Research Article

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EFFECT OF THE PESTICIDE MONOCROTOPHOS ON THE OSMOREGULATION OF A BRACKISH WATER OLIGOCHAETE, *PONTODRILUS BERMUDENSIS* (BEDDARD) IN RELATION TO SALINITY VARIATIONS

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Abstract: The toxicity of a commonly used organophosphate pesticide, Monocrotophos, on *Pontodrilus bermudensis* (Beddard) in optimum salinity was studied from a tropical brackish water environment, the Southern Lighter Channel at the Visakhapatnam Harbour. Physicochemical characteristics were determined at the habitat of the worm during the period of the study. The LC_{50} values obtained for this worm subjected to 24, 48, 72 and 96 hours exposure showed that the toxicity of the pesticide increased with the period of exposure. The high LC_{50} values indicated its endurance to the toxicant. The concentration changes found in the body fluid (C^{-}, Na+ & K+) in the worms might suggest the possible influence of the pesticide on ion pumps or ion regulatory mechanisms of the worm. The present study confirms that Monocrotophos has interfered remarkably with ion regulation of the worm.

Keywords: Pesticidal pollution, Harbor waters, Monocrotophos, Oligochaete, Osmoregulation

INTRODUCTION

Pesticidal pollution, the outcome of modern agricultural practices is never normally imagined by the modern man. The large scale use of pesticides and fertilizers presented not only health hazards to him and to his animals' also exerted eco-imbalance causing global concern. Ecologists and several non-Governmental organizations throughout the world, who have realized these dangerous hazards, promoted the global awareness and also called for the moratorium on the use of xenobiotics. The United Nations conference at Stockholm in 1972 focused and drew the attention on growing menace and hazards of environmental contamination arising out of air, water, land and noise pollution.

In order to understand how pollutant can affect an organism at sea, it is important to know the normal metabolism of the organism. Usually the population dynamics and physiological responses of organism are governed not by a single environmental factor but by many counteracting, supporting and modifying parameters. Consideration of a spectrum of natural ecological factors like salinity, temperature etc., and manmade ones (pollutants) is necessary in evaluating the environmental health and suitability to inhabitances[1, 2]. Considering the millions of species that could be adversely affected by introduction of xenobiotics into the marine environment, integrated approaches to acquire the knowledge of ecophysiology and biochemistry of diverse organisms and ecotoxicology of various xenobiotics are of paramount importance[3].

Visakhapatnam is an industrial city on the east coast of India (Latitude 17°41' N; Longitude 83°17'E), where the environmental pollution levels are alarming. Whether or not the harbor fauna are subjected to any pesticidal contamination, the harbor environment receives undoubtedly agro pollutants from the surrounding agricultural fields through the river "Mehadri" and domestic and industrial wastes from the other canals[4,5,6,7]. The harbor also encourages fouling potential of a number of organisms of high order. In order to control their infestation variety of antifouling agents containing biocidal compounds and insecticides are used. In addition, the Visakhapatnam municipal corporation also frequently uses organochlorines, organophosphates and some carbamates in its public health activities in slums surrounding the harbor and its environs which are the breeding grounds for a variety of disease spreading germs and insects. Hence, it is presumed that the pesticidal contamination is inevitable in the harbor environment, thereby exerting influence on non-target organisms.

Brackish water oligochaete *Pontodrilus bermudensis* (Beddard) occurs in large numbers in the harbor where the environmental parameters fluctuate widely[8]. The worm is a soft bodied sub surface organism and used as bait for fishing and occupies lower tropical level in the food chain and incidentally *P. bermudensis* in the Visakhapatnam harbor along with other biota may experience exposure to toxicants such as pesticides which are either transported into or present in the harbor waters and sediments. Since it

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has already been mentioned, the physiological, biochemical and toxicological responses of marine, estuarine or coastal organisms to the stresses imposed by exposure to lethal and sub lethal concentrations of pollutants are poorly understood, it is felt that such a study that depicts the effects of ecological parameters and xenobiotics on overall metabolic activities of a non-target organisms is worth undertaking. Since *P. bermudensis* experiences environmental salinity variations in the habitat and regulates body, fluid ion concentrations, the present study enlightens the combined effects of salinity and the xenobiotic compounds (pesticides) on the hydromineral metabolism of the worm.

MATERIALS AND METHODS

P. bermudensis were collected from the Visakhapatnam harbor waters and acclimated to the laboratory conditions at different sw concentrations for a period of 5 to 7 days. Both for acclimation and experimentation, the sea water used was filtered with Whatman No. 42 filter paper; worms were maintained in batches using a number of acclimation chambers. This avoids crowding effect which may results in heavy mortalities. Worms of the same size (weight 99 to 111 mg; X = 104.8 mg) were separately maintained for experimentation. The desired concentrations of test salinities ranging from 5 to 34$^{o}/_{oo}$ were prepared by mixing 100% sea water of known salinity with appropriate amounts of distilled water. A Salinity of 34.5$^{o}/_{oo}$ is considered as 100% sea water. All the experiments were conducted at a constant temperature (30 ± 1°c) using a BOD incubator. Since the rate of oxygen consumption changes with the size (weight) of the worm, two sets of experiments were conducted, one with the worms of equal size and the other with the organisms varying in size in different salinity media.

In the first set, almost equal sized worms (average weight 104 mg) acclimated to laboratory conditions, were used for experimentation in different salinities (5 to 34$^{o}/_{oo}$). The worms were transferred to test salinities and their individual oxygen uptake was measured 2 to 3 time at 2 hourly intervals at each salinity. In all, 12 worms were used at each salinity concentration. Simultaneously, control experiments were conducted at the ambient salinity concentration (25$^{o}/_{oo}$). In the second set of experiments, the respiratory measurements were made using worms of different sizes at different salinities (5 to 34$^{o}/_{oo}$). Worms of different size ranging from 70 mg to 140 mg were used in the test salinities. In both the sets, the animals were allowed to remain for at least two hours in the test media before oxygen consumption measurements were made. Salinity of the water samples were determined by Harvey's method[9]. The results are expressed as parts per thousand (‰).

The acclimated animals were exposed to 96 hr LC_{50} and sublethal concentrations of Monocrotophos at different sw media concentrations. The body fluid ion concentrations (Na^+, K^+, and Cl^-) were estimated in the warms exposed to 96 hr LC_{50} and sublethal concentrations of the selected pesticide. Simultaneously sw and the body fluid ion concentrations were determined after subjecting the worms to different sw media without pesticide. The body fluid was collected by simple incision of the body wall and by gentle draining into a small chilled glass vial. Tissue free body fluid was centrifuged at 3000 rpm for about 15 minutes and the clear supernatant was used for analyses.

The chloride (Cl^-) concentration in the body fluid was estimated by the mercumetric method of Shales and Shales[10]. The chloride contents in the body fluid of the control exposed worms at different salinities (25, 50, 75 & 100% sw) and in combination with 96 hr LC_{50} and sublethal concentrations of monocrotophos was estimated. In addition to this the chloride concentration in the different sw medic employed in experimentation was also estimated. Sodium (Na^+) and Potassium (K^+) concentrations in experimental media and body fluids of the control and exposed animals were estimated on flame photometer (ELICO). The body fluids were diluted to 200 times with 0.2 $NHNO_3$. Standard graphs were prepared by using known amounts of sodium and K^+ concentrations in the body f1uids of control and exposed worms were calculated by comparing the values with the standard graphs prepared. The worms were exposed to 96 hr LC_{50} and sublethal concentrations of Monocrotophos in 25, 50, 75 & 100% sw media and body fluid sodium and K^+ concentrations were estimated. The Na^+ and K^+ concentrations in different sw concentrations were also estimated. The results reported are the averages of 10 observations at each concentration.

RESULTS

The body fluid ion concentrations of Cl^-, Na^+, and K^+ of *Pontrodrilus bermudensis* after subjecting thorn to different concentrations of sw and 96 hr LC_{50} and sublethal concentrations (1/10 of LC_{50}) of monocrotophos in heteroosmotic media (Ranging from 25, 50, 75 & 100% sw) are presented in Tables 1-3.

Table.1: Body fluid Chloride Ion concentration (m M/l), changes in *P. bermudensis* exposed to respective LC_{50} and sublethal concentrations of Monocrotophos in different sea water media concentration (Temp: 30±1°C).

% of sea water	Controls	96 hr LC_{50} concentration	% of change	96 hrs SLC	% of change
25	133.32	153.42	15.08	103.63	-22.26
50	218.822	253.79	15.97	167.58	-23.41
75	293.889	325.188	10.65	202.67	-31.63
100	396.947	426.43	7.43	326.55	-17.73

Table.2: Body fluid Sodium Ion concentration (m M/l), changes in *P. bermudensis* exposed to respective LC_{50} and sublethal concentrations of Monocrotophos in different sea water media concentration (Temp: 30±1°C).

% of sea water	Controls	96 hr LC_{50} concentration	% of changes	96 hrs SLC	% of changes
25	289.54	305.15	5.39	195.00	-32.56
50	329.4	356	8.07	259.99	-21.07
75	372.18	398.22	7.00	269.36	-27.62
100	414.33	436.12	5.25	376.3	9.090

Table.3: Body fluid Potassium Ion concentration (m M/l), changes in *P. bermudensis* exposed to respective LC_{50} and sublethal concentrations of Monocrotophos in different sea water media concentration (Temp: 30±1°C).

% of sea water	Controls	96 hr LC 50 concentration	% of changes	96 hrs SLC	% of changes
25	20.512	25.84	25.98	26.95	31.39
50	21.534	27.174	26.209	28.87	34.05
75	26.150	30.76	17.629	32.04	22.52
100	28.720	31.79	10.389	35.89	26.965

Chloride (Cl^-):

The body fluid Cl^- concentrations of control worms in different sw media ranged from 130.8-390.6 M Eq/1th sw medium of 25-100%. The body fluid chloride concentration is almost isoionic in the worms subjected to 25% sw and hypoionic at higher salinities (50, 75, and 100% sw) (Table 1).

The body fluid Cl^- concentration in worms exposed to 96 hr LC_{50} concentration of Monocrotophos increased in all test salinities ranging from 156.14 to 423.26 M Eq/l. The body fluid Cl^- is hyperionic at 25 % sw and hypoionic at 50, 75 and 100% sw Media (Table 1). The percentage increase in Cl^- ion concentration in Monocrotophos exposed worms is 15.97, 15.08, 0.65 and 7.43 respectively in 25, 50, 75 & 100% sw media.

The 96 hr sublethal concentration of Monocrotophos exposed worms showed a body fluid Cl^- ion concentration range of 94.52 to 305.66 M Eq/l in salinity media of 25-100% sw. The body fluid Cl^- concentration of worms exposed to 96 hr sublehal concentrations of Monocrotophos decreased in all test media. The percentage decrease in Cl^- ion concentrations recorded are 22.26, 23.41, 31.03, and 17.73 in 25, 50, 75 & 100% sw media tested.

Sodium (Na^+):

The body fluid Na^+ concentrations of the worm ranged from 289.54-414.33 mµ/1 in sw medium of 25-100%. The body fluid Na^+ concentrations are hyperionic in worms subjected to 25, 50 and 75% sw media and hypolonic in 100% sw (Table 2).

In worms subjected to respective 96 hr LC_{50} concentration of Monocrotophos at different sw media showed increased concentrations ranging from 292.5 to 436.1mµ/1 and Na^+ ion is hyperionic at 100% sw medium. The percentage increases in ion concentrations recorded are 0.91 and 525 in worms tested at 25 and 100% sw. The percentage increase in Na concentration is maximum (8.07 and 7.0) in worms exposed to 50 and 75 % Sw media.

The body fluid Na^+ concentrations of the worms subjected to respective 96 hr sub lethal concentrations of Monocrotophos at different test salinities showed decreased ion concentrations and are hypoionic at all the salinities excepting in 25% sw medium. The Na^+ concentrations ranged from 195.0 to 376.3 mM/1 in sw media 25-100%. The percentage decrease in the body fluid Na concentration is minimum in worms tested at 100% sw and maximum in worms tested at 75, 50 and 25% sw media. The body fluid Na^+ concentration of worms exposed to 96 hr LC 50 concentration of is hyperionic at 25, 50 & 75% and hypoionic at 100% sw. Sodium ion concentration in the body fluid of the worms ranged from 317.8 to 452.0 mM/l in 25, 50, 75 and 100% sw (Table 2). The percentage increase in Na^+ concentration is high in worms experimented at low salinity and low in 100% sw medium. The percentage change in ion concentrations observed are marginal/minimum in worms tested at 50 and 75% sw media.

Potassium (K^+):

The body fluid K^+ concentrations in the Worms remained hyperionic to the medium concentration levels tested. The body fluid K^+ ion concentrations reported here in the control worms respectively are 20.51, 21.53, 26.15 & 28.72 m M/l in 25, 50, 75 and 100% sw media (Table 3).

In worms exposed to 96 hr L50 and sublethal concentrations of Monocrotophos at sw media, the body fluid K^+ levels increased than those recorded in the control worms k^+ concentrations recorded are 28.84 to 31.79 and 26.95 to 35.89 mM/l in 96 hr LC 50 and sublethal concentrations of Monocrotophos exposed worms and are hyperionic at all the test media. The percentage increase in K^+ concentrations more in worms exposed sublethal concentrations than to LC $_{50}$ concentration of Monocrotophos (Table 3). Increased body fluid Potassium ion concentrations of 25.98, 26.21, 17.63 & 10.69 mM/l in worms exposed to LC_{50} and 31.39, 34.05, 22.52 & 24.97 mM/1 to sublethal concentrations of Monocrotophos recorded show no pattern change.

DISCUSSION

The data obtained in this study clearly showed variations in the body fluid sodium, potassium, and Cl^- concentrations of the worm, *P, bermudensis* in response to different sw Media and in with pesticide

(Monocrotophos) selected. The body fluid Cl^- , K^+ and Na^+ in the worm showed a linear increase with increase in the external media concentration (25, 50, 75 and 100% sw) (Tables 1-3).

Body fluid Cl^- concentrations of the worm are hyperionic to 25% sw medium and hypoionic in 50, 75 and 100% sw media. Onset of hyperionicity of Cl^- was noticed in the worms exposed below 25% Sw medium, while at high salinity media tested the worm exhibited hypoionicity (Table 1). Maintenance of hyperionicity at low salinities and hypoionicity of body fluid Cl^- by this worm were also well documented earlier[6]. While the onset of hyperioncity of body fluid Cl^- in this worm did not differ from the earlier reports, surprisingly the present investigations revealed that there is a shift in the onset of hyperionicity of body fluid Cl^- in worms exposed to pesticides i.e, Monocrotophos at LC_{50} and sublethal concentrations. Further, the levels of hyperionlcity of Cl^- in Monocrotophos exposed worms at LC_{50} concentration in 25% sw medium suggested the variations in the pesticide action i.e., the differential toxicity of the pesticide used. In addition the hyperionic body fluid Cl^- concentration levels in the worm in different sw media in combination with pesticides at sublethal concentrations recorded, explain the characteristic variations of these two pesticides. The results showed that the body fluid Na^+ Concentrations hyperionic below 100% sw media and hypolonic at 100% sw in unexposed worms to pesticides, while the exposed ones to lethal concentrations exhibited hyperionic Na^+ in all sw media excepting at 100% sw medium (Tables 2). A shift in hyperionicity of Na^+ is noticed in worms subjected to SLC but the worms exposed to SLC of Monocrotophos showed hyperionic Na^+ in 25% sw media. Hyperionic regulation of K^+ in exposed and unexposed animals is in agreement with the retention of high potassium concentrations, a common phenomenon in several aquatic organisms in normal and in stress conditions[11].

The hyperionic regulation at low salinities and hypoionic regulations at high salinities has been recorded in a number of estuarine and brackish water organisms[12]. Hyperionic body fluids and hyperionicity of Cl^-, Na^+, and K^+ were reported is an estuarine polychaete *Marphsa graveltyi* during reduced salinity stress and hypoionicity when adopted to high salinities, (29.52%) has been reported by Krishna Moorthi and Krishna Swamy[13]. Hypoionic chloride regulation also has been reported in the estuarine crabs, *Upogebia affinis* and *Upogebia pugettensis*[14]. Hyper or hypoionic regulation of haemolymph and body fluids with respect to salinity variations has been reported in several invertebrate organisms[15]. Zanders [16] observed slight decrease in the blood Na^+, Cl^-, Mg^+ and SO_4^- and constancy of K^+ and Ca^{+2} in mangrove crab, *Goniopsis cruentata* adopted to external concentrations ranging from 100% down to 5% sw. An initial rapid decrease in inorganic ions of the haemolymph follow additional osmotic adjustments in the blood in response to salinity decrease is observed in the clam, *Ranea Cuneata*. Hyperionic regulation of haemolymph chloride, sodium and potassium was reported in a decapods crustacean, *Carcenus maenus*. Biote1 and Truchot[15] also observed similar changes with the same crab. High K^+ concentration and almost isoinoic concentrations of Cl^- and Na^+ in the coelomic fluids of a marine worm, *Priapulus caudatus* and hyperionic concentrations of chloride, sodium and K^+ were reported in *Sipunculus nudus* and *Golfingia vulgaris*[17].

Hypoionic Cl^- regulation was well established in semiterrestrial oligochaete species like *Lumbricas terrestries* and was also found that earthworms also behaved like fresh water organisms physiologically. Rama Murthy[18] noticed hypoionic and hyperionic regulation of chloride in a fresh water leech, *Poecilobdella* subjected to high and low salinities of sw. The pattern of chloride regulation in *Pontodrilus bermudensis* recorded in the present study although is similar to that reported pattern in earth worms and the fresh water leech, the levels however differ suggesting habitat difference i.e., the brackish water habitat.

The body fluid Na^+ in *P. bermudensis* is hypoionic in salinities above 75% sw and hyperionic to lower concentrations. Skaer[19] made a similar observation in a polychaete, *Mecirellct enigmaticta*. Zanders[16] reported, that the marine decapod, *Carcinus maenas* is nearly isoosmotic and isoionic in full strength sw. The hyperionicity of K^+ in the body fluids of worms recorded in all sw media is in agreement with those reported earlier in a number of organisms[17]. Annelids are also known to retain high K^+ levels in their body fluids. Hyperionic concentration of K^+ in several polychaetes like *Nereis succrinea, Perinereis cultifera, Mercierella enigmatica, Arenicola manina, Abaarencola calparedii* to wide changes in salinity has been reported[19]. In general K^+ concentration in several organisms vary two to four times greater than the medium[20]. Earlier studies on acute effects of salinity and temperature on *P. bermudensis* revealed hyperiornic concentration of body fluid Cl^- and Na^+ at low and hypoionic at high concentrations of SW media and hyperionic K^+ levels in the body fluid of the worm in all the sw media [4,6].

Disruption of osmoregulation was suggested to be one of the important means by which xenobiotic chemicals such as heavy metals and pesticides apart from natural ecological parameters affect a number of terrestrial and aquatic organisms[21, 22]. A number of pesticidal compounds such as organophosphates is known to exert stress in turn leading to impairments in osmoregulatory and electrolyte regulating systems in number organisms[23]. Haux and Larsson[24], have reported significant increase of blood Cl^- in flounders treated with DDT for 48 hrs and 96 hrs and also found that long term exposure of 6 weeks to DDT to induce the lowering of plasma chloride levels Verma *et al*.,[25] have recorded a consistent hyporchiorernia in *Saccobranchus fossilis* following chlordane treatment. The same fish *saccobranchus fossilis* elicited hypochloremia between 2-6 hrs and hyperchioremia between 48-96 hr to an organophosphate pesticide Fenthion indicating mode of action of different groups of pesticides as different on the same organism. Sivaprasada Rao *et al*., [26] observed that the decrease in body Na^+, K^+, and Ca^{+2} levels in melathion exposed *Tilapia mossambica*. He also observed significant decrease in the Na^+, K^+, and Ca^{+2} levels when compared to water content in methyl parathion toxified *Tilapia* sp. too suggesting the regulation of osmoionic balance is more effective through the operation of salt pump rather than the water pump.

Reduction in osmotic and ionic content of the body may be potentiating the known physiological effects of organophosproues pesticides on the nervous system and these chemicals exert their effects on nervous system by blocking the membrane (Na^+ and K^+) channels and by inhibiting the nerve ATPases[22]. Interference of xenobiotics with ionic homeostasis also may be due to altered Na^+ K^+ ATPase activity[23]. Smith[27] noticed that a high sensitivity of Na^+, K^+ ATPase in tissues of liver and kidney of *Channa gachua*. Inhibition of Na^+ K^+ ATPase in the tissues of rat colon suggests the action of the organochlorine compound DDE on the sodium pump.

In the present study, Monocrotophos exposed (96 hr LC_{50}) animals in different sw media showed elevated body fluid Cl^-, Na^+ and K^+ levels over controls. Whereas sublethal concentrations of these pesticides effectively decreased the body fluid inorganic ions excepting K^+. Blood chloride concentration have been shown to increase or decrease in fishes exposed to sublethal concentrations of organoch1oine pesticides[28]. The pattern regulation of ions exhibited by the worms is slightly different in both the chemicals tested probably due to their mode of action being different. The Cl^- which is isoionic at 25% sw medium in unexposed organisms became hyperionic in Monocrotophos exposed ones and no change in exposed ones to lethal concentrations. While Cl^- became hypoionic in worms exposed to sublethal concentrations of these two chemicals. This deviation from the general phenomenon of maintaining hyperionicity in 25% sw medium and below might be of an apparent inhibitory action of pesticides. Further the present study indicates that the sublethal concentrations as more prone to cause imbalances in ion regulation of the body fluids of the worms. The body fluid also showed a shift in Na^+ concentration in the worm subjected to higher sw medium concentration (100% sw) and with the pesticides tested. *P. benmudensis* showed hypoionic Na^+ concentration in 30% sw medium with LC 50 concentrations of Monocrotophos The body fluid Na^+ levels in the body fluids of the worms subjected to sublethal concentrations of Monocrotophos in different sw media recorded are remarkably lower to those maintained in sw media and in LC_{50} concentrations. Decrease in Na^+ and Cl^- concentrations were reported in *Crangon crangn* and *Carcinus maenas* treated with sublethal concentrations of heavy metals (Cu, Zn) in sw media at low dissolved oxygen levels[15]. Reduction in ion concentration levels were reported on exposure to sublethal concentrations of mercury at low salinities. The decreased ion concentration levels recorded in the body fluids of the worm exposed to sublethal concentrations of pesticides may elicit the earlier findings. Hyperchloremia followed by hypochioremia in the blood of cat fish, *Heteropneustes fossilis* exposed to Aldrin also do support the present study. The possible interference of monocrotophos andeither with electrolyte regulating system or with ion pumps cannot be ruled out in the present findings. Monocrotophos is also known to affect electrolyte exchange and activity of ion pumps in a number of organisms where as such reports regarding are scanty[29].

Hypoionic K^+ concentrations in exposed and unexposed worm in all sw media appear to be a normal phenomenon. In sublethal concentrations of the pesticide tested the K^+ levels in the body fluids of the worms showed enhanced levels (Table 3). But the increase in K^+ concentrations in worms exposed to sublethal concentrations of the Pesticide in all sw media tested may indicate the retention capacity of the worm. Elevated blood K^+ level and interrelated Na^+ and Cl^- levels is the general pattern of ion regulation in normal sw. Tyler[30] reported that inorganic electrolytes like Na^+ and K^+ are the most important substances influencing both distribution and retention of body water. The elevated levels of K^+ in the body fluids of Monocrotophos treated worms indicate the role of ions especially potassium and retention of potassium in the cells in stress conditions[31]. Variations in level concentrations found in these inorganic ions in *P. berrnudensis* subjected to LC_{50} and sublethal concentrations confirm the variability in the action of different pesticides as suggested by Sulekha *et al*.[32]

In general ion transport is an energy demanding process. Na^+, K^+ ATPase has a key role in osmoregulation since it provides energy for active transport of Na^+ and K^+ across the cell membrane[31]. Interference of xenobiotic substances with ionic homeostasis might result in altering Na^+, K^+ ATPase activity and disrupt energy producing metabolic pathways[23]. Inhibition of gill Na^+ K^+ ATPase activity by Monocrotophos was reported in dragon fly larva, *Pantala flavesens*[22]. The elevated body fluid Cl^- Na^+ and K^+ concentrations recorded in worm treated with lethal concentrations than in sublethal concentrations of Monocrotophos, do focus the changes in permeability properties of biological membrane systems since ATPase are exclusively located in the plasma membranes (Tables 3).

CONCLUSION

The present study clearly showed that Monocrotophos induced alterations in regulatory mechanisms in *P. bermudensis*. The prominent shifts in hyper and hypoionic levels in sublethal concentrations recorded indicate that sublethal concentrations as more hazardous to the worm than LC_{50} concentrations. A comparison of isoionicity of Na^+ and Cl^- in the worm at lethal and sublethal concentrations further suggest that Monocrotophos affected Cl^- than Na^+ regulation in the Worm.

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