Assessment of wastewater tolerant algal flora from the industrial effluents of Lahore City, Pakistan

Rizwan Sarwar[1], Rahmatullah Qureshi[1], Noshin Ilyas[1], Khalid Saifullah Khan[2]

1. Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.
2. Department of Soil Science, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Abstract- The present study was aimed at screening and identifying pollution tolerant algal species from the industrial wastewater of Lahore city. A total of 74 species belonging to 24 genera, 20 families were collected and identified. The highest numbers of families (08) were belonging to phylum Chlorophyta, followed by phylum Bacillariophyta (07) families. Identifying heavy metal tolerant species from industrial wastewater may serve to be used as biosorbent in phyto-remediation programme.

Index Terms- Alga flora, industrial wastewater, Lahore city, Pakistan.

I. Introduction

Water is indispensable to the life of all living beings and performing a key role in the economy of a nation and functioning of ecosystems in the world. It is estimated that five million deaths occurred each year globally due to the intake of polluted drinking water (Uiet, 2002). Industries contaminate water bodies due to the accumulation of an enormous amount of poisonous substances (Baroni et al., 2007). Among these poisonous substances or toxic materials, heavy metals persist in either land or water environments and hazardous for living organisms.

Algae absorb discharged carbon dioxide and put back non-sustainable resource and can contribute to climate change. Nowadays, algal biomass is used as fertilizers, feed, food, nutritional supplements, cosmetics and pharmaceuticals (Borowitzka 2013). It is foreseen that in near future, algae will be marketed for high value products through extracting the algae biomass and bulk products shall be economically viable (Vanthoor-Koopmans et al., 2013).

Microalgae are very adaptive nature and several species can heterotrophically and mixotrophically grow. Mixotrophic condition is that in which such species are capable to utilize light and organic carbon as sources of energy. Such competitive features are found in microalgae advantageous to fungi and bacteria while degrading organic pollutants (Subashchandrabose et al., 2013). Algae are pigmented organisms that store such compounds specifically present in their cell wall. The major classes are Basillariophyta Clorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta and Chrysophyta.

Western countries started cultivation of microalgae in the late 1950s due to high protein content (Chacoón-Lee & González-Mariño, 2010). These have been investigated for agricultural feed, fuel and pharmaceuticals (Olaizola, 2003). Besides, 13 of microalgae are used for remediation of N and P from wastewater. The reduction of such nutrients from wastewater may be attributed to the uptake and absorption as a result of algal growth (Larsdotter, 2006). Furthermore, such metals may also be reduced due to elevated pH stimulated by the algae (Hammouda et al., 1994). Some of the studies (Hultberg et al., 2013) have demonstrated the same phenomena by using residual water from greenhouse production treated with microalgae.

Heavy metals are believed to be one of the causative agents responsible for water pollution. Due to non-degradable materials, these amass into the environment. The accumulation of heavy metals in water bodies pollute water in which living organisms live. Besides, such polluted water is also used in irrigating vegetables and crops. As a part of food chain, if human consume fish, vegetables and cereals, the human health becomes in danger because of heavy metals accumulate through the food chain (Ali, 2020). The majority of heavy metals include arsenic, cadmium, chromium, copper, lead, nickel and zinc responsible in water pollution (Jaishankar et al., 2014). Approximately, 90% of all the wastewater containing industrial resources go unprocessed or as a whole into the fresh water bodies in many developing countries which are unsafe for human use or intake. Today, water recycling is the need of hours and a great challenge for a healthy population (Kanamarlapudi et al., 2018).

Industrial effluents contain high concentrations of inorganic and organic nutrients and are the leading cause of serious environmental problems and irreparable ecological degradation (Das et al., 2008). If these effluents are released untreated into water bodies such as rivers, lakes and streams cause microbial oxidation of organic pollutants and ammonium, deplete oxygen as a direct effect and indirectly causes eutrophication (Vieira and Volesky, 2000). Similarly these effluents lead to loss of natural habitats of aquatic life and the related ecological destruction. These effluents are coming from many sources such as energy and fuel producing installations, industries of fertilizer, pesticide, leather and steel, corrosion of water pipes, waste of dumping, etc. (Wang & Chen, 2009).

Metals are resistant to decomposition and add up in the bodies of living organisms which suffer from numerous diseases and disorders on accumulation of these metals even in trace quantities. Mercury (Hg), nickel (Ni), arsenic (As), copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn) and cobalt (Co), etc. are highly toxic and life threatening heavy metals (Wuana and Okieimen, 2011). Removal of wastes from water bodies and elimination of heavy metals from industrial wastewater is the need of the hour as these pollutants are threat to public health and affect the aesthetic quality of drinking water. Majority of effluents coming from industries have these toxic metals which are resistant to decomposition and the industrial effluents must contain tolerable levels of metals before discharging them into environment (Tchounwou et al., 2012).

This study was aimed at identifying algal flora of industrial effluents from Lahore, the 2nd largest city of Pakistan. Previously similar studies were undertaken on the algae from various water bodies of Pakistan such as Khuram et al. (2022); Shah et al. (2019); Ali et al. (2018; 2015); Ahmad et al. (2013); Shanaz *et al.* (2009); Gul *et al*., (2007, 2008); Leghari *et al*. (2008); Hussain *et al.* (2008); Leghari *et al*. (2005) and Naz *et al*. (2003).

II. MATERIALS AND METHODS

Study Area

Lahore is an historical city of Pakistan and capital of the Province of Punjab**.** It is located between 31.5204° N, 74.3587° E and the Ravi River flows from its northern side which is badly used by residents as effluent disposal water body. According to a report of Pakistan Council of Research in Water Resources, in Lahore city, 70 per cent of water is polluted and unfit for human consumption. According to WWF's Living Planet Report 2018, Lahore is amongst the badly contaminated cities in the world and serious efforts are needed to overcome this issue. Industrial sector of Lahore is very broad which is comprised of leather, chemicals, fertilizer, textile, pharmaceutical, electrical goods, paper, food, sugar, cement, automobile, light/heavy engineering, basic metal and non-metallic minerals and other agriculture related industries. These industrial processes generate huge harmful wastes which are causing disease or death to humans and impairment to both the living organisms and the environment. In order to investigate the role of algal species in removing heavy metals and phycoremediation, four sites were selected for present research. These sites were New Sharda Ravi Bridge, Motorway Bridge Lahore, Thokar Niaz Baigh and Rohi Nala Lahore, Pakistan.

Collection of Algal Samples

During field survey the algal samples were collected and labeled from four sites in the Lahore city. Algal samples were collected and labeled in sterile glass bottles from surface of water and from substrates at each site.

Preservation

To preserve the microalgae samples, 2-3% formalin was added at the spot and 4-5% formalin was added to the samples of macroalgae and filamentous types (Zarina et al., 2009).

Identification

Temporary and permanent slides were prepared for the identification of microalgae algae. Each sample was homogenized, a glass slide was taken and one drop of sample was placed on it. Then the glass slide was covered by a cover slip and observed under Leica DMLB microscope on various magnifications and photographs were taken. Identification of isolated algal strains was done upto the genus and species level (Prescott 1964, Komarek and Angnostidis, 1999, Munir et. al., 2013).

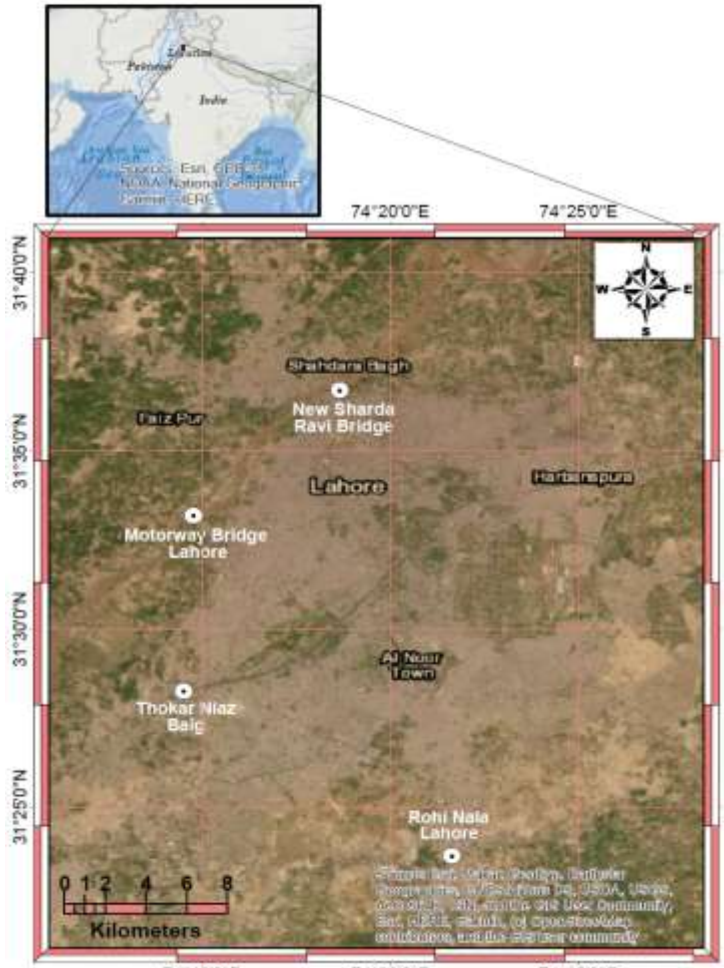


Fig. 3.1 Map of the sampling sites from Lahore, Punjab, Pakistan.

III. RESULTS AND DISCUSSION

Algal biomass has been recognized as highly economic, reliable and best alternative to the conventional water treatment methods and can be used as biosorbent for the removal of metallic elements (Wang and Chen, 2009). Identifying heavy metal tolerant species from industrial wastewater is obviously important to be used as biosorbent. Such kind of algae flora has been undertaken from various water bodies of Pakistan by Khuram et al. (2022); Shah et al. (2019); Ali et al. (2018; 2015); Ahmad et al. (2013); Shanaz *et al.* (2009); Gul *et al*., (2007, 2008); Leghari *et al*. (2008); Hussain *et al.* (2008); Leghari *et al*. (2005) and Naz *et al*. (2003). Keeping in view, this study was undertaken to record algal flora of industrial wastewater of Lahore city, Punjab, Pakistan with the view to identify algal species which are tolerant to the pollutants and biosorption of heavy metals by using the selected algal species.

From the industrial wastewater of Lahore city, a total of 74 species belonging to 24 genera, 20 families, 18 orders, 9 classes and 5 phyla were collected and identified (Table 1). The highest number of families (08) was belonging to phylum Chlorophyta (Table 2; Appendix 1), followed by phylum Bacillariophyta (07 families). The highest number of species (34) was belonging to phylum Bacillariophyta, followed by phylum Chlorophyta (19 species).

The largest family was Fragilariaceae that contributed 09 algal species (12.2%), followed by Cymbellaceae and Naviculaceae (7 spp., 9.5% each). Rest of the families contributed between 2-4 algal species (Table 3). Phylum Charophyta was represented by two families in which Zygnemataceae contributed seven algal species (9.5%) and Desmidaceae shared five algal species (6.8%). The phylum Chlorophyta was represented by eight families from which Cladophoraceae and Euglenaceae contributed equally four species (5.4% each) and other families contributed 2-3 species. Phylum Euglenophyta was represented only by the single family Euglenaceae that contained four species (5.4%). The phylum Cyanophyta represented by two families in which Oscillatoriaceae shared four species (5.4%); while Microcystaceae represented a single species (Table 3). Nineteen species of blue green algae and the Genus Planktothrix and *Anabaena viguieri* were identified and taxonomically described from different localities of Punjab (Gul *et al*., 2007, 2008). Algal species fix the carbon from various sources through photosynthetic activity, absorb different nutrients and assimilate those (Ahmad et al., 2013).

The identified genera include *Amphora*, *Chaetophora*, *Chlorella*, *Cladophora*, *Cosmarium*, *Cymbella*, *Diatoma*, *Euglena*, *Fragilaria*, *Gomphonema*, *Lyngbya*, *Microcystis*, *Navicula*, *Oedogonium*, *Oscillatoria*, *Pandorina*, *Scenedesmus*, *Spirogyra*, *Surirella*, *Synedra*, *Trochiscia*, *Ulothrix*, *Volvox* and *Zygnema*. These all genera were belonging to five phyla, Bacillariophyta, Charophyta, Chlorophyta, Euglenophyta and Cyanophyta. The phylum Bacillariophyta was the largest and most diverse phylum, represented by 34 species distributed in seven families. Our studies are in agreement with the studies carried out in different fresh water bodies of Punjab province in which 29 genera of algae belonging to 15 families of the phylum Bacillariophyta were collected and taxonomically described. Among them family Pinnulalaraceae with 19 species were most commonly found (Ali *et al*., 2010).

Table 1. Identification of Algae from Industrial water of Lahore city, Pakistan

Division	Class	Order, Family, Genus and Species name
Cyanophyta	**Cyanophyceae**	
		Order: Chroococcales
		Family: Microcystacae
		1. *Microcystis aeruginosa* Kutzing
		Order: Oscillatoriales
		Family: Oscillatoriaceae
		2. Lyngbya heieronymusii Menegh
		3. *Oscillatoria chlorina* Kuetz.
		4. *Oscillatoria acutissima* Kufferath
		5. *Oscillatoria perronata* Skuja
Bacillariophyta	**Bacillariophyceae**	**Order: Cymbellales**
		Family: Cymbellaceae
		6.*Cymbella turgida* W. Gregory
		7. *Cymbella. cesati* Rabenhorst
		8.*Cymbella parva* (W.Smith) Kirchne
		9.Cymbella tumidula Grunow
		10. Cymbella. normani Ehrenberg
		11. Cymbella. brehmii Hustedt
		12.Cymbella leptoceros Ehrenberg
		Family: Gomphonemaceae
		13.Gomphonema ventricosum Gregory
		14.Gomphonema constrictum (Lyngbye)
		15. Gomphonema acuminatum Ehrenberg.
		16.Gomphonema trucatum
		Order: Fragilariales
		Family: Fragillariaceae
		17.Synedra amphicephala Kutzing
		18.Synedra tenera Rabenhorst
		19. Synedra affinis Kuetz.
		20. Synedra acus W.Smith
		21.Fragilaria intermedia Grunow
		22.Fragillaria. pinnata Ehrenberg
		23.Fragillaria virescens Ralfs
		24.Fragillaria capucina

Division	Class	Order, Family, Genus and Species name
		Desmazieres
		25.Fragillaria construens Grun.
		Order: Naviculales
		Family: Naviculaceae
		26.Navicula citrus Krasske
		27. Navicula dicephala Ehrenberg
		28.Navicula meniscula J. Schumann
		29.Navicula exigua Gregory
		30.Navicula crucicula (W.Smith) Donkin
		31.Navicula leptoceros Krasske
		32.Navicula simplex Krasske
		Order : Surirellales
		***Family:* Surirellaceae**
		33.Surirella linearis W.Smith
		34.Surirella elegans Ehrenberg
		35.Surirella ovata Kutzing
		Order: **Tabellariales**
		***Family:* Tabellariaceae**
		36.Diatoma anceps (Ehrenberg) Grunow
		37. Diatoma vulgare (Fricke) Hust.
		Order: Thalassiophysales
		Family: Catenulaceae
		38. Amphora commutate Grun.
		39.Amphora coffeaeformis Kutzing
		Order : Chlamydomonadales
		Family: Volvocaceaea
		Pandorina morum (O.F.Muller)
		Volvox aurus Ehrenberg
Euglenophyta	**Euglenophyceae**	**Order: Euglenida Family: Euglenaceae**

Division	Class	Order, Family, Genus and Species name
		40.Euglena polymorpha P.A.Dangeard
		41.Euglena gracilis Klebs
		42.Euglena oblanga F.Schmitz
		43.Euglena acutissima Lemmermann
		Order: Sphaeropleales
		Family: Scenedesmaceae
		44.Scenedesmus abundans (O. Kirchner)
		45.Scenedesmus arcuatus Proshkina
		Order: Chlamydomonadales
		Family: Volvocaceaea
		46.Pandorina morum (O.F.Muller)
		47.Volvox aurus Ehrenberg
Chlorophyta	**Chlorophyceae**	**Order: Oedogoniales**
		Family: Oedogoniaceae
		48.Oedogonium reinschii J. Roy
		49.Oedogonium macroandrium Wittrock
		Order: Chlorellales
	Trebouxiophyceae	**Family: Chlorallaceae**
		50. Chlorella sorokiniana
		51.Chlorella ellipsoidea Gerneck
		Family: Oocystaceae
		52.Trochiscia asper Reinsch
		53.Trochiscia reticularis Reinsch.
		Order: Chetophorales
		Family: Chaetophoraceae
		54.Chaetophora spp.
		55.Chaetophora elegans C. Agardh

Division	Class	Order, Family, Genus and Species name
		56.Chaetophora pisiformis C. Agardh
	Ulvophyceae	**Order: Cladophorales**
		Family: Cladophoraceae
		57.Cladophora spp.
		58.Cladophora crispate (Roth) Kutzing
		59. Cladophora glomerata (L.) Kuetzing
		60. Cladophora elegans (L.) Hoek
		Order: Ulotrichales
		Family: Ulotrichaceae
		61.Ulothrix spp F.Weber & Mohr
		62.Ulothrix gemilata Kutzing
	Zygnematophyceae	**Order: Desmidiales**
		Family: Desmidaceae
		63. Cosmarium formosulum Corda.
		64. Cosmarium punctulatum Brebisson
		65.Cosmarium impressulum Elfving
		66.Cosmarium dydowskii Corda
		67.Closterium littorale M.Chihara
		Order: Zygnematales
		Family: Zygnemataceae
		68.Spirogyra spp.
		69.Spirogyra tetrapla Transeau
		70. Spirogyra. subsalsa Kutzing
		71.Spirogyra rhizobrachialis C-C Jao
		72.Zygnema insigne (Hassall) Kutzing
		73.Zygnema tenue Kutzing
		74. Zygnema strile Transeau.

Table 2. Percentage of different phyla from industrial wastewater of Lahore city.

S. #	Phylla	No. of Families	No. of species	%age
1	Bacillariophyta	07	34	46
2	Charophyta	02	12	16
3	Chlorophyta	08	19	26
4	Cyanophyta	02	05	6.8
5	Euglenophyta	01	04	5.4

Table 3. Percentage of different families from industrial wastewater of Lahore city.

S. #	Family	Genera	%age	Species	%age
1.	Catenulaceae	01	4.2	02	2.7
2.	Chaetophoraceae	01	4.2	03	4.0
3.	Chlorallaceae	01	4.2	02	2.7
4.	Cladophoraceae	01	4.2	04	5.4
5.	Cymbellaceae	01	4.2	07	9.5
6.	Desmidaceae	01	4.2	05	6.8
7.	Euglenaceae	01	4.2	04	5.4
8.	Fragilariaceae	02	8.3	09	12.2
9.	Gomphonemataceae	01	4.2	04	5.4
10.	Microcystaceae	01	4.2	01	1.6
11.	Naviculaceae	01	4.2	07	9.5
12.	Oedogoniaceae	01	4.2	02	2.7
13.	Oocystaceae	01	4.2	02	2.7
14.	Oscillatoriaceae	02	8.3	04	5.4
15.	Scenedesmaceae	01	4.2	02	2.7
16.	Surirellaceae	01	4.2	03	4.0
17.	Tabellariaceae	01	4.2	02	2.7
18.	Ulotrichaceae	01	4.2	02	2.7
19.	Volvocaceaea	02	8.3	02	2.7
20.	Zygnemataceae	02	8.3	07	9.5
	Total	24	100	74	100

Appendix

Appendix 1. Micrographs of Algal Flora of Industrial Wastewater of Ravi River Lahore.

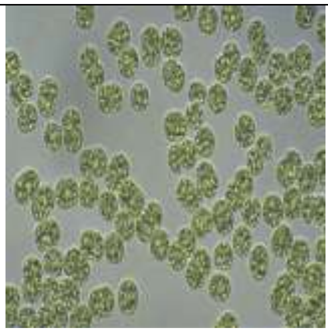


Fig. 1. *Microcystis aeruginosa*

Fig. 2. *Lyngbya heieronymusii*

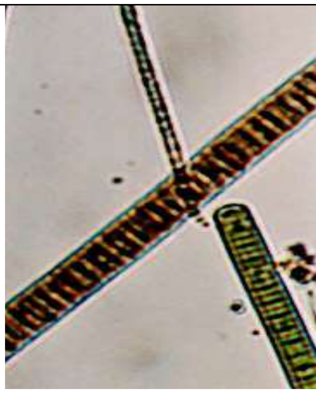


Fig. 3. *Oscillatoria chlorina*

Fig. 4. *Oscillatoria acutissima*

Fig. 5. *Oscillatoria perronata*

Fig. 6. *Cymbella turgida*

Fig. 7. *Cymbella cesati*

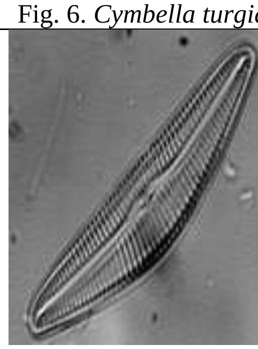


Fig. 8. *Cymbella parva*

Fig. 9. *Cymbella tumidula*

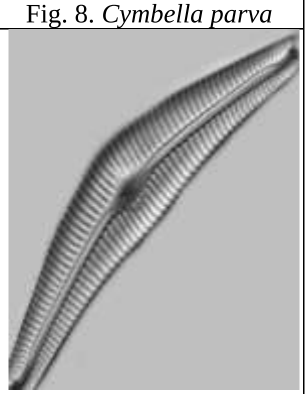


Fig. 10. *Cymbella normani*

Fig. 11. *Cymbella brehmii*

Fig. 12. *Cymbella leptoceros*

Fig. 13. *Gomphonema ventricosum*

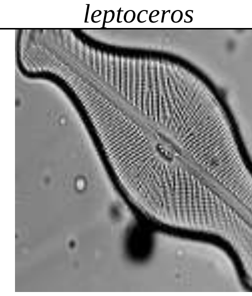


Fig. 14. *Gomphonema constrictum*

Fig. 15. *Gomphonema acuminatum*

Fig. 16. *Gomphonema trucatum*

Fig. 17. *Synedra amphicephala*

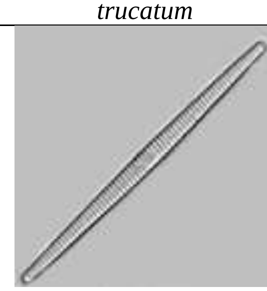


Fig. 18. *Synedra tenera*


Fig. 19. *Synedra affinis*


Fig. 20. *Synedra acus*

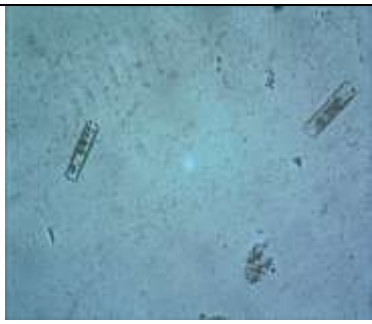


Fig. 21. *Fragilaria intermedia*


Fig. 22. *Fragilaria pinnata*

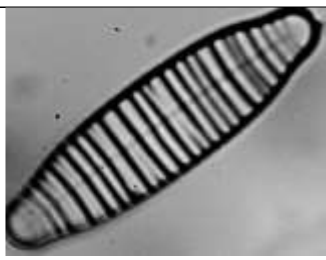


Fig. 23. *Fragilaria virescens*

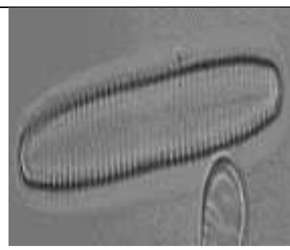


Fig. 24. *Fragilaria capucina*

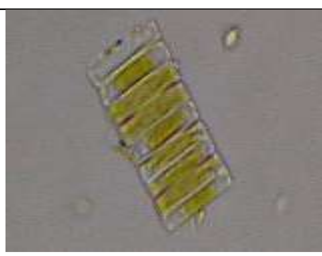


Fig. 25. *Fragilaria construens*

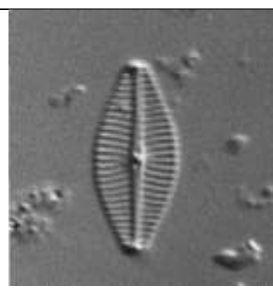


Fig. 26. *Navicula citrus*

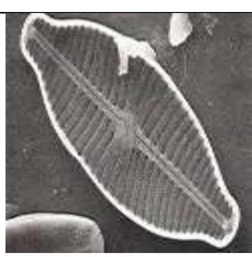


Fig. 27. *Navicula dicephala*

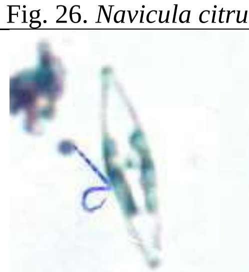


Fig. 28. *Navicula meniscula*

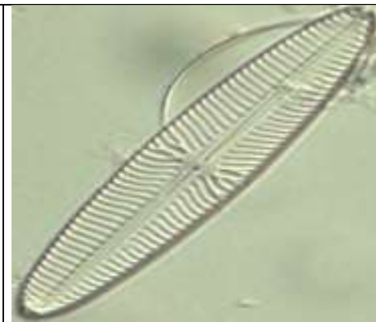


Fig. 29. *Navicula exigua*

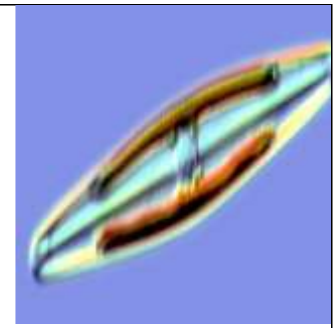


Fig. 30. *Navicula crucicula*


Fig. 31. *Navicula leptoceros*


Fig. 32. *Navicula simplex*


Fig. 33. *Surirella linearis*


Fig. 34. *Surirella elegans*


Fig. 35. *Surirella ovata*


Fig. 36. *Diatoma anceps*


Fig. 37. *Diatoma vulgare*


Fig. 38. *Amphora commutate*


Fig. 39. *Amphora coffeaeformis*


Fig. 40. *Euglena polymorpha*

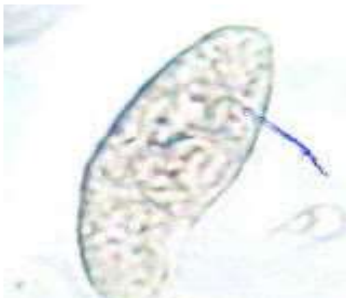


Fig. 41. *Euglena gracilis*


Fig. 42. *Euglena oblanga*


Fig. 43. *Euglena acutissima*


Fig. 44. *Scenedesmus abundans*


Fig. 45. *Scenedesmus arcuatus*

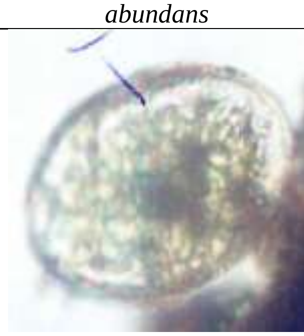
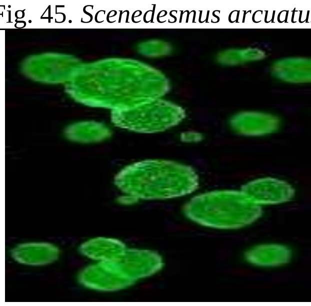


Fig. 46. *Pandorina morum*



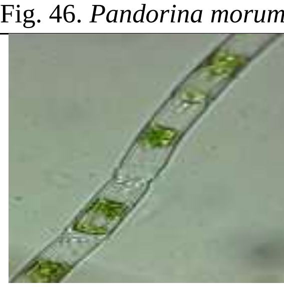


Fig. 48. *Oedogonium*

Fig. 47. *Volvox aurus*

reinschii

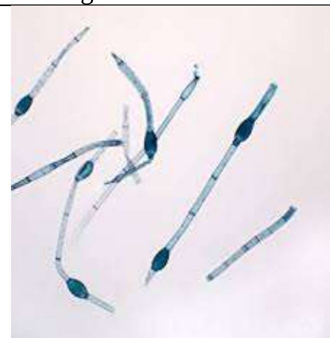


Fig. 49. *Oedogonium macroandrium*

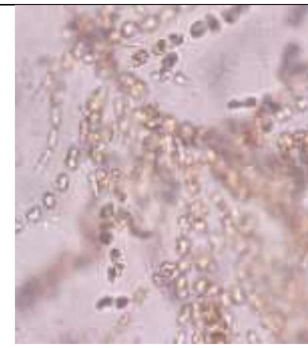


Fig. 50. *Chaetophora* sp.


Fig. 51. *Chaetophora elegans*


Fig. 52. *Chaetophora pisiformis*


Fig. 53. *Trochiscia asper*


Fig. 54. *Trochiscia reticularis*


Fig. 55. *Chlorella sorokiniana*

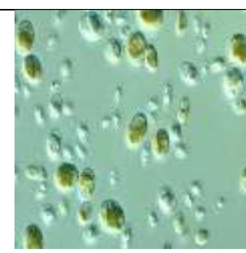


Fig. 56. *Chlorella ellipsoidea*


Fig. 57. *Cladophora sp.*


Fig. 58. *Cladophora crispata*


Fig. 59. *Cladophora glomerata*


Fig. 60. *Cladophora elegans*


Fig. 61. *Ulothrix sp*


Fig. 62. *Ulothrix gemilata*

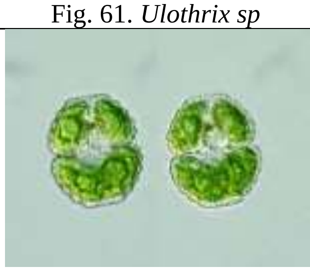


Fig. 63. *Cosmarium formosulum*


Fig. 64. *Cosmarium punctulatum*


Fig. 65. *Cosmarium impressulum*


Fig. 66. *Cosmarium dydowskii*


Fig. 67. *Closterium littorale*

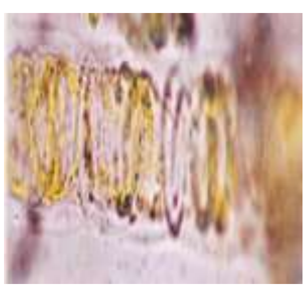


Fig. 68. *Spirogyra* sp.

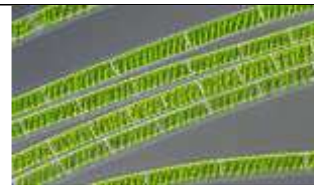


Fig. 69. *Spirogyra tetrapla*


Fig. 70. *Spirogyra subsalsa*


Fig. 71. *Spirogyra rhizobrachialis*

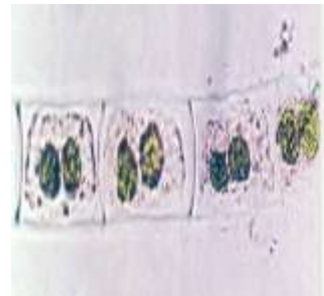


Fig. 72. *Zygnema insigne*


Fig. 73. *Zygnema tenue*

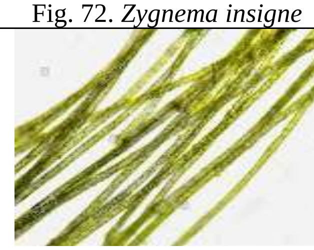


Fig. 74. *Zygnema strile*

CONCLUSION

The present work was aimed at identifying algal species tolerant to industrial effluents from Ravi River located in Lahore. A total of 74 species belonging to 24 genera, 20 families, 18 orders, 09 classes and 05 phyla were collected and identified. Identifying heavy metal tolerant species from industrial wastewater may serve to be used as biosorbent in phyto-remediation programme.

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Authors

First Author – Rizwan Sarwar, M. Phil., Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Second Author –Rahmatullah Qureshi, PhD, Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Third Author – Noshin Ilyas, PhD, Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Fourth Author – Khalid Saifullah Khan, PhD, Department of Soil Science, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan

Correspondence Author – Rahmatullah Qureshi