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Seasonal Variation in Protozoan Population in Tannery Effluents

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Abstract: Wastewater samples were collected from the ponds receiving effluents from tanneries in industrial area of Kasur to determine the seasonal variation in chromium resistant protozoan population over a period of one year from August 1999 to July 2000. During this period the pH of the pond water ranged between 7.46 and 9.66, temperature ranged between 19ºC and 34ºC, and the chromium content ranged between from 0.05ppm and 1.6 ppm. A wide variety of micro-organisms were found including protozoa, rotifers and algae. From amongst Protozoa *Vorticella, Stylonychia, Oxytricha, Tachysoma* and *Metopus* were found to dominate the protozoan population throughout the study. *Amoeba, Paramecium, Euplotes* and *Chilodonella* were not observed at 34ºC during the month of August, and *Cyclidium* and *Spathidium* were not found at 19ºC during the months of November-January, but were present in fairly large number during other months of the year. *Oxytricha, Amoeba, Plagiopyla* and *Euplotes* were not observed at pH 9.66 in May and *Paramecium, Cyclidium* and *Spathidium* were not found at pH 7.46 but were observed in other months of the study at fairly high pH range of 8.05-9.4. *Euplotes, Cyclidium, Plagiopyla, Tetrahymena* and *Euglena* were not observed at 1.6 µg/ml of chromium concentration but were observed at 0.05 µg/ml.

Key words: Protozoan population, chromium, tannery effluents, bioremediation

INTRODUCTION

Industrialization has led to increased emission of pollutants into ecosystems (Diagomanolin *et al*., 2004). Heavy metals in wastewater come from industries and municipal sewage, and are one of the main causes of water and soil pollution. Accumulation of these metals in wastewater depends on many local factors such as type of industries in the region, people's way of life and awareness of the impacts on the environment by careless disposal of wastes (Shakoori *et al*., 2004; Chisti, 2004; Chipasa, 2003). Atmospheric dust deposition can affect biogenic calcification in oceanic regions characterized by trace metal limitation (Schulz *et al*., 2004). Heavy metal contamination and the problems that it poses to the biota have been well documented (Raskin and Ensley, 2000; Meagher, 2000). Accumulation of toxic metals, *e.g*., Hg, Cu, Cd, Cr, and Zn, in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses (Thiele, 1995).

The industrial base of Pakistan has increased considerably during the past one decade. There were 315 industries in the country in 1987 and since then the industry has continued to expand. In the province of Punjab, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be major contributor to pollution (Khalil *et al*., 1991). The leather and steel industry has long been recognized as a main contributor to water pollution because of highly toxic nature of water borne components such as Cr^{6+} and Cd^{2+} of untreated industrial water wastes. The public awareness about pollution, its hazards and the need for remedy has been very poor in Pakistan. In spite of the fact that daily expanding industrial areas are just flooding our environment with toxic heavy metal ions laden effluents little is being done to save the human population from health hazards due to heavy metals.

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There are vast areas of foul smell and wastewater-inundated land in industrial areas in the vicinity of Lahore City, which are source of variety of environmental problems such as unwanted marshy areas, agricultural wastelands, generally uninhabitable areas and sources of health hazards to human and animal life. Such areas exist in Kasur, Sheikhupura and Kala Shah Kaku-industrial towns in the vicinity of Lahore City.

Industrial wastes laden with heavy metal are posing serious problems in Pakistan where the environmental awareness is very low. Waste recycling treatments and disposal of effluents is not according to the world standards. In Kasur, a small town located 54 km south east of Lahore, the effluents from tanneries are discharged in open land, rendering the atmosphere absolutely smelly, air unbreathable, and sub-soil water which is 6-7 meter deep from the soil surface, contaminated with the wastewater, particularly chromium.

In-spite of toxic elements in the wastewater, the ponds receiving these effluents have been found to contain rich population of microorganisms (Haq *et al*., 1998; Shakoori *et al*., 2000; Haq *et al.,* 2001). Apparently these microorganisms have adopted themselves to these toxic environments and developed variety of mechanisms to avoid toxicity of these contaminants (Haq and Shakoori, 2000). The populations of variety of microorganisms seem to fluctuate over the year depending upon the pH, temperature and concentration of heavy metals and other contaminants. Shakoori *et al.* (2001) have described the fluctuation in bacterial population over a period of one year in ponds receiving wastewater of tanneries.

The aims of present work are (i) to record fluctuation in the variety of protozoan fauna of ponds receiving industrial wastes from tanneries; (ii) to relate the variety of protozoan population with the changing environmental conditions; and (iii) to correlate fluctuation in protozoan population with Cr^{6+} content of the industrial waste. The specific objectives are to use this data for preparation of strategy for bioremediation of industrial waste from tanneries.

MATERIALS AND METHODS

Water sampling site and water samples

Water samples were collected in sterilized screw capped glass bottles, from five different ponds located near the Leather Service Station of Punjab Small Industries Corporation and receiving effluents from approximately 500 tanneries (Fig. 1) in industrial area of Kasur regularly on 10^{th} of every month. Monthly variation of the protozoan diversity in tannery wastewater was recorded from August 1999 to July 2000. Vegetation around these ponds was scarce, though weeds like *Suaeda fruticosa* and *Trianthema mongyna* could be seen here and there. From each pond, two samples were collected, one from the surface and other from the 0.5 to 1.00 foot depth of the pond. The water samples were quickly transferred and acclimatized to the laboratory conditions by keeping them at room temperature for 24 hours. Water samples were used for determination of protozoan diversity and estimation of chromium contents. The pH and temperature of the water samples were also recorded at the time of samples collection.

Determination of protozoans diversity

Identification of the protozoans was done by observing their body shape, other morphological features, movements and behaviour (Edmondson, 1966; APHA, 1992; Curds, 1982; Curds *et al*., 1983).

Estimation of chromium in water samples

The water samples were spun down at 450x g for 15 minutes and the supernatants were used for the estimation of Cr^{6+} by atomic absorption spectrophotometer (Varian, U.S.A) at wavelength 357.9 µm. The amount of metals in the supernatants was determined using standard curve.

RESULTS

Temperature of water samples

The extent of fluctuation of temperature in different ponds was almost the same-the maximum being 33-34ºC during the month of August and the minimum being 19-20ºC during January (Fig. 2).

pH of water samples

Generally the pond water was alkaline, the pH ranging from pH 7.46 to 9.66. In pond 1, the pH ranged between 8.10 (November) and 8.70 (September), in pond 2, it ranged between 7.71 (June) and 9.44 (August), in pond 3, it ranged between 7.73 (March) and 8.80 (September), in pond 4, it ranged between 8.10 (January) and 9.60 (April), in pond 5, it ranged between 7.46 (September) and 9.66 (May) (Fig. 3).

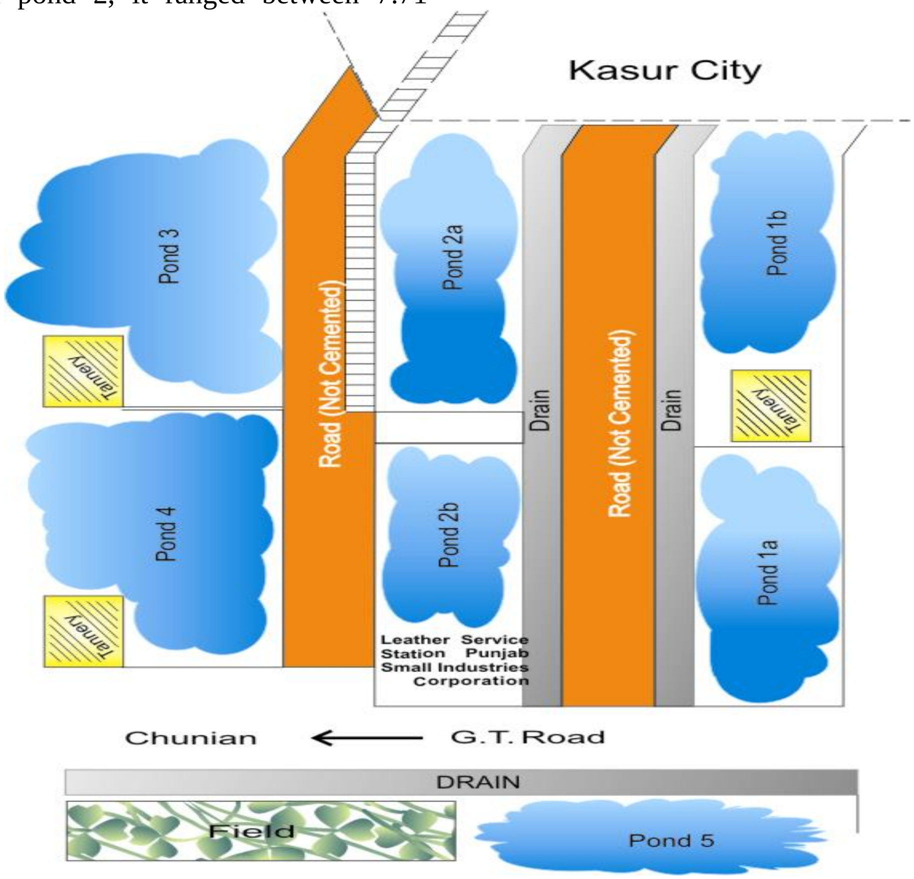

Fig.1. Location of five ponds receiving industrial wastewater from tanneries in Kasur, a small industrial town close to Lahore.

Chromium concentration

The overall chromium concentration fluctuated from a minimum of 0.05 µg/ml to a maximum of 1.6 µg/ml during the period of this study (Fig. 4). The highest Cr concentration recorded in pond 1 was 0.8 µg/ml in May, 2000, while the lowest was 0.05 µg/ml in April same year. The maximum Cr concentration found in pond 2 was 0.9 µg/ml in May and December, 2000, while the lowest was 0.2 µg/ml in October, November, January, and June. The maximum Cr concentration observed in pond 3 was 1.6 µg/ml in July, 2000, while the lowest was 0.1 µg/ml in October, November, January, March and April. The highest Cr concentration observed in pond 4 was 0.6µg/ml in September and December, 1999 and the minimum was 0.05 µg/ml in March and April. The maximum Cr concentration recorded in pond 5 was 0.35 µg/ml in February, while the lowest was 0.05 µg/ml in March. The fluctuations in chromium concentration in different ponds may be due to processing of large quantity of leather in those months and may also depend on the effluents these ponds were receiving.

Protozoan diversity

Wastewater samples from tannery effluents contained a wide variety of micro-organisms. The protozoans observed in the samples were

Paramecium, Stylonychia, Euplotes, Chilodonella, Colpoda, Cyclidium, Euplotes, Campanella, Metopus, Plagiopyla, Tachysoma, Tetrahymena, Vorticella, Oxytricha, Spirostomum, Amoeba and

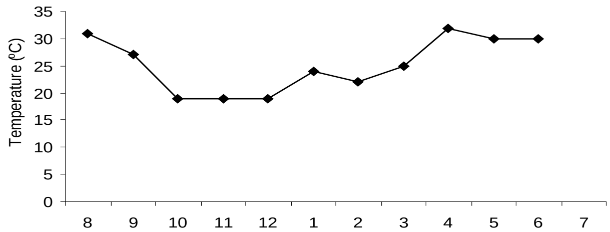

Fig.2. Variation in temperature (ºC) of wastewater collected from tannery effluent from Kasur district from August 1999 to July 2000. The numbers at the X-axis are the months of the years, for example 8 is August and 1 is January.

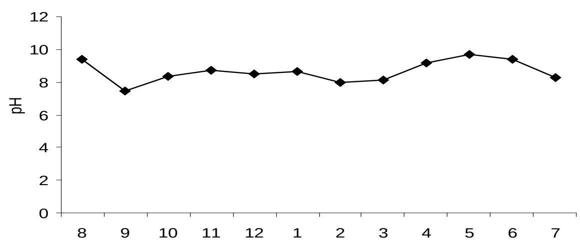

Fig. 3. Variation in pH of wastewater collected from tannery effluent from Kasur district from August 1999 to July 2000. The numbers at the X-axis are the months of the years, for example 8 is August and 1 is January.

Spathidium (Table I). The ciliate protozoa present almost throughout the year were *Vorticella*, *Stylonychia*, *Oxytricha*, *Tachysoma* and *Metopus*. *Euplotes*, *Paramecium* and *Amoeba* were not observed frequently during all the months of the year. *Paramecium* disappeared in November, 1999 and reappeared in March, 2000 and was not observed during the months of December, January and February. It may be due to low temperature in the environment. *Euplotes* was first time observed in November and could be observed till the month of May but was later not observed during the months of June-October. It may be due to high temperature in the environment.

The frequently observed algae were *Chlorella, Chlamydomonas, Arthrospora, Diatoms, Spirolina,* and *Euglena*. The metazoan micro-organisms found in the samples were some rotifers *viz.*, *Philodina roseola*, *Notommata copeus*, *Platyias patulus*, *Testudinella truncata*, and *Brachionus rubens* and a Gastrotrich *Chaetonotus brevispinosus*. The appearance of various metals and pollutant resistant micro-organisms in ponds constantly receiving toxic industrial effluents showed a high capacity of the microorganisms to evolve in response to xenobiotic stress.

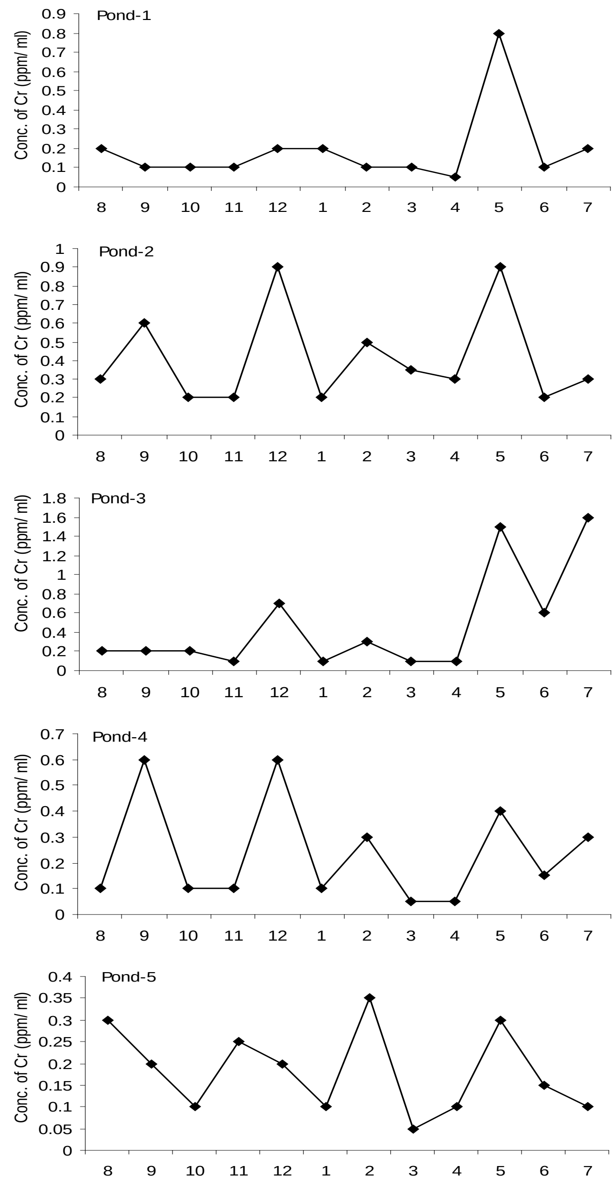

Fig. 4. Variation in Cr (µg/ml)

concentration of wastewater collected from tannery effluent from Kasur district from August 1999 to July 2000. The numbers at the X-axis are the months of the years, for example 8 is August and 1 is January.

Table I: Seasonal variation in protozoan diversity of wastewater collected from tannery effluents from Kasur district from August 1999 to July 2000.

Month	Protozoa	Algae	Rotifers
August	*Oxytricha, Oxytricha, Tachysoma, Metopus, Paramecium, Vorticella, Cyclidium, Spathidium, Stylonychia.*	*Chlorella, Chlorella, Euglena.*	*Platyias patulu, Notommata copeus, Chaetonotus brevispinosus.*
September	*Paramecium, Metopus, Vorticella, Stylonychia, Spathidium, Tachysoma, Oxytricha, Cyclidium.*	*Chlamydomonas, Euglena.*	*Testudinella truncate, Platyias patulu, Philodina roseola.*
October	*Vorticella, Metopus, Paramecium, Oxytricha, Amoeba, Stylonychia, Spathidium, Tachysoma, Cyclidium.*	*Euglena, Chlamydomonas.*	*Notommata copeus Testudinella truncata, Pomphalyx sulcata.*
November	*Vorticella, Oxytricha, Euplotes, Amoeba, Metopus, Stylonychia, Chilodonella, Tachysoma.*	*Euglena, Chlamydomonas*	*Notommata copeus, Pomphalyx sulcata*
December	*Amoeba, Oxytricha, Tachysoma, Vorticella, Metopus, Stylonychia, Euplotes, Tetrahymena.*	*Diatoms, Chlamydomonas, Eudorina elegans.*	*Testudinella truncata Philodina roseola.*
January	*Vorticella, Amoeba, Oxytricha, Metopus, Stylonychia, Euplotes Chilodonella, Tachysoma.*	*Euglena, Chlamydomonas.*	*Platyias patulu, Pomphalyx sulcata.*
February	*Cyclidium, Oxytricha, Stylonychia, Plagiopyla, Vorticella, Amoeba, Euplotes, Tachysoma.*	*Euglena, Chlamydomonas, Chlorella.*	*Testudinella truncata, Pomphalyx sulcata.*
March	*Stylonychia, Oxytricha, Paramecium, Cyclidium, Vorticella, Tetrahymena, Metopus, Euplotes.*	*Spirolina, Diatoms, Euglena.*	*Platyias patulu, Philodina roseola, Chaetonotus brevispinosus.*
April	*Vorticella, Stylonychia, Paramecium, Oxytricha, Cyclidium, Tachysomsa, Metopus, Plagiopyla, Euplotes.*	*Spirolina, Diatoms.*	*Chaetonotus brevispinosus. Testudinella truncata*
May	*Stylonychia, Metopus, Paramecium, Oxytricha, Vorticella, Tachysomsa, Amoeba, Cyclidium, Plagiopyla, Euplotes.*	*Euglena, Chlorella, Spirolina, Diatoms.*	*Notommata copeus, Chaetonotus brevispinosus.*
June	*Oxytricha, Tachysoma, Paramecium, Amoeba, Stylonychia, Metopus, Vorticella.*	*Chlorella, Chlamydomonas,*	*Chaetonotus brevispinosus, Testudinella truncate.*
July	*Paramecium, Oxytricha, Tachysoma, Amoeba, Stylonychia, Metopus, Vorticella.*	*Spirolina, Euglena. Chlorella*	*Philodina roseola Notommata copeus, Chaetonotus brevispinosus.*

DISCUSSION

The physical and chemical characteristics of these pond waters showed seasonal fluctuations interacting with one another and having a combined effect on animals and plants (Jeffries and Mills, 1990). Some environmental parameters (pH, temperature, Cr concentration) were studied on monthly basis from August 1999 to July 2000. A wide variety of micro-organisms were found in wastewater samples from tannery effluents. They were protozoa, rotifers and algae. Among these protozoa were the dominant organisms followed by algae and rotifers. During the present study the ciliate protozoa, *Vorticella, Stylonychia, Oxytricha, Tachysoma* and *Metopus* were abundant almost throughout the study period. *Euplotes, Paramecium* and *Amoeba* were not observed frequently during all the months of the year.

The minimum and maximum temperature of the industrial effluent observed was 19ºC and 34ºC during January and August, respectively. *Amoeba, Paramecium, Euplotes* and *Chilodonella* were not observed at the maximum temperature (34ºC) in August during the present study but were present in fairly large in number in other months of the year. *Cyclidium* and *Spathidium* were not found at 19ºC but observed in other months of the study. Variations in ponds' water temperature occur throughout the year with seasonal changes in air temperature, day length, sunlight, photoperiod and depth. The intensity and seasonal variations in temperature of water directly affect the productivity of these ponds. Rathore and Khangarot (2002) reported that the toxicity of cadmium, chromium, cobalt, copper, lead, mercury, nickel and zinc increased with increased temperature.

The pH 7.46 was recorded during the month of September and 9.66 during May 2000, which are well within the acceptable limits of tolerance. *Oxytricha, Amoeba, Plagiopyla* and *Euplotes* were not observed at the pH 9.66 in May, although these organisms were observed during other months of the study. *Paramecium*, *Cyclidium* and *Spathidium* were not found at pH 7.46 but were observed during other months at fairly high pH range (8.05-9.40). These physicochemical characteristics of pond waters showed that different environmental factors interact with each other to provide entirely different environment for the organisms to survive in these pond waters. This results in diversity and eventually leads to adaptation.

The minimum chromium concentration observed was 0.05 µg/ml in March-April and maximum of 1.6 µg/ml during July. *Euplotes, Cyclidium, Plagiopyla, Tetrahymena* and *Euglena* were not observed at the maximum chromium concentration (1.6 µg/ml), but were observed at the minimum chromium concentration (0.05 µg/ml). *Oxytricha* and *Tachysoma* were not observed at the minimum chromium concentration (0.05 µg/ml) observed in March. Deram *et al.* (2006) studied the seasonal variation, from January to July, of Cd and Zn in *Arrhenatherum elatius*.

The seriousness and persistence of heavy metals in water are compounded by the fact that generally they are water soluble, non-degradable, vigorous oxidizing agents and strongly bound to many bio-chemicals especially polypeptides and proteins. The heavy metals are bio-accumulated in the body of microorganisms and other aquatic plants and animals. Haq *et al*. (1998) already reported the growth and survival of two protozoans species *viz.*, *Stylonychia mytilus* and *Tetrahymena pyriformis* isolated from a tannery effluent. *Vorticella microstoma* and *Stylonychia mytilus* were also isolated from the wastewater samples of the tannery effluent (Rehman *et al*., 2005; Shakoori *et al*., 2004).

The frequently encountered algae were *Chlorella, Chlamydomonas, Arthrospora, Diatoms, Spirolina*, and *Euglena*. Rehman and Shakoori (2001) reported the isolation of chromium tolerant alga *Chlorella* from the tannery effluent. Among metazoans some rotifers *viz.*, *Philodina roseola, Notommata copeus, Platyias patulus, Testudinella truncata,* and *Brachionus rubens* and a Gastrotrich *Chaetonotus brevispinosus* were also present in the tannery effluents. Haq *et al*. (2001) reported the isolation of various rotifers and a gastrotrich from heavily polluted industrial effluents.

The industrial effluents from tanneries which are generally discharged into open fields are harmful for animal and human life and hence pose a serious threat to the environment of the region. In these areas there is a need to purify Cr^{6+} containing waste water and make it reusable at last for irrigation or fish production purposes. So in the present study protozoan diversity has been studied under various seasons and these protozoa are now proposed to be used for detoxication of industrial waste water containing chromium.

Haq and Shakoori (1998) studied different ponds in Kasur getting wastes from tanneries. The industrial wastewater in all these industrial zones harbor variety of microorganisms, which apparently thrive on this water deriving their nutrition from the components of industrial wastewater and having adapted themselves to detoxicate the contaminants. Bacteria were found dominant in these tannery effluents along with yeast and protozoa. Many of these microorganisms have developed resistance and achieved the capability of metabolizing toxic substances present in polluted soils and waters (Shakoori *et al*., 2001).

Rehman *et al*. (2006) reported that *Tachysoma pellionella* culture grown in the medium containing Cr^{6+} (10 µg/ml) could reduce 77% from the medium after 48 hours, 85% after 72 hours and 92% after 96 hours, respectively. It could also reduce 68% Pb^{2+} after 48 hours, 80% after 72 hours, and 88% after 96 hours from the medium containing Pb^{2+} at a concentration of 10 µg/ml. The presence of metal resistant ciliate in industrial effluents carrying highly toxic metal ions has indicated adaptation of these organisms to environment containing toxic metals and may be employed for metal detoxification operations.

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