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Research Article

BIOREMEDIATION OF TEXTILE DYE EFFLUENTS USING INDIGENOUS EARTHWORM *LAMPITO MAURITII* (KINBERG)

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ABSTRACT

The present study is intended to assess and monitor the Bioremediation of textile dye effluent using *Lampito mauritii* earthworm. The study focus on the bioremediation of surface water of SIPCOT dye industry Cuddalore district over a period of one year and has revealed variations among the different parameters at different study sites. For the analysis of surface water three sampling sites were selected for the study. In the area, from SIPCOT dye industry to Cuddalore district the parameters concentration was decreased and it is useful for drinking purpose. Heavy metals such as copper and zinc are essential elements for metabolic activities however they show toxic effects when exposed to higher levels whereas lead, mercury, cadmium and are not essential for metabolism which they exhibit toxic properties. The heavy metals of surface water in the study area are in the order of Zn > Cu > Pb > Cd > Hg (mg/L) respectively. Mercury, cadmium were noticed in least level in the non-industrial area whereas industrial area concentration of metals were slightly increased than the prescribed limits. The examination of water samples for bacteriological parameter includes MPN index, total heterotrophic bacterial colony count, morphological and biochemical characteristics. MPN is a suitable and widely used method to determine the microbial quality of water. Due to heavy rain the microbial load was increased during monsoon season. Based on morphology and biochemical characteristics, identify the species of the organism namely *Escherichia coli, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Based on motility, shape, size and structure, the organism was identified and based on IMVIC tests of biochemical reaction with production of acid and gas the organism was identified.

Keywords: Bioremediation, Textile dye, *Lampito mauritii,* Water, Microbial.

INTRODUCTION

Despite the potential for 400 million tonnes of vermicompost to be produced annually from waste degradation, India has yet to fully comprehend the relevance of vermiculture (Sinha *et al*., 1996). They also serve as a detoxifying agent for polluted soil, inhibiting soil-borne diseases (Davis & Taylor, 1972). The earthworm gets its name from the fact that it burrows into the ground and eats its way out. Earthworms have existed for more than 20 million years. Earthworms are found in 3920 different species all over the world. Microdrilli are aquatic earthworms, while megadrilli are terrestrial earthworms. There are around 509 species of earthworms in India, divided into 67 genera. Aside from these, India has received more than 20 species from other countries. These are referred to as 'peregrines.' Earthworms can be found in a variety of settings; organic materials such as manures, litter, compost, and other organic materials are particularly appealing to them, but they can also be found in very hydrophilic environments near both fresh and brackish water, and some species can survive under snow (Sharma *et al*., 2005) Nowadays, advent of chemical fertilizers is bringing up in agriculture day by day which ultimately destroys that fertility of soil upon long term use (Senthilmurugan *et al*., 2018).

One pair of earthworms can generate 100 cocoons in 6 weeks to 6 months under ideal conditions (Sicheri *et al*., 1997). Earthworms lay their eggs in a translucent, tiny, spherical protective capsule called a cocoon. Cocoons vary in shape, size, colour, and number depending on the

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species. A cocoon's incubation period is typically 3-5 weeks in temperate worms, 3-30 weeks in tropical worms, and 1-8 weeks in tropical worms. One of the elements determining the commencement and rate of reproduction is the quality of organic waste (Goel *et al*., 2005). Large amounts of rotting animal waste and plant litter are swallowed by epigeic earthworms. A worm's food consumption ranges from 100 to 300 mg g-1 body weight day -1 (Edwards & Bater, 1992). Among all other industries, the textile industry's wastewater effluent is considered one of the most polluting. For a long time, scientists have been studying the environmental and health implications of textile industry waste water. The chemical composition of textile manufacturing waste varies, ranging from organ chloride-based waste insecticides to heavy metals connected with dyes and the dying process (Han *et al*., 2011; Lobert *et al*., 1994). The release of reactive dyes into the environment causes significant harm since they have a significant impact on hydrophytes' photosynthetic capacity by restricting light penetration, and their breakdown products may be harmful to some aquatic animals (Choi & Wang, 2009). Water bodies, ecosystem integrity, soil fertility, and plant growth are all affected. Because of their obvious colour, biorecalcitrance, and toxicity to animals and humans, azo dyes are becoming a major source of concern (Senan & Abraham, 2004).

Water-borne disorders such as nausea, perforation of the nasal septum, ulceration of the skin and mucous membranes, renal damage, cramps, sporadic fever, haemorrhage, dermatitis, hypertension, severe respiratory tract irritation, or cancer can all be caused by coloured effluents that enter water bodies. The colours' bioaccumulation is affected by their availability and persistence in water and food, as well as their physiochemical characteristics (Mubarak Ali *et al*., 2011; Pines *et al*., 2011). The textile industry's effluent has a negative impact on soil fertility, plant development and productivity, and plant susceptibility to diseases. Germination percentage, seedling survival shoots and root length of the seedling, and other metrics for measuring plant response to contaminants include. The amount of chlorophyll in plants diminishes as a result of the textile industry's wastewater. Total protein, carbohydrate, and chlorophyll levels have all decreased, indicating that textile dye effluents are hazardous (Puvaneswari *et al*., 2006). The FDA approved the use of 3,000 tonnes of azo dyes in drugs, foods, and cosmetics in 1991. In experimental laboratory animals, several azo dyes cause liver nodules. If dye workers are exposed to excessive amounts of azo dyes, they are more likely to develop bladder cancer. Industries commonly utilise azo dyes based on benzidine. The National Institute for Occupational Safety and Health (NIOSH) in the United States published survey data on Benzidine-based dyes in laboratory animals as well as epidemiological research on dye workers in 1980. Benzidine has been linked to human urinary bladder cancer and has been shown to be tumorigenic in animals. Experimental animals (rats, dogs, and hamsters) were given benzidine and benzidine congener dyes, which revealed the presence of potentially carcinogenic aromatic amines and their N-acetylated derivatives in their urine (Puvaneswari *et al*., 2006).

The use of huge volumes of dyestuffs throughout the dyeing phases of the textile production process is the principal cause of colour in textile industry effluent (Greenfelder *et al*., 1989; Martin *et al*., 2002). Significant dye residues are frequently present in the final dyehouse effluent in hydrolised or confoxed forums because to inefficient dyeing processes (Lozano *et al*., 2016). Water, like enterprises that aim to improve human health, is an unavoidable requirement of life. Industrialization had a significant impact on human health, either directly or indirectly through the environment it created. The poisoning of water sources such as lakes, rivers, oceans, and groundwater is known as water pollution. Water pollution has an impact on the plants and other organisms that live in these bodies of water, and the effects are usually always harmful to not only individual species and populations, but also natural biological ecosystems, including humans. In the global context, water contamination is a big issue. It has been suggested that it is the biggest global cause of fatalities and diseases (Faucette *et al*., 2006; Pink *et al*., 2006) and that it kills over 14,000 people per day.

Approximately 10,000 Azo dyes are made each year, with at least 15% of them being discharged into the environment (Donlon *et al.,* 1997). Under anaerobic conditions, this organic solution will naturally decompose. Aromatic amines were produced as a result of this method. Aromatic amines are thought to be mutagenic and carcinogenic, as well as posing a major threat to aquatic and human life. Two chemical groups that are abundant in the environment are azo dyes and nitrated polycyclic aromatic hydrocarbons. Despite legislation restricting their usage in numerous countries, the impact of azodyes in the food industry and their degradation products on human health has been a source of worry for several years. Cancer, human bladder, splenic sarcomas, and hepotocorcinomas have all been related to azo dyes.

MATERIALS AND METHODS

Test animal

Lampito mauritii (Figure 1) were used as test animal in the present study. *Lampito mauritii* was purchased from a vermiculture farm in Madurai, Tamilnadu. *Lampito mauritii* was obtained from an earthworm bank (pit) in the Department of Zoology, Annamalai University, and Tamilnadu. The earthworms were maintained in control soil which was placed in a dark room at 25 + 2oC and 80% humidity for an acclimatization period of 2 weeks.

Sampling stations

The soil samples used in the present study were collected from two sites in January 2015. Both sites are located in SIPCOT, Cuddalore, and Tamilnadu. One of these two sites is highly polluted with the deposits of match industry wastes and the other site has been contaminated by wastes from automobile service station.

Figure. 1. *Lampito mauritii* morphology image**.**

Heavy metal analysis in soil and earthworm body tissue

A watch glass was placed over the liquid in a beaker, and it was allowed to reflux for 45 minutes. After that, the watch glass was removed, and 10 mL of HCl was added. Sample's Origin, Phosphates Sulphates, BOD, COD, Nitrates, Turbidity, Color, Total Solids, and selected heavy metals were determined using established methods in triplicates in the laboratory. Cooling was done until the weight of the dish with residue was constant to within 0.05mg for Total Solid (TS) determination. The weight of the total solids was calculated by subtracting the weight of the dish. Suspended Solids (SS) Determination, the weight of the suspended solids is deducted from the weight of the filter paper. Dissolved solids (DS) are determined by subtracting total solids from suspended solids. Chemical Oxygen Demand Calculation (COD) As described in Determination of Biological Oxygen Demand, two drops of ferroin indicator were added to the contents of the flask, and the leftover dichromate was titrated with standardardized ferrous ammonium sulphate. BOD the untreated effluent sample was evaluated for BOD right after it was collected. As previously stated, the biologically treated sample was also tested for BOD. The heavy metals in the sample were measured using conventional techniques as stated by using an atomic absorption spectrophotometer to detect the quantities of nitrate, sulphate, and phosphorus.

Microbiological analysis

In a water sample, this test was performed using three tube series and the method of most probable number (MPN). The results were compared to a three-tube series standard chart (WHO, 1981). Preliminary examination A negative presumptive test was defined as the absence of gas production after 48 hours. This indicates that the water sample was free of E. coli and was deemed safe (WHO 1981). Pour plate technique was utilised for bacterial population enumeration, and nutrient agar medium was used for bacterial enumeration (WHO, 2012).

RESULTS AND DISCUSSION

The concentration of heavy metals in a surface water sample from the SIPCOT Cuddalore district was determined. Heavy metal buildup varied from station to station and month to month, depending on the availability of metals in the surface water. Table 1 shows the variability in cadmium levels in surface water of the Cauvery River at fifteen distinct locations. The yearly average cadmium concentrations in the fifteen distinct sites are 2.463, 2.217, 2.276, 1.685, 1.972, 1.7752, 2.117, 1.965, 2.3498, 2.53, 2.603, 2.694, 2.688, 2.43, and 2.46, respectively, in units or ppm. The SIPCOT dye industry had the highest cadmium concentration (2.688 ig/l), whereas the SIPCOT dye industry had the lowest (1.685 (ig/1)(ig or mg) cadmium concentration. At a 5% level, the measured cadmium concentrations between stations and months are statistically significant. Copper concentrations differed from station to station and month to month. At the 5% level, the analysis variance is statistically significant. In the SIPCOT dye industry, annual average copper concentrations were 0.844, 0.825, 0.824, 0.529, 0.765, 0.771, 0.696, 0.695, 0.722, 0.751, 0.763, 0.844, 0.799, 0.74, and 0.738 ig/l, respectively (Table 1). The distribution of lead in the SIPCOT dye business is depicted graphically in Fig 21 and is documented in Table 21. For fifteen stations, the annual average lead readings were 0.248, 0.168, 0.229, 0.163, 0.219, 0.201, 0.209, 0.23, 0.2328, 0.252, 0.235, 0.233, 0.241, 0.24, and 0.244 ng/1. Stations (0.252 [xg/1) SIPCOT dye industry had a greater level of lead, while station (0.163 (ig/1) SIPCOT dye industry had a lower level. At the 5% level, the reported values are highly significant between the station and between the seasons. The estimated mercury concentration in the SIPCOT dye business in Cuddalore district varies from station to station and month to month in this study (Table 1). Among the stations, the SIPCOT dye industry (0.201 (ig/1) had a higher mercury concentration than the ICMR norm. In the surface water of Station, the annual average mercury concentration was between 2.463 and 2.46 xg/1. At a 5% level, the measured mercury levels are statistically significant.

Klebsiella pneumoniae is a bacterium that causes pneumonia. It was made up of nonmotile, capsulated rods that grew well on regular media and formed enormous, dome-shaped mucoid colonies with varied degrees of stickiness. They were short, plump, straight rods with a diameter of 1-2 x 0.5-0.8 mm. In light of these findings, this study implies that *Lampito mauritii*, as well as other novel species, could be employed for bioremediation of soils contaminated by match industry wastes and petroleum oil products. The growth patterns of two earthworm species were studied after exposure to different polluted soils in the study's second phase. The average weight of living earthworms was calculated on a regular basis, and the results are reported in Table 1. In the body tissue of *Lampito mauritii* exposed to control soil, there was a constant state of growth (Table 1). (Are there any water quality parameters in (Table 1) At the conclusion of the experiment, there was a 46.55 percent rise in weight (Table 1). The weight of *Lampito mauritii* cultivated on MS and OS showed a negative trend, as shown in Table 1. The biomass decreased significantly as the duration of the study period grew longer. *Lampito mauritii* grew rather well in OS rather than MS, according to this study (Figure 1). check According to Table 1, weight reduction in MS is 58.69 percent, whereas weight loss in OS is 32.89 percent. A similar pattern was seen with another earthworm species, as shown in Tables 1 and 2 check and Figure 1. In 28 days, earthworms grown in control soil increased their body weight by 71.87 percent. There are a number of reports that back up the conclusions of this study. According to (Eitminavičiūtė *et al.*, 2005), heavy metal concentration in the substrate inhibited development. They discovered that the amount of Cd in earthworms affected their body weight.

Table 1. Various organic and inorganic pollutants and its range in the textile industry raw effluent.

S.No	Composition	Level of composition
1	Chemical oxygen demand (mg /l)	1,512
2	Biochemical oxygen demand (mg/l)	90.64
3	Surfactants (mg/l)	3 1.1
4	Color (A559)	1.202
5	pH	10.5
6	Conductivity (mV)	109
7	Hardness (mg/l as CaCO3)	86.5
8	Cyanide (mg/l)	0.2
9	Sodium	70%
10	Phenolic compounds (mg/l)	0.077
11	Total iron (mg/l)	0.77
12	N-nitrate (mg/l)	2.0
13	Sulfate (mg/l)	345.3
14	Phosphate (mg/l)	12
15	Fluoride (mg/l)	0.64
16	Aluminum (mg/l)	<0.01
17	Arsenic (mg/l)	<0.2
18	Barium (mg/l)	<0.01
19	Boron (mg/l)	<2.0
20	Cadmium (mg/l)	<0.0006
21	Chromium (mg/l)	<0.005
22	Cobalt (mg/l)	<0.007
23	Copper (mg/l)	0.2
24	Lead (mg/l)	<0.02

Table 2. Confirmatory biochemical test results of bacteria in surface water samples in variation of SIPCOT dye industry Cuddalore earthworm gut district.

S.No.	Biochemical tests	*E. coli*	*S. typhi*	*K. pneumoniae*	*E. aerogenes*	*V. cholerae*	*P. aeruginosa*
1	Motility	+	+	-	+	-	+
2	Gram reaction	-	-	-	-	-	-
3	Gas from glucose	+	+	+	+	+	+
4	Acid from lactose	+	-	+	+	-	-
5	Acid from sucrose	+	-	+	+	+	-
6	Indole	+	-	-	-	+	-
7	Methyl red test	+	+	-	-	-	-
8	Voges-Proskauer test	-	-	+	+	-	-
9	Citrate utilization test	-	+	+	+	+	+
10	H_2S	-	+	+	-	-	-
11	Urease	-	-	+	-	-	-
12	Phenylalanine deaminase	-	-	-	-	+	+
13	Arginine dihydrolase	-	+	-	-	-	+
14	Lysine decarboxylase	+	+	+	+	+	-
15	Ornithine decarboxylase			-	-		-

'+' 90% or more positive in 1 or 2 days; '-' 90% or more negative.

Figure 2. Isolation and colony count of bacterial culture in the nutrient agar, MacConkey agar and blood agar from the surface water samples in variation of SIPCOT dye industry, Cuddalore district.

The elevated zinc concentration could be attributed to the rock unit and soil type. Zinc levels in human serum and plasma are around 1.0 ppm, however the concentration in blood is around 5 times greater due to a high concentration. Zinc is found in the human body in amounts ranging from 1.4 to 2.3 g. (Sinha *et al.*, 1996). Lead is a highly hazardous heavy metal with a lengthy half-life and high tissue accumulation. Lead builds up in the bones and tissues. Lead poisoning and kidney damage can occur as a result of lead accumulation. It's linked to miscarriages, as well as reproductive and sperm problems. Anemia is the initial symptom of chronic lead poisoning in animals, and it can also cause weight loss, constipation, and tooth loss (Lassar *et al.*, 1995). Lead prevents Ca, Zn, Mn, Cu, and Fe from being absorbed, resulting in deficits.

(Xiong *et al*., 1997)investigated the global lead levels in Pondicherry region unpolluted natural water. Lead inhibits numerous important enzymes involved in the general process of protein synthesis, resulting in the accumulation of metabolite intermediates (Atwa *et al*., 2014) looked at why there was a higher quantity of lead in oil refineries. After digestion, higher concentrations of lead may produce harmful consequences in aquatic organisms(Senthilmurugan *et al*., 2018) digestive systems. In human metabolism, lead plays no role. Furthermore, suggested that increased Pb concentrations could lead to aberrant human behaviour. Lead is a moderately toxic element that has no metabolic role in plants or animals and is extremely persistent in soils. The lead level of roadside plants and soils is high (Edwards & Bater, 1992). Lead concentrations were within the permissible limits in the non-industrial area of the current investigation. Similar investigations (Sharma *et al*., 2005)found extremely low levels of lead at the Bondamui Rourkela industrial complex. The Warangal district has also been reported to have relatively low lead concentrations. Cd levels are commonly found in leafy vegetables, grains, and cereals. Cadmium reacts as an effective enzyme inhibitor in tiny doses, but when it replaces Zn2+ in some Zn activated metallo ases, it causes cadmium toxicity. It damages testicular tissue and red blood cells, as well as causing bone marrow disorders, high blood pressure, and kidney damage (Martin *et al*., 2002).

CONCLUSION

The monitoring assessment did provide an understanding of surface water quality in the study area from the foregoing chapters it is inferred that the concentrations of all the parameters are exceeded in the non industrial area. Among SIPCOT dye industry stations, are heavily polluted, from the above study and the other stations concentrations of nutrients that water is not safe for drinking only it is used for domestic purposes. On the basis of microbiological studies, it can be conclude that the stations situated nearby to non industrial area are contaminated due to higher concentration of bacteria. In the non-industrial area, concentrations of nutrients, heavy metals and bacteria are within the prescribed limits. So public should be made aware of the water quality importance on sanitation and economical water treatment methods like filtration and boiling would prove beneficial to avoid waterborne diseases in the industrial area. The remedial measures are must immediately to safeguard and conserve the precious water resources from pollution for future generations. This is a prime solution to pollution and future imminent water wars.

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REFERENCES

Atwa, M., Ahmed, F. H., & Naguib, N. A. (2014). Treatment of peanut seeds with mint and clove essential oils to eliminate fungal load and aflatoxins production in the final yield. *Journal of Agricultural Chemistry and Biotechnology, 5*(2), 57-70.

Choi, J., & Wang, H. (2009). Stakeholder relations and the persistence of corporate financial performance. *Strategic Management Journal, 30*(8), 895-907.

Davis, L. E., & Taylor, J. C. (1972). *Design of jobs: Selected readings*: Penguin.

Donlon, B. S., Mueller Jr, R. L., Daniel, S. C., Gifford III, H. S., & Stevens, J. H. (1997). Clamp assembly and method of use: Google Patents.

Edwards, C. A., & Bater, J. E. (1992). The use of earthworms in environmental management. *Soil Biology and Biochemistry, 24*(12), 1683-1689.

Eitminavičiūtė, I., Matusevičiūtė, A., & Bagdanavičienė, Z. (2005). Remediation of lanfill soils with sewage sludge. 1. Pedobiont successions in the first stage of soil remediation. *Ekologija, 3*, 44-53.

Faucette, L. B., Risse, L. M., Jordan, C. F., Cabrera, M. L., Coleman, D. C., & West, L. T. (2006). Vegetation and soil quality effects from hydroseed and compost blankets used for erosion control in construction activities. *Journal of Soil and Water Conservation, 61*(6), 355-362.

Goel, J., Kadirvelu, K., Rajagopal, C., & Garg, V. (2005). Removal of mercury (II) from aqueous solution by adsorption on carbon aerogel: Response surface methodological approach. *Carbon, 1*(43), 197-200.

Greenfelder, L., Reil, W., Ramos, D., McGranahan, G., Hendricks, L., Olson, W., Snyder, R. (1989). Walnut hedgerow planting system. *Leaflet-University of California, Cooperative Extension Service (USA)*.

Han, F., Lin, L., Warby, S. C., Faraco, J., Li, J., Dong, S. X., Li, Q. Y. (2011). Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Annals of Neurology, 70*(3), 410-417.

Lassar, W., Mittal, B., & Sharma, A. (1995). Measuring customer-based brand equity. *Journal of Consumer Marketing*.12(*4*),11-19.

Lobert, S., Isern, N., Hennington, B. S., & Correia, J. J. (1994). Interaction of tubulin and microtubule proteins with vanadate oligomers. *Biochemistry, 33*(20), 6244-6252.

Lozano Sánchez, D. M., & Moreno Donado, Y. J. (2016). Análisis de la incidencia de agentes patógenos en la estructura de guadua de la Universidad la Gran Colombia sede Armenia.1-96.

Martin, D. F., Sierra-Madero, J., Walmsley, S., Wolitz, R. A., Macey, K., Georgiou, P., Stempien, M. J. (2002). A controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. *New England Journal of Medicine, 346*(15), 1119-1126.

MubarakAli, D., Thajuddin, N., Jeganathan, K., & Gunasekaran, M. (2011). Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids and Surfaces B: Biointerfaces, 85*(2), 360-365.

Pines, J. M., Hilton, J. A., Weber, E. J., Alkemade, A. J., Al Shabanah, H., Anderson, P. D., Ferrandiz, S. (2011). International perspectives on emergency department crowding. *Academic Emergency Medicine, 18*(12), 1358-1370.

Pink, D., Lindner, T., Mrozek, A., Kretzschmar, A., Thuss-Patience, P. C., Dörken, B., & Reichardt, P. (2006). Harm or benefit of hormonal treatment in metastatic low-grade endometrial stromal sarcoma: single center experience with 10 cases and review of the literature. *Gynecologic Oncology, 101*(3), 464-469.

Puvaneswari, N., Muthukrishnan, J., & Gunasekaran, P. (2006). Toxicity assessment and microbial degradation of azo dyes.*Indian Journal of Experimental Biology*,44(8),618-626.

Senan, R. C., & Abraham, T. E. (2004). Bioremediation of textile azo dyes by aerobic bacterial consortium aerobic degradation of selected azo dyes by bacterial consortium. *Biodegradation, 15*(4), 275-280.

Senthilmurugan, S., Sattanathan, G., Vijayan, P. P. K., & Tamizhazhagan, V. (2018). Evaluation of different concentration of vermiwash on seed germination and biochemical response in Abelmoschus esculentus (L.). *International Journal of Biology Research, 3*(1), 332-338.

Sharma, G., Verma, R., & Pathare, P. (2005). Mathematical modeling of infrared radiation thin layer drying of onion slices. *Journal of Food Engineering, 71*(3), 282-286.

Sicheri, F., Moarefi, I., & Kuriyan, J. (1997). Crystal structure of the Src family tyrosine kinase Hck. *Nature, 385*(6617), 602-609.

Sinha, M. K., Opentanova, I., Ohannesian, J. P., Kolaczynski, J. W., Heiman, M. L., Hale, J., Caro, J. F. (1996). Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *The Journal of Clinical Investigation, 98*(6), 1277-1282.

WHO, W. (2012). Guideline: daily iron and folic acid supplementation in pregnant women.

Xiong, M., Sivasankaran, R., Stankovic, J. A., Ramamritham, K., & Towsley, D. (1997). Scheduling access to temporal data in real-time databases *Real-Time Database Systems*, 167-191.