DISCUSSION
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Heavy metals get accumulated in the cells. Marine protozoan communities accumulate lead in their cells. The concentrations tested were 500 and 1000 mug cnt dot-l lead as lead acetate. The protozoan was able to bio-accumulate 27.02. 504 mug Pb cnt dot-g-1 dry weight (Fernandez et. al. 1998). *Tetrahymena* has accumulated visible amounts of gold in refractile granules after 24 hrs of exposure in 15 m M aurothiomalate (GSTM) (Nilsson 1997). Accumulation of lead and silver in estuarine Ciliate *Fabrea salina* was measured using the gamma -emitting radio isotopes 210 Pb and 110 m Ag : volume /
volume concentration factors for 110 m Ag in Ciliate ranged from 7 to 40 times. The concentration factors for 210 Pb was 2 times. Bioconcentration of some particle reactive trace metals by the estuarine microzooplanktons can serve as a source of these metals for animals which consume them (Fisher et. al. 1995). Bioconcentration can occur either through ingestion of contaminated food organisms or by direct absorption through integument.

Behavioural changes was observed in *Euglena gracilis* on exposure to high concentration of copper, mercury, cadmium and lead. The precision of orientation was decreased due to frequent deviations of the cells from straight paths. High concentrations also decrease the swimming speed of the cells (Stallwitz and Haeder 1994). Behavioural changes was also observed in *Tetrahymena pyriformis* on exposure to mercury ions at the lowest effective concentration of 0.0001 mg/l for 24 hrs. The rate of cell divisions multiplication of *Tetrahymena pyriformis* decreased with the increase of concentrations of mercury ions. High concentrations of the chemical caused deformation, shrinkage, abnormal movement and even death of the cell (Xu and Xiu 1993).

Mercury compounds are effective SH-enzyme inhibitors (Barron and Levine 1952 and Jennette et. al. 1975). Mercury
compounds can also inhibit non SH-enzyme (Friesell and Hellerman, 1957 and Green and Neurath, 1953). The SH enzymes are more sensitive to every mercury compound tested than non-SH enzyme (Waku and Nakazawa, 1979). In C-mitosis all mercury compounds inactivates spindle fibre mechanism at cell division resulting in aneuploidy / polyploidy (Ahmed and Grant, 1972). Chang and Hartmann (1972) in their investigations by electron microscope histochemical techniques showed mercury to be bound to plasma membrane, lysosomes, endoplasmic reticulum, mitochondria, Golgi complex and the nuclear envelope. Cell bound organo mercurials was found to effect DNA replications and protein synthesis (Gruenwedel et al. 1981).

Mercury is mainly used in agriculture and industries. Refined lead is extensively used in manufacture of lead acid batteries, cables, pigments, alloys etc. Arsenic is used in glass industry, electronics industry and as wood preservatives.

The aquatic organisms vary greatly in their sensitivity and susceptibility to heavy metals. Fargasova (1994) investigated the toxicity of Hg^{2+}(HgCl_2), Cr^{6+} (1) (CNH-4)-2 CrO-4) Cr^{6+} (2) (Cr O_3), Cd^{2+} (CdCl_2 Cnt dot 2, S 4- 20 ), Pb^{2+} (Pb CH-3COO)-2 cnt dot 3 H_2O) on the sensitivity and survival of Daphnia magna and Tubifex.
tubifex. For Daphnia magna, 48 hrs LC-50 rank order toxicity was Hg\textsuperscript{2+} gt Cr-\textsuperscript{6+}(2) gt Cd\textsuperscript{2+} = Cr\textsuperscript{6+} (1) gt Pb\textsuperscript{2+} gt As \textsuperscript{3+} = Cr \textsuperscript{6(1)} gt Pb\textsuperscript{2+} gt Cd\textsuperscript{2+} gt Cr-\textsuperscript{6}(2)gt Cr-\textsuperscript{6}(6) gt Pb\textsuperscript{2+} gt As\textsuperscript{5+}. For Tubifex 96 hrs L C 50 rank order toxicity was Hg-\textsuperscript{2+}gt Cd\textsuperscript{2+} gt Cr\textsuperscript{6+}(2) gt Cr\textsuperscript{6(1)} gt Pb \textsuperscript{2+} gt As \textsuperscript{5+} Daphnia magna was a more sensitive organism than T. tubifex its LC 50 values for all metals were several times lower than LC 50 values rank order toxicity was Hg\textsuperscript{2+} gt Cd \textsuperscript{2+} gt Cr\textsuperscript{6+}(2) gt Cr\textsuperscript{6-1)} gt Pb\textsuperscript{2+} gt As\textsuperscript{5+}. D. magna was a more sensitive orgnaism than T. tubifex and its LC 50 values for all metals were several times lower than LC 50 values for T. tubifex.

In the present investigation for Epistylis rotans L C 50 (24 hrs) rank toxicity order is Hg- \textsuperscript{2+}gt Pb\textsuperscript{3+}gt As\textsuperscript{3+}. The L C 50 values are 0.04 ppm mercury, 4 ppm lead and 30 ppm arsenic. The 24 hrs L C 50 for arsenic is unusually high when compared to mercury and lead. The organism are severely affected at lower dose but 50% are killed only in 30 ppm concentration of arsenic. Heavy metals and related toxic compounds that enter the aquatic ecosystem through rain water and effluents from nearby industries may lead to a reduction of Ciliates population there by affecting food chain and also regeneration of nutrients, upsetting nutrient cycle and also at times altering species composition of Ciliate communities (Lavanya 1974 ).
The heavy metals and trace metals at sub-lethal concentrations are absorbed rapidly and concentrated to many times in the cellular organisation. The Ciliates ability to concentrate heavy metals would enable them to enter aquatic food chain thereby enhancing the toxicants to pass on and probably exert their effect at higher trophic level (Biological magnification) (Jensen and Jernelov 1969, Bhatnagar et.al. 1988).

Temperature, food, pH and chemical composition of media etc. tremendously influence the growth and distribution of Protozoa. The field investigations have shown a close relationship between the density of population of different Ciliates that exist in a particular environment. It has been ascertained that the water temperature, pH, dissolved carbon dioxide dissolved oxygen, silicates, phosphates, nitrite, nitrates, and dissolved organic matter has greater influence on the density of Ciliate population. (Sreedharan 1985).

Noland (1925) studied more than 65 species of fresh water Ciliates with respect to various factors and came to the conclusion that the nature and amount of available food has more to do with the distribution of these organism than any other one factor. Euryphagous protozoa which feed on a variety of food organisms are widely distributed, while stenophagous forms that feed on a few species of
food organisms are limited in their distribution. Thus the density of Ciliate population depends upon the type of food and concentration of the food (Bond 1933, Loefer 1938a, 1938b, Reich 1936, Rottier 1936 and Barlow and Finley 1976).

Many investigators have suggested various recipe for the culturing of protozoans (Woodruff 1912, Glaser and Coria 1930, Hall 1933a, Mast 1939, Pace and Belda 1944, Wichterman 1949, Finley et al. 1959 and Ranganathan 1969). Thus the growth and density of Ciliate population is directly influenced by the food media. The three different food media studied were pea broth, hay infusion and PPCW. The pea broth and hay infusion supports polyxenic population and were easily contaminated and turned turbid and hence abandoned. Where as the PPCW, the optimal concentration was 1:1:10 for the Ciliate *Epistyris rotans* and the growth and development was excellent.

The majority of protozoa seems to prefer a certain range of pH for the maximum metabolic activity. The pH of the medium greatly influence the enzyme activities of the Ciliates (Lawrie 1937). A slight variation in the pH of the medium bring about considerable change in the mophology (Loefer 1938 b) and growth rate which is reflected in the volume of the cell (Kasturi Bai and Tara 1974). A number of
investigators have studied optimum pH for different Ciliates (Saunders 1924, Jerkins 1927, Darby 1930, Loefor 1935, Mast and Pace 1938 and Wichterman 1948).

The optimal pH in the present investigation was found to be pH 7. It was confirmed that slight deviation from the acceptable and optimal limits of pH diminished the density. Thereafter the optimal pH was maintained with the help of 0.1 N NaOH and 0.1N HCl.

The growth of Ciliate population is interrelated to another important factor the temperature. The density of Ciliate population is also temperature dependent. The rate of food intake increases with the increase in temperature it attains a peak and decreases there after (Rogerson 1981). The rate of reproduction is also temperature dependent. The temperature plays an important role in the reproduction rate (Finley 1977) and various aspects of metabolism (Scherbaum 1964, Rosenbaum et. al. 1966, Calkins and Gunn 1967).

In the present investigation the optimal temperature for good growth of *Epistylis rotans* was 25° C.

The *Epistylis rotans* on exposure to the sub-lethal concentration of mercury, lead and arsenic showed a deviation from their normal structure.
At low concentration the effect was negligible but with increase in chemical concentration most of the cells were affected and exhibited changes in morphology and change in oral ciliary activities. Shrinkage of the body was evident with the increase in concentration of the chemical from 0.01 ppm to 0.03 ppm mercury, lead 1 ppm to 3 ppm and arsenic 7.25 ppm to 29 ppm. Dying cells were swollen and rounded with total immobility. Slow contractile activity of the body, change in timings for formation of food and contractile vacuoles was also seen. In all the treated cases the timings for formation of contractile vacuole and food vacuole increased.

Similar morphological changes in fully hydrated Chilomonas paramecium was observed when exposed to copper. The Ciliates on exposure to 2.5 mg/l of copper rounded up within minutes of exposure and were almost spherical within 20 minutes (Abraham 1998).

Euglena gracilis on exposure to Cu gtorey 50 Mu-M, cadmium 3 Mu-M; mercury 1 Mu-M and lead above 300 Mu-M, decreased the swimming velocity and precision of the orientation compared to the control due to frequent deviation of the cells from straight paths (Stallwitz and Haeder 1994).
The Ciliate *Dileptus margaritifer* when exposed to high Potassium concentration are depolarised and are often disfigured by rounding up of previously tapered cell extremities which lead to formation of tailess, hump back or astomous cells. (Golinska - 1996). Similar changes in *Colpidium* and *Vorticella* was observed when exposed to above 500 mg/l of lead acetate. In this study it was observed that a range of distinct changes of motility and morphology like increased excitation and sometimes simultaneous reflexed alterations occurred. The rounding of the bodies occurred also, indicating probably an attempt on the part of the protozoan to resist the toxic effect by reducing the contact surface. Body deformation due to the endo and ectoplasmic evagination is often caused by appearance of “hyaline drops”. During this phase certain changes in the sol-gel structure of the ectoplasm takes place. An increasing volume of pulsating vacuoles appear as the result of an effort to evacuate larger quantities of toxic agents from the cell. Finally plasmolysis, breaking up of cells, disintegration of the pellicle and spilling out of the cytoplasm occurred (Apostol 1973).

A good number of investigators have done extensive studies on the influence of heavy metals on the genetic apparatus and have reported the consequent changes in the nuclear morphology. Ultrastructural studies with reference to the nuclear alterations has

Light microscopic studies showed certain changes in the morphology of nucleus like elongation, condensation, vacuolization, clumping, breakage, beaded nature, diminished affinity towards Feulgan staining etc, in *Epistylis rotans* exposed to all the three heavy metals mercury, lead and arsenic.

Carbohydrates play a vital role in the life of cell organisms as they are indispensable in the growth media for the optimum production of cells. Glycogen has been time and again demonstrated to be a major constituent of Ciliate cytoplasm. The presence of glycogenous bodies in *Paramecium, Glaucoma, Vorticella, Stentor* and parasitic ciliates like *Ophryoscolecidae, Nyctotherus* and *Balantidium* has been reported by Faure - Fremiet and Thaureaux (1944). Remjantez and Wernel (1925) showed the presence of glycogen in *Actinosphaerium*.

Proteins are extremely diversified allowing each animal species and each tissue to have its own kind of proteins. The proteins play a vital role to a great extent enhancing the functions of the living
matrix. Almost all the chemical reaction of cells are catalyzed by enzymes that are proteins (Holter and Kopac 1937), proteinase etc. Lipids are also an important component of Ciliates e.g., Lipids account for 12% of the dry weight of Noctiluca scintillans (Pratje 1921), 3.55 percent of lipid content of Eimeria gadi (Panzer 1913).

The biosynthesis of carbohydrates, proteins, and lipids are integrated and is responsible for the all round process of growth and division of individual cells.

A significant decline in the liver and muscle glycogen on exposure of fish Cyprinus carpio to sub-lethal concentration of chromium (15 mg and 25 mg cntdot 1-1,) was reported by Al-Akel and Shamsi (1996) The liver and muscle glycogen decreased by prolonged exposure and with increasing concentration of chromium.

A decline of carbohydrate was noted in the liver of fish Oesochromius mossambicus exposed to sub-toxic dose of 0.003 ppm, 0.004 ppm and 0.065 ppm of endosulphan after 4,7,8 and 10 days of treatment (Ganeshen et. al. 1989).

A decline in glycogen level occurred in the tissues of fish Cyprinus carpio on exposure to sub-lethal dose of mercury (0.01 mg/l and 0.1 mg/l), nickel (2 and 20 mg/l) and chromium (2 and 20 mg/l) for seven days. Following the metal exposure the glycogen content
in the liver and muscle of fish were measured. Results were statistically compared with a control group which was kept in the same condition without any metal addition. Glycogen levels in the tissues of all metals exposed Carps significantly decreased. When compared to the control levels, mercury caused highest depletion of glycogen in the tissues (upto 96%) and was followed by nickel (upto 80%) and chromium (upto 75%) (Canli 1996). Similar changes caused by different toxic chemicals has been reported by Umminger (1970), Manoharan and Subbiah (1982) Kalarani et. al. (1984), Ramalingam (1988) and Lomte and Muller (1992).

The total carbohydrate content of *Epistyliis rotans* treated with sub-lethal doses of mercury registered a decline from 8.33 μgs ± 0.33 S.D. to 2.08 μgs ± 0.57 S.D. (8.33 μgs ± 0.33 S.D. in controls to 6.04 μgs ± 0.24 S.D in 0.01 ppm, 3.54 μgs ± 0.29 S.D. in 0.02 ppm and 2.08 μgs ± 0.57 S.D. in 0.03 ppm of concentration.

In the case of *Epistyliis rotans* exposed to lead the total carbohydrate content decreased from 8.12 μgs ± 0.50 S.D. in controls to 2.15 μgs ± 0.51 S.D. (8.12 μgs ± 0.50 S.D. in controls to 6.24 μgs ± 0.29 S.D. in 1 ppm, 4.12 μgs ± 0.42 S.D. in 2 ppm, 2.15 μgs ± 0.51 S.D. in 3 ppm).

Similarly, it was evident that the animals treated with arsenic
showed a decline of total carbohydrate content from 8.54 μgs ± 0.29 S.D. in control to 7.36 μgs ± 0.26 S.D. in 7.25 ppm, 4.16 μgs ± 0.29 S.D. in 14.5 ppm and 1.59 μgs ± 0.30 S.D. in 29 ppm.

A greater utilization of carbohydrate occurs under stress conditions in the species with increase in concentration of the chemicals, mercury, lead and arsenic. This in turn causes a decline in carbohydrate content. This fact has been supported by Umminger (1970) that carbohydrates represent the principal and immediate energy precursors for animals exposed to stress conditions.

A decline in the tissue protein content of *Cyprinus carpio* on exposure to sub lethal concentration of mercury (0.01 and 0.1 mg/l), chromium (2 and 20 mg/l) and nickel (2 and 20 mg/L) was noted (Canli 1996). There was a significant decline in the total protein contents in the tissues of metal-exposed animals after seven days over control values, except in the liver and gills of chromium exposed animals. In the heat-treated homogenates, percent protein loss differed between control and metal-exposed fish. Highest protein loss after the heat treatment occurred in chromium exposed fish (up to 48%) and it was followed by nickel (upto 43%) mercury (upto 41%) and control (upto 33%) fish. Except in the chromium experiment, highest percentages of protein loss were found in the muscle, and
were followed by the liver and gills (Canli 1996).

The effect of heavy metals on the blood protein of the fish Cyprinus carpio was investigated: The fish were exposed to two non-essential (Hg and Pb) and two essential (Cu and Ni) heavy metals salts at lethal and sub-lethal concentrations. Blood serum total protein, serum globulin and serum albumin was analysed every 2 hrs for 72 hrs and again at 48 and 72 hrs. Serum protein and globulin level showed a decline from 21 hrs onwards that extended over a period of 72 hrs. Serum albumin showed an initial immediate decline from 2 to 4 hrs followed by an intermittent period of recovery and declined that extended over a period of 72 hrs (Gopal et. al. 1997).

The tissue protein content of Sarotherdon mossambicus decreased on exposure to sub-toxic dose of mercury and other chemicals like malathion and DDT also. There was a steady drop in tissue protein content after 7 and 15 days of exposure when compared with 24 hrs of treatment. In the initial period of exposure the protein content remains almost unaltered due to resistance exhibited by the fish to the modified situation for a definite period, but ultimately succumbs as a result of inability to adopt continuously (Ramalingam and Ramalingam 1982). Similar changes have been reported by Fry (1921), Shakoori et. al. (1970), Mc Leay and Brown.
In the present investigation the total protein content decreased significantly after 24 hrs of exposure.

The *Epistylis rotans* exposed to sub-lethal concentration of mercury showed a steady decline of total protein content from 8.72 mgs ± 0.14 S.D. to 2.61 mgs ± 0.36 S.D. In control, the total protein content was 8.72 mgs ± 0.14 S.D. but declined at 0.01 ppm to 7.76 mgs ± 0.41 S.D. at 0.02 ppm to 4.08 mgs ± 0.50 S.D. and at 0.03 ppm to 2.61 mgs ± 0.36 S.D. In the case of animals exposed to lead a decline in the total protein content was evident with the increase in concentration of the chemicals. A steady decline was recorded from control 8.32 mgs ± 0.34 S.D. 1 ppm - 6.43 mgs ± 0.34 S.D. 2 ppm 4.17 mgs ± 0.29 S.D. and 3 ppm - 2.77 mgs ± 0.30 S.D. Similar results was evident for animals exposed to arsenic. The total protein content decreased from 8.91 mgs ± 0.13 S.D. in controls to 6.49 mgs ± 0.23 S.D. in 7.25 ppm, 4.24 mgs ± 0.30 S.D. in 14.5 ppm and 2.93 mgs ± 0.34 S.D. in 29 ppm in experimental.

A decline in lipid contents was noted in the liver and kidney of *Heteropneustes fossilis* exposed to the toxic effect of lead nitrate (PbNO₃) and mercury nitrate (HgNO₃) on the activity of a few lipids like phospho lipids, neutral lipids in the liver and kidney. Impact of
these heavy metals on hepatic and nephric tissue interfere with lipids metabolism (Gautam and Parihar 1996).

Investigations were done on the toxic effects of selected sub-lethal concentrations of mercuric chloride (HgCl$_2$ 0.05 mg cnt L$^{-1}$), methyl mercuric chloride (CH$_3$ HgCl, 0.04 mg cnt L$^{-1}$) and Emisan 6 (Organic mercurical fungicide, 0.5 mg cnt L$^{-1}$) on levels of lipids - total lipids - TL, Phospho lipids - PL free cholestrol, FC; esterified cholestrol EC. and free fatty acids FFA. of the ovary and liver in *Clarius batrachus* exposed for 45, 90 and 180 days. The total lipid level decreased significantly only in the ovary of 180 days Hg groups. The ovarian PL and FC levels showed a significant reduction at all durations of Hg exposure. The EC level in the ovary showed a significant decline after both 90 and 180 days of Hg treatment. Where as in the liver it decreased significantly only after 180 days HgCl$_2$ groups. The results suggest that the decrease in ovarian lipid levels is largely due to their immobilization from the liver and the reduction in vitellogenin content may be due to both the inhibition of its synthesis in the liver and subsequent incorporation into the ovary (Kirubagaran and Joy 1995).

The total lipid content of treated *Epistyliis rotans* with mercury showed a decline from 4.76 µgs ± 0.49 S.D. to 1.45 µgs ± 0.05 S.D.
and 3.45 µgs ± 0.16 S.D. in 0.01 ppm, 2.26 µgs ± 0.15 S.D. in 0.02 ppm and 1.45 µgs ± 0.05 S.D.in 0.03 ppm.

Like wise *Epistylis rotans* exposed to lead showed a decrease in total lipid content from 4.52 µgs to ± 0.33 S.D. to 1.58 µgs ± 0.08 S.D. and 3.53 µgs ± 0.11 S.D. in 1 ppm, 2.05 µgs ± 0.09 S.D. in 2 ppm and 1.58 µgs ± 0.08 S.D. in 3 ppm.

Similarly, it was evident that the animals treated with arsenic registered a decline of total lipid content from 4.64 µgs ± 0.29 S.D. in controls to 1.73 µgs ± 0.08 S.D., 3.60 µgs ± 0.20 S.D. in 7.25 ppm, 2.42 µgs ± 0.13 S.D. in 14.5 ppm, 1.73 µgs ± 0.08 S.D. in 29 ppm.

A gradual decline in total lipid content in the species with increase in concentration of all the three chemicals is due to failure of the cells to synthesize lipids and utilization of lipids for the energy demand associated with the situation of stress. This supports the view of Harpert et. al. (1977), Rao and Rao (1981) and Gautam and Parihar (1996).

Further, a measurement of oxygen consumption of medium with animals exposed to heavy metals indicate that oxygen tension is affected. The growth of protozoans is tremendously influenced by the oxygen tension of the medium.
Imel (1915), Juday (1919), Hall (1933b), Rottier (1936a), Moore (1939), Sprugel (1951) and Finley and Mc Laughlin (1963) pointed out that the Peritrich population fluctuates with the rise and fall of amount of dissolved oxygen. The oxygen consumption of *Mystus gulio* was altered due to exposure to different concentration of CuSO₄. Copper was proved to be potent respiratory inhibitor than zinc (Sultana and Devi 1995). The effects of low oxygen on 24 hrs survival rates of *Goby* and *Bay anchovy* was tested. Naked *Goby* and *Bay anchovy* larvae strongly avoided dissolved oxygen concentration in 1 mg/l, which were lethal within 24 hrs at 25 to 27°C. In addition naked *Goby* larvae, whose behaviour was tested at a wider range of dissolved oxygen concentration also showed a reduced preference of an oxygen concentration of 2 mg/l, which leads to reduced survival during long-term exposures and to reduced feeding rates Klewkoski and Zivgzds (1971) Sigmond (1979) and Brietburg (1994) confirms that oxygen consumption is a useful measurement that can be used as a indicator of over all physiological state. Zinc concentration > 89 µg/L in river Yamuna around Delhi (decreased algal biomass) decrease dissolved oxygen concentration (Katiyar 1997). When *Daphnia pulex* was treated with 5.0 µg cadmium, the rate of oxygen consumption was almost doubled to that of the control (Dean et. al. 1980).
In the present investigation the dissolved oxygen content of the medium alone was $8.62 \text{ ml} \pm 0.08 \text{ S.D./l}$ and it decreased in the control with *Epistylys rotans* to $5.37 \text{ ml} \pm 0.42 \text{ S.D.}$. At 0.01 ppm of mercury the D.O. content decreased to $7.53 \text{ ml} \pm 0.82 \text{ S.D.} /l$. There was a further decrease in D.O. content to $6.73 \text{ ml} \pm 0.35 \text{ S.D./l}$ at 0.02 ppm and $4.93 \text{ ml} \pm 0.26 \text{ S.D./l}$ at 0.03 ppm.

In the case of lead there was a decrease of D.O. content from $8.62 \text{ ml} \pm 0.02 \text{ S.D.} /l$ in medium alone to $5.23 \text{ ml} \pm 0.09 \text{ S.D.} /l$ in the controls. At 1 ppm it decreased to $7.82 \text{ ml} \pm 0.22 \text{ S.D./l}$ There was a further gradual decrease to $6.84 \text{ ml} \pm 0.18 \text{ S.D./l}$ at 2 ppm and $5.48 \text{ ml} \pm 0.35 \text{ S.D./l}$ at 3 ppm was evident.

Similar situation was evident with respect to *Epistylys rotans* in arsenic medium, where the dissolved oxygen content was inversely proportional to the increase in concentration of the metals. The medium alone had $8.62 \text{ ml} \pm 0.02 \text{ S.D.} \text{ D.O./l}$. The D.O. of the control was $5.27 \text{ ml} \pm 0.21 \text{ S.D./l}$. At 7.25 ppm of arsenic the D.O. decreased to $7.70 \text{ ml} \pm 0.25 \text{ S.D./l}$. There was a further sudden decrease to $6.93 \text{ ml} \pm 0.16 \text{ S.D./l}$ at 14.5 ppm and $5.75 \text{ ml} \pm 0.20 \text{ S.D./l}$ at 29 ppm.

Decrease in the dissolved oxygen content in the final stage of chemical treatment that is 0.03 ppm of mercury, 3 ppm of lead and
29 ppm of arsenic containing the same number of Epistyliis rotans indicates clearly the fact that in the initial stages the organism will be under sudden stress and later the oxidative process increases where by there will be a decrease in oxygen content.