduced.

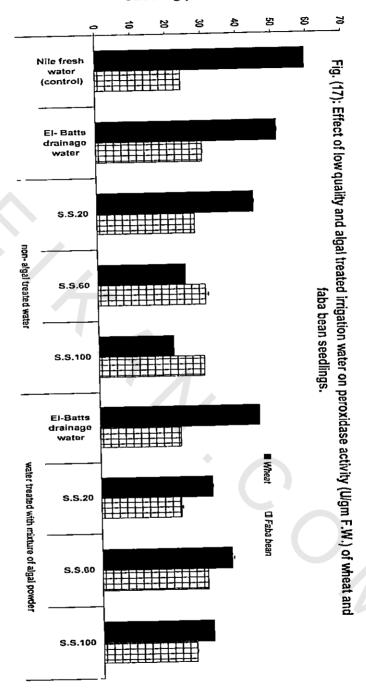
Table (17): Effect of low quality and algal treated irrigation water on final percentage germination (%) of seeds and seedling enzymes of wheat and faba bean.

			Wheat			Faba bean	
			Seedling	Seedling	Final	Seedling	Seedling
Water treatments		Final	Peroxidase	Catalase (U/gm.	germination	0	Catalase (U/gm.
		germination(%)	(U/gm. F.W)	F.W)	(%)	(U/gm, F.W)	F.W)
Nile fresh water (control)	(control)	100.00±0.58	59.51 ±0.08	6,00±0,01	100.0 ±0.58	24.179 ±0.02	30.000 ±0.43
El-Batte drainage water	se water	_	51.32*** ±0.09	2.36*** ±0.02	83.33* ±4.4	30.127*** ±0.04   14.000*** ±0.07	14.000*** ±0.07
	500	╄			70 00*** 10 0	OO 670*** +0 03	© 707*** +0 0K
	S.S.20	66.67*** ±3.3	44,40*** ±0.17	1.10*** ±0.04	60.00*** ±2.9	29.6/9*** ±0.03 8./2/*** ±0.00	8. /2/*** ±0.00
Non-algal	S.S.60	63.33*** ±3.3	24.72*** ±0.05	$1.09*** \pm 0.04$	60.00** ±5.8	30.492*** ±0.69 20.000*** ±0.10	20.000*** ±0.10
treated water	S.S.100	56.67*** ±3.3	21.23*** ±0.01 1.46*** ±0.01	1.46*** ±0.01	46.67*** ±4.4	46.67*** ±4.4 29.892*** ±0.03 33.161** ±0.08	33.161** ±0.08
	El-Batts	86.67n.s. ±3.3	45.21*** ±0.07   1.00*** ±0.01	1.00*** ±0.01	96.67n.s. ±3.3	96.67n.s. ±3.3   22.725*** ±0.14   18.180*** ±0.02	18.180*** ±0.02
Water treated	S.S.20	76.67 n.s. ±3.3	31.57*** ±0.11	2.91*** ±0.09	73.33n.s. ±3.3	73.33n.s. ±3.3   22.620*** ±0.47   14.000*** ±0.10	14.000*** ±0.10
with mixture of	S.S.60	76.67* ±3.3	36.67*** ±0.66	3.09*** ±0.01	73.33n.s ±3.3	73.33n.s ±3.3   29.861n.s. ±0.08   34.910*** ±0.04	34.910*** ±0.04
aigai pon uci	S.S.100	70.00n.s. ±5.8	31.18*** ±0.09; 4.09*** ±0.02	4.09*** ±0.02	60.00n.s, ±2.9	60.00n.s. ±2.9   26.502*** ±0.05   21.818*** ±0.02	21.818*** ±0.02
Non significant n.s.	at P > 0.05	.05	High	Highly significant		at P ≤ 0.01	
Significant (*) at F \( \) 0.05 S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd. 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)	lution of heavy	eavy metals (4 ppm	of Cd, 4 ppm of	pm of Pb, 4 ppm of Ni, 4 ppm c	4 ppm of Zn and	_	
S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu) S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu)	dution of he	eavy metals (12 pp heavy metals (20 p	m of Cd, 12 ppm pm of Cd, 20 ppn	ot Pb. 12 ppm of a of Pb, 20 ppm o	('Ni, 20 ppm of Z	n and 20 ppm of Cu	u)
Mixture of algal powder (10 gm of powder of U. lucluca, C. mediterranea and J. rubens)	der (10 gm	of powder of U. k	icluca, C. meditei	ranea and J. Fane	ns)		

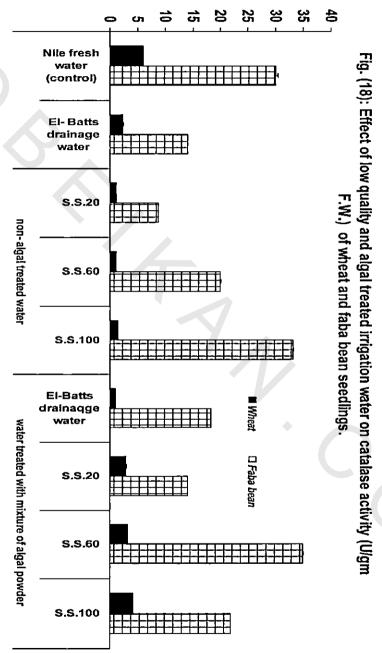




#### Seedling peroxidase (U/gm F.W.)







## 4.4.3 Vegetative growth parameters:

The data obtained and the mean values/plant were computed for shoot height, root length, fresh and dry weights, leaf number and leaf area/ plant.

#### 4.4.3.1 Wheat:

### 4.4.3.1.1 Shoot height (cm):

The results presented in Table (18 and 19) and Figure (19)= indicated that shoot height of wheat plants were significantly affected by different irrigation treatments used at 5 and 9- weeks old plants. The maximum shoot height values at 5 and 9- weeks old plants (16.8 and 35.07 cm) were attained from irrigation with the Nile fresh water (control). Irrigation with El- Batts drainage water before algal treated significantly decreased shoot height at 5 and 9weeks old plants by 20.24 and 16.65%, respectively, compared\_ with the control, however, algal treated water of El- Batts drainage= decreased the shoot height at 5 and 9- weeks old plants by 15.48and 6.67%, respectively, compared with the control. Using the synthetic solutions contained 20, 60 and 100 ppm heavy metals caused very highly significant reductions in shoot height at 5- weeks= old plants by 31.55, 32.74 and 40.48%, respectively, and at 9weeks old plants by 19.87, 22.81 and 28.63%, respectively, ascompared to the control. Generally, irrigating wheat plants with El-Batts drainage water before or after algal treated increased the shoot height at 5 and 9- weeks old plants than all the synthetic solution. (20, 60 and 100 ppm heavy metals) before or after algal treating.

However, high significant differences in shoot height were noticed at 5 or 9- weeks old plants between synthetic solutions of 20 ppm and 60 ppm heavy metals, either before or after algal treatment, but increasing heavy metal concentrations in irrigated water from 20 to 100 or from 60 to 100 ppm before or after algal treatment significantly decreased shoot height at 5- weeks old plants by 32.74 and 40.48% and at 9- weeks old plants by 22.81 and 28.63%, respectively, compared with the control. The corresponding values of heavy metal concentrations decrease shoot height of wheat plants irrigated with algal treated water at 5- weeks old plants by 24.40 and 33.33%, and at 9- weeks old plants by 13.89 and 22.07%, respectively.

It is obvious that increasing heavy metals concentration in irrigated water significantly decreased the shoot height of wheat plants at 5 or 9- weeks old plants. Irrigation with algal treated low quality water showed significant increases in shoot height of all water treatments, but still less than the Nile fresh water.

# 4.4.3.1.2 Root length (cm):

Data represented in Table (18 and 19) and Figure (20) point out that the root length of wheat plants irrigated with Nile fresh water was significantly higher than those irrigated with El- Batts drainage water or synthetic solutions of 20, 60 and 100 ppm heavy metals at 5- weeks old plants by 20.16, 42.92, 42.92 and 68.84%, respectively, and at 9- weeks old plants by 17.74, 25.0, 38.12 and 64.11%, respectively. Also, root length of wheat plants irrigated with El- Batts drainage water increased than those irrigated with

synthetic solution of 20 ppm heavy metals at 5 and 9- weeks by—19.18 and 6.17%, respectively.

It is clear that as the heavy metals concentration increased in irrigated water the reduction in root length increased significantly—However, the bioremediation of heavy metals from irrigated water—(algal treated water) leads to an increase in root length of wheat—plants over the non- algal treated water.

These results concluded that irrigating wheat plants with low-quality water caused adverse effects on the root length at the two-growth stages (5 and 9- weeks old). Also, the reduction in the root-length was significantly increased with increasing the heavy metals—concentration in irrigation water, compared to the control. In-addition, treated algal water increased the root length at 5 and 9—weeks old plants over the non-algal treated ones.

## 4.4.3.1.3 Shoot fresh weight (gm):

As can be noticed from Table (18 and 19) and Figure (21) that the shoot fresh weight of wheat at the two growth stages (5 and 9- weeks old) appeared to decrease due to low quality water irrigation before or after algal treatment, as compared to the Nile fresh water irrigation.

Irrigation with El- Batts drainage water before or after algal treatment significantly reduced the shoot fresh weight at 5- weeks-old plants by 28.95 and 18.86%, respectively, and at 9- weeks old plants by 16.18 and 8.22%, respectively, when compared with Nile fresh water (control).

Treatment of synthetic solution (20, 60 and 100 ppm heavy metals) with algae increase shoot fresh weight at 5- weeks old plants by 12.42, 20.0 and 23.88%, respectively, and at 9- weeks old plants by 15.76, 6.53, and 12.93%, respectively, over the non- algal treated synthetic solutions. Increasing heavy metals concentration in irrigated water to 100 ppm gave the lowest fresh weight of wheat shoot (1.340 and 2.243 gm) at 5 and 9 weeks plant old.

### 4.4.3.1.4 Shoot dry weight (gm):

The data listed in Table (18 and 19) and Figure (22) reflects the effects of different irrigation treatments on the shoot dry weight of wheat plants.

The maximum shoot dry weight at 5 and 9- weeks old plants, i.e. 0.375 and 0.807 gm, respectively, were obtained from the Nile fresh water, whereas irrigation with synthetic solution of 100 ppm heavy metals gave the minimum shoot dry weight values of wheat plants (0.261 and 0.423 gm).

Irrigation with El- Batts drainage water increased shoot dry weight at 5- weeks old plants by 6.46, 12.59 and 19.92% and at 9-weeks old plants by 11.34, 28.89 and 62.41%, than the shoot dry weight of plants irrigated with synthetic solutions contained 20, 60 and 100 ppm of heavy metals, respectively.

On the other hand, treating El- Batts drainage water or synthetic solutions of 20, 60, and 100 ppm heavy metals with algae caused increases in shoot dry weight more than the non- treated ones at the two growth stages, but the values obtained still below the control value.

### 4.4.3.1.5 Leaf number/ plant:

The data illustrated in Table (18 and 19) and Figure (23) clearly show that using the Nile fresh water (control) for irrigation increase the leaf number/ plant at 5 and 9- weeks old plants (5.33 and 7.67 leaves/ plant), whereas high significant reductions in the leaf number/ plant (2.66 and 4.33 leaves/ plant) at 5 and 9- weeks old plants, were observed when using the synthetic solution of 100 ppm heavy metal concentration for irrigation.

In case of using El- Batts drainage water in irrigation, the leaf number/ plant was decreased non- significantly at 5 and higher significantly at 9- weeks old plants by 18.76 and 30.51% respectively, compared with the control. However using El- Batts algal treated water reduced the leaf number/ plant at 5 and 9- weeks old plants than the Nile fresh water by 12.57 and 13.04% respectively, without significant effect.

Using algal treated synthetic solution of 100 ppm heavy metals—in irrigation reduced the leaf number/ plant at 5 weeks plant old by—25% than algal synthetic solutions of 20 or 60 ppm heavy metals. Similar finding were obtained at 9- weeks old. Algal treatment of El- Batts drainage water and synthetic solutions of 20, 60 and 100—ppm heavy metals concentration increased the leaf number/ plant than the non- algal treated ones at 5- weeks old plants by 7.62,—20.12, 20.12 and 12.78%, respectively, and at 9- weeks old plants by 12.57, 12.57, 13.40 and 15.47%, respectively.

## 4.4.3.1.6 Leaf area/ plant (cm<sup>2</sup>):

The results presented in Table (18 and 19) and Figure (24) revealed that irrigation with the Nile fresh water produced the maximum leaf area at 5 and 9- weeks old plants, i.e. 116.53 and 181.27 cm<sup>2</sup>, respectively, whereas, the minimum leaf area/ plant at 5 and 9- weeks old plants (57.27 and 81.57 cm<sup>2</sup>) were resulted from irrigation with synthetic solution contained 100 ppm heavy metal concentration.

The leaf area/ plant at the two growth stages, obtained from irrigation with El- Batts drainage water (80.23 and 133.53 cm<sup>2</sup>) exceeded those observed from irrigation with synthetic solutions of 20, 60 and 100 ppm by 20.88, 23.05, and 40.09 %, respectively, at 5- weeks stage and by 12.66, 24.18 and 63.70%, respectively, at 9-weeks stage.

Using synthetic solutions of 20, 60 and 100 ppm heavy metals in irrigation gave significant differences in leaf area/ plant at 5 and 9- weeks old plants.

Algal treating of El- Batts drain water and synthetic solution 20, 60 and 100 ppm heavy metals concentration increased leaf area/plant than the non- algal treated ones at 5- weeks stage by 20.03, 17.01, 15.84 and 15.89%, respectively. At 9- weeks of treatment the corresponding increment values were 19.43, 19.49, 26.17 and 31.21%, respectively.

It can be concluded that as the concentration of heavy metals in irrigation water increased, the leaf area/ plant of wheat significantly decreased, but treating these water with algae can induced an inhibition to this reduction in leaf area/ plant at the two growth stages.

Table (18): Effect of low quality and algal treated irrigation water on some vegetative growth parameters of wheat plants at 5- weeks from planting.

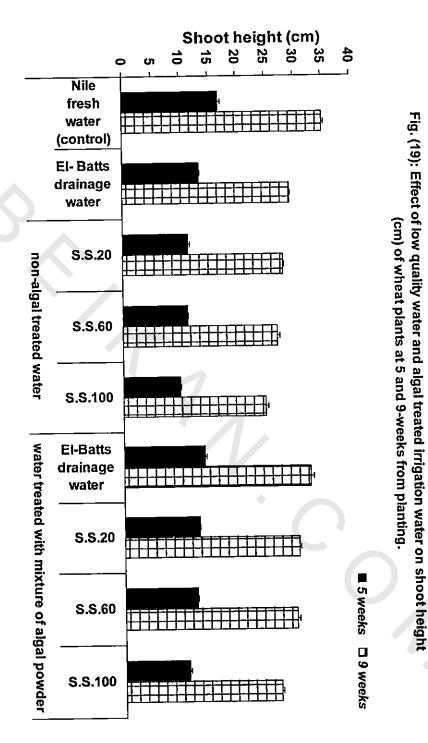
				S weeks	ales		
				Shoot fresh	Shoot dry		Leaf area per
treatments	ents	Shoot height	Root length	weight	weight	plant	plant (cm²)
		\ \ \		(gill)	(5)		
Nile fresh water	ı water rol)	16.8 ±0.44	5.97 ±0.12	2.28 +0.03	0.375 ±0.01	5.33 ±0.33	116.53 ±6.9
El-Batts drainage	rainage	13,4** ±0.17	4.97* ±0.23	1.62*** ±0.03	0.313 n.s. ±0.02 4.33 n.s. ±0.33	4.33 n.s. ±0.33	80.23* ±7.3
water	GI.	11 0444 10 25	4 17*** ±0.07	1.61*** ±0.04	0.294** ±0.001	3.33** ±0.33	66.37** ±2.3
Non-algal	3.3.20	1	_		0.01 **87¢	3.33** ±0.33	65.20** ±3.4
treated	5.5.00	- 1	1, 1 L C L		0.001***10.00	3 K7** +0 33	57.27** ±3.6
water	S.S.100	10.0*** +0.09	3.53*** ±0.23	1.54	0.401		
Water	El-Batts	14.2 n.s. ±0.29	5.43 n.s. ±0.19	1.85** ±0.04	$0.354 \text{ n.s.} \pm 0.03 \text{ 4.67 n.s.} \pm 0.33$	4.67 n.s. ±0.33	96.30 n.s. ±3.2
treated	S.S.20	13.1** ±0.12	4.83** ±0.12	$1.81** \pm 0.01$	$0.317 \text{ n.s.} \pm 0.01 \text{ 4.00 n.s.} \pm 0.58$	4.00 n.s. ±0.58	77.6/* ±1.3
with	S.S.60	12.7*** ±0.12	4.77 n.s. ±0.18	1.74* ±0.02	0.305 n.s. ±0.01 4.00 n.s. ±0.58	4.00 n.s. ±0.58	75.53 n.s. ±2.5
mixture						200	66 37 5 6 47 6
of algal	S.S.100	11.2** ±0.23	4.03 n.s. ±0.15	1.66** ±0.04	0.297 n.s. ±0.01  3.00 n.s. ±0.50	3.00 n.s. ±0.58	00.3 / fl.S. ±2.4
Japanor						01	
Non significant n.s. Significant (* Significant (* S.S. 20 = Synthetic S.S. 60 = Synthetic S.S. 100 = Syntheti	ant n.s. (*) ,nthetic solv ynthetic solv Synthetic solval powdo	It p > 0.05  Highly significant (**) at P ≤ 0.01  Very highly significant (***) at P ≤ 0.001  Very highly significant (***) at P ≤ 0.001  S.S. 20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)  S.S. 60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)  S.S. 100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu)  S.S. 100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu)  S.S. 100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu)	Highly significant Very highly signif tals (4 ppm of Cd, 4 ppr tals (12 ppm of Cd, 12 pen tals (20 ppm of Cd, 20 pens) etals (20 ppm of Cd, 20 pens)	Highly significant (**) Very highly significant (***) ppm of Cd, 4 ppm of Pb, 4 ppn 12 ppm of Cd, 12 ppm of Pb, 12 (20 ppm of Cd, 20 ppm of Pb, 2 U. lactuca, C. mediterranea an	at P ≤ 0.01 ** ) at P ≤ 0.001 ** ppm of Ni, 4 ppm of ppm of Ni, 12 pp b, 20 ppm of Ni, 20 pp and J. rubens)	0.01 0.001 m of Zn and 4 pr 12 ppm of Zn and , 20 ppm of Zn au	ppm of Cu) and 12 ppm of Cu) and 20 ppm of C

Table (19): Effect of low quality and algal treated irrigation water on some vegetative growth parameters of wheat plants at 9- weeks from planting.

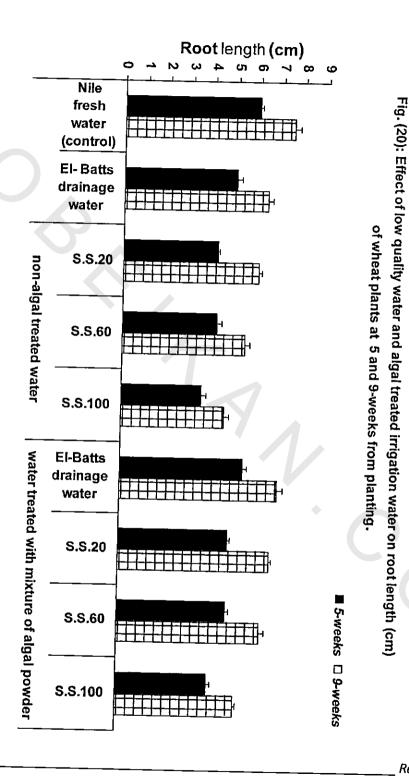
Water	1			9 weeks	eks		
treatments		Shoot height	Root length	Shoot fresh weight Shoot dry weight	Shoot dry weight	I onfine /mlant	Leaf area/plant
	700.00	(cm)	(em)	(gm)	(gm)	Leal no. /plant	(cm²)
Nile fresh water (control)	water ol)	35.07 ±0.38	7.50 ±0.26	4.053 ±0.06	0.807 ±0.01	7.67 ±0.33	181.27 ±3.7
El-Batts drainage water	ainage r	29.23*** ±0.24	6.37* ±0.23	3.397*±0.19	0.687*** ±0.01	5.33**±0.33	133.30*** ±4.8
Non- S	S.S.20	28.10*** ±0.17	6.00** ±0.12	2.957*** ±0.06	0.617***±0.02	5.33**±0.33	118.53*** ±4.6
algal S	S.S.60	27.00*** ±0.46	5.43** ±0.20	2.860*** ±0.06	0.533*** ±0.02	5.00* ±0.58	107.53*** ±3.1
water S.	S.S.100	25.03*** ±0.47	4.57*** ±0.20	2.243*** ±0.04	0.423*** ±0.02	4.33** ±0.33	81.57*** ±2.5
Water El	El-Batts	32.73** ±0.55	6.97 n.s. ±0.27	3.720 n.s. ±0.10	0.747* ±0.01	6.00 n.s. ±0.58	159.47** ±1.7
_	S.S.20	30.67*** ±0.24	6.67* ±0.12	3.423** ±0.03	0.693** ±0.01	6.00 n.s. ±0.58	141.63** ±1.7
	S.S.60	30.20** ±0.40	6.30* ±0.23	3.047 n.s. ±0.04	0.603* ±0.01	5.67 n.s. ±0.33	135.67** ±2.7
of algal S.S.100 powder	S.100	27.33** ±0.26	5.20* ±0.12	2.533* ±0.07	0.497**±0.01	5.00 n.s. ±0.58	107.03** ±2.5
Non significant Significant S.S.20 = Synthe	ant n.s. (*) nthetic sol	s. at $P > 0.05$ *) at $P \le 0.05$ solution of heavy r	netals (4 ppm of (	Highly significant (**) Very highly significant (***)  7d. 4 man of Ph. 4 man of Ni	( ** ) cant ( *** )	at $P \le 0.01$ at $P \le 0.001$ of $Zn$ and $A$ now of	
S.S.ZO = SYI	inenc s	S.S.20 = Symmetric solution of heavy $r$	netals (4 ppm of	S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Nt, 4 ppm of Zn and 4 ppm	opm of Ni, 4 ppm o	t Zn and 4 ppm of	of Cu)

Mixture of algal powder (10 gm of powder of *U. lactuca*, *C. mediterranea* and *J. rubens*)

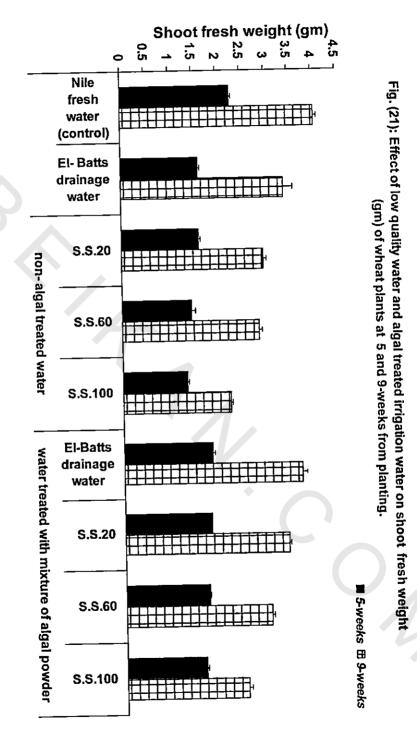
S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)



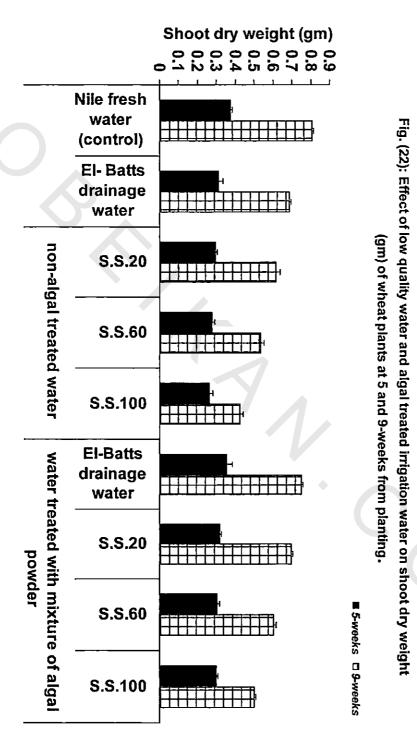
Results



Results



Results



Results

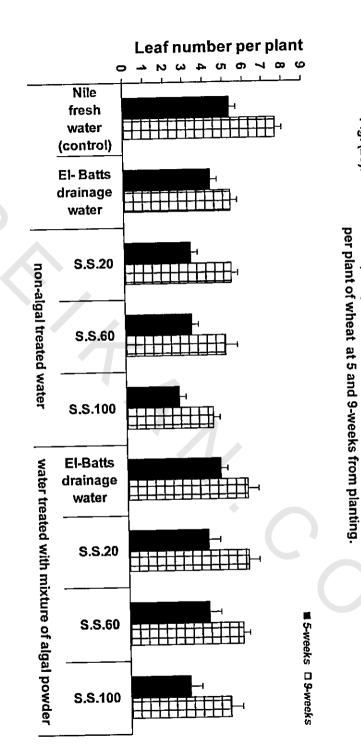


Fig. (23): Effect of low quality water and algal treated irrigation water on leaf number

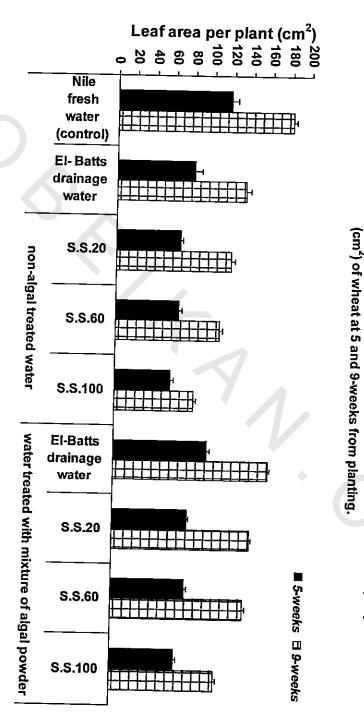


Fig. (24): Effect of low quality water and algal treated irrigation water on leaf area per plant

#### 4.4.3.2 Faba bean:

#### 4.4.3.2.1 Shoot height (cm):

It is clear from data recorded in Table (20 and 21) and Figure (25) that irrigating faba bean plants with El- Batts drainage water caused significant decreases in shoot height at 5 and 9- weeks old plants than the control by 8.11 and 6.33%, respectively. However, the shoot height of faba bean irrigated with algal treated of El-Batts decreased than the control by 3.38 and 3.10%, respectively. These results revealed that using treating low quality water with algae in irrigation caused an remarkable increases in shoot elongation as a result of heavy metals removal by algal powders.

The adverse effect of heavy metals in irrigated water on fababean shoot height can be noticed clearly when the synthetic solutions contained 20, 60 and 100 ppm heavy metals were used in irrigation. The shoot height at 5- weeks old of faba bean plants irrigated with El- Batts drainage water before algal treatment was superior than those obtained from synthetic solutions of 20, 60 and 100 ppm heavy metals concentration by 1.38, 3.57 and 8.09%, respectively, whereas after algal treatment the superiority of El-Batts water over the synthetic solutions (20, 60 and 100 ppm) in shoot height reached 2.69, 5.0 and 8.39%, respectively. Similar results were achieved at 9- weeks old plants, where shoot height

resulted from El-Batts drainage water before algal treatment was higher than those obtained from synthetic solutions 20, 60 and 100 ppm by 2.64, 3.27 and 6.64%, respectively. After algal treatment, shoot height of El-Batts was higher by 2.35, 3.73 and 7.34%, respectively, for algal treated synthetic solutions of 20, 60 and 100 ppm heavy metals.

It could be concluded that irrigation faba bean plants with low quality water significantly decreased shoot height of plants at 5 or 9- weeks old plants, when compared with Nile fresh water irrigation, but the reduction at 9- weeks old plants was less than at 5- weeks old plants. However, algal treating for low quality water induced pronounced increase in shoot height than non- algal treated water.

## 4.4.3.2.2 Root length (cm):

Table (20 and 21) and Figure (26) indicated that root length of faba bean plants was varied due to different treatments of irrigated water at the two growth stages, i.e. 5 and 9- weeks old.

The maximum root length at 5 and 9- weeks old plants (11.17 and 13.97 cm) was observed with the Nile fresh water, whereas the lowest ones (6.47 and 8.80 cm) were detected from irrigation with synthetic solution of 100 ppm heavy metals.

Irrigation with El- Batts drainage water significantly—decreased root length at 5 and 9 weeks old plant by 12.26 and 8.88%, respectively, compared with the control. However, irrigation with El- Batts algal treated water decreased the root length at 5 and 9 weeks by 7.52 and 4.08%, respectively, when compared with Nile—fresh water, but without any significant differences.

Algal treatment of synthetic solutions 20, 60 and 100 ppm heavy metals leads to an increase in the root length of 5- weeks old plants, over non- treated ones by 13.36, 19.40 and 12.83%, respectively, whereas the corresponding values in root length at 9-weeks old plants were 7.31, 8.37 and 7.16%, respectively.

It is obvious that treating low quality water by algal powder resulted in an increase in root length, but this increment are still below the control values.

### 4.4.3.2.3 Shoot fresh weight (gm):

The results presented in Table (20 and 21) and Figure (27) revealed that the highest fresh weight of faba bean at 5 and 9- weeks old plants, i.e. 26.07 and 46.40 gm, respectively, were observed from irrigation by Nile fresh water (control). However, irrigation with non- treated synthetic solution at level 100 ppm heavy metals

gave the lowest shoot fresh weight (18.38 and 35.07 gm, respectively).

Irrigating faba bean plants with algal treated water of El-Batts or synthetic solutions of heavy metals resulted in increases in shoot fresh weight at 5- weeks old plants than the non- algal treated water by 4.82, 5.72, 4.84 and 6.91%, respectively, and by 4.02, 4.62, 5.09 and 3.22%, respectively for 9- weeks old plants.

These results proved that the faba bean shoot fresh weight significantly decreased at the two growth stages as heavy metals concentration increased in irrigated water and this adverse effect can be reduced by treating low quality water used in irrigation with algal powder.

## 4.4.3.2.4 Shoot dry weight (gm):

Table (20 and 21) and Figure (28) showed that using the Nile fresh water in irrigation gave the highest shoot dry weight at the two growth stages, i.e. 4.620 and 9.766 gm, respectively.

Irrigation with El- Batts drainage water significantly decreased shoot dry weight at 5 and 9- weeks old plants by 20.35 and 12.62%, respectively, comparing to those obtained from the Nile fresh water. However, the differences between shoot dry weight of plants irrigated with algal treated, observed from El- Batts

drainage water and synthetic solution of 20 ppm heavy metals at 5—and 9- weeks old plants were non-significant.

Algal treated of low quality water, i.e. El- Batts drainage—water, synthetic solutions contained 20, 60 and 100 ppm heavy—metals led to increases in shoot dry weight than the same non- algal\_treated ones at 5- weeks old plants by 7.34, 7.37, 10.30 and 10.39%, respectively, and at 9- weeks old plants by 7.79 ,11.02,—12.55 and 7.60%, respectively.

It is evident from the previous results that using algae in treating of low quality water is a beneficial method in raising the shoot dry weight of faba bean plants at the two growth stages.

### 4.4.3.2.5 Leaflets number/ plant:

Table (20 and 21) and Figure (29) clarified that irrigating faba bean plants with synthetic solution of 20, 60, and 100 ppm heavy metals concentration significantly decreased the leaflet number/ plant at 5- weeks old plants by 13.94, 17.42 and 22.30%, respectively, and at 9- weeks old plants by 19.61, 20.98 and 26.86%, respectively, compared to irrigation with Nile fresh water (control).

On the other hand, algal treated El- Batts drainage water or synthetic solutions of 20, 60 and 100 ppm heavy metals induced increases in the leaflets number/ plant than non- algal treated ones at

\_\_\_\_\_\_ Results

5- weeks old plants by 7.69, 9.31, 12.66 and 12.11%, respectively, whereas the corresponding values of increment at 9- weeks old plants were 6.67, 6.58, 7.44 and 6.43%, respectively.

It is evident that the sharpest reduction in the leaflets number/ plant at 5 and 9- weeks old plants was occurred when plants were irrigated with non- algal synthetic solution of 100 ppm heavy metals, compared with the control, whereas treating this water with algae reduced the inhibition effects of heavy metals on leaflets appearance at 5 and 9- weeks old plants by 12.11 and 6.43%, respectively.

### 4.4.3.2.6 Leaflets area/ plant (cm<sup>2</sup>):

The data recorded in Table (20 and 21) and Figure (30) indicate that the leaflets area/ plant in this study were significantly varied from each other at the two vegetation growth stages, due to the effect of different irrigation treatments.

Very highly significant reductions in leaflets area/ plant were occurred when plants were irrigated with low quality water either at 5 and 9- weeks old plants, comparing to the Nile fresh water.

In the case of irrigation with El- Batts drainage water, the leaflets area/ plant at the two growth stages were significantly reduced by 45.61 and 21.57%, respectively, than the control. However, the leaflets area/ plant, obtained from El- Batts drainage water were superior than those detected from irrigation with synthetic solutions of 20, 60 and 100 ppm heavy metals

concentration by 12.99, 20.82 and 32.11%, respectively, at 5- week old plants and by 27.21, 30.70 and 45.46%, respectively, at 9—weeks old plants.

On the other hand, treating the low quality water (i.e. El—Batts drain, synthetic solutions contained 20, 60 and 100 ppm heavy—metals) with algae increase the leaflets area/ plant than the non—algal treated ones at 5- weeks old plants by 62.70, 51.72, 53.13 and 47.70%, respectively, and at 9- weeks old plants by 15.66, 21.52—16.40 and 15.52%, respectively.

Table (20): Effect of low quality and algal treated irrigation water on some vegetative growth parameters of faba bean plants at 5- weeks from planting.

1				5 weeks	IS.		
water treatments	ıts	Shoot height (cm)	Root length (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Leaflets no. /plant	Leaflet area/plant
Nile fresh water (control)	vater l)	29.60 ±0.40	11.17 ±0.15	26.07 ±0.73	4.640 ±0.13	28.7 ±0.88	602.9±15
El-Batts drainage	inage						
water	o o	27.20* ±0.46	9.80** ±0.12	21.13** ±0.37	3.680* ±0.22	26.0 n.s. ±0.58	8 327.9*** ±8.4
Non-algal	S.S.20	26.83** ±0.18	8.23*** ±0.19	20.29** ±0.29	3.680** ±0.12	24 7** +0 33	200 2*** +7 1
treated	S.S.60	26.23** ±0.29	6.70*** ±0.21	19.83**+0.41	3 300** +0 14		271 4*** 12
water s	S.S.100	S.S.100 25.00*** +0 12	6 47*** +0 24	10 10*** 10 11 11 11 11 11 11 11 11 11 11 11 11	3 000444	-	五11.4 王13
1			-0.2	10.00	5.060*** ±0.0/   22.3** ±0.88		248.2*** ±6.8
Water	I-Batts	EI-Batts 28.60 n.s. ±0.50	10.33 n.s. ±0.23	22.18 n.s. ±0.23	3.950 n.s. ±0.17 28.0 n.s. ±0.58	-7.7	533.5*** ±12
treated with S.S.20	S.S.20	27.83* ±0.22	9.33* ±0.23	21.47 n.s. ±0.43	3.930 n.s. ±0.12 27.0* ±0.58	$\rightarrow$	440 3*** +0 4
mixture of S.S.60		27.17 n.s. ±0.38	8.00 n.s. ±0.45	20.79 n.s. ±0.54	3.640 n.s. ±0.05 26 7** ±0 33	_	717 *** 717
ulgal powder S.S. 100	.S.100	26 20* +0 32	730 n = +0 10	_L		+-	T1.0.0
		20.20	7.50 n.s. ±0.40	19.65 n.s. ±0.45	3.400 n.s. ±0.10 25.0 n.s. ±0.58		366.6*** ±8.1
Non significant		at P > 0.05	High	Highly significant	(**)	at P ≤ 0.01	
S 20 = Syntha		at P \( 0.05	Very	Very highly significant ( ***	( *** )	at $P \le 0.001$	
S.60 = Synther	tic solut	ion of heavy metal	s (12 num of Cd. 4)	S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd. 12 ppm of Pb. 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)	f Ni, 4 ppm of Zn	and 4 ppm of Cu	<b>.</b>

Mixture of algal powder (10 gm of powder of U. lactuca, C. mediterranea and J. rubens)

S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)

able (21): Effect of low quality and algal treated irrigation water on some vegetative growth parameters of faba bean plants at 9- weeks from planting.

	_			9 weeks	eks		
Water treatments	ts	Shoot height (cm)	Root length (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)	Leaf lets no. /plant	Leaf lets area/plant (cm²)
Nile fresh water (control)	rater )	52.60 ±0.31	13.97 ±0.18	46.400 ±1.1	9.767 ±0.23	51.0±1.7	1271.50 ±19
El-Batts drainage water	inage	49.27** ±0.38	12.73* ±0.26	41.533* ±0.75	8.567* ±0.20	45.0 n.s. ±1.7	997.20** ±46
Non-algal S	S.S.20	47.97*** ±0.15	9.57*** ±0.33	37.667** ±0.52	7.567** ±0.30	41.0**±0.58	783.87***±17
treated \$	S.S.60	47.67*** ±0.23	9.20*** ±0.23	37.300** ±0.69	7.433** ±0.30	40.3** ±0.88	762.97*** ±20
water S	S.S.100	46,00*** ±0,45	8.80***±0.26	35.066***±0.60	51.0± *** ±0.15	37.3** ±0.88	685.53*** ±33
Water	Cl-	50.97* ±0.38	13.40 n.s. ±0.26	13.40 n.s. ±0.26 43.200 n.s. ±0.53	9.233 n.s. ±0.19	48.0 n.s. ±1.7	1153.40* ±36
with	S.S.20	49.77*** ±0.18	10.27 n.s. ±0.22	39.400* ±0.26	8.400 n.s. ±0.26	43.7* ±0.33	952.60** ±17
읔	S.S.60	49.07* ±0.35	9.97 n.s. ±0.26	39,200 n.s. ±0.32	8.367 n.s. ±0.32 43.3 n.s. ±0.88	43.3 n.s. ±0.88	888.07* ±28
powder S	S.S.100	47.23 n.s. ±0.26	9.43 n.s. ±0.18	36.200 n.s. ±0.52	7.167 <b>*</b> ±0.12	39.7 n.s. ±0.67	791.90 n.s. ±31
Non significant Significant	nt n.s. (*)	at $P > 0.05$ at $P \le 0.05$	1	Highly significant (**) Very highly significant (***)	) -	at $P \le 0.01$ at $P \le 0.001$	1

S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)

S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd. 4 ppm of Pb. 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)

Mixture of algal powder (10 gm of powder of U: lactuca, C: mediciranea and J: rubens)

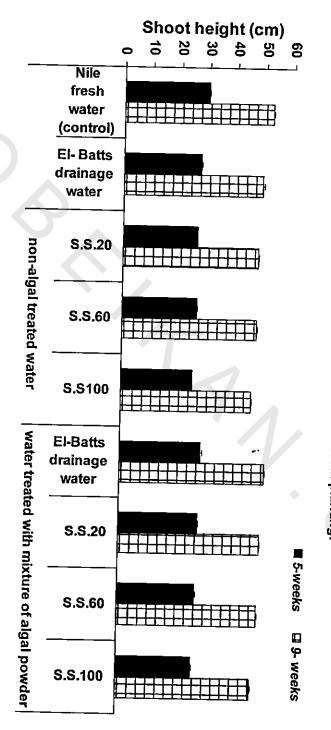
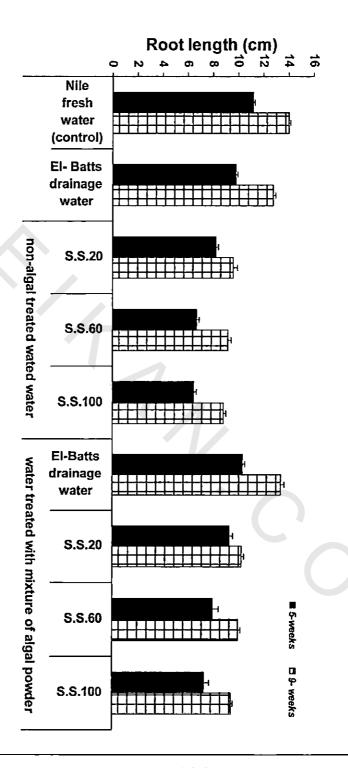


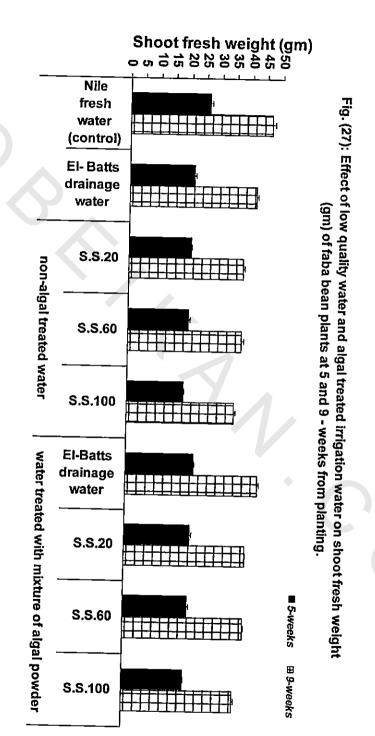
Fig. (25): Effect of low quality water and algal treated irrigation water on shoot height (cm) of faba bean plants at 5 and 9 - weeks from planting.



Results

Fig. (26): Effect of low quality water and algal treated irrigation water on root length

(cm) of faba bean plants at 5 and 9-weeks from planting.



### Shoot dry wieght (gm)

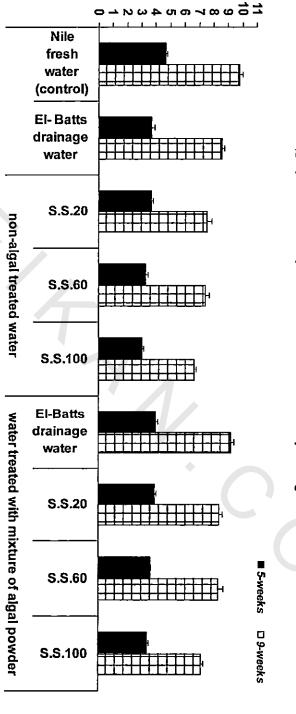


Fig. (28): Effect of low quality water and algal treated irrigation water on shoot dry weight (gm) of faba bean plants at 5 and 9 weeks from planting.

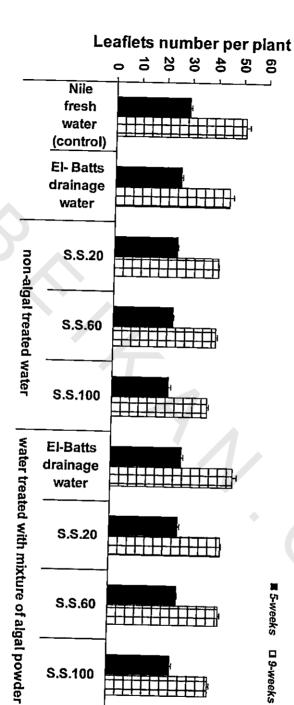


Fig. (29): Effect of low quality water and algal treated irrigation water on leaflets number per plant of faba bean at 5 and 9-weeks from planting.

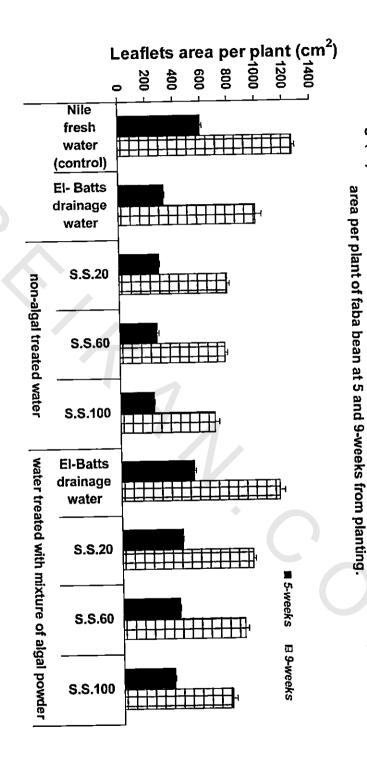


Fig. (30): Effect of low quality water and algal treated irrigation water on leaflets

### 4.4.4 Photosynthetic pigments:

#### 4.4.4.1 Wheat:

Photosynthetic pigment contents, i.e. chlorophyll a, b, carotenoids and total pigments in relation to irrigation with treated and non- algal treated drainage and synthetic solutions were estimated in wheat leaves at 5 and 9 weeks old plants are presented in Table (22 and 23) and graphed in Figures (31 and 32). It is clear from the results that, chlorophyll a and b, a+b, carotenoids and total pigments at the two vegetative growth stages (5 and 9- weeks old plants) appeared to decrease by increasing heavy metals concentration when irrigated with low quality water. However, there was an increase in all pigments content as a result of the irrigation with algal treated water of all heavy metal concentrations (El-Batts, synthetic solutions 20, 60 and 100 ppm) compared with non-treated ones.

Maximum values of chlorophyll a at 5 and 9- weeks old plants were observed in plants irrigated by Nile fresh water (2.730 and 7.627 mg/gm F.W.), followed by El-Batts algal treated water (2.423 and 6.137 mg/gm F.W.), then algal treated synthetic solution of 20 ppm heavy metals (2.13 and 4.84 mg/gm F.W.).

Concerning, chlorophyll b at 5 and 9- weeks old plant irrigation with Nile fresh water gave the highest values, i.e. 1.34 and 3.86% mg/gm F.W., respectively. However irrigation with non-treated El-Batts water or synthetic solutions caused highly significant reduction in chlorophyll b at 5-weeks old plants by

61.44, 63.41, 66.05 and 78.67%, respectively, when compared with the control. At 9-weeks old plants the corresponding reduction percentage were 29.66, 42.44, 64.96 and 89.22%, respectively. One the other hand, treating El- Batts water or synthetic solutions of 20, 60 and 100 ppm with algae induced very highly significant decreases in chlorophyll b than the control at 5- weeks old plant by 3.32, 14.53, 19.64 and 32.67%, respectively. At 9- weeks old plant the reduction percentage than the control were 15.46, 32.05, 43.33 and 73.09%, respectively.

Regarding the carotenoids content at 5 and 9- weeks old—plants, the maximum values were detected from irrigation with Nile fresh water (2.880 and 5.340 mg/gm F.W.). Irrigation with algal treated drainage and synthetic solutions gave non-significant increases in the carotenoids content than non-algal treated ones at 5 and 9- weeks old plants; but they were lower than the control.

With respect to total pigments, the highest total pigment values at 5 and 9- weeks old plants (6.954 and 16.830 mg/gm F.W.) were obtained from irrigation with Nile fresh water, whereas the lowest ones (3.641 and 7.240 mg/gm F.W.) were obtained from non-algal synthetic solution of 100 ppm heavy metals. The algal treated of El- Batts drainage water significantly increased the total pigments at 5 and 9- weeks old plants by 32.34 and 18.72%, respectively, compared with the non-algal treatment drainage. These obtained results were found to be true for the algal treated synthetic solutions of 20, 60 and 100 ppm heavy metals concentration at 5 and 9- weeks old plants.

Table (22): Effect of low quality and algal treated irrigation water on leaf pigments content of wheat plants at 5- weeks from planting.

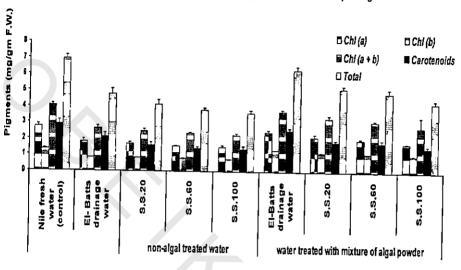
				5 weeks		
Water treatments	, 	Chlorophyll (a) (mg/gm F.W.)	Chlorophyll (b) (mg/gm F.W.)	Chlorophyll (a + b) (mg/gm F.W.)	Carotenoids (mg/gm F.W.)	Total pigments (mg/gm F.W.)
Nile fresh water (control)	control)	2.730 ±0.16	1.340 ±0.047	4.070 ±0.11	2.880 ±0.28	$6.954 \pm 0.22$
El-Batts drainage water	e water	1.813* ±0.17	0.830*** ±0.013	2.643** ±0.18	2.110 n.s. ±0.24	4.753** ±0.37
N'	S.S.20	1.677** ±0.12	0.820*** ±0.012	2.497*** ±0.13	1.630* ±0.17	4.123** ±0.30
water	S.S.60	1.563**±0.05	0.807*** ±0.008	2.370*** ±0.05	1.427** ±0.13	3.797**±0.14
77 H (C)	S.S.100	11.0± **005.1	0.750*** ±0.007	2.250*** ±0.10	1.390** ±0.18	3.641*** ±0.16
	El-Batts	2.423* ±0.12	1.297*** ±0.004	3.720** 0.12	2.571 n.s. ±0.14	6.292*±0.25
water treated with	S.S.20	2.130 n.s. ±0.19	1.170* ***0.019	3.301*±0.19	1.830 n.s. ±0.12	5.131* ±0.20
nowder	S.S.60	1.980** ±0.09	1.120*** ±0.020	3.100** ±0.09	1.787 n.s. ±0.22	4.887* ±0.26
***	S.S.100	1.750 n.s. ±0.05	1.010*** ±0.024	2.765** ±0.64	1.513 n.s. ±0.15	4.273 n.s. ±0.20

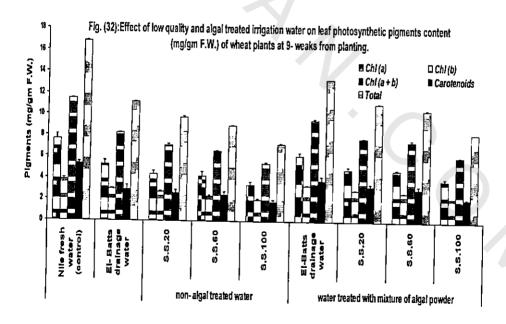
Significant (\*) at  $P \le 0.05$  Very highly significant (\*\*\*) at  $P \le 0.001$  S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu) S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) Mixture of algal powder (10 gm of powder of U. lactuca, C. mediterranea and J. rubens) Non significant n.s. nt n.s. at P > 0.05(\*) at  $P \le 0.05$ Highly significant at P ≤ 0.01

Table (23): Effect of low quality and algal treated irrigation water on leaf pigments content of wheat plants at 9- weeks from planting.

				9 weeks		
Water		Chlorophyll (a)   Chlorophyll (b)	Chlorophyll (b)	Chlorophyll (u + b)	Carotenoids	Total pigments
treatments		(mg/gm F.W.) (mg/gm F.W.)	(mg/gm F.W.)	(a + b) (mg/gm F.W.)	(mg/gm F.W.)	(mg/gm F.W.)
Nile fresh water (control)	control)	7.627 ±0.45	3.860 ±0.12	11.490 ±0.08	5.340 ±0.20	16.830 ±0.030
FI-Batts drainage water	water	5.267** ±0.37	2.977** ±0.06	8.247*** ±0.04	2.950**±0.41	11.197*** ±0.06
	S.S.20	4.390** ±0.31	2.710** ±0.10	7.100*** ±0.13	2.610** ±0.32	9.710*** ±0.12
Non-algal treated	S.S.60	4.193** ±0.42	2.340*** ±0.10	6.536*** ±0.06	2.433** ±0.35	8.966*** ±0.02
water	S.S.100	3.413** ±0.30	2.040*** ±0.07	5.450*** ±0.09	1.790*** ±0.25	7.240*** ±0.03
	El-Batts	6.137 n.s. ±0.32	3.343** ±0.06	9,483*** ±0.11	3.813 n.s. ±0.37	13.293*** ±0.02
Water treated with	S.S.20	4.840 n.s. ±0.25 2.923 n.s. ±0.05	2.923 n.s. ±0.05	7.763**±0.07	3.317 n.s. ±0.21	11.080***±0.03
mixture of algal	S.S.60	4.790 n.s. ±0.10	2.693* ±0.08	7.483** ±0.18	3.017 n.s. ±0.35	10.503*** ±0.09
powder	S.S.100	3.863 n.s. ±0.24 2.230 n.s. ±0.02	2.230 n.s. ±0.02	6.090**±0.04	2.143 n.s. ±0.11	8.233*** ±0.01
Non significant n.s.	at P > 0.05	.05	Highly significant	ant (**)	at P ≤ 0.01	•
tic so	(*) at P ≤ 0.05 slution of heavy me solution of heavy me solution of heavy me der (10 gm of powe	.05 metals (4 ppm of C metals (12 ppm of V metals (12 ppm of V metals (20 ppm of V owder of U. Incluca	Very highly sig d, 4 ppm of Pb, 4 Cd, 12 ppm of Pb, f Cd, 20 ppm of Pb , C. mediterranca	Very highly significant (***) 4 ppm of Pb, 4 ppm of Ni, 4 ppm of , 12 ppm of Pb, 12 ppm of Ni, 12 pp d, 20 ppm of Pb, 20 ppm of Ni, 20 p ; mediterranca and J. rubens)	at P ≤ 0.001 Zn and 4 ppm of Cu; m of Zn and 12 ppm pm of Zn and 20 ppn	u) n of Cu) m of Cu)

Fig. (31): Effect of low quality and algal treated irrigation water on photosynthetic pigments content (mg/gm F.W.) of wheat plants at 5- weaks from planting.





#### 4.4.4.2 Faba bean:

The data recorded in Table (24 and 25) and Figures (33 and 34) demonstrated that the photosynthetic pigments in faba beam leaflets (chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoids and total pigments) at 5 and 9- weeks old plants from planting were significantly affected by different untreated irrigation treatments applied in this study.

It is evident that irrigating faba bean plants with low quality water, i.e. El-Batts drainage water or synthetic solution of 20, 60 and 100 ppm heavy metals caused considerable reduction in chlorophyll a at 5- weeks old plants by 27.98, 48.09, 49.63 and 55.50%, respectively, when compared with plants irrigated by Nile fresh water. However, the corresponding reduction values in chlorophyll a at 9-weeks old plants were 21.57, 29.57, 30.32 and 34.22%, respectively.

The results revealed that treating El-Batts drainage water with algae gave non-significant difference in chlorophyll a at 5 and 9- weeks old plants, as compared with the control.

Increasing heavy metal concentrations in irrigated water before algal treatment from 20 to 60 or 100 ppm decreased significantly chlorophyll a at 5 and 9-weeks old plants. Also, the treated water of 20, 60 and 100 ppm heavy metals increased chlorophyll a content in leaflets at 5 and 9- weeks old plants, but without significant differences.

Regarding chlorophyll b in leaflets of faba bean, results indicated that the maximum values were resulted from irrigating with Nile fresh water at 5 and 9-weeks old plants.

Irrigating with El-Batts drainage water reduced chlorophyll b by 34.89 and 23.06% at 5 and 9- weeks old plants than the control. More reduction in chlorophyll b were noticed as heavy metals concentration in irrigated water increased at 5 and 9- weeks old plants.

Irrigation with algal treated water of El-Batts drainage or synthetic solutions of 20, 60 and 100 ppm heavy metals at 5 and 9-weeks old plants caused increases in chl b than the non-treated water.

Concerning the carotenoids content of faba bean leaflets, data illustrated in Table (24 and 25) and Figures (33 and 34) show gradual decreases in carotenoids with increasing heavy metals concentration in irrigation water. The maximum carotenoids content at 5 and 9- weeks plants old were observed from using Nile fresh water, followed by algal treated water of El-Batts drainage. The lowest carotenoids values at 5 and 9- weeks plants old (1.050 and 3.717 mg/gm F.W.) were detected from irrigation with synthetic solution contained 100 ppm heavy metals.

The results indicated that irrigation with El-Batts drainage water at 5-weeks old plants significantly increased carotenoids than irrigation with synthetic solution of 20 ppm heavy metals. On the other hand, there was highly significant increase in carotenoids at 9-

weeks old plants to a value near that obtained from with Nile freshwater irrigation due to algal treatment of El-Batts drainage water and synthetic solution of 20 ppm heavy metals.

With respect to total pigments at 5 and 9- weeks old plants—
the results revealed that increasing heavy metals in irrigation water—
induced substantial role in total pigments reduction in faba bean—
leaflets. Total pigments were significantly decreased at 5 and 9—
weeks old plants by irrigation with El-Batts drainage water—
compared to the control.

Irrigation by treated low quality water, i.e. El-Batts and synthetic solutions of 20, 60 and 100 ppm heavy metals with algaeresulted in significant increases in total pigments than non-treated ones at 5- weeks plants by 29.89, 37.30, 34.09 and 33.38%, respectively, whereas the corresponding values at 9- weeks old plants reached 12.43, 12.25, 13.1 and 8.02%, respectively.

As a general trend, the obtained results pointed out that photosynthetic pigments content of faba bean leaflets irrigated with algal treated water increased as plant age increased from 5 to 9-weeks. Also, the photosynthetic pigments were significantly inhibited with using low quality water contained different concentrations of heavy metals, comparing with Nile fresh water. However, bioremediation of heavy metals (algal treated water) caused remarkable increases in photosynthetic pigments than the non-algal treated ones, but this increment did not reached the levels that obtained by using Nile fresh water, either at 5- weeks or at 9-weeks old plants.

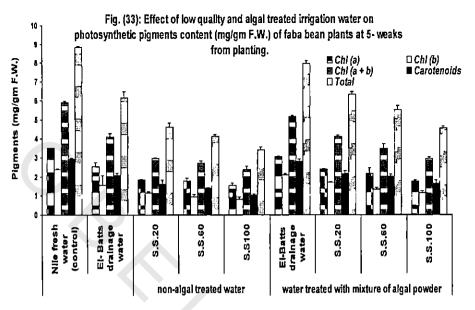
Table (24): Effect of low quality and algal treated irrigation water on leaf pigments content of faba bean plants at 5- weeks from planting.

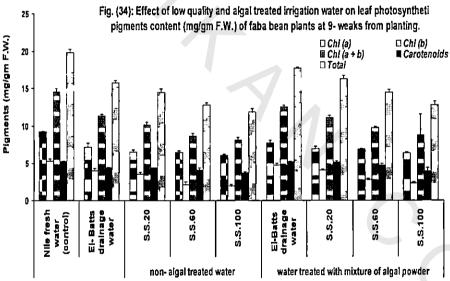
Water				5 weeks		
freatments	its	Chlorophyll (a)	Chlorophyll (b)	Chlorophyll (a)   Chlorophyll (b)   Chlorophyll (a + b)	Carotenoids	Total pigments
		(mg/gm F.W.)	(mg/gm F.W.)	(mg/gm F.W.)	(mg/gm F.W.)	(mg/gm F.W.)
Nue iresh water (control)	(control)	3.506 ±0.02	$2.396 \pm 0.03$	5.903 ±0.05	2.930 ±0.05	8.833 ±0.06
El-Batts drainage water	ge water	2.525** ±0.22	1.560*** +0.51	4.089*** +0 19	2 067** ±0 15	C 155## 1004
			10.00	#.009 ±0.19	2.06/** ±0.15	6.155** ±0.34
Non-algal treated	5.5.20	1.820*** ±0.05	1.160*** ±0.05	2.980*** ±0.01	1.623**±0.22	4.603*** +0.22
0	S.S.60	1.767*** +0 17	0 950*** +0 12	0 717*** 10 10	1 1001	1011
water	25.00	1.707 ±0.17	0.10± ±=0.13	2,/1/*** ±0.12	1.400*** ±0.04	$4.117*** \pm 0.10$
	3.3.100	1.560*** ±0.12	0.813*** ±0.14	2.373*** ±0.21	1.050*** ±0.04	3.423*** ±0.17
Water treated	El-Batts	3.055 n.s. ±0.03	2.113** -0.06	5.168** ±0.06	2.827** ±0.10	7.995** +0.15
יי שנכי נו כמוכם	S.S.20	2.430*** +0.03   1.710*** +0.03	1 710*** +0 02	$\downarrow$	3	10110
with mixture of		2 127 20.02	1./10 ** ±0.03	4.140*** ±0.05	2.180 n.s. ±0.13	6.320** ±0.17
algal powder	5.5.60	2.157 n.s. ±0.31   1.340 n.s. ±0.10	1.340 n.s. ±0.10	3.496* ±0.22	2.023** ±0.12	5.519** +0.19
	S.S.100	S.S.100 1.767 n.s. ±0.06 1.157 n.s. ±0.11	1.157 n.s. ±0.11	2.923 n.s. ±0.11	1.647* ±0.18	4.567** ±0.07
Non significant n	n.s. at P > 0.05	).05	Highly significant	ant (**)	at P < 0.01	

Significant (\*) at  $P \le 0.05$  Very highly significant (\*\*\*) at  $P \le 0.001$  S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu) Mixture of algal powder (10 gm of powder of U. lactuca, C. mediterranea and J. rubens) S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd. 20 ppm of Pb. 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)

Table (25): Effect of low quality and algal treated irrigation water on leaf pigments content of faba bean plants at 9- weeks from planting.

				9 weeks		;
Water		Chlorophyll (a)	Chlorophyll (a) Chlorophyll (b)	Chlorophyll	Carotenoids	Total pigments
o eachiells		(mg/gm F.W.)	(mg/gm F.W.) (mg/gm F.W.)	(a + b) (mg/gm F.W.)	(mg/gm F.W.)	(mg/gm F.W.)
Nile fresh water (control)	ontrol)	9.243 ±0.10	5.303 ±0.35	14.547 ±0.44	5.243 ±0.06	19.790 ±0.50
El-Batts drainage water	water	7.240± ±0.52	4.080* ±0.26	11.320** ±0.23	4.423*** ±0.07	15.743** ±0.35
Van algal treated	S.S.20	15:07 ***015	3.590** ±0.23	10.100**±0.39	4.397*** ±0.03	14.497** ±0.42
water	S.S.60	6.443*** ±0.27	2.183** ±0.39	8.627*** ±0.37	4.163* ±0.27	12.790*** ±0.31
	S.S.100	6.080*** ±0.16	6.080*** ±0.16 2.037*** ±0.21	8.117*** ±0.33	3.717**±0.22	11.833*** ±0.45
Water tracted with	El-Batts	7.703 n.s. ±0.42	7.703 n.s. ±0.42 4.803 n.s. ±0.23 12.507* ±0.22	12.507* ±0.22	5.197** ±0.08	17.703** ±0.17
mixture of aloal	S.S.20	6.950 n.s. ±0.34	4.137 n.s. ±0.12	6.950 n.s. ±0.34 4.137 n.s. ±0.12 11.087 n.s. ±0.34 5.187** ±0.16	5.187**±0.16	16.273* ±0.50
powder.	S.S.60	6.907 n.s. ±0.11	2.823 n.s. ±0.17	6.907 n.s. ±0.11   2.823 n.s. ±0.17   9.730 n.s. ±0.19   4.733 n.s.±0.20	4.733 n.s.±0.20	14.463* ±0.39
<u>.</u>	S.S.100	6.380 n.s. ±0.14	6.380 n.s. ±0.14 2.407 n.s. ±0.13 8.787 n.s. ±2.8	8.787 n.s. ±2.8	4.003 n.s. ±0.43	12.790 n.s. ±0.49
cant n.s.	at P > 0.05		Highly significant	(**)	at P < 0.01	
S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)  S.S.60 = Synthetic solution of heavy metals (4 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)	at r s 0.05 n of heavy m n of heavy m	ictals (4 ppm of C ictals (12 ppm of)	very nighty significant (***) d, 4 ppm of Pb, 4 ppm of Ni, 4 Cd. 12 ppm of Pb. 12 ppm of Ni	Iteant (***) ppin of Ni, 4 ppin of Ni, 12 i	at P ≤ 0.001 of Zn and 4 ppm o nnm of Zn and 12	fCu)
S.S.100 Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 Mixture of algal powder (10 gm of powder of U. luctuca. C. mediterranea and J. rubane)	on of heavy i	metals (20 ppm of vder of <i>U. lactucu</i>	S.S.100 Synthetic solution of heavy metals (20 ppm of Cd. 20 ppm of Pb, 20 ppm of Ni Mixture of algal powder (10 gm of powder of U lactuca, C. mediterranea and J. rubens)	and <i>J. rubens</i> )	ppm of Zn and 20	) ppm of Cu)





# 4.4.5 Chemical components of seeds:

# 4.4.5.1 Total carbohydrate content (%):

### 4.4.5.1.1 Wheat:

The results presented in Table (26) and Figure (35) reveal that the total carbohydrate content values in wheat grains were varied significantly due to the different low quality water, before and after algal treatment.

The highest carbohydrate content value (77.98%) was—obtained from the Nile fresh water (control), whereas the lowest one—(40.21%) was detected from irrigating wheat plants with synthetic—solution at level 100 ppm of heavy metals concentration.

Irrigating wheat plants with El-Batts drainage water—significantly reduced the total carbohydrate content of grains by—27.0%, however algal treatment of El-Batts reduced the grains—carbohydrate content by 22.29%, when compared with the control treatment. These results proved that the application of algal powder—to treat El-Batts drainage water caused a significant decrease in the adverse effect of low quality water on the total carbohydrate content of grains.

It is evident that increasing the heavy metals concentration in irrigation water from 20, 60 and 100 ppm, resulted in very high significant decreases in the total carbohydrate content of wheat grains, than those obtained from the control by 37.87, 44.40 and 48.44%, respectively.

The algal treated of El-Batts drainage water and synthetic solutions of 20, 60 and 100 ppm of heavy metals concentration caused highly significant increases in total carbohydrate content, than those obtained from non- algal treated ones by 6.46, 13.95, 15.4 and 9.82%, respectively.

### 4.4.5.1.2 Faba bean:

The data illustrated in Table (27) and Figure (35) indicate that irrigating faba bean plants with the Nile fresh water gave the highest value of total carbohydrate content in seeds (65.36%), whereas the lowest value (35.3%) was resulted from irrigation of synthetic solution of 100 ppm heavy metals concentration.

Irrigating faba bean plants with El-Batts drainage water and synthetic solutions of 20, 60 and 100 ppm levels of heavy metals concentration significantly decreased the total carbohydrate in seeds by 25.0, 35.0, 41.0 and 46.0%, respectively, when compared with Nile fresh water.

The reductions in total carbohydrate content of seeds, obtained from plants irrigated with algal treated El-Batts drainage water and synthetic solutions of 20, 60 or 100 of ppm of heavy metals concentration reached about 21.0, 29.0, 34.0 and 40.0%, respectively, compares with Nile fresh water.

It is evident that treating the low quality water of El-Batts drainage and synthetic solutions of 20, 60 or 100 of ppm heavy metals with algae induced very high significant increases in total

carbohydrate of faba bean seeds by 4.0, 6.0, 7.0 and 6.0%—respectively, than the same values before algal treatment.

These results reveal the synergetic effect of algal treatment—on improving the quality of irrigation low quality water and its rol—in reducing the adverse effects of heavy metals in water.

# 4.4.5.2 Total protein content (%):

### 4.4.5.2.1 Wheat:

The results shown in Table (26) and Figure (36) reveal that water treatments have significant effects on the total protein content of wheat grains. Irrigating wheat plants with the Nile fresh water significantly increased the total protein content in grains than those resulted from irrigation with El-Batts drainage water and synthetic solutions of 20, 60 and 100 ppm of heavy metals concentration by 17.69, 25.07, 36.93 and 47.02%, respectively. However, the total protein content of grains resulted from irrigation with Nile fresh water was surpassed those obtained from algal treated water of El-Batts drain and synthetic solutions of 20, 60 or 100 ppm of heavy metals concentration by 12.33, 19.04, 28.14 and 38.96%, respectively.

On the other hand, treating El-Batts drain water and synthetic solution of 20, 60 or 100 ppm of heavy metals concentration significantly increased the total protein content of wheat grains over the non- algal treated ones by 4.77, 5.07, 6.86 and 5.30%, respectively.

\_\_\_ Results

### -4.4.5.2.2 Faba bean:

The results of Table (27) and Figure (36) show that irrigating faba bean plants with Nile fresh water gave the highest protein content of seeds (20.7%), whereas the lowest protein content in seeds was observed from irrigation with synthetic solution of 100 ppm level of heavy metals concentration (12%). Irrigating with El-Batts drainage water decreased the total protein content of seeds by 4.55 than that obtained from Nile fresh water. Also, irrigating faba bean plants with algal treated water of El-Batts drain showed very highly significant effect on total protein content of seeds when compared with the non-algal treated water of El-Batts drain.

Regarding the effects of irrigation with synthetic solutions of 20, 60 or 100 ppm of heavy metals concentration on total protein content of seeds, the data indicate that the total protein content of faba bean seeds were significantly reduced by 6.0, 6.83 and 8.7%, respectively, compared with that obtained from Nile fresh water.

On the other hand, a very weak increases in total protein content were resulted from using algal treated synthetic solutions of 20, 60 and 100 ppm level of heavy metals concentration, as compared with the non-algal synthetic solutions treatments.

# 4.4.5.3 Heavy metals content (mg/kg):

#### 4.4.5.3.1 Wheat:

The effects of low quality water before and after alga treatment on the heavy metals content, i.e.  $Cd^{+2}$ ,  $Pb^{+2}$ ,  $Ni^{+2}$ ,  $Zn^{+2}$  an  $Cu^{+2}$  in wheat grains are presented in Table (26) and Figure (37).

It is clear that the lower heavy metals content in wheat grain (Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup>) were resulted from irrigation with the Nile fresh water (control).

The values of Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup> in wheat grainswere varied significantly from each other due to the low qualitywater content of these heavy metals either before or after algaletreating.

Irrigation with El-Batts drainage water after algal treating induced very high significant decrease in wheat grains content from Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup> by 22.0, 21.77, 22.05, 17.41 and 60.62%, respectively, than irrigation with raw El- Batts drainage water.

The heavy metals content in wheat grains (Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup> = Zn<sup>+2</sup> and Cu<sup>+2</sup>) were significantly increased by 10.31, 69.89, 2.64, 40.15 and 64.77%, respectively, by increasing these heavy metals-concentration in irrigation water from 20 to 60 ppm and before algal treating. On the other hand, increasing the heavy metals-concentration from 20 to 60 ppm in synthetic solutions after algal treatment resulted in significant increases of Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup>

\_ Results

and Cu<sup>2+</sup> content in wheat grains by 9.54, 17.42, 1.15, 7.88 and 65.51%, respectively. These results reveal that the using of treated low quality water in irrigation caused pronounced reduction in heavy metals content of wheat grains than non-algal treated water even though the concentration of heavy metals increased in irrigation water, except for Cu<sup>+2</sup> content.

It is obvious that rising the heavy metals concentration in irrigation water to 100 ppm, either for non-algal treated or algal treated water resulted in the higher values of heavy metals content in wheat grains, but the increase of heavy metals content of wheat grains resulted from algal treated water was less than that obtained from irrigation with non-algal treated water.

## 4.4.5.3.2 Faba bean:

The results recorded in Table (27) and Figure (38) reveal that the heavy metals content of faba bean seeds were significantly affected by low quality water used in irrigation before and after algal treatment.

The minimum heavy metals content, i.e. Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup> and Cu<sup>2+</sup> in faba bean seeds were observed from irrigation with Nile fresh water (control), whereas irrigating with synthetic solution of 100 ppm heavy metals gave the maximum faba bean seeds content from heavy metals.

Irrigating faba plants with algal treated water of El-Batts drain showed very high significant decrease in heavy metals content of seeds, i.e. Cd<sup>+2</sup>, Pb<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cu<sup>+2</sup> by 20.71, 31.56, 26.53,

20.29 and 40.12%, respectively, compared with irrigation by non—treated El- Batts drainage water.

Increasing heavy metals concentration in synthetic solution from 20, 60 and 100 ppm, significantly increased the faba bear seeds content from Cd<sup>+2</sup> by 2.68 and 17.61%, Pb<sup>+2</sup> by 46.21 anc 93.21%, Ni<sup>+2</sup> by 0.47 and 15.18%, Zn<sup>+2</sup> by 24.44 and 35.26% anc Cu<sup>+2</sup> by 35.71 and 92.0%, respectively.

On the other hand, there was a very high significant decrease—in heavy metals content of faba bean seeds, i.e.  $Cd^{+2}$ ,  $Pb^{+2}$ ,  $Ni^{+2}$   $Zn^{+2}$  and  $Cu^{+2}$  by 27.17, 43.42, 25.34, 21.04 and 55.42%—respectively, after using algal treated water of 20 ppm heavy metals—in irrigation, in comparison with non-algal treated corresponding—ones. However, using algal treated water contained 60 ppm heavy—metals in irrigation caused very high significant decrease in  $Cd^{+2}$ — $Pb^{+2}$ ,  $Ni^{+2}$ ,  $Zn^{+2}$  and  $Cu^{+2}$  in faba bean seeds by 17.98, 33.36, 24.03—31.98 and 10.51%, than the same heavy metals resulted from—irrigation with non-algal treated water of 60 ppm heavy metals—significantly decreased  $Cd^{+2}$ ,  $Pb^{+2}$ ,  $Ni^{+2}$ ,  $Zn^{+2}$  and  $Cu^{+2}$  content infaba bean seeds than the same ones resulted from irrigation with—non-algal treated water by 15.32, 45.02, 28.54, 37.21 and 22.18%,

It could be concluded that algae play an effective role in reducing heavy metals effects of low quality water.

respectively.

Table (26): Effect of low quality and algal treated irrigation water on some chemical components of wheat grains at harvesting.

Water				Wheat			
reatments	Total Carboby-	Total protein			Heavy metal		
7000	drate (%)	(%)	Cd <sup>+2</sup>	Pb <sup>+3</sup>	Ni <sup>+2</sup>	Zn <sup>+2</sup>	Cu-1
Nile fresh water (control)	77.98 ±0.026	9.38 ±0.012	0.412 ±0.026	0.436 ±0.059	1.410 ±0.12	22.00 ±0.034	1.097 ±0.006
El-Batts drainage water	56.92*** ±0.059 7.97*** ±0.021 6.946*** ±0.12 15.640*** ±0.093 13.613*** ±0.021	7.97*** ±0.021	6.946*** ±0.12	15.640*** ±0.093	13.613*** ±0.021	20.850** ±0.23	1.699** ±0.098
Non- S.S.20	48.45*** ±0.11	7.50*** ±0.10	9.120*** ±0.14	22.205*** ±0.26	15.407*** ±0.019	$7.50*** \pm 0.10$ 9.120*** $\pm 0.14$ 22.205*** $\pm 0.26$ [15.407*** $\pm 0.019$ ] 2.930*** $\pm 0.09$ ] 2.027*** $\pm 0.019$	2 027*** +0 11
nlgal S.S.60	43.36*** ±0.13	6_85*** ±0.025	9.990*** ±0.27	37.725*** ±0.091	15.812*** ±0.042	6.85*** ±0.025 9.990*** ±0.27 37.725*** ±0.091 15.812*** ±0.04232.140*** ±0.034 3.340*** ±0.067	3 340*** +0 067
water S.S.100	40.21*** ±0.16	6.36*** ±0.035	11.318*** ±0.21	50.600*** ±0.089	17.560*** ±0.21	6.36*** ±0.035 11.318*** ±0.2150.600*** ±0.089 17.560*** ±0.21 37.520*** ±0.021 4.623*** +0.013	4.623*** +0.013
Water El- treated Batts	60.60*** ±0.015	8.35***±0.023 5.418**±0.18	5.418** ±0.18	12.235*** ±0.073   10.610*** ±0.042   17.220*** ±0.14	10.610***±0.042	17.220*** ±0.14	810'0∓ ***699'0
with S.S.20	55.21*** ±0.031	7.88*** ±0.012	7.135*** ±0.034	7.88*** ±0.012 7.135*** ±0.034 17.190*** ±0.087 12.180*** ±0.031 18.585*** ±0.15	12.180*** ±0.031		1.238** +0.059
c of S.S.60	50.04*** ±0.030	7.32*** ±0.015	7.814*** ±0.048	7.32*** ±0.015 7.814*** ±0.048 20.185*** ±0.12 12.314*** ±0.03220.086*** ±0.048	12.314*** ±0.032		2.049*** +0.75
powder S.S. 100	S.S.100 44.16*** ±0.017 6.75*** ±0.017	6.75***±0.017	9.615** ±0.16	34.545*** ±0.11	14.120*** ±0.14		3.101*** ±0.035
Non significant Significant	n.s. at $P > 0.05$	.05 05	Highly si	Highly significant (*	(*)	at P ≤ 0.01	
S.20 = Syntheti	9	matale (A pam	Sur (12)	Comments againstant (		at P ≤ 0,001	

Significant (\*) at P < 0.05 very nignty significant (\*) at P < 0.05 very nignty significant (\*). S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb, 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu).

S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu) S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb, 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)

Mixture of algal powder (10 gm of powder of U. lactuca, C. mediterranea and J. rubens)

Table (27): Effect of low quality and algal treated irrigation water on some chemical components of faba bean seeds at harvesting.

Water	ter	Total Carlsohv-			Faba bean	Uassanatal		
treatments	nents	Total Carbohy- drate (%)	Total protein (%)	Cd+2	Pb <sup>+2</sup>	Heavy metal	Zn+2	Cu <sup>+2</sup>
Nile fresh water (control)	h water trol)	65.36 ±0.026	20.70 ±0.006	0.689 ±0.080	0.462 ±0.080	2.126 ±0.068	13.940 ±0.11	1.050 ±0.007
FI-Batts o	drninage ter	49.02*** ±0.044	16.15*** ±0.012	6.260*** ±0.053	18.865*** ±0.12	12.830*** ±0.074	El-Batts drninage 49.02*** ±0.044 16.15*** ±0.012 6.260*** ±0.053 18.865*** ±0.12 12.830*** ±0.074 17.995*** ±0.073 1.163*** ±0.004	1.163*** ±0.
Non-	S.S.20	42.48*** +0.040	14.70*** ±0.015	8.894*** ±0.11	23.880*** ±0.039	42.48*** ±0.0±0 14.70*** ±0.015 8.894*** ±0.11 23.880*** ±0.039 14.6±0*** ±0.27 20.395*** ±0	20.395*** ±0.034	0.034 1.750*** ±0.019
treated	S.S.60	38.56*** ±0.035	13.87*** ±0.046	9.132*** ±0.071	34.915***±0.13	38.56*** ±0.035 13.87*** ±0.046 9.132*** ±0.071 34.915*** ±0.13 14.710*** ±0.09125.380*** ±0	25.380*** ±0.042	0.0422.375*** ±0.042
water	S.S.100	35.29*** ±0.012	12.00*** ±0.017	10.460*** ±0.044	46.140*** ±0.50	35.29*** ±0.012 12.00*** ±0.01710.460*** ±0.01446.140*** ±0.50 16.980*** ±0.06627.586*** ±0	27.586*** ±0.031	0.0313.360*** ±0.003
Water treated	El- Batts	51.63*** ±0.025	16.97*** ±0.025	5.186*** ±0.065	14.340*** ±0.13	51.63*** ±0.025 16.97*** ±0.025 5.186*** ±0.065 14.340*** ±0.13 10.140*** ±0.11 14.960*** ±0	14.960*** ±0.083	0.083 0.830*** ±0.024
with	S.S.20	46.40*** ±0.036	15.52*** ±0.017	6.994*** ±0.076	16.650*** ±0.11	46.40*** ±0.036 15.52*** ±0.017 6.994*** ±0.076 16.650*** ±0.11 11.680*** ±0.077 16.850*** ±0	16.850*** ±0.053	0.053 1.126*** ±0.046
of algal	S.S.60	43.14*** ±0.064	14.70*** ±0.015	7.740*** ±0.073	26.180*** ±0.15	11.860*** ±0.096	43.14*** ±0.064 14.70*** ±0.015 7.740*** ±0.073 26.180*** ±0.15 11.860*** ±0.096 19.230*** ±0.13 2.148* ±0.052	2.148* ±0.0:
powder	S.S.100	39.22*** ±0.031	12.62*** ±0.021	9.070***±0.003	31.816*** ±0.12	powder S.S.100 39.22*** ±0.031 12.62*** ±0.021 9.070*** ±0.003 31.816*** ±0.12 13.210*** ±0.026 20.105*** ±0.000 31.816*** ±0.026 20.105*** ±0.026 20.105*** ±0.026 20.105*** ±0.026 20.105*** ±0.026 20.105*** ±0.026 20.105***		0.093 2.750*** ±0.01
Non significant	icant	n.s. at P > 0.05	1.05	Highly significant	gnificant (**)	*	at P ≤ 0.01	

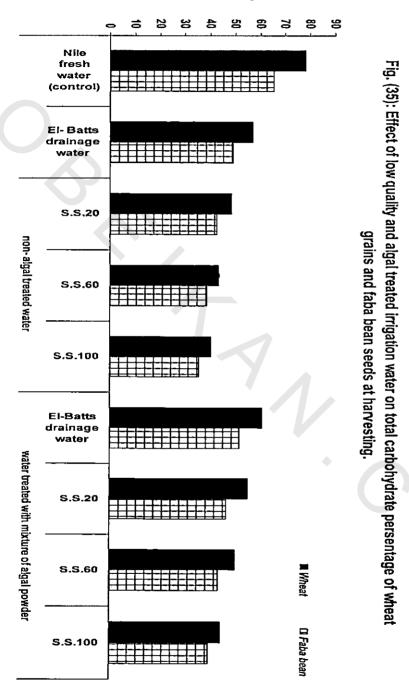
S.S.100 = Synthetic solution of heavy metals (20 ppm of Cd, 20 ppm of Pb, 20 ppm of Ni, 20 ppm of Zn and 20 ppm of Cu)

Mixture of algal powder (10 gm of powder of U. lactuca, C. mediterranca and J. rubens)

S.S.60 = Synthetic solution of heavy metals (12 ppm of Cd, 12 ppm of Pb. 12 ppm of Ni, 12 ppm of Zn and 12 ppm of Cu)

Significant (\*) at  $P \le 0.05$  Very highly significant (\*\*\*) at  $P \le 0.001$  S.S.20 = Synthetic solution of heavy metals (4 ppm of Cd, 4 ppm of Pb. 4 ppm of Ni, 4 ppm of Zn and 4 ppm of Cu)

#### Total carbohydrate content%



Results

#### Total protein content%

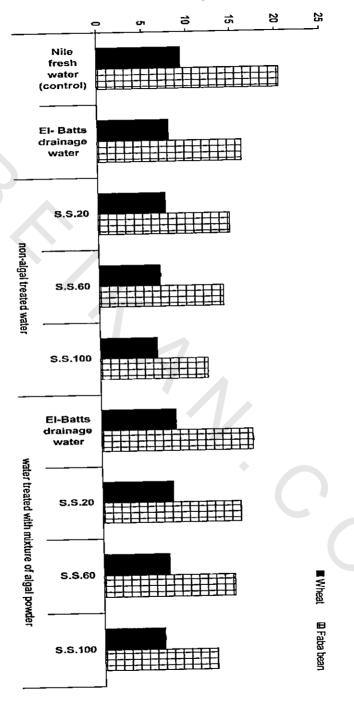


Fig. (36): Effect of low quality and algal treated irrigation water on total protein percentage of wheat grains and faba bean seeds at harvesting.

