Effect of Humic Acid, Treated Sewage Effluent and Radiation on Canola Growth and Oil Production

Abstract

A greenhouse experiment was carried out to evaluate the role of Humic acid (H) applied at different rates i.e. 0, 5, 10, 15, and 20 mg kg^{-1} in improving canola (*Brassica napus* L.) growth when irrigated with treated sewage effluent. Before planting, Canola seeds were exposed to different doses of gamma rays (0, 100, 200, 300, 400 and 500 Gy). Response of canola plant to humic acid rates and irradiation doses have the following rank, 15 > 20 > 10 > 5 > 0 mg.kg^{-1} and 300 > 200 > 100 > 400 > 0 > 500 Gy, respectively. The positive effect of irradiation was indicated by R_2 (200 Gy) and R_3 (300 Gy) treatment enhancement of both seeds and oil canola growth and micro-nutrients (Fe, Zn, Mn and Cu) uptake.

Key words: Canola, Humic acid, Gamma ray, Treated sewage effluent.

Introduction

Rapeseed as a raw material for oil production is cultivated in area of 32 million hectare (**FAO, 2010)**. It provides about 16% of world vegetable oil and occupies the third position as the most important oilseed crop worldwide **(Fediol, 2014)**. Rapeseed production represented 10 - 15 percent of the world oil crop production between years 2000 and 2009. During the last decade, European rapeseed production increased sharply from 12 million tons in 2000 to 20 million tons in 2010 **(FAOSTAT, 2014),** mainly due to higher demands for biofuel. The small, round rapeseed seed contains 38 to 45 percent oil. In addition to high oil content, rapeseed seeds contain approximately 17 - 26% protein (**Uppstrom, 1995**). Mainly, the importance of rapeseed is considered from the food sector viewpoint **(Hidalgo and Zamora, 2006)**.

Water is becoming an increasingly scarce resource in arid and semi-arid countries and planners are forced to consider any source of water that might be used economically and effectively to meet increasing demands for water **(Ahmadifard and Kalbasi 2014).** Therefore, low quality water resources including wastewater are considered a solution to minimize dependence on agricultural fresh water requirement **(Galavi *et al.*, 2010)**.

Precautions should be taken into consideration when planning to reuse the contaminated or wastewater resources. In this regard many researches have dealt with identification of contamination and low quality of such water resources **(Chong et al., 2010; Zeng et al., 2011, 2013a and b, Deng et al., 2013; Chen et al., 2013, Lesmana et al., 2009, Tang et al., 2012; Rahman and Islam, 2009).**

Humic substances compose up to 80% of soil organic matter **(Brady & Weil, 2008)**. Carbon, oxygen, hydrogen, nitrogen, and sulfur are the most common elements in humic substances **(Pettit, 2004).** Humic acids are water soluble in alkaline conditions **(Pettit, 2004).** High portions of the humic acids structure are carbon rings and carbon chains **(Pettit, 2004).** Humic acids are typically composed of 54 to 58% carbon, 33 to 38 % oxygen, 36 % hydrogen, 0.8 to 4.3% nitrogen and 0.1 to 1.5% sulfur **(Steelink, 2002).**

The aim of the present study is to evaluate the effect of seed irradiation and humic acid on stimulation of canola growth and oil production.

Materials and Methods

Pot experiment was carried out during 2017 / 2018, in randomized complete block design with 5 replicates under greenhouse conditions. The latitude and longitude of the experiment site are 30o 24` N, 31o 35` E, respectively, while the altitude is 20 m above the sea level One part of the experiment was irrigated with fresh water and another one was irrigated with treated sewage effluent. Experimental factors were: Factor 1: Canola (*Brassica napus* L.) seeds Irradiation with gamma rays at 0, 100, 200, 300, 400 and 500 Gy; symbol as R 0, R 1, R 2, R 3, R 4, and R 5, respectively. Gamma irradiation was conducted using 60Co gamma source at a dose rate of 100, 200, 300, 400 and 500 Gy; for seed of canola (Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Egypt.

). Factor 2: Humic acid addition (H); 0, 5, 10, 15, and 20 mg kg^{-1} symbol as H_0, H_1, H2, H_3 and H_4, respectively. Humic as contented 90% Humic and10% potassium. Poly-vinyl chloride (PVC) pots with dimensions, 30 cm width and 30 cm depth, filled with 10 kg soil per pot were used. Seeds were sown 10 per pot thinned to 5 after 10 days from seeding.

Extraction of oil for seeds by Soxhlet extractor was carried out as described by **Akbar et al. (2009).** Some of chemical and physical properties of the experimental soil were determined according to the standard methods outlined by **Hamdy (2005),** and presented in Table (1). Some of chemical properties of treated sewage effluent used for irrigation are presented in Table (2).

Table 1. Some chemical and physical properties of experimental soil

EC dS m^{-1}	pH	OC (mg kg^{-1})	(mg kg^{-1})			Coarse sand%	Clay %	Silt %	Sand %	Soil texture
			N	P	K					
1.21	7.85	4	3.21	1.58	1.65	4.0	7.0	2.5	86.5	Sand

EC= Electrical Conductivity of saturation extract ,, OC= Organic Carbon %, FC=Field Capacity

Table 2. Main properties of the sewage water

EC ds/m	pH	BOD	COD	OC gkg^{-1}	(mg L^{-1})							
					N	P	K	Fe	Zn	Mn	Cu	Pb
1.62	7.31	190	375	36	22.5	4.5	1.87	1.55	0.11	0.12	0.07	0.08

BOD= Biochemical oxygen demand, and COD= Chemical oxygen demand

Results and discussion

Seeds yield:

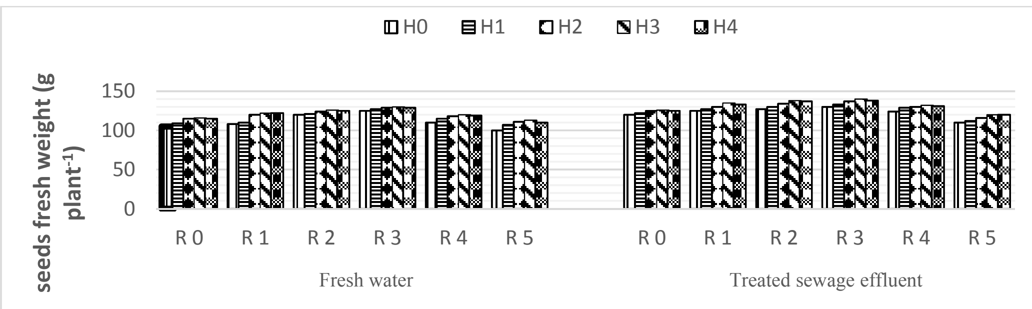
Seeds yield as affected by irrigation water, humic acid rate and different irradiation doses is presented in Fig (1). Plants irrigated with treated sewage effluent produced higher seed yield than those recorded with fresh water irrigation. This phenomenon was true under all radiation and humic acid treatments. Slight increase in fresh weight of seeds was detected with application of humic acid comparing to the untreated plants. In this regard, there was no remarkable variation between humic acid rates. Concerning the effect of radiation, data reflected the superiority of R_2 (200 Gy) and R_3 (300 Gy) over other radiation doses. On the other hand, the lowest values were recorded with 400 Gry, 0 Gry and 500 Gry, respectively. Under irrigation with treated sewage effluent, the effect of gamma irradiation of seed fresh weight could be arranged as following: 300 Gry > 200 Gry > 100 Gry > 400 Gry > 0 Gry > 500 Gry recording 136,133,130,129,124 and 116 g $plant^{-1}$, respectively. In case of fresh water, values could be rank as following: 300 Gry > 200 Gry ≥ 100 Gry > 400 Gry > 0 Gry > 500 Gry representing 128,123,116,116,112 and and 108 g $plant^{-1}$, respectively.

Seeds fresh weight yield as affected by humic acid rates indicated that the best treatments could be ranked as following: 15 mg kg^{-1} > 10 mg kg^{-1} > 20 mg kg^{-1} > 5 mg kg-1 > 0 mg kg^{-1} humic acid, recording 132,129,124,126and 123 g $plant^{-1}$, respectively when plants irrigated with treated sewage effluent.

In case of fresh water irrigation, effect of humic rates have the following order: 15 mg kg^{-1} > 10 mg kg^{-1} ≥ 20 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1}, recording 121,120,120,115and 111 g $plant^{-1}$, respectively.

In this respect, many research have indicated the positive effect of reuse of sewage effluent on canola biomass and seed yield which attributed to the nutrients contained in such water resources (**Chen and Cutright, 2001; Peralta-Videa et al, 2002).**

Nasiri et al. (2017) reported that applications of humic acids increased seed yield and seed oil of oil crops. The efficiency of humic substances depends on their origin and the processing methods **(Senesi, 2007).** The number of seeds per plant and seed weight per plant were reported by **Kafeel et al. (2011)** to increase with irrigation using sewage water .



Notes: **R_0, R_1, R_2, R_3, R_4 and R_5 are 0 , 100 , 200 , 300, 400 and 500 Gy respectively. H_0, H_1, H_2, H_3 and H_4 are 0, 5, 10, 15 and 20 mg humic acid kg^{-1} respectively**

Fig1. Effect of humic acid and gamma ray irradiation on seed fresh weight (g $plant^{-1}$) irrigated with sewage effluent and fresh water.

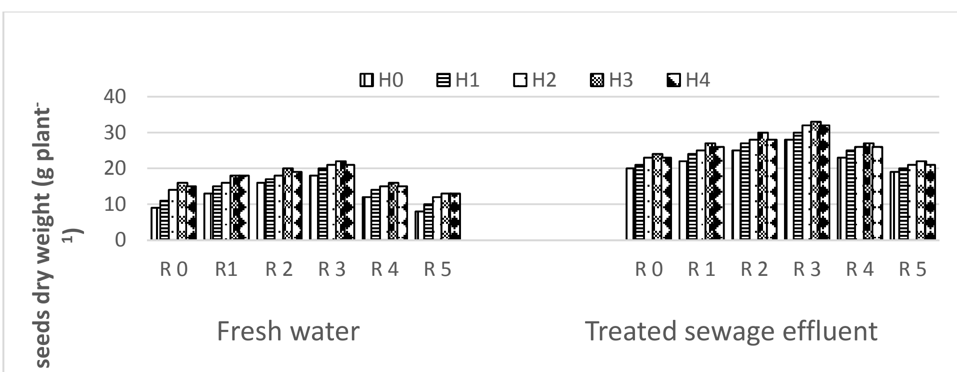
Canola dry weight (g $plant^{-1}$) as affected by application of humic acid rate and canola seeds were exposed to different doses of gamma rays under two water tube (Fresh water irrigation and treated Irrigation with sewage effluent)was presented in Fig (2). Generally, under treated Irrigation with sewage effluent, it was clear that application of doses of gamma rays treatment either solely or in combination with humic acid enhanced Canola dry weight, in most treatments, comparing to the non-addition humic acid. dry weight yield of canola under doses of gamma rays treatment with two water tube (Fresh water irrigation and treated Irrigation with sewage effluent), the best treatments could be ranked as following: Treated Irrigation with sewage effluent:- 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry recorded, 31,27,25.4,24.8,22.2 and and 20.6 g plant^{-1} respectively.

Fresh water irrigation: - 300 Gry > 200 Gry > 400 Gry > 100 Gry > 0 Gry > 500 Gry recorded, 20.4,18, 16,14.4,12.8 and and 11.2 g plant^{-1} respectively.

Dry weight yield of canola under humic acid rate with two water tube (Fresh water irrigation and treated Irrigation with sewage effluent), the best treatments could be ranked as following: Treated Irrigation with sewage effluent: - 15 mg kg^{-1} > 20 mg kg^{-1} > 10 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1} humic acid, recorded 27.1, 26.0, 25.8, 24.5and 22.8 g plant^{-1} respectively.

Fresh water irrigation: - 15 mg kg^{-1} > 20 mg kg^{-1} >10 mg kg^{-1} > 5 mg kg^{-1} > 0 mg kg^{-1} humic acid, recorded 17.3, 16.8, 16.2, 14.5and 12.6 g plant^{-1} respectively.

Approximately, similar trends were noticed when seeds dry weight as affected by experimental treatments was considered **(Table 4).** In accordance, **Schiavon et al., (2010); Berbara and Garcia, (2014)** reviewed some different mechanisms responsible for enhancement of plant growth as affected by humic acid addition**.** In harmony**, Shakeel Ahmad et al., (2016)** reported that growth and yield of canola were increased by application of humic acid.

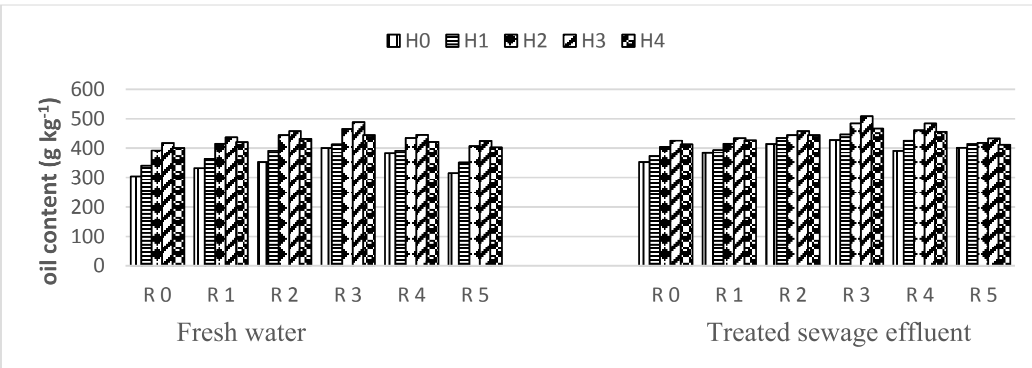


<u>Notes:</u> R_0 , R_1 , R_2 , R_3 , R_4 and R_5 are 0 , 100 , 200 , 300, 400 and 500 Gy respectively. H_0 , H_1 , H_2 , H_3 and H_4 are 0, 5, 10, 15 and 20 mg humic acid kg-^{1} respectively

Fig2. Effect of humic acid and gamma rays on canola seeds dry weight (g plant^{-1}) irrigated with sewage effluent and fresh water.

Oil yield

As show in Fig (3), humic acid rates as well as irradiation doses have a positive effect on production of oil. Values of oil content were varied among humic acid rates. The best values were recorded with H_3 (15 mg kg^{-1}), H_2 (10) and H_4 (20), respectively comparing to H_0 and H_1 (5) treatments. Concerning the irradiation treatments, the best values of oil content were recorded with R_3 (300 Gy), R_2(200) and R_4 (400), respectively. This holds true under fresh water and treated sewage effluent but the values were to some extent, higher in case of treated sewage effluent than those recorded with fresh water irrigation. These findings are consistent with those reported by (**Oregani et al.2014**) who indicated municipal wastewater irrigation affected significantly on the biomass and yield of canola.



See footnotes of Fig 1 for treatment designations.
Fig 3. Effect of humic acid and gamma rays on oil content (g kg^{-1}) in canola seeds under irrigation with treated sewage effluent and fresh water irrigation

Metal content

Micronutrients content in canola plants as affected by irrigation sources, humic acid and gamma irradiation are presented in Tables (3, 4, 5 and 6), for Fe, Zn, Mn and Cu, respectively. Iron content (Table 3), was enhanced by irradiation doses and values recorded with R_1, R_2 and R_4 (mean value) were nearly closed to each other while the best one was recorded with R 3, under fresh water irrigation. Similar trend, but to somewhat higher extent, was with treated sewage effluent irrigation. Application of humic acid induced significant increases in Fe content as compared to the untreated plants. Application at rate of 15 mg kg^{-1} resulted in the highest Fe value either under fresh water or treated sewage effluent treatments. In general, both irradiation and humic addition reflected positive effects on plant growth and consequently it's content of iron. Many researchers had explained the mechanisms related to enhancement of metal content.

Aiken et al., (1985) reported that humic acids contain acidic functional groups such as phenolic, hydroxyl and carboxyl groups which can bind to many metal ions, and they exist in soil and aquatic environments**.** In presence of metal ions, the resulting humic acids could complex with them and provide plants with many microelements **(Lobartini et al., 1998).** Humic acids serve as carriers of Fe (II) and Fe (II)-HA **(Rose and Wait, 2003and Willy et al., 2008).**

Table 3. Effect of humic acid and gamma rays on **Fe** content in canola shoot (mg kg^{-1}) under treated sewage effluent and fresh water irrigation.

	Humic acid "mg kg^{-1}" (H)					
Irradiation (R)	**H_0**	**H_1**	**H_2**	**H_3**	**H_4**	**Mean**
			Fresh water			
R_0	556	594	618	650	640	611
R_1	600	627	649	675	670	644
R_2	619	645	661	689	680	658
R_3	645	672	688	699	685	677
R_4	620	650	670	680	675	659
R_5	570	622	643	654	650	627
Mean	601	635	654	674	666	
LSD	**R= 4.530 H = 4.135 R x H = 10.13**					
			Treated sewage effluent			
R_0	726	750	775	790	785	765
R_1	755	775	790	841	810	794
R_2	796	824	842	885	825	834
R_3	850	877	895	950	844	883
R_4	820	855	860	880	835	850
R_5	770	836	850	862	828	829
Mean	786	819	835	868	821	
LSD	**R= 9.78 H = 8.92 R x H = 21.86**					

See footnotes of Fig 1 for treatment designations.

Zn content (Table 4), followed the same trend recorded with Fe but in very low values. Also, it was, to somewhat extent, high in case of sewage effluent irrigation. Both irradiation and humic addition had enhanced Zn uptake by treated plants. On line, humic acids found to be as enhancer for Cu, Cd and Zn uptake since they are organic and inorganic chelates **(Lesage et al. 2005, Meers et al. 2005, Finžgar et al. 2006).**

Table 4. Effect of humic acid and gamma rays on Zn content of canola (mg kg$^{-1)}$ under sewage effluent and fresh water irrigation.

Humic acid "mg kg^{-1}" (H)						
Irradiation (I)	**H_0**	**H_1**	**H_2**	**H_3**	**H_4**	**Mean**
Fresh water						
R_0	3.5	4.2	5	5.6	5.3	4.7
R_1	3.7	4.8	5.3	6.1	5.9	5.1
R_2	4.1	5.3	6.2	7	6.5	5.8
R_3	4.4	5.9	6.5	7.4	7.1	6.2
R_4	4.2	4.6	5.8	6.9	6.5	5.6
R_5	3.2	4.1	5.2	5.8	5.4	4.7
Mean	3.8	4.8	5.6	6..4	6.1	
LSD	**R= 0.86 H = 0.79 R x H = 0.194**					
Treated sewage effluent						
R_0	5.5	6.8	7.5	8.2	8	7.2
R_1	6.7	7.5	8.3	8.7	8.5	7.9
R_2	8.4	8.8	9.4	9.9	9.4	9.1
R_3	9.9	10.1	12.1	13.4	9.7	11.0
R_4	8.2	8.4	9.3	11.5	8.8	9.2
R_5	6.1	7.1	7.4	9.7	8.5	7.7
Mean	7..4	8.1	9	10.2	8.8	
LSD	**R= 0.12 H = 0.11 R x H = 0.27**					

See footnotes of Fig 1 for treatment designations.

Table 5. Effect of humic acid and gamma rays on **Mn** content of canola (mg kg^{-1}) under treated sewage effluent and fresh water irrigation.

Humic acid "g kg^{-1}" (H)						
Irradiation (R)	**H_0**	**H_1**	**H_2**	**H_3**	**H_4**	**Mean**
Fresh water irrigation						
R_0	12	16	19	22	21	18
R_1	15	20	22	25	23	21
R_2	18	23	27	30	28	25
R_3	21	25	29	35	33	28
R_4	17	22	24	26	25	22

R_5	13	15	18	20	20	17
Mean	16	20	23	26	25	
LSD	**R= 0.632 HA = 0,577 R x HA = 1.415**					
Treated sewage effluent						
R_0	80	85	89	97	93	88
R_1	87	98	125	129	120	111
R_2	110	124	136	140	127	127
R_3	117	140	152	158	138	141
R_4	96	122	144	145	141	129
R_5	88	114	131	134	130	119
Mean	96	113	129	1133	124	
LSD	**R= 1.47 H = 1.34 R x H = 3.28**					

See footnotes of Fig 1 for treatment designations.

Table 6. Effect of humic acid and gamma rays on **Cu** content of canola (mg kg^{-1}) under treated sewage effluent and fresh water irrigation.

Humic acid "g kg^{-1}" (H)						
Irradiation (R)	**H_0**	**H_1**	**H_2**	**H_3**	**H_4**	**Mean**
Fresh water						
R_0	3	6	9	10	8	7.2
R_1	5	7	8	11	9	8
R_2	8	9	12	13	10	10.4
R_3	9	10	13	15	12	11.8
R_4	6	8	9	10	8	8.2
R_5	4	7	8	9	8	7.2
Mean	5.8	7.8	9.8	11	9	
LSD	**R= 0.233 H = 0.213 R x H = 0.522**					
Treated sewage effluent						
R_0	8	10	13	15	14	12
R_1	9	13	16	17	15	14
R_2	12	15	18	20	17	16
R_3	15	18	21	23	20	19
R_4	12	13	15	17	16	14
R_5	9	11	12	13	11	11
Mean	10.8	13.3	15.8	17.5	15.5	
LSD	**R= 0.18 H = 0.16 R x H = 0.40**					

See footnotes of Fig 1 for treatment designations.

Conclusion

Irradiation and humic acid were effective in enhancing canola growth, seed yield, oil content and micronutrients i.e Fe, Zn, Mn and Cu. Applications of moderate humic acid rate and irradiation doses resulted in remarkable values of plant growth, seed and seed oil yields. Recent results proved that humic acid could be considered beneficial either applied individually or in combination with gamma irradiation for plant and oil production.

Therefore, we recommend further field research for good explanation and more discussion of the responsible mechanisms of humic acid and radiation impacts on plant.

References

Ahmadifard S. and Kalbasi M 2014. Effect of irrigation with municipal wastewater effluent on selected chemical properties of three calcareous soils and heavy metals concentration in corn. IJB, 5 (3) : 133- 138.

Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. 1985. In: Humic substances in soil, sediment and water-geochemistry, isolation and characterization. John Wiley and Sons, New York.

Akbar E, Yaakob Z, Kamarudin S, Ismail M 2009. Characteristics and Composition of *Jatropha curcas* oil seed from Malaysia and its potential as Biodiesel Feedstock. Eur. J. scientific Res. 29: 396-403.

Behzad Sani 2014. Foliar Application of Humic Acid on Plant Height in Canola. 4th International Conference on Agriculture and Animal Science (CAAS 2013)

Berbara, R. L. L. and A. C. Garcia. 2014. Humic substances and plant defense metabolism. In: Physiological mechanisms and adaptation strategies in plants under changing environment. (Eds.): Ahmad, P. and M.R. Wani. Springer Science + Business Media, New York, pp. 297-319.

Brady, N.C. and R. R. Weil. 2008. The Nature and Properties of Soils. Pearson Prentice Hall, Upper Saddle River, NJ.

Chen, G.Q.; S. Guan; G. M. Zeng; X. D. Li; A. W. Chen and C. Shang. 2013. Cadmium removal and 2,4-dichlorophenol degradation by immobilized Phanerochaete chrysosporium loaded with nitrogen-doped TiO nanoparticles. Appl Microbiol Biotechnol. 97:3149–57.

Chen, H. and T. Cutright. 2001. EDTA and HEDTA effects on Cd, Cr and Ni uptake by Heli anthus annus Chemosphere, 45: 21–28.

Deng, J. H.; X. R. Zhang; G. M. Zeng; J. L. Gong; Q. Y. Niu; J. Liang. **2013**. Simultaneous removal of Cd(II) and ionic dyes from aqueous solution using magnetic graphene oxide nanocomposite as an adsorbent. Chem Eng J ;226:189–200.

FAO. 2010. Data statistics. http://faostat.fao.org.

FAOSTAT, 2014. Food and agriculture organization of the United Nations. Statistics Division 2014.Accessed August 14, 2014. http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E

Fediol. About our products – general overview: World Production Data. Copyright **2014.** Accessed August 11, **2014.** http://www.fediol.eu/web/world%20production %20data/1011306087 /list1187970075/f1.html

Galavi M, Jalali A, Ramroodi M, Mousavi SR, Galavi H. 2010. Effects of treated Municipal wastewater on soil chemical properties and heavy metal uptake by Sorghum (*Sorghum Bicolor L.*). Journal of Agricultural Science **2(3),** 235-241.

Hamdy, A. 2005. Soil, Water and Plant analysis manual for arid and semiArid countries of the mediterranean.210 p. IAM-Bari, Italy.

Hidalgo, F. J.; Zamora, R. 2006. Peptides and proteins in edible oils: stability, allergenicity, and new processing trends. Trends in Food Science & Technology, 17: 56 - 63

Kafeel, A.; A. M. Azam; Z. I. Khan; M. Ashraf; F. Al-Qurainy; A. Fardous; S. G. A. R. Bayat and E. E. Valeem. 2011. Lead, Cadmium and Chromium Content of Canola Irrigated with Sewage Water. *Pak. J. Bot*., 43(2): 1403-1410.

Lesage, E.; Meers, E.; Vervaekle, P.; Lamsal, S.; Hopgood, M.; Tack, F.M.G.; Verloo, M.G., 2005. Enhanced phytoextraction: II. Effect of EDTA and citric acid on heavy metal uptake by Heliantus annus from a calcareous soil. Int. J. Phytoremed. 7: 143–152

Lesmana, S. O; N. Febriana; F. E. Soetaredjo; J. Sunarso and S. Ismadji. 2009. Studies on potential applica- tions of biomass for the separation of heavy metals from water and wastewater. Biochem Eng J., 44(1):19–41.

Lobartini, J.C., Tan, K.H., Pape, C. 1998. Dissolution of aluminum and iron phosphate by humic acids. *Comm. Soil Sci. Plant Anal.* **29**, 535-544.

Meers, E.; Unamuno, V.; Vandegehuchte, M.; Vanbroekhoven, K.; Geebelen, W.; Samson, R.; Vangronsveld, J.; Diels, L.; Ruttens, A.; Laing, G.D.; Tack, F., 2005. Soil-solution speciation of Cd, as affected by soil characteristics in unpolluted and polluted soils. Environ. Toxicol. Chem., 24: 499-509**.**

Nasiri, A.; M. Samdaliri; A. Shirani-Rad; A. M. Mirkale and H. Jabbari. 2017. Influence of Humic Acid, Plant Density on Yield and Fatty acid Composition of some Rapeseeds Cultivars during Two years Academia Journal of Agricultural Research 5(5): 103-109.

Oregani K E, Ali Gholami, Zahra Kazemi Esfeh, Hossein Lari Yazdi 2014. The effect of municipal wastewater irrigation on yield and biomass of canola (Brassica napus L.) and soil chemical properties. IJB. 4 (8) : 144- 151.

Peralta-Videa JR1, Gardea-Torresdey JL, Gomez E, Tiemann KJ, Parsons JG, Carrillo G. 2002. Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environ Pollut.119(3):291-301.

Pettit, R.E. 2004. Organic Matter, Humus, Humate, Humic Acid, Fulvic Acid and Humin: Their Importance in Soil Fertility and Plant Health [Online]. Available at http://fertiorganicos.com/english/images/lib/ *CTI Research*. 1-15.

Rahman, M.S. and M. R. Islam. 2009. Effects of pH on isotherms modeling for Cu(II) ions adsorption using maple wood sawdust. Chem Eng J;149(1–3):273–80.

Schiavon, M.; D. Pizzeghello; A. Muscolo; S. Vaccoro; O. Francioso and S. Nardi. 2010. High molecular size humic substances enhance phylpropanoid metabolism in maize (*Zea mays* L.). *J. Chem. Ecol.,* 36(6): 662-669.

Senesi, N.; C. Plaza; G. Brunetti and A. Polo. 2007. A Comparative Survey of Recent Results on Humic- like Fractions in Organic Amendments and Effects on Native Soil Humic Substances. Soil Biology & Biochemistry. 39 (6): 1244-1262.

Shakeel A.; I. Daur; S. G. Al-Solaimani; S. M. A. Ahmed; B. M. H. Madkour and M. Yasir. 2016. Effect of rhizobacteria Inoculation and Humic Acid Application on Canola (*BRASSICA NAPUS* L.) Crop. Pak. J. Bot., 48(5): 2109-2120.

Steelink, C. 2002. Investigating Humic Acids in Soils. Analytical Chemistry. 74 (11): 326A-333A.

Tang, W. W.; G. M. Zeng; J. L. Gong; Y. Liu; X. Y. Wang and Y. Y. Liu. 2012. Simultaneous adsorption of atrazine and Cu(II) from wastewater by magnetic multi-walled carbon nanotube. Chem Eng J;211–212:470–8.

Willy, J.D., Kieber, R.J. Seaton, P. J., Miller, C. 2008. Rainwater as a source of Fe(II)-stabilizing ligands to seawater. Limnol. Oceanogr. 53, 1678-1684.

Zeng, G. M.; M. Chen and Z. T. Zeng. 2013a. Shale gas: surface water also at risk. Nature ;499(7457):154.

Zeng, G. M.; M. Chen and Z. T. Zeng. 2013b. Risks of neonicotinoid pesticides. Science;340 (6139): 1403-1409.

Finžgar, N., A. Žumer and D. Leštan, 2006. Heap leaching of Cu contaminated soil with [S,S]-EDDS in a closed process loop. *J. Hazardous Materials B*, 135: 418−422

Zeng, G.M.; X. Li; J. H. Huang; C. Zhang; C. F. Zhou and J. Niu. 2011. Micellar-enhanced ultrafiltration of cadmium and methylene blue in synthetic wastewater using SDS. J Hazard Mater 185(2–3):1304–10.