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Investigation of toxic algae populations (cyanobacteria and diatoms) in some selected drinking water plants in Baghdad

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Abstract

Toxic and non-toxic algae diagnosed and identified which found in final tanks of drinking water of three water supplies at Baghdad represented by Al-Wathba and Al-Rasheed plants supplied with water from Tigris river. It became obvious from the recognized results the phylums of green algae, blue-green algae and two orders of bacillus algae (diatoms) centrales and pennales represented in all plants with dominance of bacillus algae phylum in water tanks more than another algae species, also a toxic algae species identified in final tank of drinking water with increasing represent of *Chroococcus minor*, *Microcystes aergenasa* and *Oscillatoria limnetica*, Blue - green filaments that belong to blue-green algae phylum which produce toxins found in study station along study period. while controlled adhered algae more than plankton and other algae such as *Lyngbya connectens, M. aergenasa, O. tenuis, O. geitleriana, O. formosa, O. subbrevis, Nostoc linka and Phormidium tenue. Anabena* spp. are found in all study station along study period with biomass with of vital number range 82- 888 cell/l, In addition some species of diatoms may can produce domic acid found in range between 15-423 cell/l which represented by *Nitzchia liongissima, N. acicularis, N. vitrea, N. rostellata, N. dissipata, N. minutula, N. linearis, N. fruticosa, N. umbonata, N. palea, N. clausii* and *N. obtuse* are found in all study stations.

Key words: Toxic algae, Cyanobacteria, Diatoms, Drinking water, Baghdad city.

Introduction

Water considered as a good solvent for a lot of materials also some materials which no dissolve in water still as suspended colloids locks like solutions. One of water resources is rain water which polluted by new additives because of urban effects such as NH_4, NO_3 and PO_4 reasons of acidic rain water that represented as global problem (Dillon and Chanton, 2005). Also industrial, agricultural and wastewater, which consider as nutrients media of some microalgae for such as blue- green algae (cyanobacteria), looks like another living being creature produce metabolites compounds because of creature vital activity which produce these compounds from building and inhibition inside living cells, some of these compounds is cold algal toxins (costa, 2006). There for cyanobacteria secretions going too far of negative effect for water quality from bad smell and taste to edge of toxicity and gravity. These toxic materials may be associated with death and decomposing of toxic algae, this means that the water treatment systems perhaps cause toxin releasing after using devices of algae removing, if these compounds released in water, it's not easy to remove them, also cannot controlling released algal toxins in water by processing of treatment and chlorine using, also treatment by charcoal is not successes to remove algal toxins (Mazur, 2001). A few of new studies refers to using aluminum sulfate Al_2 (SO_4) because of Al2 (SO4) to remove alga from water by precipitation and coagulation at water treatment plant with insurance of non-releasing algal toxins in water with detecting algae in water by light microscope the detecting of toxic algal compounds and limitation of these toxic compounds concentration in drinking water considered as non-routine and important analysis where world health organization (WHO) fixed limited concentration of algal toxins which no become over than 0.5-1.0 µg / decimeter ^{3} of drinking water (WHO, 989). Many methods were used to detect and imitate concentrations of these toxins lick thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and ELIZA (Wright, 1995).

One of these toxins is domoic acid (DA). Which recognized at first time in 1987 in Prince Edward Island, Canada which cause amnesic shellfish poisoning (ASP), was produced by the diatom species *Nitzschia pungens f. multiseries* (Hallegraeff, 1995). In addition to that there are several toxic species of diatoms have been recorded such as *Amphora coffeaeformis, Nitzschia navis – varingica, Pseudo-nitzschia australis, P. calliantha, P. cuspidata, P. delicatissima, P. fraudulenta, P. galaxiae, P. multiseries, P. multistriata, P. pungens, P. seriata* and *P. turgidula.* The bloom of toxic species of *Pseudo-nitzschia* probably become a recurrent phenomenon so it is important to determine if there is a seasonal variation or spatial predictability (Jessim, 2009). In 1958, domoic acid was originally isolated from the red alga called "doumoi" or "hanayanagi" (*Chondria armata*) in Japan (Takemoto and Diago, 1958). *Pseud-nitzschia* and *Nitzschia* produce domoic acid including freshwater species (Lundholm and Moestrup, 2000). DA belong to the kainoid class of compounds (Wright and Quilliam, 1995). DA is a potent neurotoxin, it is a class of excitatory neurotransmitters that bind to specific receptor proteins in neuronal cells cause continual depolarization of the neuronal cell until cell rupture occurs (Wright, 1995). In another hand there were more toxic materials can be found in many parts of world (Jessim, 2009). Water body's and coasts (Jessim, 2009). In addition microcystins are one group of toxins which It is more productive by algae and more widespread internationally (Carmichael *et al.*, 1997). Microcystins are a cyclic peptides containing seven amino acids, the more abundant a toxic blue-green algae problem more than 80 isomers (Butler *et al.*, 2009). The aim of present study Investigation and detection of local toxic algae in three water plants located at Baghdad by microscopic diagnosis of these toxic algae.

Materials and Methods

Samples of water are collected form three water plants located at Baghdad city, east of Tigris river, Al-Wathba and Al-Rashed plants (Figure 1).

Figure (1): Locations of studied area

from final tank of drinking water for chemical analysis and adhered algae from first tank of participation for each plant separately, after fixing of algae samples by Lugl's solution as conservation material to keep algae population as a genuine situation like in sampling station, stored at 4 Cº and transported to laboratories of environment and water directorate for analysis. Samples precipitated depending on (Furet, 1982).

Diagnosis and cells numbering: Phytoplankton total cells counted by using sedimentation method (Ohtani, 1992).One letter of water took from each station and put in graduated cylinder volume 1 litter and kept after adding droops of Lugol's solution then the algae cells counted by using hemocytometer results unit counted as cell/l.

Chemical analysis:

- a- Water temperature; measured locally by graduated mercuric thermometer from 0-100 Cº.
- b- pH; measured by pH meter after calibration pH meter with buffer solutions 4, 7 and 9.
- c- Electrical conductivity; measured by conductivity meter, results reached with units µS/cm.
- d- Nitrate NO_3; limitation of NO3 depend on method of (APHA, 1998). took 50 ml of water sample after filtration for removing suspended materials, then added 1ml of HCl (1 normal), mixed well then concentration measured by spectro-photometer on wavelength 220 nm. Results reached by unit of mg/l.
- e- Nitrite NO_2; method of (APHA, 1998) followed to determine NO_2, took 10 ml of filtered samples, diluted by 50 ml of distilled water, 1 ml of sulphanil amid with shaking gently, after two minutes added N-1- dihydrochloride naphthyl Ethelendi amin. Sample left 5 minutes then measured absorption of produced ping color by spectrophotometer on wavelength 543 nm. Results reached by unit of mg/l.
- f- Phosphate PO_4; for PO_4 limitation method of (APHA, 1998) followed, 8 ml of combined reagent made from (ammonium para molbedate $[(NH_4)_6 Mo_7O_{24}.4H_2O]$, potassium antimony tartrate K_5bo. C_4 H_4 O_6 and ascorbic acid $C_6H_8O_6$) added to 50 ml of filtrated water sample to change color of mixture to blue. Wavelength measured by spectrophotometer on wavelength 860 nm, also results reached with unit of mg/l.

Results and Discussion

Figure (2) showed that there were 42 diagnosed of toxic species in final tank (Drinking water) distributed for all study period, highly number as a biomass from 15- 634 cell/l, 13 species belong to Bacillariophayceae with ratio 30.95 % from a list of total a count of genus *Nitzschia* for all species especially producers of domoic acid, as in studies which refers to existence of highly biomass of domoic acid diatoms producers density 15 x 10^3 cell/l, toxin concentration will be 0.8 mg/l is active to make nerve paralysis at warm temperature between 15-20 Cº arrived to 25 Cº sometimes with biomass density increased to 3 x 10^5 with major and minor nutrition availability like silicate in less than 0.62 mg/l with phosphate as adenosine tri phosphate (ATP) to editing energy for algal cells production. Domoic acid considered more active three times than the activity of neuroexcitator toxins and 100 time more than glutamic and kainic acids by toxins excretion through external membrane of *Nitzscia*sp. (Congestri, 2008). Domoic acid and its analogous are kainoids (Clayden *et al.*, 2005).

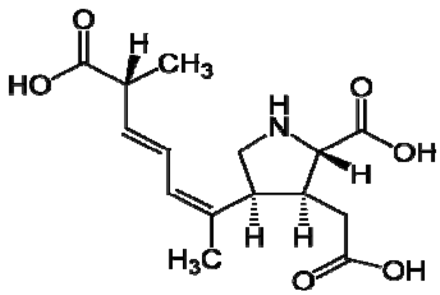

Figure (2): General structure of domoic acid

Figure (2) showed a toxic algae species which produce microcystins, microcystins are a cyclic peptides containing seven amino acids, the more abundant the toxic blue-green algae problem more than 80 isomers, it is noted in the overall shape to be fitted to every microcystins share in places X, Z, R1, R2 (Butler *et al.*, 2009).

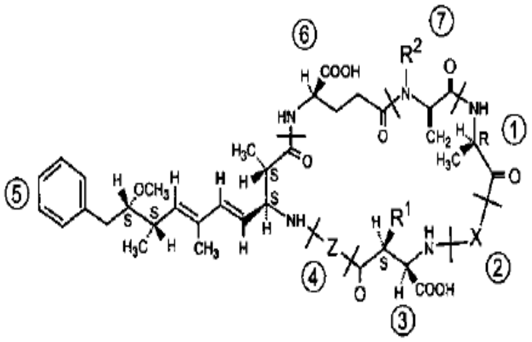

Figure (3): General structure of microcystin

Toxic algae species diagnosed in drinking water in Al-Rashed water plant such as *Mycrocystes aerogenosa* which considered as one of most dangerous of algae species, different vital number of *M. aergenosa* recorded in year seasons, in Autmn 198 cell/l, but in spring 169 cell/l and in summer 122 cell/l as adhered in high density. At same plant, *Chroococcus minor* which known as a toxic species alga presented in autumn season, recorded as vital number as 227 cell/l, and 222 cell/l in winter, also 127 cell/l in spring with number of cells 321 cell/l in summer also found adhered form, according to (Fastner, 2001), higher level of toxins when there is 1- 2 mg of microcystis which produce 100 – 400 cell / liter toxic algae at water environments, this might cause increased risk warning in the aquatic environments while the water is potable where is concentration of microcystis less than 1 mg/l according to (WHO, 1998). If 5-50 mg of microcystis cumulatively cause severe liver ulcer, also 29 diagnosed species belong to division of Cyanophyceae with ratio of 69.04% representing by species *Microcystes aerogenosa, Nostoic carneum, Nostoc linka, Aphanocapsa biformis, Lyngbya spirulinoides, Oscillatoria limnetica, O. limosa, O. pseudogeminata, O. formosa, O. subbrevis Pandorina morum, and Phormidium tenue* as in Table (1).

But there are 46 diagnosed algal species at final tank (drinking water) of east Tigris plant, distributed along study period as highly density as bio mass from 50 to 587 cell/l in addition to adhered algae as a boom of *Chrococcus turgids*, *Anabena* spp., *Oscillatoria proboscidea*, *Merismopedia eiegans*, *Nitzschia acicularis* and *Haemato coccuslacustris*. Where 16 species diagnosed belong to division of Bacillariophyceae (diatoms) in ratio of 34.78 % from total account of the genus *Nitzschia* spp. for all species which represent a hazard of producing domoic acid (Fernández, 1995). In another hand 30 species diagnosed belong to the division of blue green algae Cyanophyceae with ratio of 65.21% from followed species, *Micrcystes aerogenosa*, *Merismopedia eiegans*, *Nostoc linka*, *Aphanocapsa biformis*, *Lyngbya spirulinoides*, *L. connectens, Oscillatoria limnetica, O. limosa, O. homogenea, O. amoena, O. subbrevis, Panorina morum* and *P. Mucicola.* as in Table (2).

Table (1): Diagnosed algae and algae cells number (cells/l) in drinking water in Al-Rasheed plant, along study period 2011-2012.

Species of algae	Studied period			
	Autumn	Winter	Spring	Summer
Class: Cyanophyceae				
Aphanocapsa bioformis	193	-	-	231
Anabena spp.		*	188*	112*
Blue-green filamntes	225*	402	127	453
Chroococcus spp.	78	-	-	90*
C. minor	227	222*	170	321*
C. turgidus	52	-	-	65*
Gloeocapsa aeruginosa	-	190*	-	53
Haematococcus lacustris	-	*	-	*
Lyngybya connectens	*	193*	422*	213
L. spirulinoides	189*			324
Microcystes spp.	42*	200*	150	154*
M. aergenosa	422	198*	169*	122
Nostoclinka	-	191*	-	*
Nostoccarneum	-	-	-	132*
Oscillatoria limnetica	*	-	-	66
O. limosa	78*	-	-	435
O. pseudogeminata	406	-	-	243*
O. curviceps	*	-	-	453*
O. tenuis	-	400	422	536
O. vizagapatensis	-	-	-	243*
O. okeni	-	-	-	241*
O. Formosa	-	423	403	120
O. agardhii	-	403	-	*
O. subbrevis	-	-	-	-
O. princeps	-	-	-	-
O. geitleriana	-	-	-	-
O. perornata	421	-	-	-
Pandorina morum	190	-	*	-
Phormidium tenue	*	-	423	45
Class Bacillariophyceae				
Nitzschialongisima	52*	-	*	34*
N. acicularis	58	117*	58*	-
N. vitrea	90	-	-	-
N. denticula	43	-	-	-
N. rostellata	15	*	-	-
N. dissipata	-	58	-	-
N. minutula	-	72	-	432
N.linearis	-	58	81*	*
N. Fruticosa	-	423	-	23*
N. sigmoidea	-	-	-	*
N. multiseries	-	-	-	342*
N. tryblionella	-	-	-	48
N. palea	-	-	-	*

*Adhered algae.

Table (2): Diagnosed algae and algae cells number (cell/l) in drinking water in east Tigris plant, along study period 2011-2012 .

Species of algae	Studied period			
	Autumn	Winter	Spring	Summer
Class: Cyanophyceae				
Anabena spp.	-	*	317*	732*
Blue-green filamntes	422	*	400	458*
Chroococcus spp.	-	398	-	546
C. minor	-	211	-	
C. turgidus	-	*	-	*
Gloeocapsa aeruginosa	-	42	-	-
Haemato coccuslacustris	*	-	-	-
Lyngbya connectens	-	190	*	-
L. spirulinoides	*	-		-
Merismopedia eiegans	-	-	211*	-
Microcystes spp.	421	-	169*	875*
M. aerogenosa	-	127*	741*	435
Nostoclinka	-	-	*	*
Oscillatoria limnetica	400	423	-	647*
O. limosa	*	190	-	354
O. tenuis	-	412	-	534
O. limnetica	-	421	-	590*
O. curviceps	-	400	-	
O. homogenea	190	-	-	123*
O. sancta	404	-	-	*
O. amoena	189	-	-	312*
O. proboscidea	*	-	-	435
O. pseudogeminata	422	-	-	*
O. formosa	-	-	73*	135
O. ornate	-	-	63*	425*
O. subbrevis	420	-	85*	*
Pandorinamorum	180	*	411	*
Phormidium amibiguum	185	-	190	
P. mucicola	420	*		435
P. tenue	-	*	*	423*
Class Bacillariophyceae				
Nitzschiaacicularis	417	117*	175	254
N. linearis	*	234	81*	
N. vitrea	187	-	-	423
N. parvula	*	-	-	212
N. apiculata	*	*	193	*
N. subcapitellata	187	-	-	*
N. rostellata	*	-	-	213
N.multiseries	-	408	-	534*
N. pusilla	-	58	-	132
N. dubia	-	*	-	435
N. vermicularis	-	192	-	50*
N. gracilis	-	-	161	-
N. intermedia	-	-	422	-

*Adhered algae.

Also results showed presence of toxic algae in both of plants, Al Wathba plant in drinking water with cell number 127 – 888 cell/l which belong to division of Cyanophyceae, the responsible of most toxins producers such as *Anabena* spp. *Blue- green filaments*, *Chroococcus minor, Lyngbya connectens, L. Gardner, Microcystes aerogenosa, Nostoic carneum, Oscillatoria tenue, O. Mucicola, O. subbrevis, O. limnetica, O. formosa, O. princeps,* and *Phormidium* spp. which produce microcystin, nodularin, anatoxin-a, hepatoxins and homo anatoxin-a (Callieri, 2007). In addition that the toxins which producing by some toxic species in division diatoms, there were 12 toxic species of *Nitzschia* spp. such as *Nitzschia australis*, *N. multiseries*, *N. pseudodelicatissima*, *N. cusppidata*, *N. serata*, *N. calliantha* and *N. navis-varingica* (Lundholm, 2000). In addition *Amphora coffeaeformis* (Congestri, 2006).

In final tank of drinking water at east Tigris plant, counted highly biomass of 43 diagnosed algae species algae distributed along study period in range of 30-888 cell/l in addition to adhered species as a booming form, like *Chroococcus*, *Anabena* spp., *Merismopedia eiegans*, *Nitzscia acicularis*, *N. turgidus*, *Oscillatoria proboscidea* and *Haemato coccuslacustris*. The 16 diagnosed species belong to division Bacillariophyceae with ratio of 37.20 % of *Nitzschia* total number for all species because of domoic acid hazard, also there are 27 diagnosed species belong to Cyanophyceae with ratio of 62.79 % from total algae species ratio, represented by following species, *Microcystes aerogenosa*, *Nostoclinkia*, *Merismopedia eiegans*, *Aphanocapsa bioforms*, *Lyngbya connectens*, *L. spirulinoides*, *Oscillatoria limnetica* and *O. limosa*as represent in Table (3).

Chemical and physical factors was measured along study period for each plant separately which showed the deference among recorded results from Autumn to Summer for temperatures, conductivity and Nitrate [NO_3], but nitrite [NO_2] and phosphate [PO_4] nearly in same range except spring and summer for all plants separately as shown in Table (4).

Recorded identified species refers to several toxic species in all plants along study period in drinking water (final tank) according to international studies as in Table (5).

Table (3): Diagnosed algae and algae cells number (cell/l) in drinking water in Al-Wathba plant, along study period 2011-2012 .

Species of algae	Studied period			
	Autumn	Winter	Spring	Summer
Class: Cyanophyceae				
Blue-green filamntes	70	212*	260	563*
Chroococcus spp.	-	-		423
C. minor	30	465*	*	276
C. turgidus	-	85	400	342*
Gloeocapsa aeruginosa	-	-	-	321*
Haemato coccuslacustris	312	-	-	122*
Lyngybya gardner			389	439
L.connectens	419	-	-	534
L. spirulinoides	*	408	-	*
Microcystes spp.	-	-	42*	553
M. aerogenosa	-	127*	888*	425
Nostoclinka			*	245
Oscillatoria limnetica	403	42*	385	438*
O. limosa	*	-	-	324*
O. perornata	-	-	137	324*
O. pseudogeminat	390	-	-	132

O. geitleriana	*	-	-	*
O. subbrevis	403	422	377	142
O. tenuis	-	*	390	241*
O. ornata	-	190	-	*
O. perornata	183	-	-	*
O. formosa	-	*	411	426
O. curviceps	*	-	-	233
O. princeps	378	390	*	
O. tanganyika	187	-	-	342
Pandorina morum	*	-	-	211*
Phormidium amibiguum	190	-	189*	534
Class Bacillariophyceae				
Nitzschia acicularis	117*	-	*	221
N. closterium	872	-	197	342*
N. fruticosa	72	432	-	-
N. paleacea	78	-	398	-
N. longissima	*	-	-	-
N. multiseries	188*	-	178*	-
N. commutata	*	-	-	-
N.palea	-	81*	161	*
N. dissipata	-	323	219	342
N. linearis	-	189*	416	435*
N. gracilis	-	*	-	324
N. intermedia	-	184*	-	-

*Adhered algae.

Table (4): Measured chemical and physical factors along study period

Tests	Studied plants											
	Al-Wathba				East of Tigris				Al-Rasheed			
	Study period				Study period				Study period			
	Aut.	Win.	Spr.	Sum.	Aut.	Win.	Spr.	Sum.	Aut.	Win.	Spr.	Sum.
Temp.	23	12.6	26	36	23.6	13	28	32	23.6	15	29	35
pH	7.3	7.5	7.9	7.8	7.6	7.6	7.7	7.9	7.4	7.4	7.8	7.7
Cond.	932	1008	941	1044	733	856	880	955	895	1000	986	1033
NO_3	0.59	0.84	1.76	2.7	0.60	0.68	1.2	1.9	0.736	0.76	1.84	3.01
NO_2	0.01	0.01	0.2	0.4	0.01	0.01	0.2	0.3	0.3	0.2	0.2	1.2
PO_4	0.05	0.05	0.3	0.5	0.1	0.1	0.4	0.8	0.1	0.1	0.3	0.6

Table (5): Diagnosed toxic algae species in drinking water (final tank) of water plants along study period 2011-2012.

Algae groups	Species	References
Cyanophyceae	*Anabena* spp.	(Chorus and Bartram, 1999; Sivonen and Jones, 1999; Nishizawa *et al.*, 2000; Agrwal *et al.*, 2012)
	Blue-green filamentous	(Rothhaupt, 2007)
	Chroococcus spp.	(south, 1996)
	C. turgidus	(Mohsin and AL-Amoudi, 1989)
	Lyngbya spp.	(Agrwal *et al.*, 2012)
	Lyngbya connectens	(Chorus and Bartram, 1999)
	Microcystis spp.	(Rinta-Kanto *et al.*, 2005)
Cyanophyceae	*M. aerogenosa*	(Agrwal *et al.*, 2012)
	Nostoc spp.	(Agrwal *et al.*, 2012)
	Nostoc carneum	(Chorus and Bartram, 1999)
	Oscillatoria spp.	(Chorus and Bartram, 1999; Sivonen and Jones, 1999; Nishizawa *et al.*, 2000; Agrwal *et al.*, 2012)
	Oscillatoria formosa	(Chorus and Bartram, 1999)
	Phormidium sp.	(Bellinger, 2010)
Bacillariophyceae	*Nitzschia multiseries*	(Hallegraeff, 1995)

Conclusions

Periodical investigation and detection for toxic algae in addition to toxins detection in final tanks of drinking water plants, detection and diagnosis of toxins types and toxins concentration by HPLC, ELISA, GC Mass and new modern methods for give toxins concentration in drinking water. Booming and blooming highly density of toxic algae in summer because of highly temperature that causes disintegration complicated organic materials to simple structures which algae can absorb these simple materials easily, thus helps to increasing biomass of algae population.

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