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RESEARCH ARTICLE

Dietary Magnesium Requirement of *Cyprinus carpio* VAR. *Communis.*

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ABSTRACT

The requirement of *Cyprinus carpio* for dietary magnesium was investigated by feeding them with a basal diet containing ($MgCo_3$: 0.1 to 1.0 /100g) different levels of magnesium, in the laboratory condition for the period of 28 days.The basal diet of 50% contained waste silkworm pupae used as the main source of protein with fishmeal and oilcake.The highest growth rate (7.905 mg/g live fish /day) occurred in the fish fed on a diet supplemented with 0.079% magnesium.The proximate composition (protein, fat, ash and water), content of the tested fishes were varied according to the dietary magnesium levels. Judging from the growth rate of the fish, adequate magnesium content of the diet of *Cyprinus carpio* was estimated to be 0.079%.

Key words: *Cyprinus carpio,* Silkworm pupae, Magnesium, Dietary requirement.

INTRODUCTION

In several countries where hatchery and culture practices are better-established supplementary feeding with the feedstuffs formulated into pellets form the backbone of the intensive fish culture. Considerable work has been done in India on supplementary feeding as a fish culture practice. In India attention is being given for silk production. In silk reeling centres, the silkworm pupae are discarded.This silkworm pupae is rich in protein as well as fat [7,18,13].Hence, it is felt that the silkworm pupae may be utilized to replace the fishmeal in formulating fish diet. Jeyachandran and Paul Raj (1977)[8] have stated that the silkworm pupae as raw materials induced the growth of carps when used instead of fishmeal in the diet. According to Venkatesh et al (1986)[30] the diet containing silkworm pupae enhances the growth of catfish when compared to meat meal and groundnut oilcake. Quality of food is the nutritional level of food. The fish food should contain all the essential nutrients as components. The fishes, which are reared on such food, will grow normally without any deficiency diseases. Hence it is stated that the efficient and economically effective fish husbandry depends on fish nutrition and diet development. Research and development organizations focus their attention on the basic nutritional requirements of cultivated species and the development of least count formulations based on commonly available ingredients. So much attention is being given to basic and

applied fish nutrition to furnish useful data for formulating artificial fish diets. Minerals required by fish are calcium, magnesium, phosphorus and a number of trace elements such as copper, iodine, iron, manganese, selenium and zinc [14,15,23].Magnesium is essential for the activation of a great number of enzymes involved in protein and carbohydrate metabolism [16,17] and for the maintenance of intra and extra cellular homeostasis in fish[12]. Feeding efficiency ratio and dietary protein utilization were significantly influenced by dietary requirements of magnesium for *Cyprinus carpio*, *Salmo gairdneri* and *Ictalurus punctatus* range from 0.04 to 0.08 of dry weight [3,19,20,5]. Fishmeal, the major source of protein in formulated diet contains 0.01 to 0.07% magnesium.

This satisfies the level of the suggested magnesium requirement for fish. But Satoh et al (1983a,b)[25,26] have found poor growth and feed efficiency together with deficiency symptoms in fish, which was fed on diet without magnesium supplementation. Lall (1979)[16] has stated that the uptake of magnesium from the environment is insufficient to satisfy the metabolic demand of freshwater fishes. The survey of literature on the requirements of magnesium for fishes indicates that the supplementation of magnesium is necessary. Hence, the present study was conducted to determine the dietary magnesium level and their effects on the growth and proximate composition of *Cyprinus carpio*.

MATERIALS AND METHODS

Feeds: The percentage composition and biochemical composition of basal diet are presented in Table 1. The basal diet was formulated from 50% silkworm pupae, 20% fishmeal, 19% oil cake, 10% mydha and 1% vitamin premix. These four components are dried in sunlight and subsequently dried at 60^{o}C for 8 hours in hot air oven. Then they were powdered and sieved. The basal feed mixture was added to source for magnesium to prepare different diets as given in Table 2.These components were mixed and pelleted in a pelletizer, without steam and stored in bins.

Table 1.Composition and biochemical composition of basal diet

Ingredient	Percent	Biochemical composition	Percent
Silkworm pupae	50	Moisture	08.2 ± 0.20
		Protein	45.1 ± 0.31
Fish meal	20	Lipid	23.6 ± 0.21
		Ash	21.5 ± 0.08
Oil cake	19	**Minerals**	
		Magnesium	00.051
Mydha	10	Calcium	01.097
		Phosphorus	00.714
Vitamin Premix	01	Energy value (KJ)	22.363

Table 2. Diets with basal feed mixture and Magnesium supplementation and their total Magnesium content.

Diets with Magnesium supplementation	Feed mixture (g)	Magnesium source supplemented ($MgCo_3$) (g)	Total Magnesium Content (%)
M1 (Control)	100	0.0	0.051
M2	100	0.1	0.079
M3	100	0.2	0.109
M4	100	0.4	0.165
M5	100	0.6	0.223
M6	100	0.8	0.279
M7	100	1.0	0.337

Experimental Design: Juveniles of *Cyprinus carpio* collected from Aliyar dam near Pollachi, TamilNadu, were brought to the laboratory and acclimated to the laboratory condition and feeding schedule, keeping them in fibre glass (acrylic) aquaria(60x30x30cm) at room temperature (28 ±1^{O}C). The acclimation period lasted for 15 days. Individuals showing signs of disease or injury were removed during the acclimation period. Stockfishes were fed ad-libitum with chopped pieces of fresh goat liver twice a day for a period of two hours at each time (8 –10 AM & 6-8 PM) and gradually switched over to formulate diet. Un-eaten food was removed from the tank after the feeding schedule. Aquarium water was changed daily to keep it clean. Well-acclimated juveniles of *Cyprinus carpio* range of 1.228 to 1.754 g were selected from the stock. Six different groups (Table 2) (C-M1, C-M2, C-M3, C-M4, C-M5, C-M6 and C-M7), each with 10 individuals were introduced into rectangular acrylic tanks (60X30X30cm) containing 20 litres of tap water. The six different groups were fed on an ad-libitum diet of formulated diets (approximately 5% of its live body weight and the food given was adjusted according to the increment in the weight of fish) with different levels of magnesium supplementation such as M1, M2, M3, M4, M5, M6 and M7 (Table 2). Triplicate was maintained for each diet. The test fish were fed twice a day at 8 AM and 6 PM for two hours at each time. The aquarium water was renewed daily to keep it clean. The rearing experiment was carried out for 28 days. Each group was weighed accurately on the first day of experimentation for their live weight. To find out the initial dry weight of test individuals, five sample individuals of similar body weight and experimental state were sacrificed and dried to weight constant in hot air oven. The test individuals were weighed periodically on 15 days interval period.On the day of termination of experiment, the test individuals were weighed in live condition and dried to weight constant to estimate the final dry weight of test individuals. The dried samples of test fish were stored in a desicator for subsequent biochemical analysis.

Data processing and evaluation of growth

Growth = Final live weight – Initial live weight.
Growth rate = Wt. gained by the fish / Initial Wt. of fish X No. of expt. days

Analyses: The feeds and tested fishes were assayed for moisture [2], Protein[9], Fat (Soxhlet with Chloroform and Methanol), Ash (560^{O}C), Energy[6,10], Phosphorus [11], Calcium and Magnesium [27] method, respectively.

Statistical analysis: Data from the magnesium requirement studies were subjected to analysis of variance and Student Newman - Keul's Multiple Range Test (SPSS/PC) to determine differences in means ($P< 0.05$).

RESULTS AND DISCUSSION

Cyprinus carpio grows well on the diet of M2.The results revealed that the fish exhibited maximum growth rate i.e., 7.905 mg live weight/ g live fish/ day when fed on diet M2. Then there was a gradual decrease in the growth rate of fish as a function of increase in the magnesium content of diet and reached the level of 4.341 mg live weight/ g live fish/ day when the fish was fed on diet with 0.337% magnesium. (Table 3). To be more appropriate, the growth rate of *Cyprinus carpio* when reared on different diets supplemented with different levels of magnesium is expressed in terms of energy value in Table 3. In terms of energy also *Cyprinus carpio* exhibited the same trend of growth as observed in terms of live weight. The fish group, which exhibited maximum growth in terms of energy value, found as M-M2 i.e., the fish group which received 0.079% magnesium when compared to all other fish groups, which received diets with higher levels of magnesium supplementation (Table 3).

One- way analysis of variance of growth rate of different groups of individuals revealed that the different diets have significant effects on growth and hence, the groups of fish were heterogenous. Further, the growth rate data were subjected to Student Newman - Keul's test and classified in to two homogenous subsets at 0.05 level of significance. From this, it is clear that the diet C-M2, which gave maximum growth, might have contained the sufficient amount of magnesium. Hence, it is concluded that the diets of that groups with 0.079 mg were considered as the optimum requirement for *Cyprinus carpio*. Hence, this diet was considered as optimum one. The optimum requirement of these fishes is compared to that of other fishes studied. The optimum magnesium requirement for *O.niloticus* ranges from 0.059 to 0.077% for better growth and feed conversion [4]. Further, they have stated that growth increased with increasing magnesium concentration up to 0.1% in the diet. For *O.aureus*, the magnesium requirement ranges from 0.05 to 0.065% for better growth rate [24].

The magnesium requirement for the catfish is 0.04%[5]. When compared to these values, the optimum dietary requirement of magnesium for *Cyprinus carpio* is greater. This may be due to the fact as suggested by Dabrowska et al (1989b)[4]. They have stated that the magnesium requirement increases with increase in protein content of diet. In our present investigation, the protein content of diet is around 45%. Hence, the magnesium requirement of *Cyprinus carpio* through diet is greater.The proximate chemical composition of *Cyprinus carpio*, which was reared on diets supplemented with different levels of magnesium, was presented in Table 4 and Fig.1.The fish group C-M2, which was fed on diet with 0.079% magnesium, exhibited protein content 57.20%. This level was more when compared to other fish groups, which received diets with higher levels of magnesium supplementation. The fat content of different fish groups did not have any definite relationship with protein content; it had inverse relationship with water.

Generally, the fast growing fishes contain more amount of protein. In the present observation also the fishes, which grow fast, contain more amount of protein. It appeared that the fish groups, which received optimum level of dietary magnesium supplementation, contained more protein. Viola et al (1986a)[31] have reported that the protein content of tilapia was high when it was reared on optimum level of phosphorus. According to them, this may be due to activation of protein synthesizing enzymes. Further they have stated that the fat content was correspondingly decreased and this may be due to the inhibition of breakdown of fat by β oxidation. Similar observation has been made by a number of authors in different species of fishes [28,32]. Takeuchi & Nakazoe (1981)[29] have also observed that the fat content decreases as the phosphorus level increases in the diet[28,1]. This fact also conformed by the present observation on the water content and fat content of fishes, which are reared on diets containing different levels of magnesium. The ash content did not vary significantly (Fig.1). The ash content does not show any significant variation as observed by Arunachalam (1986)[1] in *M.vittatus*, which were reared at different environmental conditions.

Table 3. Effects of different levels of magnesium supplementation in diets on growth (live weight gained) of *Cyprinus carpio* (1.228 ± 0.450g). Each value is the average (± SD) performance of 3 groups, consist of 10 individuals.

Diets	Magnesium (%)	Total growth (mg)	Growth rate (mg/ g live fish	Percentage increased
C-M1 ©	0.051	1785.95 ± 14.15	7.205 ± 0.75[b]	172.90
C-M2	0.079	1885.11 ±13.62	7.905 ± 0.05[a]	189.79
C-M3	0.109	1120.27 ± 21.02	4.564 ± 0.06[d]	109.53
C-M4	0.165	1464.38 ± 22.38	4.873 ± 0.10[c]	116.95
C-M5	0.223	1126.62 ± 21.02	4.624 ± 0.08[d]	110.98
C-M6	0.279	1010.45 ± 25.31	4.341± 0.09[c]	104.18
C-M7	0.337	1059.25 ± 26.15	4.341 ± 0.10[c]	104.20

©=Control Diet; Means in columns that have different superscripts are significantly different (P<0.05).

Table 4. Effects of dietary magnesium on body composition, enzymes and whole body mineral content of *Cyprinus carpio*.

Fish group	DM (%)	Water (%)	Protein (%)	Fat (%)	Ash (%)	Whole body mineral content (%)		
						P	Ca	Mg
C-M1 ©	0.051	79.20 ± 2.12	56.50±1.56	22.30 ±1.07	17.90 ± 0.71	1.986[ab]	3.143[bc]	0.120[a]
C-M2	0.079	79.20± 1.97	57.20±1.19	21.15 ± 1.20	17.72 ± 0.83	2.080[b]	3.120[bc]	0.127[b]
C-M3	0.109	78.20 ±2.00	54.70±1.19	23.20 ± 1.43	17.80 ± 0.79	1.990[ab]	3.261[c]	0.132[bc]
C-M4	0.165	78.00± 1.12	53.20±1.16	23.80 ± 1.25	18.10 ± 0.86	2.016[ab]	3.015[ab]	0.139[cd]
C-M5	0.223	77.98± 1.25	52.96±1.79	24.68 ± 1.25	18.05 ± 0.56	1.914[a]	2.963[a]	0.141[d]
C-M6	0.279	77.28± 1.16	51.82±1.23	24.95 ± 1.35	18.11 ± 0.68	2.106[ab]	3.054[ab]	0.143[4d]
C-M7	0.337	76.40± 1.98	50.60±1.30	25.80 ± 1.38	18.12 ± 0.79	2.018[b]	3.252[d]	0.146[d]

© = Control Diet, DM (%) = Dietary magnesium

Means in columns that have different superscripts are significantly different (P<0.05).

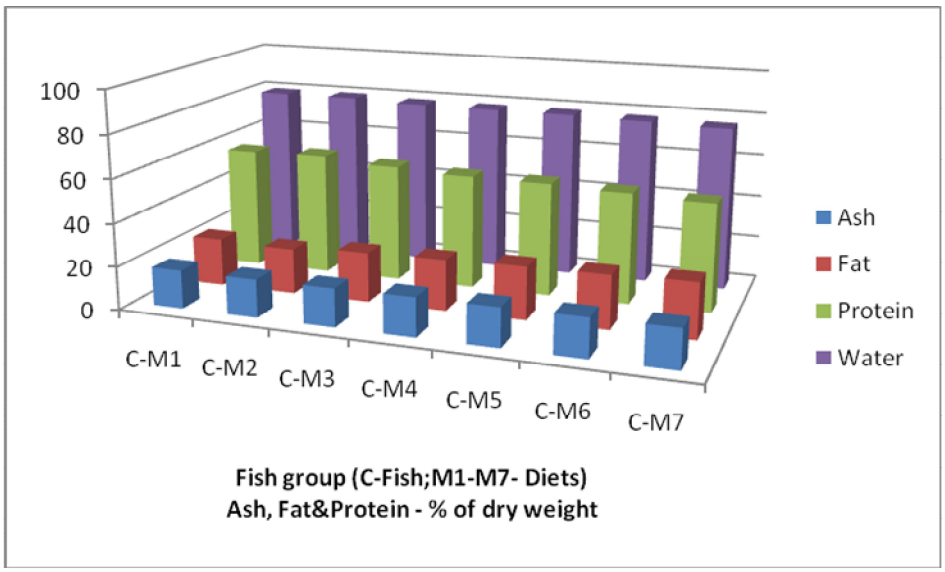

Fig. 1. Effect of Magnesium supplementation to diet on body composition of *Cyprinus carpio*.

From the whole body mineral retention studies in *Cyprinus carpio* which was 0.120% in the fish receiving control diet with 0.051% magnesium increased up to 0.146% when the dietary magnesium level increased to 0.337% (Table 4). As per the statistical analysis (One-way Anova and Student Newman – Keul's test), the dietary magnesium levels have the significant effects on magnesium retention in *Cyprinus carpio*. But the variation in whole body phosphorus and calcium was not consistent with the level of magnesium (Table 4). Similar observations have been made on whole body magnesium content of fishes, fed on graded levels of magnesium. Ogino & Chiou (1976)[21], who have studied the effect of dietary magnesium level on whole body content of magnesium content increased with dietary magnesium levels and that the other minerals like calcium and phosphorus did not show any consistent changes in relation to dietary magnesium levels.

Ogino et al (1978)[20]have also reported that the whole body magnesium content of rainbow trout increased from 0.084 to 0.146%, when it was fed on diets supplemented with increasing levels of magnesium from 0.046% to 0.779%, while other minerals did not show drastic changes. Dabrowska et al (1989b)[4] have found that the dietary magnesium levels did not affect calcium and phosphorus concentration. In the present observation, it is derived that the mineral, which was given as supplementation with diet, increased in the body according to the levels of supplementation, while other minerals did not show any consistent changes. The body mineral content is more than the dietary level of the same, and this may be for normal health and mineralisation [21,22].

REFERENCES

1. Arunachalam, S.1986. Physiological studies on Freshwater catfish. Ph.D.Thesis.Madurai Kamaraj University, Madurai,India,148 p.
2. Association of Official Analytical Chemists (AOAC), 1975. Official Methods of Analysis Washington. DC.USA.P.1094.
3. Dabrowska, H., Gunther, K. D. & Meyer – Burgdorff, K. 1989a. Availability of various magnesium compounds to tilapia (*Oreochromis niloticus*). Aquaculture, 76: 269 – 276.

4. Dabrowska, H. Meyer – Burgdorff, K. & Gunther, K. D. 1989b. Interaction between dietary protein and magnesium level in tilapia (*Oreochromis mossambicus*). Aquaculture, 76: 269 – 276.
5. Gatlin, D. M., III.Robinson, E. H., Poe, W. E. & Wilson, R. P.1982. Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. Journal of Nutrition, 112:1182 – 1187.
6. Jana, B. 1980.Calorific values as functions of mainbody constituents in some freshwater teleosts. Indian Journal of Fisheries, 27: 269 – 272.
7. Jeyachandran, P. & Paul Raj, S. 1976. Experiments with artificial feeds on *Cyprinus carpio* fingerlings Journal of Inland fisheries Society India, 8:33 – 37.
8. Jeyachandran, P. & Paul Raj, S. 1977.Formulation of pelleted feeds and feeding trials with common carp. Journal of Inland fisheries Society India, 9:46-52.
9. Jeyaraman, J. 1981. Laboratory manual in biochemistry. Wiley Eastern Ltd. p.77-78.
10. Fisher, Z. 1979.Selected problems of fish bioenergetics. PP.17 – 44. In: Proceedings of World Symposium on Finfish Nutrition and Fishfeed Technology, Vol 1 Eds: J.E.Halver and K.Tiews H.Heenemann GMBH and Co, Berlin, Germany.
11. Fiske,C.H.&Subbarow,Y. 1925.The colorimetric determination of calcium.Journal of Biological Chemistry, 66: 375 – 400.
12. Houston, A. H. 1985.Erthrocyte magnesium in freshwater fishes. Magnesium, 4: 106 - 128.
13. Kandeepan, C. & Arunachalam, S. 2003. Phosphorus supplementation to fish diet containing silk worm pupae as a protein source.Proceedings of International Seminar on Recent Advances In Biotechnology, 24th and 25th Feb.2003.P.R.College, Tanjore, India p.48 – 55.
14. Kandeepan,C. & Arunachalam, S. 2005.Dietary phosphorus requirement of *Oreochromis mossambicus*.Journal of Ecobiology, 17(6):571 – 579.
15. Kandeepan, C & Poongulali,C.2009. Dietary magnesium requirement of the *Oreochromis mossambicus*. Journal of Natural Science and Technology – Life Sciences and Bioinformatics,1:67 – 72.
16. Lall, S. P. 1979. Minerals in finfish nutrition. p.85-97. In Proceedings of World Symposium on Finfish Nutrition and Fish feed Technology, Eds:J.E.Halver and K. Tiews, Vol I, H. Heenemann GMbH and Co, Berlin, Germany.
17. Muthukumar, P. & Kandeepan, C. 2009. Effects of diets containing different proportions of chicken intestine waste on growth of *Mystus cavicius*. Journal of Natural Science and Technology – Life Sciences and Bioinformatics, 1:78–80.
18. Nandeesha, M. C., Srikanth, G. K., Kesavanath, P., Vargesh, J. & Shetty, H. P. C. 1990. Growth performance of *Cyprinus carpio var communis* fed on diets containing different levels of deoiled silkworm pupae, Pp 271-274. In: Proc. Second Asian Fiseries Forum, Eds.R.Hirano and I.Hanyu. Asian Fish Soc.Manila, and the Philippines.
19. Ogino, C. & Chiou, J. Y. 1976.Mineral requirements in fish II.Magnesium requirement of carp.Bulletin of the Japanese Society of Scientific Fisheries, 42: 71-75.
20. Ogino, C., Takashima, F. & Chiou, J. Y. 1978. Requirement of Rainbow trout for dietary magnesium. Bulletin of the Japanese Society of Scientific Fisheries, 42: 793 – 799.
21. Ogino,C. & Takeda, II.1976. Mineral requirements in fish III.Calcium and Calcium requirements in carp. Bulletin of the Japanese Society of Scientific Fisheries, 42: 793- 799.
22. Oser, B. L. 1965 Hawk's Physiological Chemistry 14th Ed. Tata McGraw Hill Publishing Co., Ltd.Bombay.NewDelhi.p.1-1472.
23. Poongulali, C. & Kandeepan, C.2008. Dietary calcium requirement of the catfish *Mystus vittatus*. Journal of Ecobiology, 23(2):187 – 195.
24. Reigh, R. C., Robinson, E. H. & Brown, P. B. 1991. Effects of dietary magnesium on growth and tissue content of blue tilapia *Oreochromis aureus* Journal of World Aquaculture Society, 2(3):192-199.

25. Satoh, S., Yamamoto, H., Takeuchi, T. & Watanabe, T.1983a. Effects on growth and mineral composition of rainbow trout of deletion of trace elements or magnesium from fish meal diet. Bulletin of the Japanese Society of Scientific Fisheries, 49:425 – 429.
26. Satoh, S., Yamamoto, H., Takeuchi, T. & Watanabe, T. 1983b.Effects on growth and mineral composition of carp of deletion of trace elements or magnesium from fish meal diet. Bulletin of the Japanese Society of Scientific Fisheries, 49:431 – 435.
27. Tacon, A.G. J. & De Silva, S.S. 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture, 31: 11-20.
28. Tacon, A.G. J., Knox, D. & Cowey, C. B. 1984. Effects of different dietary levels of salt mixtures on growth and body composition in carp. Bulletin of the Japanese Society of Scientific Fisheries, 50: 1217-1222.
29. Takeuchi, M. & Nakazoe, J. 1981.Effects of dietary calcium on lipid content and its composition in carp. Bulletin of the Japanese Society of Scientific Fisheries, 47:347-652.
30. Venkatesh, B., Mukerji, A. P., Mukhopadhyay, P. K. & Dehadraai, P.V. 1986. Growth and metabolism of the catfish *Clarias batrachus* (Linn.) fed with different experimental diets. Proceedings of Indian Academic Science, 95 (4): 457 – 462.
31. Viola, S. Zohar, G. & Arieli, Y. 1986a. Phosphorus requirement and its availability from different sources for intensive pond culture in Israel part 1.Tilapia.Bamidgeh 38 (1): 3 – 12.
32. Wooten, I. D. P. 1964. In: Microanalysis in medical biochemistry. J and A Churchill Ltd., London W.1.

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RESEARCH ARTICLE

Characterization of Biosynthesized Silver Nanoparticles and its Antibacterial Activity for Detection of Food -Borne Pathogens.

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ABSTRACT

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Besides the rather established chemical and physical production methods of silver nanoparticles, biological production systems are of special interest due to their effectiveness and flexibility.Silver nanoparticles are attractive as these are non toxic to human body at low concentration and having broad spectrum antibacterial nature. Synthesis using biological method and characterization using SEM, AFM, FTIR, UV-Vis spectroscopy,XRD were previously reported[1][2].In this present study, TEM (Transmission Electron Microscopy), Antibacterial activity of silver nanoparticles have been obtained.TEM Interpretation and analysis shows that the particles are uniformly dispersed with an average size of 30-40nm and the shape are more or less spherical. Further, antibacterial activity shows that the synthesized silver nanoparticles could effectively inhibit against various pathogenic organisms found in food such as *Escherichia coli*, *Pseudomonas aurignosa*. From the results obtained, silver nanoparticles are efficient growth inhibitors and can be used for further research and development along with sensor scheme to detect pathogens in food.

Key words: Transmission Electron Microscopy, silver nanoparticles, Antibacterial activity.

INTRODUCTION

Nanotechnology is enabling technology that deals with nano sized objects. Nano biotechnology combines biological principles with physical and chemical procedures to generate nano sized objects with unique functions. Green synthesis of small silver nanoparticles with experimental processes is evolving into an important branch of nano technology.Today, nano metal particles like silver is emerging their unique and extensive application in the advanced

developmental areas of electronics, medicine and in material sciences at nano scale level [4]. Human beings are often affected by the diseases spread by bacteria and viruses in their environments. Even though many scientists researched to develop new effective antibacterial agents to eliminate the resistances caused by microorganisms and are cost effective. This led to the proclamation of silver based antiseptics which produce silver ions that can be known to exert strong inhibitory and bactericidal effects.

Biological methods of synthesis of silver nanoparticles using microorganisms, enzymes, from plant extracts have been proposed as the environmentally friendly alternatives to physical and chemical methods [2]. The extracellular synthesis and characterization of silver nanoparticles using *Escherichia coli* have been reported previously [1]. Biologically synthesized silver nanoparticles were widely used in biosensor, biolabeling, cancer therapeutics and in the coating of medical appliances [3].Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to smaller size. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surfaces. Ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that DNA loses its replication ability once bacteria treated with silver ions [5].

The aim of the present study is to characterize and analyze the size and structure of silver nanoparticles using TEM. The average size of the silver nanoparticles thus obtained was measured in the range of 30-40nm and the shape found to be in spherical.Antibacterial effect of silver nanoparticles was evaluated against six pathogenic bacteria including *Escherichia coli, Staphylococcus aureus, Pseudomonas aurignosa,* and *Vibrio cholera, Micrococcus leutus, Salmonalla typhi* using the common agar disc diffusion method. This was conducted for six microorganisms to measure the diameter of zone of inhibition of silver nanoparticles.

MATERIALS AND METHODS

TEM Analysis

For TEM measurement for particle shape, size and its distribution, a 50 µl drop of solution containing biosynthesized silver nanoparticles was placed on the carbon- coated copper grids and subjected to vacuum desiccation before loading onto a specimen holder. TEM micrographs were taken by analyzing the prepared grids on TEM instrument having a low voltage (100 kV) construction [6].

Antibacterial Activity

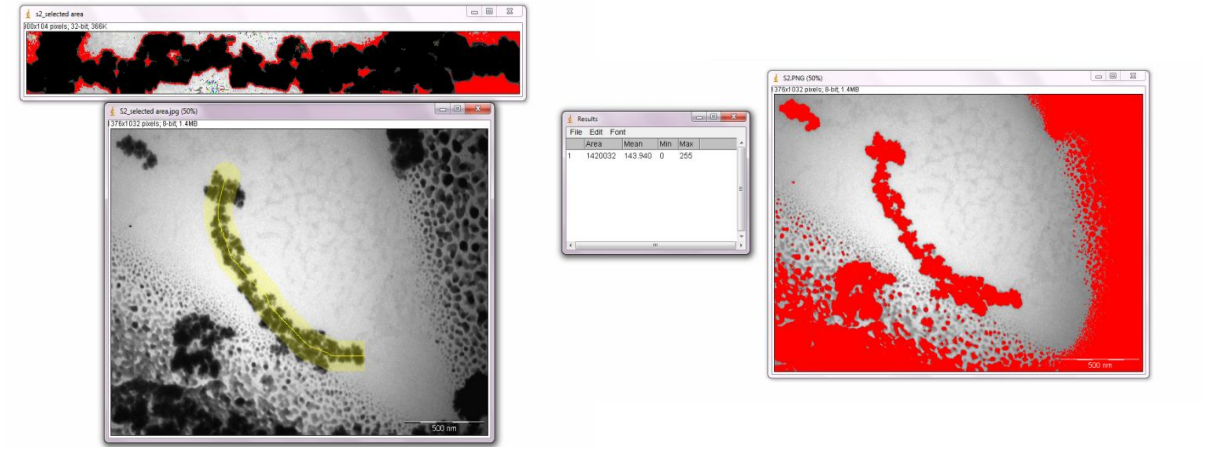
The Antibacterial activities of silver nanoparticles were carried out by Agar disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification, the bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10ug/ml) and placed in the nutrient agar plate and kept for incubation at 37^0C for 24 hours. The diameter of zones of inhibition for control, silver nanoparticles, antibiotics and antibiotics with silver nanoparticles were measured using ruler.

Silver nanoparticles synthesized using microbial filtrate is tested for its potential antibacterial activity against six bacterial strains *Escherichia coli, Staphylococcus aureus, Micrococcus leutus, Salmonella typhi, Pseudomonas aurignosa,* and *Vibrio cholera.,*were used as the test organisms. The antibiotic Amoxylin (500mg Dosage) was used as antibacterial drug for comparative study against silver nanoparticle.

RESULTS AND DISCUSSION

TEM Image Analysis

TEM analysis revealed that the biosynthesized nanoparticles are stable in solution at room temperature. The size of nanoparticles ranges from 30-40nm (Fig: 1), the decrease in anisotropy and particle size is evident from the images. The TEM images revealed equal spherical shape and orthorhombic crystals.



Slice	Count	Total Area	Average Size(nm)	Shape
For selected area	53	1998	37.698	Spherical

Fig: 1. TEM Image Analysis of Silver Nanoparticles

Antibacterial Activity

Zone of Inhibition in the plate showed that silver nanoparticles synthesized using filtrate has the antibacterial activity against test pathogens namely *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aureginosa, Vibrio cholera,* and *Micrococcus leutus.* On comparison with the antibiotic (Amoxylin(500mg Dosage) silver nanoparticles outperformed in the antibacterial effect. According to antibacterial activity method the silver nanoparticles from *microbes (Escherichia coli &Pseudomonas aureginosa)* showed the Inhibition zone equal or more than that of Antibiotic (Maximum: 8.5mm in mL).

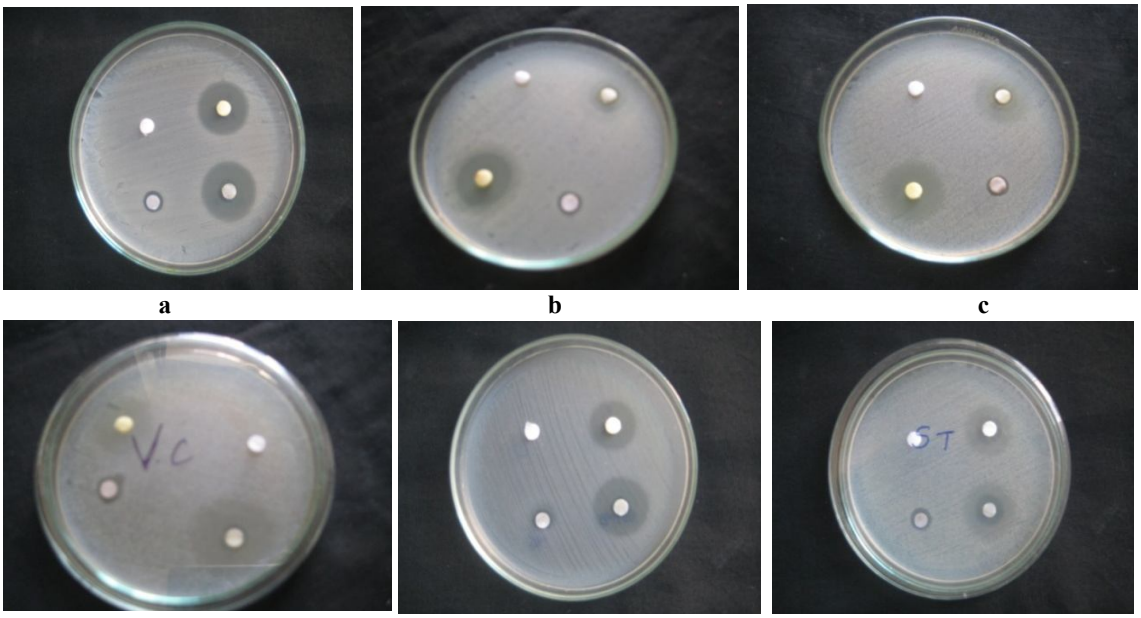


Fig: 2. a) ***Escherichia coli, b) Pseudomonas aurignosa, c) Micrococcus leutus, d) Vibrio cholera, e) Staphylococcus aureus, f) Salmonella typhi***

Table: 1.Antibacterial activity against six bacterial strains, the diameter of inhibition of zone was measured in mm.

Sl.No.	Organism	Control (c)	Antibiotics (ab)	Nano-particles (nps)	Antibiotics+ Nanoparticles (ab+np)
1	*Escherichia coli(E.C)-*	10	22	9	23
2	*Pseudomonas aurignosa (P.A)-*	9	18	9	22
3	*Vibrio cholera(V.C)-*	9	24	11	26
4	*Micrococcus leutus(M.L)+*	10	25	10	26
5	*Staphylococcus aureus (S.A)+*	9	24	9	25
6	*Salmonella typhi (S.T)_*	10	27	10	29

CONCLUSION

TEM results in the formation of monodispersed spherical nanoparticles of the size range 30-40 nm. The nanoparticles have been shown to have a potent antimicrobial effect against human pathogen *E.coli.* From the table: 1 it is observed that the silver nanoparticles with antibiotics shown 90% of zone of inhibition. From bacterical effect or cell viability test, by increasing the concentration of silver nanoparticles the optical density (O.D) decreases gradually. It is also believed that silver nanoparticles after penetration into the bacteria have lost the activity of their enzymes, generating hydrogen peroxide and caused bacterial cell death [4]. These silver nanoparticles thus obtained will be in future for detection of food borne pathogens with a scheme of biosensor.

REFERENCES

1. V.Manonmani,Vimala Juliet, "Biosynthesis of Ag Nanoparticles For The Detection Of Pathogenic Bacteria In Food", 2011 International Conference on Innovation, Management and Service, IPEDR vol.14(2011) IACSIT Press, Singapore.

2. V.Manonmani,Vimala Juliet, "Synthesis and Characterization of Silver nanoparticles from Escherichia Coli MTCC 118",CiiT International Journal of Biometrics and Bioinformatics,Vol 4,No: 1,January 2012.

3. Maribel G. Guzmán, Jean Dille, Stephan Godet, "Synthesis of silver nanoparticles by chemical reduction method and their antibacterial Activity", International Journal of Chemical and Biological Engineering 2:3 2009.

4. Sridhara veeranna,Ali Burhanuddin,Satapathy Lakshmi Narayana and Khandelwal Pratima, "Biosynthesis and Antibacterial activity of silver nanoparticles", Research of Biotechnology,Vol. 8(1),January(2013).

5. Gaikwad Sagar and Bhosale Ashok ,"Green Synthesis of Silver Nanoparticles Using *Aspergillus niger* and Its Efficacy Against Human Pathogens", European Journal of Experimental Biology, 2012, 2 (5):1654-1658

6. Ratan Das, Sneha Gang, Siddhartha Sankar Nath, "Preparation and Antibacterial Activity of Silver Nanoparticles", *Journal of Biomaterials and Nanobiotechnology*, 2011, 2, 472-475 doi:10.4236/jbnb.2011.24057 Published Online October 2011.

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RESEARCH ARTICLE

Labiatae- Potential Sources of Antifungal Compounds.

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ABSTRACT

Labiatae alludes to the flowers typically having petals fused into an upper and lower lip, the flower thus having an open mouth.The present study was undertaken as a part of research to find out the efficacy of crude extract of 3 plants belonging to the family lamiaceae *Anisochilus carnosus*, *Ocimum canum* and *Hyptis suaveolens* , members of labiatae on fungi. The efficacy of the essential oils of a number of plants of Lamiaceae has been reported against bacteria, viruses, pests, and in the control of myriads of human diseases. The methanol extract at the concentration of 50 and 100 µg/ ml was more effective against *Aspergillus ochareceous* than the positive control Fluconozole. The results indicate that the crude aqueous extract of *Anisochilus carnosus, Hyptis suaveolens,* and *Ocimum canum* did not exhibit antifungal activity but the acetone and methanol extract of *Anisochilus* and *Hyptis* did exhibit anti fungal activity.

Key words: Labiatae, Fluconozole, Lamiaceae, anti –fungal activity.

INTRODUCTION

The family labiatae was established by De Jussieu in 1789 as the Order Labiatae. The name Labiatae alludes to the flowers typically having petals fused into an upper and lower lip, the flower thus having an open mouth. Although Labiatae is an acceptable, botanists more often use Lamiaceae after the genus *Lamium.* The family is divided into several subfamilies and tribes of which subfamily Nepetoideae has the most genera. The Lamiaceae is closely related to the family Verbenaceae.

The present study was undertaken as a part of research to find out the efficacy of crude extract of 3 plants belonging to the family lamiaceae *Anisochilus carnosus*, *Ocimum canum* and *Hyptis suaveolens* , members of labiatae on fungi. The chemicals present in plants are classified as primary and secondary metabolites. The primary metabolites are biochemicals that are essential for plant growth and survival. These include sugars, amino acids, nucleic acids and

chlorophylls. Secondary metabolites make up the remaining plant chemicals, and includes alkaloids, phenols etc. Many members of lamiaceae are known for the presence of essential oils. The compositon of phytoconstituents of the essential oils havebeen known to vary. The efficacy of the essential oils of a number of plants of Lamiaceae have been reported against bacteria, viruses,pests, and in the control of myriads of human diseases. It is difficult for a layman to get the essential oils. Most people in villages depend on traditional medicines, and resort to taking medicines in the form of infusion, powders and chew raw leaves.

The term fungi refer to a wide range of life forms that grow on both living and dead organic marerials. The 3 large classes of fungi a layman recognizes are Mushrooms, yeasts and molds. Fungi are essential players in the ecosystem, participating in the recycling of dead organic matter.Fungi as infecting parasites can be hostile to other living creatures – plants and animals, large and small. Human beings use fungi in food production and are hosts to resident species such as *Candida*. Fungi produce disease in different ways. The most obvious are skin infections. Surface infections of the scalp produce itching and Scaling (dandruff) and are a life – long feature of most humans. Invasive fungal infections can be life threatening and are more difficult to diagnose and treat than bacterial infections. The fungi chosen for study were Aspergillus niger and Aspergillus ochareceous, Candida albicans, Fusarium semitectum and *Trichophyton rubrum*.

Most fungicides that can be bought retail are in liquid form. A very common active ingredient is sulfur. Present at 0.08% in weaker concentrates and as high as 0.05% for more potent fungicides. Fungicides in powdered form that are usually around 90% sulfur are very toxic. Indiscriminate use of Synthetic fungicides pose a number of problems regarding its toxicity, efficacy, cost and has also resulted in emergence of resistant strains. Public pressure to reduce the use of synthetic fungicides in agriculture has increased. Fungicide residues have been found on food for human consumption, mostly from post – harvest treatments. Some fungicides are dangerous to human health such as Vinclozin, which have been removed from use [2]. It has become necessary to emphasize the use of phytochemicals for the control of Fungi in order to address the concerns raised about the impact on strain on the environment and potential health risk related to the use of these compounds.

The test fraction of crude extract of Mentha spicata showed considerable anti fungal activity against *Trichophyton longifusus, Microsporum canis, Candida albicans, Aspergillus flavus,Fusariu solani, Candida glaberata*[2,3] Labdane diterpenoids hispanolone[5,6], dehydrohispanolone[5]and ballonigrine[5,6] isolated from Ballota inaequidens and .saxatilis subsp. Saxatilis were reported as antifungal agents against *Candida albicans* and *C. krusei*. The abietane diterpenoids 2,3-dehydrosalipisone and 7-oxo-royleanone [4] isolated from Salvia sclarea and primarane diterpenoid sandracopimaric acid showed moderate activity against Candida albicans[7]. The antifungal activity of pisiferic acid against rice blast fungus was reported by Kobayashi,etal.,[8] and carnosic acid 12-methyl ether against Alternaria[9].

MATERIALS AND METHODS

Antifungal activity

The use of a simple extraction procedure of Olukoya et al., was followed to prepare aqueous and organic extracts of plants tested. To prepare aqueous extracts, 1.1g of plants' leaves (previously dried at 50 c and ground into fine powder) was steeped in 10ml distilled water at 30-32 c for five days. The organic extracts were prepared by steeping 1.2g of plant material in 5 m of 40% ethanol. Extracts were then passed through Hemmings filters (BTI UK) and the resulting sterile filterates were aseptically transferred to sterile bottles. The organic extracts were subsequently reconstituted with phosphate buffered saline solution (ph 7.2) to nullify the effect of ethanol on the tested organism.

Dilution method

Minimum Inhibitory Concentration (MIC) was carried out by agar dilution method [1]. The powder samples were dissolved in acetone, methanol and aqueous at the following concentrations: 25, 50 and 100 µg/ml. The antifungal assay was carried out on PDA medium for 48-72 hours at 27°C. Observations were performed in duplicate and results (MIC) expressed as the lowest concentration of plant extract that produced a complete suppression of colony growth.

Antibiotic sensitivity test on microbes (Positive control)

The antibiotic sensitivity test using standard antibiotic (fluconozal) were analysed by following the method.The sterilized PDA medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh fungal cultures with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic namely fluconozal (25µg/ml) were placed on the fungal culture plates. Then, the plates were incubated for 27°C for 48-72 hours. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

Antifungal effects of solvents (Negative control)

The antifungal activities of acetone and methanol solvents were tested against the selected fungal strains. The sterilized PDA medium was poured into each sterile petriplates and allowed to solidify by using a sterile cotton swabs, a fresh fungal culture with known population count was spread over the plates by following spread plate technique. A well known quantity of acetone and methanol and distilled water. Then, the plates were incubated for 27°C for 48-72 hours. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

RESULTS AND DISCUSSION

The results indicate that the crude aqueous extract of *Anisochilus carnosus*, *Hyptis suaveolens* , and *Ocimum canum* did not exhibit antifungal activity but the acetone and methanol extract of *Anisochilus* and *Hyptis* did exhibit anti – fungal activity. The acetone extract of *Anisochilus* at concentration of 100µg/ ml was almost as effective as the positive control against *Aspergillus niger*. The methanol extract at the concentration of 50 and 100 µg/ ml was more effective against *Aspergillus ochareceous* than the positive control Fluconozole. The extracts were also ineffective in control of *Candida albicans*. The acetone extract at 100 µg/ ml conc. was effective in controlling *Fusarium semitectum*. The acetone extract at 50and 100 µg/ ml and methanol extract at the conc. 100 µg/ml was more effective against Trichophyton rubrum when compared to the positive control. The Acetone and methanol extract of Hyptis suaveolens at the conc. 100 µg/ml was effective against *Aspergillus niger*, and the methanol extract at the conc.100µg/ml was almost as effective as the positive control against *Fusarium semitectum* while the acetone and methanol extract at the conc.50 and100 µg/ml was more effective than the positive control against *Trichophyton rubrum.*

The Acetone extract of *Ocimu canum* at the conc. 100 µg/ml was effective the positive control against *Fusarium semitectum* while the acetone at the conc.50 and 100 µg/ml and the methanol extract at the concentration of 100µg/ ml was more effective against T. rubrum when compared to the positive control.The acetone extract of *Ocimum* at 25 and 50µg/ml conc. did not show inhibitory effect on *Aspergillus niger* and *Aspergillus ochareceous.* From the above, it can be concluded that many plants of this family could be viewed as natural, easily accessible, potential sources of anti-fungal agents . These could be regarded as relatively safe alternative to the synthetic fungicides.

REFERENCES

1. Mitscher, LA, Lev R, Bathala MS, Wu W, Beal JL (1972). Antimicrobial agents from higher plants. Introduction, rational and methodology. Lioydia. 35: 157-166
2. Hrelia, 1996- The genetic and non-genetic toxicity of Fungicide Vinclozolin Mutagenesis, vol 11, 445-453.
3. Naseem Ullah , , Muhammud Khuram, Muhammad Usman A min, Hamid Hussain Afridi,Farhat Ali Khan, Shibzada Muhammud Khayam, Saleem Ullah, Umberin Najeb, Javid Hussain and M. Asif Khan, comparison of phytochemical constituents and anti microbial activities of Mentha spicata from four Northern districts of Khyber Pakhtunkhwa, Journal of Applied Pharmaceutical Science 01(17);2011,72-76.
4. Ulubelen .G, Topcu. C, Eirs u, Sonmez.M, Kartal.S., Kurucu and C. Bozok Johansson(1994), Trpenoids from Salvia Scalarea, Phytochemistry 36, 971-974.
5. G. Citoglu, M. Tanker, B. Sever, J. Englert, R. Anton and N. Altanlar (1998) . Antibacterial activity of diterpenoids isolated from Ballota saxatilis subsp saxatilis, Plata Med . 64, 484-485.
6. G. Citoglu, B. Sever, S. Antus, E. Baitz- Gacs and N. Altanlar(2004), Antifungal diterpenoids and flavonoids from Ballota inaquidens, Pharma Biol. 42, 659-663 .
7. G.Topcu, R. Erenler, O. Cakmak, C.B. Johansson, C. Celik, H.B. Chai and J.M. Pezzuto(1999), Diterpenes from berries of Juniperus exelsa, Phytochemistry, 50,1195-1199.
8. K. Kobayashi, C. Nishino, H. Tomita and M. Fukushima(1987), Antifungal activity of pisiferic acid derivatives against rice blast fungus , Phytochemistry, 26, 3175-3179.
9. M. Miyakado, T.Kato, N. Ohno,T.J. Mabry(1976), Pinocembrin and (+)- β-eudesmol from Hymenoclea monogyra and Bacchris glutinosa. Phytochemistry15, 846.

Tab.1. Antifungal activity of sample-1

S.No.	Name of the Organisms	Zone of inhibition at different concentration (µg/ml) (diameter in mm)								
		Acetone			Methanol			Aqueous		
		25	50	100	25	50	100	25	50	100
1.	*Aspergillus niger*	5	10	12	7	9	10	-	-	-
2.	*Aspergillus ochareceous*	2	3	5	5	15	20	-	-	-
3.	*Candida albicans*	7	7	10	5	8	10	-	-	-
4.	*Fusarium semitectum*	3	5	10	3	5	8	-	-	-
5.	*Trichophyton rubrum*	6	9	11	5	6	12	-	-	-

Tab.2.Antifungal activity of sample-2

S.No.	Name of the Organisms	Zone of inhibition at different concentration (µg/ml) (diameter in mm)								
		Acetone			Methanol			Aqueous		
		25	50	100	25	50	100	25	50	100
1.	*Aspergillus niger*	5	10	12	8	10	13	-	-	-
2.	*Aspergillus ochareceous*	3	5	8	2	3	5	-	-	-
3.	*Candida albicans*	3	7	12	3	5	10	-	-	-
4.	*Fusarium semitectum*	2	3	5	2	4	10	-	-	-
5.	*Trichophyton rubrum*	7	10	13	6	10	12	-	-	-

Tab.3.Antifungal activity of sample-3

S.No.	Name of the Organisms	Zone of inhibition at different concentration (µg/ml) (diameter in mm)								
		Acetone			Methanol			Aqueous		
		25	50	100	25	50	100	25	50	100
1.	*Aspergillus niger*	-	-	5	3	5	7	-	-	-
2.	*Aspergillus ochareceous*	-	-	5	-	3	5	-	-	-
3.	*Candida albicans*	3	7	10	3	5	7	-	-	-
4.	*Fusarium semitectum*	3	5	10	5	5	12	-	-	-
5.	*Trichophyton rubrum*	5	9	14	3	5	13	-	-	-

Tab.4.Effect of standard Antibiotic (Positive control)

S.No.	Name of the organisms	Zone of inhibition (mm)
		Fluconozole
1	*Aspergillus niger*	12
2	*Aspergillus ochareceous*	11
3	*Candida albicans*	13
4	*Fusarium semitectum*	10
5	*Trichophyton rubrum*	7

Tab.5.Effect of solvents (Negative control)

S.No	Name of the Organisms	Zone of inhibition (mm)		
		Acetone	Methanol	Aqueous
1.	*Aspergillus niger*	-	-	-
2.	*Aspergillus ochareceous*	-	-	-
3.	*Candida albicans*	-	-	-
4.	*Fusarium semitectum*	-	-	-
5.	*Trichophyton rubrum*	-	-	-

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RESEARCH ARTICLE

Impact of Mercury Chloride and Lead Nitrate on Haematological Profile of Fresh Water Fish, *Catla catla.*

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ABSTRACT

The present study was to evaluate heavy metal toxicity stress symptoms in fish blood during short-term exposure of sublethal concentration of mercuric chloride ($HgCl_2$) and lead nitrate (Pb(NO3)2). Lead and mercury may alter tissue biochemical parameters in fishes. *Catla catla* was exposed to different sub-lethal concentrations of selected metal lead nitrate and mercuric chloride @ 10% and 20% level. LC_{50} values for 96 hr was determined for each metal and found to be 5.0mg/l for lead nitrate, 75µg/l for mercuric chloride. To investigate hematological parameters on 2^{nd}, 4^{th} and 6^{th} day of exposure on Lead and mercury. To decline in the total number of RBC and haemoglobin content on exposure to lead nitrate and mercury chloride and slightly increase WBC, when treated with lead nitrate and mercuric chloride. Reduction of RBC and haemoglobin leads to haemolysis erythropoitic disorders, haemodilution and hypochronic microlytic anaemia. Increase of WBC suggestive of gradual loss of defensive mechanism against infection, lymphocytosis and inflammatory reactions.

Keywords: *Catla catla*, Blood Parameters, Lead nitrate, Mercury chloride.

INTRODUCTION

Industrial developments in last few decades have increased the concentration of lead, mercury and some other heavy metals in river and lake, affected fishes and deplete natural resources. Some of the industrial effluents release inorganic mercuric compounds like mercuric chloride, which is converted into more toxic organic form *viz*., methyl

mercury, through bacterial action [23]. Heavy metals have become major environmental hazards, although they have great biological significance as micronutrients. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and on the diversity of aquatic organisms [3,4,5,7]. Major sources of Mercury chloride ($HgCl_2$) and Lead nitrate ($Pb(NO_3)_2$) in aquatic environment are sewage and industrial effluents. Lead and Mercury toxicity to fishes had already been reported by many workers [14,15,16,25]. Fishes are the simple and reliable biomarker of lead and mercury pollution of aquatic bodies. This metal are present in water enters the fish body and gets accumulated in various organs like liver and kidney [2,6,20]. The blood parameters have been used as sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, etc. These heavy metals are the probable major cause of the physiological abnormalities in fish. The metals responsible for toxicity are those whose toxicity and or mobility are enhanced by the depression of pH, hardness and alkalinity that typically accompany these events. A variety of heavy metals including Mercury chloride ($HgCl_2$) and Lead nitrate ($Pb(NO_3)_2$) are in use from the ages for industrial progress. Both flora and fauna including human beings have suffered a loss on account of heavy metal pollution of water resources throughout the globes. The present investigation is carried out to evaluate the changes Hb, RBC and WBC parameters of the freshwater fish, *Catla catla* after exposing to sublethal concentration of Mercury chloride ($HgCl_2$) and Lead nitrate ($Pb(NO_3)_2$).

MATERIALS AND METHODS

Test species

Specimens of *Catla catla were* collected from Paalaru dam near Palani, Dindigul District, and Tamilnadu, India. Specimens were acclimatized to laboratory conditions for 15 days. Water was changed daily and fish were fed *oilcake* with flour pellets. For experimental studies, fish ranging from 9 to 14 cm in length and weighing 14.5 to 18 g were selected.

Chemicals

Lead nitrate and mercury chlorides were obtained from Merck [Merck Company, Darmstadt, Germany] Glasxo India Limited, Bombay, India and used without further purification. All chemicals used were of analytical grade.

Experimental design

The qualities of the water was determined according to the method of Apha et al. (1976) and were as follows: dissolved oxygen 5.4 ± 0.02 mg/L; pH 8.6 ± 0.2; water temperature 28.0 ± 2.0°C; salinity 38 ± 0.07 ppt; total hardness 8.2 ± 2.0 mg/L; calcium 5.0 ± 0.1 mg/L; magnesium 3.0 ± 2.0 and total alkalinity 16.0 ± 06 mg/L. Preliminary studies were carried out to find out the sub lethal concentration (LC_{50}) of lead and mercury for 96 h by Probit analysis method of Finnery (1978)[8]. The concentration at which 50% survival/mortality occurred was taken as a sub lethal concentration (LC_{50}) for 96 h, which was 5.0mg/l for lead and 75µg/l for mercury. Acute toxicity studies were conducted for 96 h with a sampling interval of 24 h with three replicates of one treatment. All the tanks were filled with 20 L of water and 5.0mg/l for lead and 75µg/l for mercury was added to each tank. Ten fish were introduced into each tank. A common control was also maintained. The experimental set up had 3 replicates. Toxicant was renewed daily in all the experimental tanks.

Collection of blood samples

At the end of every 24 h, live fishes were taken from the experimental medium and blood was drawn from the caudal vein by using a disposable hypodermic syringe. EDTA was used as anticoagulant. The blood was used for the analysis of various heamatological parameters. RBC count was made by diluting 200 time of blood with Hendrick's solution[11]; WBC count was made by diluting 20 time of blood with acetic acid solution containing two drops methyl blue per 100 ml solution. Hb content of blood was estimated using Shali's Haemogloinometer with

permanent coloured glass comparison standard. For the assessment of short-term toxicity of the Lead nitrate and Mercury chloride, two different concentrations of each metal representing 10% and 20% of the LC_{50} level in 96hr.

RESULTS AND DISCUSSION

In the present study, exposure of fish to sub-lethal concentration of lead nitrate and Mercury chloride for every 24 hr (2, 4 and 6 days) caused significant alterations in haematological parameters of Indian freshwater fish, *Catla catla*. For the assessment of short-term toxicity of the Lead nitrate and Mercury chloride, two different concentrations of each metal representing 10% and 20% of the LC_{50} 96hr. The concentration at which 50% survival/mortality occurred was taken as a median lethal concentration (LC_{50}) for 96 h, which was 5.0mg/l for lead and 75µg/l for mercury. Table 1 and Figure 1 shows the changes in the haemoglobin (Hb), RBC and WBC content of fish exposed to lead and mercury. The Hb and RBC content decreased ($p < 0.05$); whereas the WBC content increased throughout the exposure period showing minimum percent decrease of at the end of 6th day. The same trend was obtained Oreochromis mossambicus to sub-lethal concentrations of lead has been shown to produce haemolytic anaemia due to lysis of erythrocytes with concomitant decrease in Hb% [19]. Lead have been reported to alter the properties of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable [12,22,24]. which probably results in cell swelling deformation and damage observed. Rai and Qayyum (1984)[18] noted a gradual decrease in RBC and Hb concentration due to toxification of lead in catla stated that anemic condition of fish attributed to haemolysis or erythropoitic disorder. So, Lead nitrate exposure of this study might be due to haemolysis or erythropoitic disorder and haemodilution as suggested.

Table.1: Haematological Parameters of *Catla catla* on different Exposure of Lead nitrate.

% of the treatment	2nd day			4th day			6th day		
	Hb*	RBC*	WBC*	Hb*	RBC*	WBC*	Hb*	RBC*	WBC*
Control	8.53±0.28	3.06±0.14	4.06±0.05	8.48±0.14	3.02±0.11	4.04±0.11	8.46±0.18	3.02±0.09	4.01±0.07
10% Treated	8.23±0.08	2.86±0.12	4.13±0.11	8.03±0.13	2.73±0.05	4.19±0.16	7.56±0.09	2.63±0.05	4.24±0.08
20% Treated	8.18±0.04	2.63±0.05	4.21±0.17	7.83±0.15	2.56±0.08	4.26±0.11	6.63±0.30	2.36±0.08	4.37±0.21

Values significantly different from control ($p < 0.05$)

Hb* measured = g/100ml , RBC* measured = 10^6 cells/mm^3 , WBC* measured = 10^3cells/mm

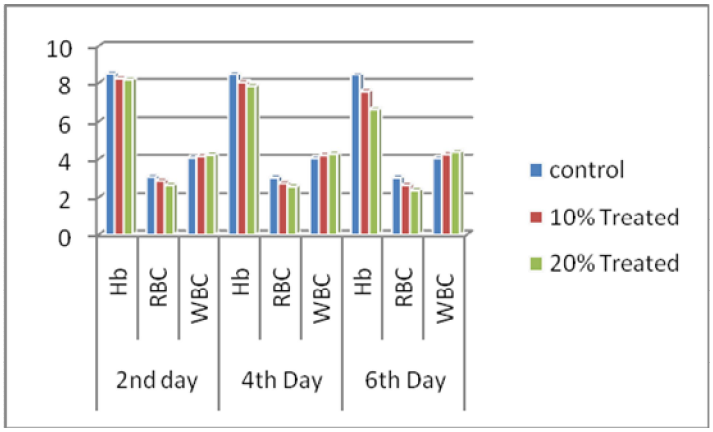

Fig.1: Effect of sub-lethal concentration of lead nitrate haematological parameter in *Catla catla*.

Table 2 and Figure 2 shows the changes in the haemoglobin (Hb), RBC and WBC content of fish exposed to mercury chloride. The Hb and RBC content decreased ($p < 0.05$); whereas the WBC content increased throughout the exposure period showing minimum percent decrease of at the end of 6th day. A fall in RBC count, Hb% and enzymatic action, in the fish, Channa punctatus upon treatment with both lead and mercury was noticed along with acute anaemia [6,21].The decreased haemoglobin synthesis might be of cytolysis due to disturbances in RNA synthesis. S.S.Patil and P.V.Jabde (1998)[17] suggested that on acute exposure to mercuric chloride, cellular changes like anisocytosis, poikilocytosis and hypocromia as well as condensation of nuclei were observed in some erythrocytes leading to anaemia.

Table.2: Haematological Parameters of *Catla catla* on different Exposure of Mercury chloride.

% of the treatment	2nd day			4th day			6th day		
	Hb*	RBC*	WBC*	Hb*	RBC*	WBC*	Hb*	RBC*	WBC*
Control	8.53±0.28	3.06±0.14	4.03±0.05	8.48±0.23	3.03±0.12	4.06±0.35	8.43±0.24	2.93±0.19	4.06±0.06
10% Treated	7.56±0.56	2.93±0.32	4.11±0.11	7.46±0.39	2.63±0.33	4.13±0.08	6.73±0.41	2.13±0.37	4.36±0.08
20% Treated	7.43±0.45	2.86±0.33	4.18±0.07	6.26±0.47	2.33±0.49	4.24±0.07	5.63±0.54	1.86±0.08	4.47±0.11

Values significantly different from control ($p < 0.05$)

Hb* measured = g/100ml,RBC* measured = 10^6 cells/mm^3 , WBC* measured = 10^3cells/mm

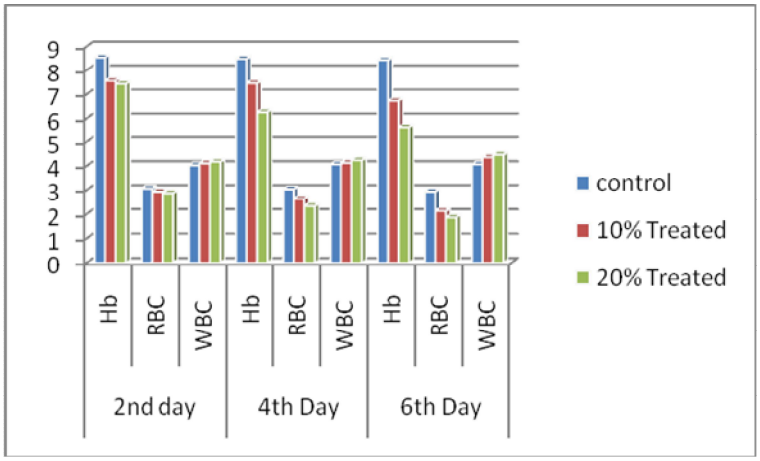

Fig.2: Effect of sub-lethal concentration of Mercury chloride haematological parameter in *Catla catla*

When the adequate exposure of mercury is known to alter the hematologic system of hosts by inhibiting the activities of several enzymes involved in heme biosynthesis. Once absorbed, it is distributed particularly to the liver, kidney, heart and gonads, as well as to the immune system. Similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals have been reported previously by Goel et al. (1985)[10] and Goel and Sharma (1987)[9]. Joshi et al. (2002)[13] suggested that heavy metal exposure also decreased the RBC and Hb% due to impaired intestinal absorption of iron. Anaemia is an early manifestation of acute and chronic intoxication of heavy metals. Reduction of RBC and haemoglobin leads to haemolysis erythropoitic disorders, haemodilution and hypochronic microlytic anaemia. Increase of WBC suggestive of gradual loss of defensive mechanism against

infection, lymphocytosis and inflammatory reactions. High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. Allen (1994)[1] suggested that haematological measurements have been used as indicators of the state offish health condition and as a biochemical method for assessing the possible mode of action of stressors. Thus, it is concluded that the haematological parameters are the most sensitive parameters in monitoring the toxicity of lead and mercury especially at sub-lethal concentrations.

REFERENCES

1. Allen, P. (1994): Changes in the haematological profile of the cichlid *Oreochromis aureus* (Steindochner) during acute inorganic mercury intoxication. Comp. Biochem. Physiol., *108* C (1): 117-121.
2. Al-Mohanna, M.M.: (1994). Residues of some heavy metals in fishes collected from (Red Sea coast) Jizan, Saudi Arabia. J. Environ. Biol., 15, 149-157
3. APHA, AWWA, WPCF (1976). Standard Methods for the Examination of Water and Waste Water. 14th Edn, American Public Health Association. Washington, USA.
4. APHA.: (2005).Standard methods for the examination of water and wastewater. 21st Edn., Washington, D.C.
5. Christensen, G.M., J.M. McKim, W.A. Brungs and E.P. Hunt: (1972).Changes in the blood of the brown bullhead (Ictalurus nebulosus (Lesueur)) following short and long term exposure to copper (II). Toxicol. Appl. Pharmacol., 23, 417-427.
6. Dharam Singh, Kamlesh Nath, S.P. Trivedi and Y.K. Sharma.2008, Impact of copper on haematological profile of freshwater fish, Channa punctatus, Journal of Environmental Biology. 29(2) 253-257.
7. Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R . 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River, *Int. J.Environ.Res. Public Health*, 4 (2): 158 -165.
8. Finney DJ (1978). Statistical Methods in Biological Assay. 3rd Edn. Griffin Press, London. 508 p.*gariepinus)* exposed to a metal finishing company effluent, Indian Journal of Science and Technology Vol. 5 No. 4 ;2510-2514.
9. Goel, K.A. and S.D. Sharma: (1987). Some haematological characteristics of Clarias batrachus under metallic stress of arsenic. Comp. Physiol. Ecol., 12, 63-66.
10. Goel, K.A., K. Gupta and M.L. Sharma: (1985). Haematological characteristic of Heteropneustes fossilis under the stress of zinc. Ind. J. Fish., 36,186-188 .
11. Hessor.E.F. (1960). Methods of routine fish haematology, Frog. Fish Culture, 22:164-171.
12. J. A. Adakole (2012). Changes in some haematological parameters of the African catfish (*Clarias*
13. Joshi, P.K., M. Bose and D. Harish: (2002). Haematological changes in the blood of Clarias battrachus exposed to mercuric chloride. Ecotoxicol. Environ. Monit., 12, 119-122.
14. K. Roy George, N. A. Malini and G. O. Sandhya rani, 2012. Biochemical changes in liver and muscle of the cichlid, *Oreochromis mossambicus* exposed to sub-lethal concentration of mercuric chloride, *Indian J. Fish.,1 59(2)* : *147-152.*
15. Magdy A, Salah El-Deen, Reda E. Saleh, Tay E Abd El-Razik and E I Saiad K. Abo-Hegab 2001. Recorded changes in some biochemical indicators of grasscarp *Ctenopharyngodon Idella* exposed to mercury and zinc, *Egypt. J. Aquat. Biol & Fish., VoL5, No. 2 :* 57-81.
16. Oliveira-Ribeiro CA, Neto FF, Mela M, Silva PH, Randi MAF, Rabitto IS, Alves Costa JRM, Pelletier E. (2006). Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. Environ. Res. 101:74-80.
17. Patil, S.S., Jabde, P.V.: (1998). Eff ect of mercury poisoning on some haematological parameters from a fresh water fi sh, *Channa gachua*. Pollut. Res., 17: 223-228.
18. Rai, R. and M.A. Qayyum. 1984. Haematological responses in a freshwater fish to experimental lead poisoning. Jour. Environ. Biol. 5:53-56.
19. Sampath, K., R. James and K.M. Akbar Ali: (1998). Effects of copper and zinc on blood parameters and prediction of their recovery in Oreochromis mossambicus. Ind. J. Fish., 45, 129-139.

20. Shukla, V., M. Dhankhar, J. Prakash and K.V. Sastry: (2007). Bioaccumulation of Zn,Cu and Cd in Channa punctatus. J. Environ. Biol., 28, 395-397.
21. Singh, M. (1995).Haematological responses in a fresh water teleost, Channa punctatus to experimental copper and Cr poisoning. J. Environ. Biol., 16, 339-341.
22. Vinodhini R and Narayanan M. (2009). The Impact of toxic heavy metals on the hematological parameters in common Carp (*Cyprinus carpio* L.*) Iran. J.Environ.Health. Sci. Eng.* 6(1), 23-28
23. Wiener, J. G. and Spry, D. J. 1996. *Toxicological significance of mercury in freshwater fish,* Lewis Publishers, Baca Raton, Fla, 265 pp.
24. Witeska M and Kosciuk B. (2003). Changes in common carp blood after short-term zinc exposure.*Environ. Sci. Pollut. Res*. 3, 15–24.
25. Zeynab Abedi, Fatemeh Hasantabar, Mohammad K. Khalesi and Sedigheh Babaei. 2013; Effect of Sublethal Concentrations of Cadmium, Lead and Chromium on Some Enzymatic Activities of Common Carp; *Cyprinus carpio.* World Journal of Zoology 8 (1): 98-105, 2013.

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RESEARCH ARTICLE

Physico – Chemical Analysis and Biodiversity of Microbes in Tannery Effluent.

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ABSTRACT

An investigation was carried out to assess the tannery effluent collected from Trichirappalli district, Tamil Nadu, India (April 2010 – March 2011) on physico – chemical parameters and microbial diversity viz. bacteria and fungi. One year study revealed that seven species of bacteria and nine species of fungi were observed from the effluent. Among bacteria, *Pseudomonas* and *Bacillus* with two species and others with single species each were recorded. Among fungi, *Aspergillus* was dominant group (4 species) were recorded. High amounts of phosphates and nitrates with sufficient amount of oxidisable organic matter, and slightly alkaline pH were probably the factors favouring the growth of microbes especially bacteria.

Key words: Tannery effluent, Physico – chemical parameters, Microbial diversity, Bacteria

INTRODUCTION

The tanning process is almost wholly a wet process that consumes high amount of water estimated to be 34 – 56 m^3 of water per tone of hide or skin processed [18], where out of the total water consumed, 85% is discharged as a wastewater [32]. The characteristics of the wastewater vary considerably from tannery to tannery depending upon the size of the tannery, chemicals used for the specific process, amount of water used and type of final product produced by a tannery. According to Seyoum Leta *et al*. (2003)[27], a composite tannery wastewater has BOD (1900 - 4800 mg/l), COD (7900 - 15200 mg/l), sulphide (325 - 930 mg/l) and total chromium (12 - 64 mg/l).

Another study in Pakistan also indicated BOD (840 - 18620 mg/l), COD (1320 - 54000 mg/l), SS (220 - 1610 mg/l), TN (236 - 350 m/l), sulphate (800 - 6480 mg/l), sulphide (800 - 6480 mg/l) and chromium (41 – 133 mg/l)[12]. The variations of effluent characteristics also occur through each working day in a tannery. According to Cristina *et al*. (2007)[7], average COD and pH analysed in one day were 2010 mg /l (± 516) and 6.98 (± 0.05), respectively, whereas 2068 mg/l (± 446) and 7.93 (± 0.08) respectively, in another day.

Micro-organisms are nature's original recyclers, converting toxic organic compounds to harmless products, often carbon dioxide and water. Ever since it was discovered that microbes have the ability to transform and/or degrade xenobiotics, scientists have been exploring the microbial diversity, particularly of contaminated areas in search for organisms that can degrade a wide range of pollutants. Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand. Diversity is composed of two elements: richness and evenness, so that the highest diversity occurs in communities with many different species present (richness) in relatively equal abundance (evenness) [13].Micro-organisms represent the richest repertoire of molecular and chemical diversity in nature, as they comprise the most diverse forms of life. Over millennia, they have adapted to extremely diverse environments and have developed an extensive range of metabolic pathways. This metabolic wealth has traditionally been exploited by man in processes such as fermentation, production of antibiotics, vitamins, etc. More recently, this largely unexplored reservoir of resources has begun to be harnessed for innovative applications useful to mankind. These include the use of micro-organisms for bioproduction of novel as well as difficult-to-synthesize compounds, monitoring pollutant levels and biodegradation of xenobiotic pollutants. Taking the above facts into consideration, a survey was undertaken in tannery effluent to explore the physico – chemical characteristics and the nature of microbial flora such as bacteria and fungi and to exploit bacteria as a tool in treating tannery effluent.

MATERIALS AND METHODS

Tannery effluent was collected from Sembattu, Trichirappalli district, Tamil Nadu, India. A sampling programme consists of a series of monthly water quality and microbial survey was conducted for one year (April 2010 – March 2011). Population of bacteria and fungi were identified and isolated from the effluents samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and various biochemical studies as given by Bergey (1984)[5]. Fungi were identified by using standard manuals [8,9]. Effluent samples were collected in duplicate from the same area in pre sterilized bottles and returned to the laboratory for the analysis of the physico – chemical characteristics. Physico – chemical characteristics of the effluent were done according to the standard methods. pH was recorded at the collection site with BDH indicator paper. At laboratory the pH was checked again with (Elico-India) pH meter[4]. Carbonate, bicarbonate, Calcium and Magnesium were determined titrimetrically [19] and expressed in mg/l. TDS, BOD and COD were estimated as per the standard methods [3]. Nitrates, Nitrites and Chloride were determined according to the method of Strickland and parsons (1972)[30]. Ammonia nitrogen was estimated following the method of Solórzano (1969)[28]. Total phosphate was measured by the method of Menzel and Corwin (1965)[21]. Inorganic phosphate was determined by the method of Murphy and Riley (1962)[24].

RESULTS

The results of physico-chemical analysis of the effluent are presented in the Table1. The effluent was slightly alkaline and contained high amounts of nitrate, nitrite and ammonia; inorganic phosphate, calcium and magnesium in all months were examined (Table 1). High levels of BOD and COD were recorded in the study period. Total dissolved solids were high in October-January followed by February-May and June-September. Carbonate was observed in all the months. Nutrients such as nitrate, nitrite was high in October-January and inorganic phosphate was high in October-January. Bicarbonate level was high in February-May followed by October-January and June-September.

Most of the parameters were tested were slightly high in February-May than October-January and June-September.Bacteria isolated from the tannery effluent were identified based on the colony morphology, gram staining and various biochemical characteristics. Totally 7 different bacteria were isolated from the effluent (Table 2) sample. The species isolated were *Pseudomonas putida, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Bacillus cereus, Bacillus subtilis* and *Micrococcus species*. All the species were recorded in all the months.Fungi from the effluent sample were isolated based on serial dilution technique. Totally 9 different species of fungi belonging to 6 genera were isolated from the tannery effluent (Table 3).Among the fungi recorded, *Aspergillus* was found to be dominant with the species viz., *Aspergillus niger, Aspergillus flavus, Aspergillus luchensis* and *Aspergillus terrus*. The rest of the genera such as *Penicillium, Trichoderma, Fusarium, Rhizopus* and *Verticillium* were recorded with single species each. All the species were recorded in all the months.

DISCUSSION

The physico-chemical analysis of the effluent revealed its slightly alkaline nature and also the presence of high quantity of organic as well as inorganic nutrients in all the months examined (Table 1). Though BOD and COD levels in the present study were high as per IS standards, their levels were not so much high as compared to other types of effluent such as paper [28,20], distillery [33,14] and dye industry effluent (Sulaiman *et al*.,2002). Most of the parameters tested were slightly higher in February-May than in June-September and October-January. Somasekar and Ramasamy (1983)[29] reported similar results with paper mill effluent at different months of a year. They recorded objectionable record of BOD and COD, Total dissolved solids and organic nutrients such as ammoniacal nitrogen, nitrate nitrogen, silicates, phosphates and calcium. Such a trend was observed in the tannery effluent also. Sahai *et al*., (1998)[25] analysed pollution load of four different effluents such as fertilizer, sugar, distillery and sewage. Among these, highly objectionable amounts of various pollutants including BOD and COD were recorded in distillery followed by sugar, fertilizer and domestic sewage. While studying the variations in physico-chemical parameters of chemical industry effluent and paper mill effluent, Agarwal and Kumar, (1978)[2] found a direct relationship between temperature and organic matter. High organic matter was observed during February-May and low during October-January. This is in keeping with the observations that the course of decomposition of organic matter depends on temperature [17,22,23]. In the present study also, a high amount of organic matter was observed during February-May followed by October-January and June-September (Table 1). In general the results observed in the present study coincided with the earlier findings.

Bacterial diversity has not been studied in detail in waste water. However, a few reports are available on the bacterial flora of certain waste water. Jain *et al* (2001)[14] isolated three different bacterial species such as *Bacillus megaterium, Bacillus cereus* and *Xanthomonas fragariae* from distillery wastes in order to carry out biodegradation of distillery effluents. Similarly, Abed *et al* (2002)[1] isolated bacteria belonging to different groups, mainly the *Cytophaga-Flavobacterium* – Bacterioides group, γ and β subclass of the class proteobacteria, and the green non sulphur bacteria, from a heavily polluted site in a coastal stream. In the present study also, seven different bacteria were isolated from the tannery effluent in all months (Table 2). Most of the isolated genera were potential pathogens. Some of these bacteria have previously been reported to be present in waste waters [6] and oil polluted sites [35]. However, Sulaiman *et al* (2002)[31] isolated only two bacterial genera such as *Derxia* and *Beijerinckia* from dye effluent drenched soils. The less number of bacterial communities was apparently due to the environmental stress caused by the high level of pollutants, which allowed only a restricted number of species that tolerated such conditions.

Fungi occurred over all months of the year (Table 3). Of the fungal genera, *Aspergillus* was dominant with other species. Kouser *et al* (2000)[16] reported 23 species of fungi from dye effluent drenched soil and found *Aspergillus, Curvularia, Penicillum* and *Trichoderma* were the dominant genera. Saxena *et al* (1990)[26] also observed fifteen pathogenic species of fungi from the effluents of gelatin factory with *Aspergillus* as the dominant genus with six species, since it occurred in most of the months studied thereby lending support to the present observation. Higher concentration of chlorides, total dissolved solids and Biological oxygen demand were the reasons for their frequent

occurrence [15,34]. Hasija and Khan (1987)[11] reported the most of the aquatic fungi preferred low temperature between 15ºC to 30ºC, whereas higher temperature retarded the growth. In the present study also, the temperature of the tannery effluent favouring the growth of fungi. BOD of the effluent was higher during February-May, than October-January and June-September (Table 1). This has direct correlation with distribution of fungi[11]. In the present investigation, maximum numbers of species were recorded in all the months. Goldstein (1960)[10] reported that nitrogen content of the water along with other seasonal changes in the environment affects the occurrences and distribution of fungi.

Fresh water fungi generally grow at pH 7.0 to 8.5[11]. Saxena *et al* (1990)[26] observed species of *Aspergillus, Fusarium* and *Curvularia* between pH 7.9 and 8.3. Similarly, the pH of the effluent in the present study ranges from 8.0 to 8.7 and fungi such as *Aspergillus flavus* and species of *Curvularia* were recorded. This agrees with the findings of Saxena *et al* (1990)[26]. Among the species of fungi *Aspergillus* was the dominant one, which occurred in all the months. In stagnant water bodies, the temperature may raise during February-May, as a result, the species diversity could be reduced as pointed out by Hasija and Khan, (1987)[11]. Contrary to this, in the present investigation, the temperature of the effluent was not varied much (Table 1) as it was collected from the running stream and hence the observed variation. Dominant and persistent occurrences, most of the species of *Pseudomonas* and *Aspergillus* indicate their capacity to thrive in the type of manmade habitat.

CONCLUSION

The cheapest sources of nutrients for the mass culturing of microbes are undoubtedly sewage and other organic industrial wastes. The bacteria that are isolated from an effluent stream could be grown on large scale in controlled waste stabilisation ponds and thus pollution is taken care of to certain extent. Several investigators [36] have pointed out that the indicator species could be used to monitor pollution in controlled waste stabilisation ponds. In this investigation, *Pseudomonas sp* and *Aspergillus sp* was observed dominant in all the months of the year (Table 2 & 3) and hence it could be treated as indicator species of tannery effluent. On the basis of this fact, it is suggested that the indicator species could be used for pollution abatement programmes.

REFERENCES

1. Abed,R.M.M., Safi,N.M.D., Koster,J., De Beer,D., El-Nahhal,Y., Rullkotter,J. and Garcia-Pichel,F.,(2002). Microbial diversity of a heavily polluted microbial mat and it community changes following degradation of petroleum compounds, A*ppl. Environ. Microbiol*., 68(4);1674-1683.
2. Agarwal,M.and Kumar,H.D.,(1978). Physico-chemical and phycological assessment of two mercury polluted effluents, *Indian J. Environ Hlth.,* 20(2):141-155.
3. APHA, (1991). Standard Methods for the Examination of Water and Wastewater, (18th edn.)American Public Health Association, Washington D.C.
4. APHA, (1989). Standard Methods for the Examination of Water and Waste water, (17th edn.). Amerian Public Health Association, Washington,D.C.
5. Bergey's Manual, (1984). *Bergey's Manual of Systematic Bacteriology.* Williams & Wilkins, Baltimore: USA.
6. Cho,J.C and Kim,S.J.,2000. Increase in bacterial community diversity in subsurface aquifiers receiving lives stock wastewater input, boil.,*Appl.Environ.Microbiol*.,66:956-965.
7. Cristina, S. C. C., Rangel, A. O. S. S. and Castro, P. M. L. (2007). Constructed wetland system vegetated with different plant applied to the treatment of tannery wastewater. *Elsevier* Ltd.
8. Ellis, M.B., (1971). Dematiaceous Hypomycetes, Commonwealth. *Mycological Institute Pub*., Kew Surrey, England.

9. Gillman, J.C., (1957). A manual of soil fungi, Revised 2nd edn., Oxford and I.B.H. Publishing company (Indian reprint), Calcutta, Bombay, New Delni., 250.
10. Goldstein,S.R.,(1960).Effect of temperature on aquatic fungi, *Hydrobiologia*,27:151-156.
11. Hasija,S.K. and Khan,M.A.,(1987). Seasonal occurances and distribution of aquatic fungi in relation to the water quality of Jabalpur lakes, *Perspec. Mycol.Res.,* 1:79-87.
12. Haydar, S., Aziz, J. A. and Ahmad, M. S. (2007). Biological treatment of tannery wastewaterusing activated sludge process. *Pakistan Journal of Engineering and Applied Science.* 1, 61 – 66.
13. Huston, M. A. (1994).Biological Diversity: The Coexistence of Species on Changing Landscapes, *Cambridge University Press*, Cambridge,UK,.
14. Jain,N.,Nanjundaswamy,C.,Minocha,A.K.andVerma,C.L.,(2001).Isolation, Screening and Identification of bacterial strains for degradation of pre-digested distillery wastewater, *Indian J. Exp.Biol.*,39;490-492.
15. Kothandaraman,V.,Thergaonkar,V.P.,Koshy,T.and Ganapati,S.V.,(1963).Physico- chemical and biological aspects of Ahmedabad sewage*, Indian J. Environ Health., 5*:1-8.
16. Kousar,N.,Sheshikala,D. and Singaracharya, M.A.,2000. Decolourisation of textile dyes by fungi, *Indian J. Microbial*., 40:191-197.
17. Krauss,H.R.,(1962). Investigation of the decomposition of organic matter in natural waters, *FAO Fish Biol.Rep.,* 34:14-21
18. Ludvick, J. (2000). The scope for decreasing pollution load in leather processing. Regional programme for pollution control in the tanning industry in South East Asia. *UNIDO*.US/RAS/92/120/11-51.
19. Mackereth,F.J.H.,(1963). Some methods of water analysis for Limnologists, Scient. Publ. *Freshwater Biol*. ASS.,21.
20. Manoharan, C and Subramanian, G., (1992b). Interaction between paper mill effluent and the cyanobacteria *Oscillatoria pseudogeminata* var. *Unigranulata*, *Poll. Res.,* 11(2): 73-84.
21. Menzel,D.W. and Corwin,N.,(1965). The measurement of total phosphorous in sweater based upon the the liberation of organically bound fractions by persulphate oxidation, *Limnol. Oceanogr.,* 10:280-282.
22. Munawar,M.,(1970a). Limnological studies on freshwater ponds of Hyderabad, India. II. The Biotype, *Hydrobiologia,* 35(1):127-162.
23. Munawar,M.,(1970b). Limnological studies on freshwater ponds of Hyderabad, India.II. The biocoenose-distribution of unicellular and colonial phytoplankton in polluted and unpolluted environments, *Hydrobiologia,* 36(1):105-128.
24. Murphy,J. and Riley,J.P., (1962). A modified single solution method for determination of phosphate in natural waters, Anal. Chem.Acta., 27:31-36.
25. Sahai,R., Saxsena,P.K.and Jabeen,S.,(1998). Ecological survey of Algal flora of polluted habitats of Gorahpur, *Phykos*, 24:4-11.
26. Saxsena,D., Singhal,P.K and Hasija,S.K.,(1990). Prevalence of pathogenic fungi in a gelatin factory effluent, *Indian J. Environ Health.,*32(2):148-152.
27. Seyoum Leta, Fassil Assefa and Gunnel D. (2003). Characterization of tannery wastewater and assessment of down stream pollution profiles along Modjo river in Ethiopia. *Ethiopian Journal of Biological Sciences.* 2 (2), 157 – 168.
28. Solorzano,L.,(1969). Determination of ammonia in natural waters by the phenol hypochlorite method, *Limnol. Oceanogr.,* 14:799-801.
29. Somasekar,R.K. and Ramasamy,S.N.,(1983). Algal indicators of paper mill waste water, *Phykos*, 22; 161-166.
30. Strickland, J.D.H. and Parsons,T.R.,(1972). A Practical hand book of Sea water analysis, 2nd edn., *Bull. Fish. Res. Bd*.Canada., 167: 310.
31. Sulaiman,S.M.,Prabakar,J.and Purushothaman.D.,(2002).Population dynamics of microbes in dye factory effluent contaminated soil, *J.Ecotoxical.Environ.Monit.,*12(1):31-34.

32. Teodorescu, M. and Gaidau, C. (2007). Possible steps to follow for filling the gap between requirements and the real condition in tanneries. *Journal of cleaner production*. 16 (5), 622 – 631.
33. Venu,P.,Kumar,V,Sardana,R.K. and Bhasin,M.K.,(1984). Indicatory and functional role of phytoplankton in the effluents of Rangpo distilleries of Sikkim Himalayas, *Phykos*,23(1-2); 38-44.
34. Wetzel,R.G., (1975). Limnology, *W.B.Saunders Co.*, Philadelphia, pp.7430.
35. Whitely,A.S. and Bailey,M.J.,(2000). Bacterial community structure and physiological state within an industrial phenol bioremediation system, *Appl. Environ. Microbial.*,66:2400-2407.
36. Whitton B.A., (1975). Algae and higher plants as indicators of river pollution. In: Biological indicators of water quality.Ed: James A and Erison and John Wiley and Sons, New York.

Table 1: Physico-chemical characteristics of Tannery effluent for the period from April - 2010 to March - 2011. (Mean ± SD values of 4 observations in a year).

Parameters	June-September	October-January	February-May
p^H	8.45 ± 0.27	8.1 ± 0.09	8.6 ± 0.19
Temperature	20.85 ± 0.43	19.55 ± 0.41	20.73 ± 0.54
Total dissolved solids	4798 ± 17.08	4873±15	4830±21.61
Carbonate	2.7±0.19	2.45±0.13	2.43±0.13
Bicarbonate	211±4.73	222±6.89	223±7.9
Nitrate	230±2.16	229±0.82	233.75±1.70
Nitrite	166.75±0.96	166.25±0.96	169.75±0.96
Ammonia	87.65±1.18	89±0.98	89.95±0.58
Total phosphate	173.53±1.29	175.03±0.72	173.73±0.73
Inorganic phosphate	81.55±1.75	82.73±1.00	82.55±1.05
Total hardness	418.60±1.05	443.23±50.54	420.35±0.83
Magnesium	133.18±0.99	133.88±0.59	134.60±1.134
Calcium	285.43±0.73	284.60±1.33	285.75±0.591
Chloride	1085.53±0.94	1083.82±0.42	1085.48±0.869
Free carbon dioxide	25.5±0.58	23±1.20	24±0.82
Biological oxygen demand	234.25±3.798	230.10±1.809	236.95±2.072
Chemical oxygen demand	501.50±2.413	524.27±49.162	503.10±0.942

Note: All values are expressed in mg / L, except pH and Temperature.

Table 2: Observed Bacterial species from the tannery effluent.

Name of the Bacteria	June-September	October-January	February-May
Pseudomonas putida	+	+	+
Pseudomonas aeruginosa	+	+	+
Klebsiella pneumoniae	+	+	+
Escherichia coli	+	+	+
Bacillus cereus	+	+	+
Bacillus subtilis	+	+	+
Micrococcus sp.	+	+	+

Note: +: observed in all four months.

Table 3: Observed fungal species from the tannery effluent.

Name of the Fungal	June-September	October-January	February-May
Aspergillus niger	+	+	+
Aspergillus flavus	+	+	+
Aspergillus luchensis	+	+	+
Aspergillus terrus	+	+	+
Penicillium sp.	+	+	+
Fusarium sp.	+	+	+
Verticillium s.p	+	+	+
Trichoderma viride	+	+	+

Note: +: observed in all four months.

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RESEARCH ARTICLE

Water Quality Assessment of Sasthamkotta Lake, Kerala, India.

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ABSTRACT

Lakes are sometimes insisted to different wastewater discharges originating from different sources like bathing, washing and Agricultural runoff etc., Chemicals such as nitrogen, phosphorus and carbon in certain concentrations might distort and disrupt aquatic ecosystems. The study was conducted from January 2009 to December 2011 at Sasthamkotta Lake situated in Kerala district. The present paper deals with physicochemical aspects of water. The parameters analyzed are water Temperature, pH, Turbidity, TDS,DO, Total Hardness, Chloride, Sulphate, Fluoride, Sodium, Potassium and iron. Monthly analysis over the period of thirty six months suggests that the Lake water is highly suitable for intensive use. Changes in water quality parameters of this Lake by months were determined. This study indicates that Sasthamkotta Lake has not reached the polluted stage yet.

Keywords: pH, Turbidity, TDS,DO, Total Hardness, Agricultural runoff.

INTRODUCTION

Sasthamkotta Lake, the largest fresh water lake in Kerala lies between $9^0$11′ and $9^0$41′ Northern latitude and $76^0$36′ and $76^0$40′ Eastern longitude. It spreads to an area of about 4.64 km^2. Except on the southern side, all other sides of the Lake are bounded by steeply sloping hillrocks. The southern side, on the other hand is a low lying land of alluvial sediments which is now used for paddy cultivation. The paddy fields end in the western boundary of the Kallada River. The lake is separated from the paddy fields by an artificial barrier (bund). The total drainage area (land area) of this Lake is 4.40 km^2 and total catchment area is 9.04 km^2 of which parts of Sasthamkotta, West Kallada and Mynagapally Panchayats of Kunnathur Taluk. Kollam District was included. The Lake lies 29 km North-East of Kollam town.

Water level in the Lake recorded during April-May period is lowest and the level starts increasing with the on set of monsoon and by the end of south-west and North-East monsoons, the level comes to a peak in October-November.

This increase in level is around 1.5m depending on the amount of rainfall. Later it keeps on declining, indicating thereby that monsoon rain is the major source of water in the lake. Water loss takes place through evaporation and daily pumping out of water for drinking purpose. Water is mostly calm, occasional wind action produces waves on the water surface and occasionally froth at the border.

METHODOLOGY

Water Sampling

To characterized water quality throughout the main basin of Lake, five permanent stations for monthly sampling were established and marked within the inflow (S1), out flow (S2), mid-Lake region (S3) and corners (S4 & S5). The sampling points were selected on the basis of their importance. Water samples were collected from the five stations every month (January 2009 to December 2011) by grab sampling method at almost 0.5m depth from the water surface in cleaned 2 litres plastic cans after rinsing sufficiently in the water. Temperature, pH and dissolved oxygen were measured on-site, using mercury-in-glass thermometer; portable hand pH meter and the azide modification of the Winkler's method respectively.The collected samples are immediately transferred and analyzed in the laboratory. Various water quality parameters are determined according to procedures outlined in the Standard Methods for the Examination of Water and Wastewater, 20th edition (APHA, 1998) [1]. The analyzed water quality parameters (physico-chemical) are listed in the Table - 1.

RESULTS AND DISCUSSION

The monthly variations of physicochemical parameters of water of Sasthamkotta lake samples were recorded during the period of investigations are shown in Table 2. Water temperature was not showing much variation in different months of the study period. The highest water temperature was recorded 28^{0}C. The lowest water temperature was 25^{0}C. Temperature is one of the essential and changeable environmental factors, since it influences the growth and distribution of flora and fauna. Water temperature ranging between 13.5 and 32^{0}C is reported to be suitable for the development of the planktonic [5&6].

pH in the Lake varied from 6.2 to 8.6. The lowest pH mean value was recorded in the month of June '11 and the highest in the month of April'10. This variation may be due to richness of flora. There was a decline in pH following the above said months. A similar trend in pH reduction was reported by Devaraj *et al.*, (1998) [3] in Hemavathy Reservoir. The depletion of pH during summer may be due to the excess of CO_2 in water resulting from the increased rate of decomposition of organic matter. According to Soruba (2002) [14] and Srivastava *et al.,* (2004) [15] high pH was more productive than low pH. pH of water is treated as most important in the life processes of aquatic organisms. No regular pattern in values was observed during the present investigation. However the alkaline pH formed throughout the year reveals that it is a reservoir with potential for high production characteristics.

Turbidity is a principle physical characteristic of water. It is caused by suspended matter or impurities include clay, silt, finely divided inorganic and organic compounds that interfere with the clarity of the water [11]. Clarity is important in drinking water for human consumption. The turbidity ranged between 1.9 to 11.4 NTU with the maximum mean value of 8.96 NTU. The maximum and minimum turbidity values were noted during June'09 and October'11 respectively. The sewage influx and settleable solids have a tremendous effect on the aquatic environment by increasing the turbidity which in turn decreases productivity and photosynthesis [2].Total Dissolved Solids (often abbreviated TDS) is an expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended forms. TDS of water samples collected from the study area lies in the range of 22 - 93mg/L. The permissible limit of TDS is 100mg/L and in the study area it not exceeded the permissible limit. Sewage pollution is likely to increase the TDS value. Open air

defecation practice in Kollidam River near Thirumalapadi in Ariyalur district caused the TDS to rise to around 1000 mg/L [8]. Increase in total dissolved solids due to urban anthropogenic impact can be often complicated by intense local agricultural activity leading to local spatial and temporal variability in run off [10].

Hardness of water is an important consideration in determining the suitability of water for domestic and industrial uses. Hardness is caused by multivalent metallic cations and with certain anions present in the water to form scale. The principle hardness-causing cations are the divalent calcium, magnesium, strontium, ferrous iron and manganous ions. The total hardness of water samples was showed minor concentration of 8 mg/L to 20 mg/L. The values are within the permissible limit of WHO: 200-600 mg/L.The range of dissolved oxygen in all sampling stations is from 1.2 to 7.2 mg/L. The DO amounts may decrease due to the decomposition of organic compounds in the lake at a particular sampling station. The rich DO of the water may be due to wind velocity, aquatic plants and water flow. The minimum value of DO was noted in River Ganga during summer and maximum DO was reported during rainy seasons. The low DO at some stations in summer is due to contamination of River Ganga with sewage from various points, which decrease the oxygen content of the water [16].

According to Zafar (1964) [21] chloride can be considered as one of the basic parameters of classifying lakes polluted by sewage into different categories. The chlorides value oscillated between 22 mg/L and 40 mg/L with the mean value of 31.2 mg/L. The high chloride content might be attributed to the presence of large amount of organic matter of both allochthonous and autochthonous origin [13]. High chloride content of water indicates organic pollution of animal origin also [17].

Sulfate (SO_4^{-2}) can be found in almost all natural waters. The origin of most sulfate compounds is the oxidation of sulfite ores, the presence of shale, or the industrial wastes. Sulfate is one of the major dissolved components of rain. High concentrations of sulfate in the water, we drink can have a laxative effect when combined with calcium and magnesium, the two most common constituents of hardness. Bacteria, which attack and reduce sulfates, form hydrogen sulfide gas (H_2S). Some soils and rocks contain sulfate minerals. As river water moves through these, some of the sulfate is dissolved into the water [4&7].The sulfate concentration in Sasthamkotta Lake fluctuated between 0.08 mg/L and 16 mg/L throughout the period of study. The sulfate concentration reached its high value during June'09 while the minimum value was noted during December'11. The mean sulfate concentration was recorded as 8.4 mg/L. Sulphate produces an objectionable taste at 300 – 400 mg/L concentration. Above 500 mg/L a bitter taste is produced in the water. At concentrations around 1000 mg/L. it has a laxative effect and causes gastro intestinal irritation [18].

The values of sodium are in the range of 2 mg/L to 6 mg/L in the water samples. The sodium values was not exceed the desirable limit of WHO (1992) in the samples. The samples, which are very near to the channel, have maximum sodium values than that for the samples collected far away from the channel. Percolation of channel water containing high ionisable salts and the intrusion of domestic sewage probably enhances the sodium concentration. Sodium is found in association with high concentration of chloride resulting in salinity. Sodium concentrations are also influenced by the cation exchange mechanism [12]. Potassium remains mostly in solution without undergoing precipitation. Potassium of water samples collected from the study area lies in the range of 0-2.0mg/L. The permissible limit of potassium is 10mg/L and in the study area it not exceeded the permissible limit. Sodium is often associated with chloride. It finds its way into lakes from road salt, fertilizers, human and animal waste. Potassium is the key component of commonly used potash fertilizer, and is abundant in animal waste. Soils retain sodium and potassium to a greater degree than chloride or nitrate. Therefore, sodium and potassium are not as useful as pollution indicators. Increase in sodium and potassium values over time can mean there are long-term effects caused by pollution. Although not normally toxic themselves, these compounds strongly indicate possible contamination from more damaging compounds [20].

Iron is the fourth most abundant element, by weight, in the earth's crust. Natural waters contain variable amounts of iron depending on the geological area and other chemical components of the waterway. Iron in groundwater is

normally present in the ferrous or bivalent form [Fe^{2+}] which is soluble. It is easily oxidized to ferric iron [Fe^{3+}] or insoluble iron upon exposure to air. This precipitate is orange-colored and often turns streams orange. The current aquatic life standard is less than 1.0 mg/L based on toxic effects. (It is one of the few for which the criterion is not calculated based on hardness). The iron content of the water samples was 0.02 mg/L to 0.09 mg/L. Iron is known to promote the growth of iron bacteria in water and also makes the water distasteful. Apart from its unpleasant taste, iron forms rust in water and it can cause clogs and stains pipes. WHO, 2006 states that below 0.3mg/L of iron does not affect the taste of water.

Fluoride at a lower concentration at an average of 1 mg/L is regarded as an important constituent of drinking water. The minimum value was recorded in July 2011 and while the maximum value was recorded in Feb' 2009 for the water. The values are lower than the prescribed value. But as its high concentration cause serious health problem in that concern it is well below. Surface water generally contains less than 0.5 mg/L fluoride. However, when present in much greater concentration, it becomes a pollutant. Areas exist where the fluoride content of water ranges from 1.5 to 6 mg/L, for example in the Kurnool district of Andhra Pradesh.

CONCLUSION

In the present study, it is found that water quality problems associated with Sasthamkotta Lake includes severe dissolved oxygen depletion, moderate water clarity and high level of algae growth, and dense beds of aquatic microphyte. There is therefore a need to properly manage wastes in the surrounded bund width and control as well as monitor human activities in order to ensure that such activities have minimal negative effects on lakes. Awareness, proper understanding, planning and management of environmental resources are essential to prevent environmental degradation of these surface water resources (lakes).The physico-chemical parameter of Lake is reasonable but in case of anthropogenic activities, quality of water is maintain for a longer period is not good.

Table : 1. Physico-chemical examination of water samples

Sl.No.	Parameters	Methods
1	Temperature (^oC)	
2	pH	Electrometric method
3	Total Dissolved Solids (TDS)	Dessicator method
4	Turbidity	Nepheleo turbidity meter
5	Total Hardness (TH)	Volumetric method
6	Dissolved Oxygen (DO)	Winkler's method
7	Chloride (Cl^-)	Argentometric method
8	Sulfate (SO_4^{-2})	Colorimetric method
9	Fluoride (Fl^-)	Colorimetric method
10	Sodium (Na^+)	Flame Photometric method
11	Potassium (K^+)	Flame Photometric method
12	Iron (Fe^{+2})	Colorimetric method

Table : 2. Physico-chemical characteristics of Sasthamkotta lake during the year 2009-2011

	pH	TDS	Turbidity	TH	DO	Cl^-	Na^+	K^+	Fe^{+2}	SO_4^{-2}	F^-
Min	6.60	22.60	1.90	8.00	1.20	22.00	3.00	0.00	0.01	2.40	0.11
Max	7.80	93.00	5.00	14.00	7.20	32.00	6.00	2.00	0.28	16.00	0.80
Mean	7.22	37.70	3.48	11.61	4.57	26.75	4.01	0.36	0.04	6.32	0.52
σ	0.25	14.19	0.58	1.47	1.91	2.42	1.15	0.47	0.04	2.15	0.08
Min	6.20	28.10	3.60	8.00	1.20	26.00	2.00	0.00	0.02	0.60	0.40
Max	8.60	49.10	4.10	16.00	7.10	40.00	4.00	2.00	0.05	6.80	0.50
Mean	7.20	37.83	3.86	9.79	4.06	27.28	2.83	1.17	0.02	3.33	0.46
σ	0.45	3.50	0.09	2.04	1.94	2.04	0.70	0.60	0.01	2.04	0.05
Min	6.80	28.80	2.40	8.00	1.20	24.00	2.00	1.00	0.02	0.08	0.40
Max	8.60	63.00	11.40	20.00	7.10	40.00	4.00	2.00	0.04	6.10	0.70
Mean	7.10	42.32	4.63	11.77	4.51	27.23	3.52	1.65	0.02	3.01	0.44
σ	0.93	11.63	2.10	3.42	2.01	4.37	0.75	0.48	0.01	2.05	0.08

*All the parameters are in mg/L except Turbidity (NTU) and pH.

REFERENCES

1. APHA., 1998. "Standard method for the examination of water and waste water" (20th edn.) American Public Health Association. Washington.
2. Bartsch , A.F., 1960. Settleable Solids , turbidity and light penetration as factor affecting water quality, In biological problem in water pollution, transactions of the 1959 seminar, U.S. Department of health education and welfare.
3. Devaraj, K.V. and M.Z. Mahadeva and A.A. Pazal. 1998. Hydrobiology of the Hemavathy reservoir, In: M. Mohan Joseph (Ed). The first Inidan Fisheries Forum., Proceedings. Asian Fisheries Society, Indian Branch, Mangalore. pp 323-327.
4. Doctor, P.B., *et al*., 1998. Physico-chemical and microbial analysis of dye contaminated river water. Indian J. Env. Health. 40(1): 7 – 14.
5. Gaikwad, S.R., K.N. Ingle and S.R. Thorat. 2008. Study of zooplankton patter and resting egg diversity of recently dried water bodies in north Maharashtra region. J. Environ. Biol., 29, 353-356.
6. Kamat, S.V. 2000. Hydrobiological studies of two temple ponds in Ponda Taluk, Goa. Ecol. Environ. Cons., 6, 361-362.
7. Kataria, H.C., Gupta and O.P. Jain. 1995. Water quality of bore wells in BHEL area of Bhopal, M.P. Poll. Res., 14(4): 455 – 462.
8. Kulasekaran, A. 2002. Water Quality Survey. TWAD Board, Chennai.
9. Mahananda, M.R., Mohanty, B.P. and N.R. Behera. 2010. Physico-chemical analysis of surface and ground water of bargarh district, Orissa, India. IJRRAS 2 (3).
10. Martin, P. and Haniffa, M.A. 2003. Water Quality Profile in the South Indian River Tamiraparani. IJEP. 23 (3): 286 – 292.
11. Mithra, J. and R. Bashkaran. 2012. Groundwater quality in and around Thanjavur town in Tamilnadu–India. International Journal of Current Research. 4(1) : pp.061-064.
12. Narain, S., and Chauhan, R. 2000. Water quality status of river complex Yamuna at Panchnada dist. Etawah, U.P. (India). An Integrated management approach. Pollution Research, 19(3), 351–364.

13. Pandit,A.K. 1999. Freshwater Ecosystems of the Himalaya. Parthenon Publishing. NewYork. London.
14. Soruba, R. 2002. Ecology of water bodies formed by limestone mining in and around Ariyalur. Ph.D., Thesis, Bharathidasan University.
15. Srivastava, A Indranigupta and R.N. Singh. 2004. Water Quality modelling at Mumbai. Poll.Res., 23(2) : 317-326.
16. Tiwary, R.K. *et al.,* 2005. Water Quality Assessment of Ganga River in Bihar Region, India. J. Env. Sci. & Engg. 47 (4): 326 – 335.
17. Thresh, J.C., Suckling, E.V. and Beale, J.F. 1994. The examination of water and water supplies. 6 Edition (E.W.Taylor.editor).
18. Vimala, G.R. *et al.,* 2006. Study on the Water Quality of Kudamurutti River and Nearby Area in Woraiyur of Tiruchirappalli District. IJEP. 26 (2): 159 – 162.
19. World Health Organization (WHO) 1992 & 2006. International Standards for drinking water, Geneva, Switzerland.
20. Yalçın Tepe, Aysun Türkmen, Ekrem Mutlu, Alpaslan Ate. 2005. Some Physicochemical Characteristics of Yarseli Lake, Hatay, Turkey. Turkish Journal of Fisheries and Aquatic Sciences 5: 35-42.
21. Zafar, A.R. 1964. On the ecology of algae in certain fishponds of Hyderabad, India. Physico-chemical Complexes. , 23:179-195.

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Books and other Monographs

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