Assessment of the toxicity of three analgesics on P. reticulata and D. rerio using Multispecies Freshwater Bio monitoring

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***Abstract*- The present work has been conducted to study the acute toxicity of three micro pollutants on P. reticulata and D. rerio. The pollutants selected for the study were acetaminophen (paracetamol), carbamazepine and ibuprofen which are widely used as drugs. Both the species were exposed to different concentrations of the pollutant for 24 hours. Their behaviour in the control water and various concentrations were observed using Multispecies Freshwater Bio monitor© developed by LimCo International, GmbH, Konstanz, Germany. The study showed that there is a direct impact on fish species with increase in concentration of toxicant. Acetaminophen was found to be more toxic compared to other pollutants tested.**

Index Terms- Acetaminophen (paracetamol), bio monitoring, carbamazepine, D. rerio, ibuprofen, P. reticulata.

1. INTRODUCTION

Over the last few decades, the occurrence of micro pollutants in the aquatic environment has become a worldwide issue of increasing environmental concern. Luo et.al (2014). Micro pollutants are referred to those materials which are toxic, persistent and bio accumulative and have negative effect on ecosystem. These are present in many of the products which we consume daily (drugs, cosmetics, insecticides etc.) at the home or industries. These are present in aquatic environment at extremely low concentrations, in the order of nanogram per litre or microgram per litre. Some of these can have potentially chronic direct or indirect effect on ecosystem. Micro pollutants reach the environment mainly through municipal or industrial waste waters and hospital activities etc. Some micro pollutants come through natural background generation. For example, the drugs can reach directly through the discharge of unused medicines or indirectly through urine and faeces of human, since only a portion of the drug is synthesized by the body. Fuhrmann (2012).

Pollution of water bodies have been of great concern in the recent times. The monitoring of the water bodies has been conventionally done by physical and chemical methods. This does not give any real time values due to its discontinuity in sampling. Biological monitoring in combination with the physio-chemical monitoring can give a better idea on the pollution status of the water body. But conventional biomonitoring concentrates only on the lethal concentration at which 50% of the replicates are dead. A recent innovation in this field is the online biomonitoring system. Online biological monitoring can not only integrate synergistic or antagonistic effects of chemicals in the effluent but can also provide an advance warning of insiduous toxic conditions. Morgan (1988). Since it is a continuous monitoring system, we can get the real time status of the water body and can take measure to prevent its further pollution.

It is well known that fish are ideal test organism for investigation of behavioural toxicity of chemicals in water. Little and Finger (1990). This is an acute toxicity study in which an instrument called Multispecies Freshwater Bio monitor© (MFB) developed by LimCo International, GmbH, Konstanz, Germany is used to study the behavioural changes of two different species when exposed to varying concentration of pollutants. The test species were placed individually in chambers in which they can move freely and these chambers are exposed to various concentrations of different pollutants. By studying the various behavioral patterns, we can predict the concentrations which imposed stress as well as death of the organism.

2. MATERIALS AND METHODOLOGY

2.1 Test species

Poecilia reticulata (guppy) and Danio rerio (zebra fish) were used as test species for this study. Both species were in their juvenile stage. Both guppy and zebra fish belong to the eight recommended model organisms in ecotoxicology testing established in the guidelines set by the Organization for Economic Cooperation and Development (OECD). Doleželová (2008). These species were selected due to the availability in same size and age. The fishes were cultured in the laboratory.

2.2 Test Chemicals

Three drugs, Acetaminophen (commonly known as paracetamol), carbamazepine and ibuprofen were used for the test. Dolo (650 mg), Tegrital (400 mg) and Ibugesic (400 mg) tablets were used for acetaminophen, carbamazepine and ibuprofen respectively. These tablets were diluted in water and filtered using pressure filter to obtain desired concentrations. The water used for experiments is same as that used to culture the fishes.

2.3 Multispecies Freshwater Bio monitor©

Multispecies Freshwater Bio monitor© is an automated, biological early warning system developed by LimCo International GmbH, Konstanz, Germany, that can be used to monitor the water quality of any water body continuously. It consists of chambers in which the test species are placed. These chambers are connected to an impedance convertor which is in turn connected to a computer with MFB software. MFB works on the principle of Quadrapole Impedance conversion. Gerhardt and Svensson (1994). The test species are placed individually in a chamber in which they are free to move. Each chamber has two pairs of electrodes. One pair of electrodes carry current and an electric field is formed when current passes through it. When the organism moves inside the chamber, the electric field is disturbed, and this disturbance is recorded by the other pair of electrodes which is non-current carrying. The locomotory and non-locomotory behaviour of the organism can be recorded. The signals are recorded as voltage Vs time graph. It is then converted to percentage of movement Vs frequency through Fast Fourier Transformation (FFT) provided in the software. If the frequency is between 0-2 Hz, it is a locomotory behaviour and if the frequency is in between 2.5 – 5Hz, it is a non-locomotory behaviour. This can act as an early warning system to predict the stress occurring on the organism.

2.4 Methodology

Visual observation was conducted to find the LC50 (24 hours) in which four replicates each of P. reticulata and D. rerio were introduced to different concentrations of acetaminophen, carbamazepine and ibuprofen. The LC50 values were found through trial and error method. With these values, behavioural analysis was carried out for 24 hours to find if there was any early warning given by the organism at any concentration before its death.

3. STATISTICAL ANALYSIS

The raw data on movement activity of P. reticulata in control and various concentrations of acetaminophen, carbamazepine and ibuprofen were statistically analyzed and plotted using Sigma Plot 13.0 (Systat Inc.). Normally distributed data of behaviour measurements of P. reticulata was analyzed with Friedman Repeated Measures Analysis of Variance on Ranks followed by Normality Test (Shapiro-Wilk): Failed ($P < 0.050$) or Tukey test for nonparametric data. There was a statistically significant difference ($P =< 0.001$) in the median values among the treatment, which is plotted in the graph (Fig. 1).

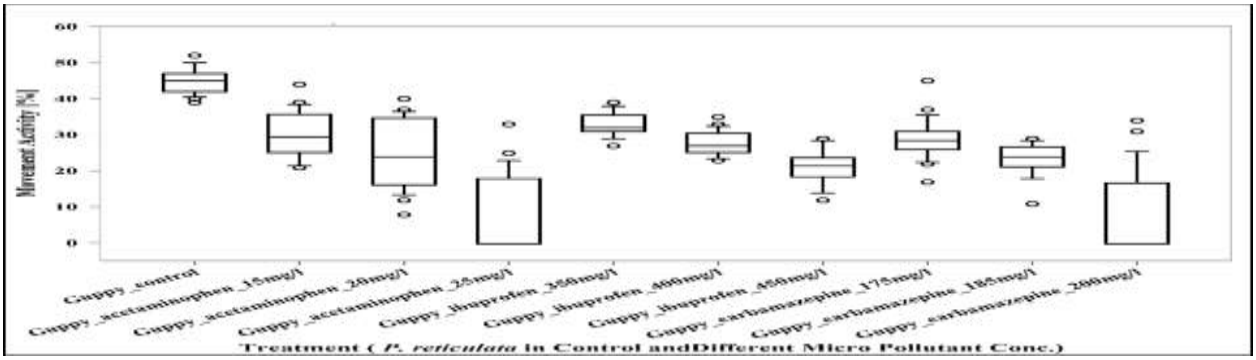

Fig. 1: Statistical analysis (box plot) of movement of P. reticulata in control and various concentrations of pollutants

The raw data on movement activity of D. rerio in control and various concentrations of acetaminophen, carbamazepine and ibuprofen were statistically analyzed and plotted using Sigma Plot 13.0 (Systat Inc.). Normally distributed data of behaviour measurements of D. rerio were analyzed with Friedman Repeated Measures Analysis of Variance on Ranks followed by Normality Test (Shapiro-Wilk): Failed (P < 0.050) or Tukey test for nonparametric data. There was a statistically significant difference (P =< 0.001) in the median values among the treatment, which is plotted in the graph (Fig. 2).

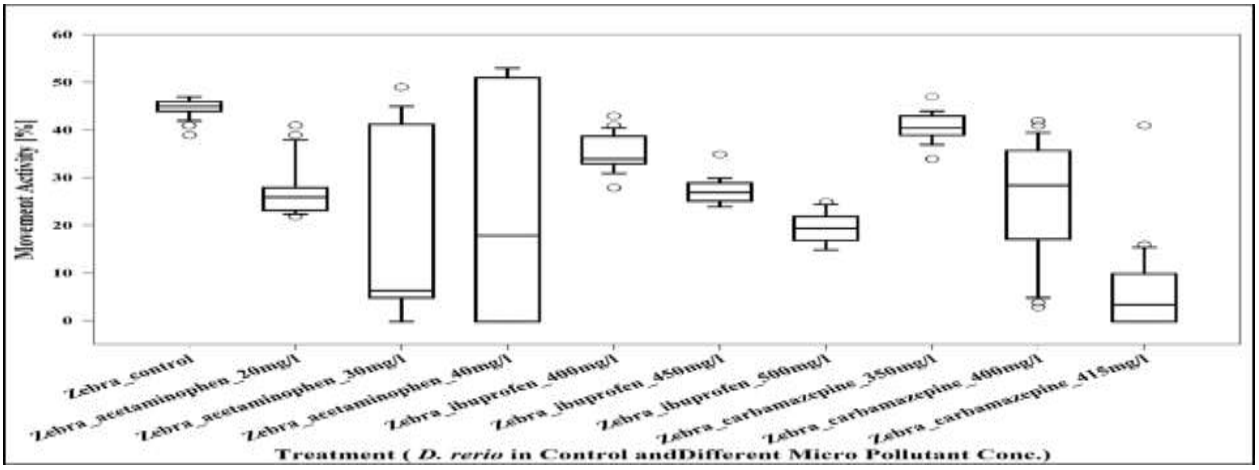

Fig. 2: Statistical analysis (box plot) of movement of D. rerio in control and various concentrations of pollutants.

4. RESULTS AND DISCUSSIONS

The locomotors and non- locomotors activities of both species in control and different pollutant concentrations with respect to time were plotted using sigma plot 13.0. (Systat Inc.). The movement activity of P. reticulata in control and different concentrations of acetaminophen for 24 hours exposure time is shown in (Fig. 3).

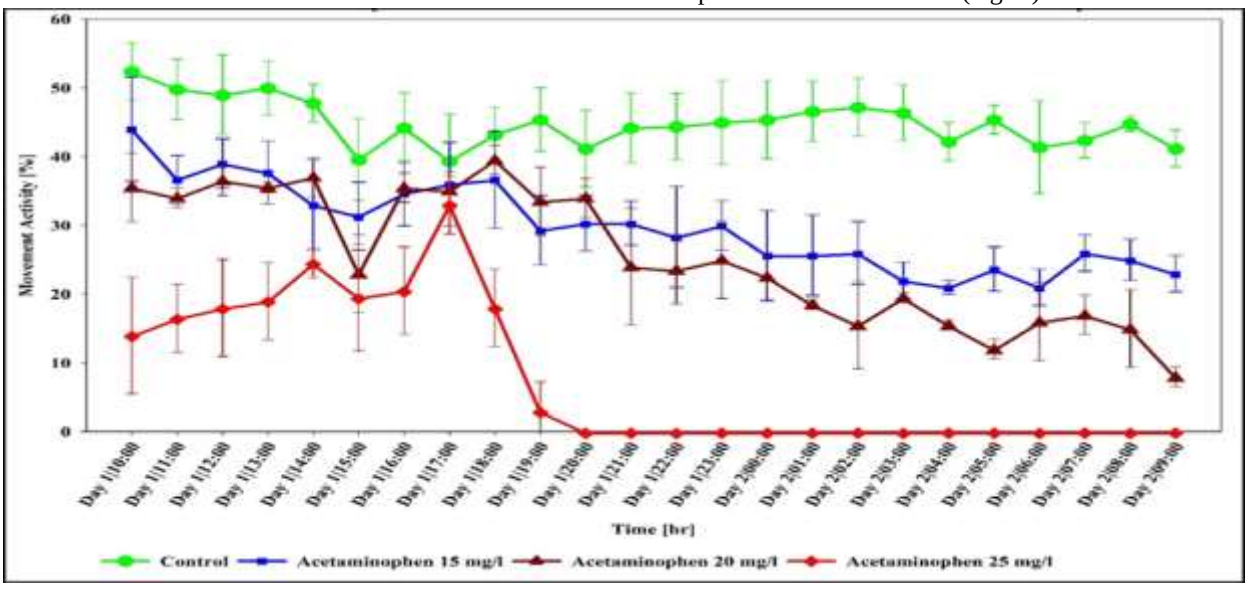

Fig 3: Movement activity of P. reticulata in control and various concentrations of acetaminophen

At acetaminophen concentration of 15 mg/l, there was a reduction in locomotory activity as the exposure time increased (< 30% occurrence of 0 – 2Hz on 2nd day), whereas the organism in control had > 40% occurrence of 0-2 Hz almost all the time. At 20 mg/l, initially there was an increased stress followed by the reduction in its movement (< 20% occurrence of 0-2 Hz). At 25 mg/l, the fish showed a

reduction in its movement (< 20% occurrence of 0-2 Hz), then an increased stress ventilation (occurrence of signals having 2.5 – 5 Hz frequencies) followed by its mortality after 11 hours of exposure. This showed that acetaminophen has an acute effect on P. reticulata. The graph showing the movement activity of P. reticulata in various concentrations of carbamazepine is given in (Fig. 4).

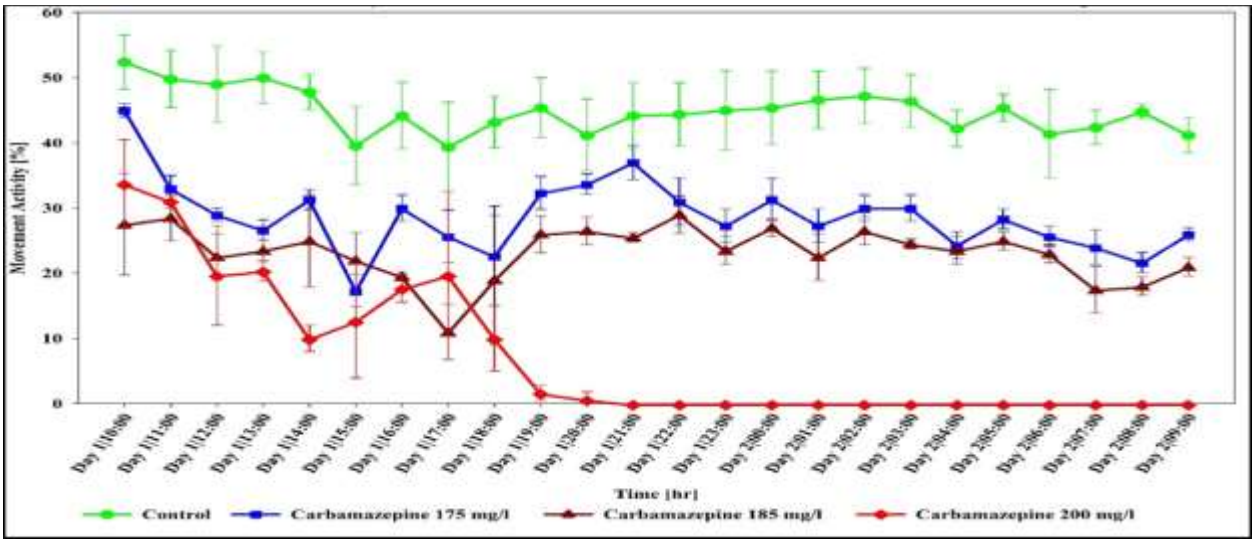

Fig 4: movement activity of P. reticulata in control and various concentrations of carbamazepine

At carbamazepine concentration of 175 mg/l, there was a reduction in movement (< 40% occurrence of 0 – 2 Hz) when compared to the movement in control (40 – 55% occurrence of 0 – 2 Hz). At 185 mg/l, there was again a reduction in movement (< 30% occurrence of 0-2 Hz). At 200 mg/l, there was an increased stress ventilation (occurrence of signals having 2.5 – 5 Hz frequencies) followed by their mortality after 10 hours of exposure. There is an acute effect of carbamazepine toxicity in P. reticulata. The graph showing the movement activity of P. reticulata in various concentrations of ibuprofen is given in (Fig. 5).

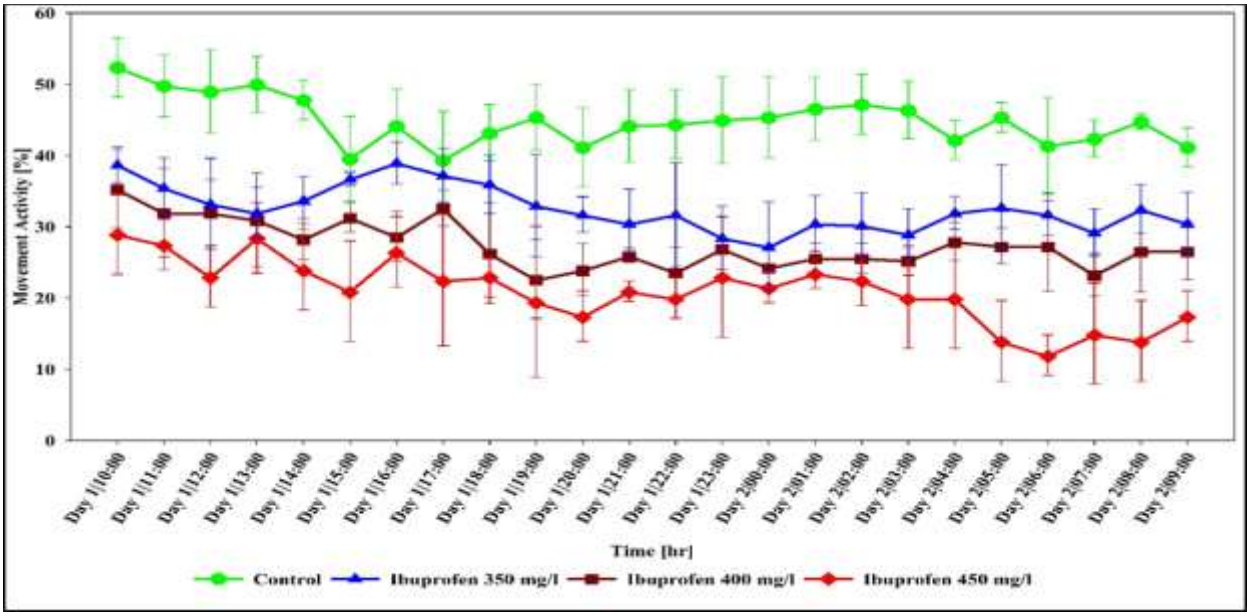

Fig 5: Movement activity of P. reticulata in control and various concentrations of ibuprofen

There was a reduction in movement observed in all the concentrations of ibuprofen. At 450 mg/l, there was only < 20% occurrence of 0-2 Hz nearing to the end of exposure time. Only visual observations were conducted for higher concentrations of 500 to 800 mg/l due to lack of time. Visually there was a

reduction in movement, but no mortality was observed for any of the concentrations. P. reticulata showed movement reduction as an early warning of acute toxicity of ibuprofen. The movement activity of D. rerio in various concentrations of acetaminophen is shown in (Fig 6).

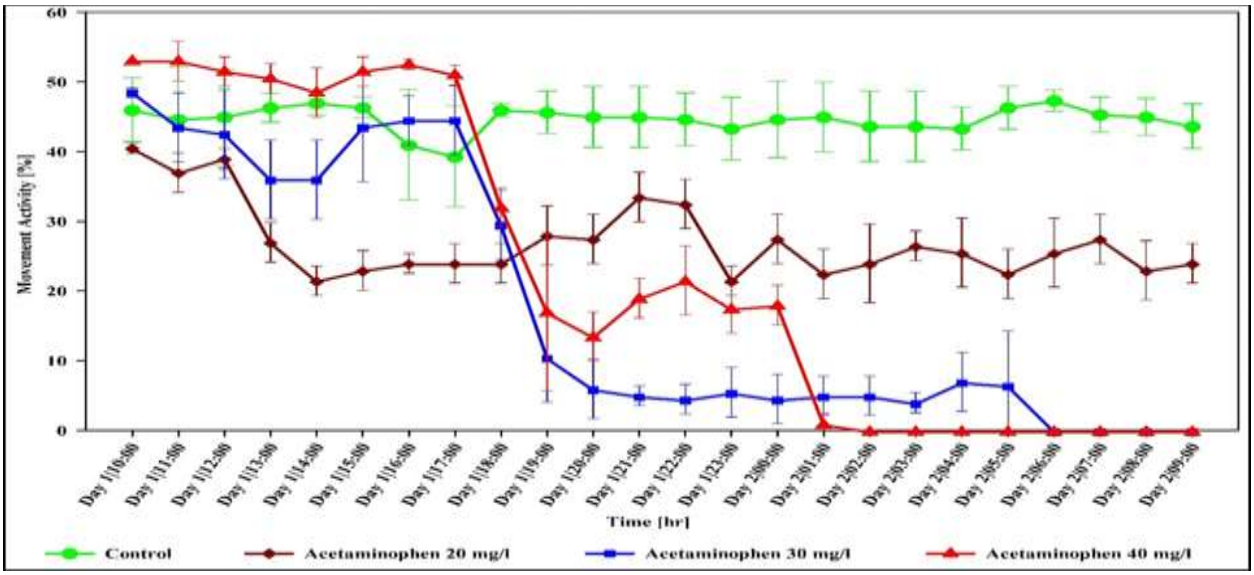

Fig 6: movement activity of D. rerio in control and various concentrations of acetaminophen

At acetaminophen concentration of 20 mg/l, there was a rapid reduction in movement (< 30% occurrence of 0-2 Hz). At 30 mg/l, the organism showed avoidance behaviour (> 50% occurrence of 0 – 2Hz) followed by stress ventilation (2.5 – 5 Hz) and mortality after 21 hours of exposure. At 20 mg/l, the organism showed a rapid reduction in movement (< 10% occurrence of 0-2 Hz) after the initial avoidance behaviour followed by mortality after 16 hours of exposure. The movement activity of D. rerio in various concentrations of carbamazepine (Fig 7).

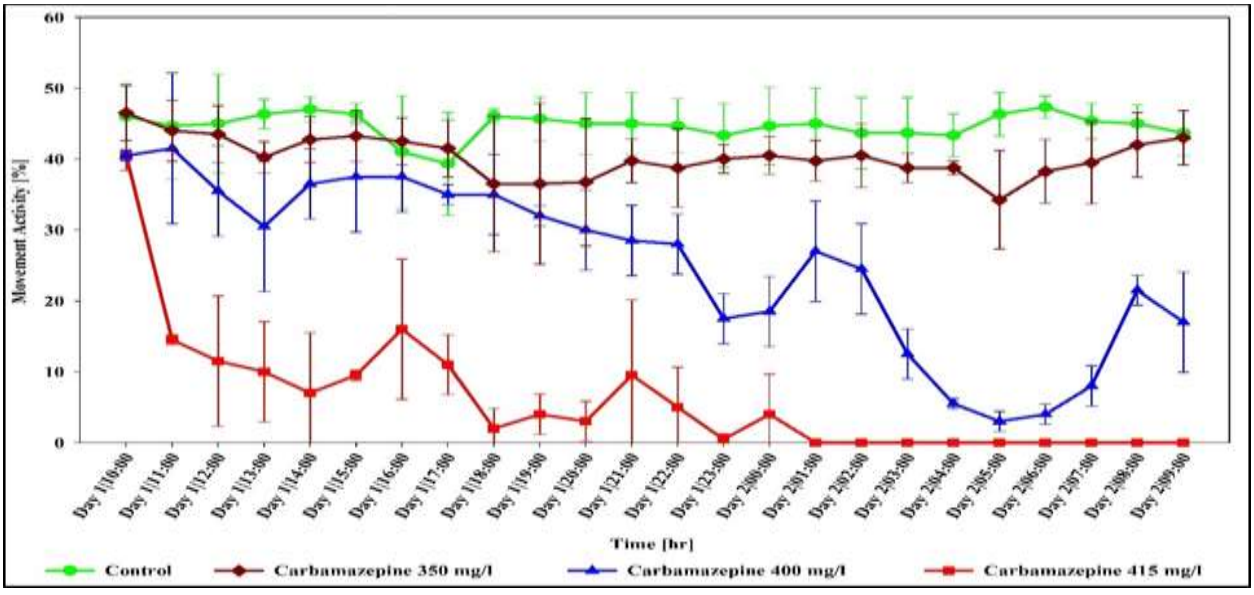

Fig 7: Movement Activity of D. rerio in Control and Various Concentrations of Carbamazepine

At carbamazepine concentration of 350 mg/l, there is a very slight reduction in movement activity. At 400 mg/l, the organism showed an initial stress ventilation (2.5-5 Hz) followed by a reduction in its movement (< 30% occurrence of 0-2 Hz). At 415 mg/l, there is a drastic reduction in movement of the organism and mortality was detected after 15 hours of exposure. The movement activity of D. rerio various concentrations of ibuprofen (Fig.8)

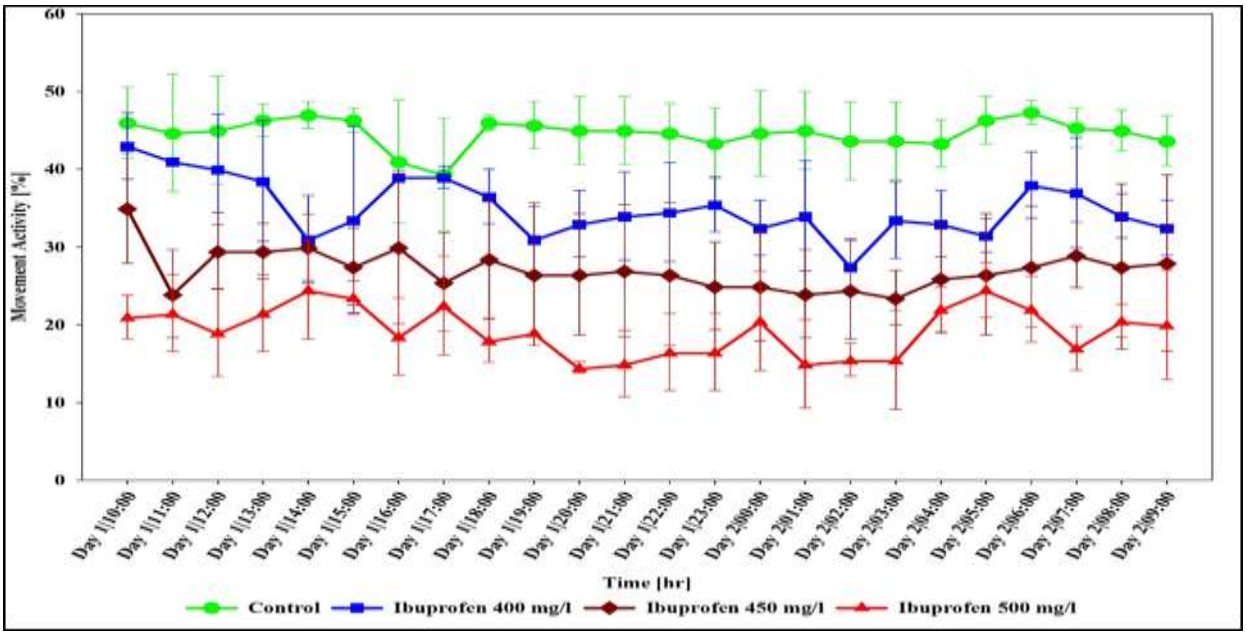

Fig 8: movement activity of D. rerio in control and various concentrations of ibuprofen

There was a reduction in movement observed in all the concentrations of ibuprofen. At 500 mg/l, there was only < 25% occurrence of 0-2 Hz during the time of exposure. Only visual observations were conducted for higher concentrations of 550 to 800 mg/l due to lack of time. Visually there was a reduction in movement, but no mortality was observed for any of the concentrations. D. rerio showed movement reduction as an early warning of acute toxicity of ibuprofen.

It has been observed through several studies that there are differences in sensitivity of different species to a given chemical compound. Gallo et.al (1995), Vittozzi (1991), Doleželová et.al (2011). Pharmaceuticals are being extensively and increasingly used and they are emerging as a serious source of water pollution. These chemicals are designed to have a specific mode of action, and many of them for some persistence in the body. Fent (2006). Due to this fact, there is a serious need to study the toxicity of these chemicals to aquatic and terrestrial life. But there is a little knowledge of the acute and chronic effect of these pharmaceuticals. Aquatic animals are the most affected due to these pollutants as eventually they reach the water bodies in different concentrations and the animals are exposed to these pollutants throughout their life. In many countries, traces of micro pollutants have been found in the municipal water and waste water treatment plants. Buser et.al (1999), Roberts et.al (2005), Ternes (1998).

From the experiments conducted, it was observed that there is a significant impact of these pharmaceuticals on both the species tested. Different pharmaceuticals target different receptors in humans. It can target the similar receptors in smaller vertebrates and invertebrates. Knowledge about similar targets exists primarily for fish. Fent (2006).

In acetaminophen, P. reticulata showed reduction in movement in 15 and 20 mg/l (20 – 35% occurrence of 0-2 Hz) and showed mortality after 11 hours of exposure in 25 mg/l. D. rerio showed reduced movement 20 mg/l (<10% occurrence of 0-2 Hz) and mortality at 30 mg/l after 16 hours of exposure. The mode of action of paracetamol is not yet fully defined. In humans, overdose of acetaminophen can affect the functioning of liver. Marta Jozwiak (2014). Similarly, in fishes there can be organ damage due to overdose. Mortality of the organism must have been due to high dosage.

Carbamazepine has been extensively used in the treatment of epilepsy, as well as in the treatment of neuropathic pain and affective disorders. However, the mechanisms of action of this drug are not completely elucidated. Ambrósio et.al (2012). In humans, over dosage can affect the cardiovascular and nervous systems. The LC50 values obtained for P. reticulata and D. rerio were 200 and 415 mg/l respectively. Both the species showed a stress

ventilation. This might be because higher concentration has affected the nervous system of the organisms.

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). It works by reducing hormones that cause inflammation and pain in the body. In humans, regular intake of ibuprofen can increase the risk to heart attacks and stroke. Higher doses can cause stomach and intestinal bleeding. As observed from the experiments, there was only reduction in movement even when the dosage was increased to 800 mg/l. The acute toxicity of ibuprofen was found to be 200mg/l. Fent (2006). Since pure form of ibuprofen was not available, ibuprofen tablets were diluted and filtered to get the desired dosage. There might be interaction of other compounds in the drugs which affected the action of ibuprofen alone.

4. CONCLUSION

Micro pollutants are a major concern with respect to water pollution. Aquatic life is adversely affected by these pollutants because of their long-term contact. The effect of three pharmaceutical pollutants was studied and it showed that there is some stress induced by these pollutants on the aquatic life. With the help of multispecies freshwater biomonitoring, the behavioural changes in the organism with varying concentrations of pollutant could be observed. This helps in understanding the condition of the organism before mortality. Online bio monitoring, along with physio-chemical test can be a better way to analyze and manage the water quality. There are several other micro pollutants which are persistent in water bodies. These micro pollutants can be tested using online monitoring to understand the stress on aquatic life.

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