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A Comparative Account of the Osmotic Response in *Etroplus suratensis*, A Brackish Water Cichlid Fish Exposed to Selected Heavy Metals and Pesticides

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ABSTRACT

Fishes are the most sought after food source throughout the world. But anthropogenic interventions have polluted their habitats affecting their health. The current study focussed on the effect of selected heavy metals viz; cadmium chloride, lead nitrate and mercuric chloride, and pesticides viz; sevin, nuvan and lindane on the branchialosmoregulatory enzyme Na^+/K^+-ATPase (NKA) and related ions in serum Na^+ and K^+. Wild *Etroplus suratensis* collected from two sites of Ashtamudi lake was exposed to sub-lethal concentrations of these pollutants while maintaining the saline conditions of either site. Results revealed an increase in NKA activity in both the sites. Noticeable changes in serum Na^+, an increase in Site I and decrease in Site II were observed. Appreciable increase in K^+ ions was also noticed in both the sites. These changes may be indicative of the health status of the fish in its normal habitat.

Key words: *Etroplussuratensis, Na^+/K^+-ATPase, Serum ions, Heavy metals, Pesticides*

Introduction

Aquatic resources around the world are under threat of degradation due to accumulation of anthropogenic generated wastes. These wastes may be mainly categorised as domestic, industrial and agricultural wastes – the latter two being the major source of heavy metal and pesticide contamination. The adverse effects of these contaminants on aquatic organisms, especially fishes, have gained much importance over the years, and hence fishes have now been considered as 'bioindicators' capable of determining the health status of the organism itself, and also of the aquatic system it inhabits (Whitfield and Harrison, 2014).

It has been found that the persistent nature of certain heavy metals and pesticides has resulted in the bioaccumulation of these substances in fish tissues, causing noticeable changes in its behaviour, morphology, anatomy, physiology, reproductive behaviour, etc (Clotfelter *et al*., 2004; Scott and Sloman, 2004; Aronzon *et al*., 2014). These changes may be effected due to the direct exposure of the skin or gill of the fish to these contaminants. Gills are the major osmoregulatory apparatus of fishes and disruption of the branchial architecture is known to affect its ionic and osmoregulatory function. Osmoregulation in fishes is carried out by maintaining a balance in the concentration of sodium and potassium ions across the branchial membrane (Nelson and Cox, 2005), and the Sodium-Potassium ATPase enzyme (NKA enzyme) is primarily responsible for the active transport of these ions (Patrick *et al*., 2006; Evans, 2008). For the effective transfer of Na^+ ions between the intracellular and extracellular fluids across the basolateral membrane of the gill, K^+ ions

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are necessary. Hence K^+ serves as a determining factor in the maintenance of Na^+ ion concentration in the body fluids, blood, etc. The enzyme Na^+/K^+-ATPase is crucial for maintaining ion balance in aquatic species, especially in fish gills. Its activity (increase or decrease) serves as a crucial marker for acceptable levels of environmental pollutants as well as a potential sign of toxic stress (Torreblanca *et al*., 1989; Thaker *et al*., 1996; Atli and Canli, 2007). Additionally, as the suppression of this enzyme occurs prior to severe osmoregulatory malfunction (Stagg *et al*., 1992), which results in an imbalance in fish ionoregulatory function, Na^+/K^+-ATPase activity can be considered as an early indicator of contaminants.

The Ashtamudi lake, a tropical estuarine Ramsar Site, receives fresh water from the River Kallada and pours its waters into the Arabian sea. Munrothuruthu (Site I) is located closer to the point where fresh water influx into the lake occurs while Mukkad (Site II) is located at the confluence point of Ashtamudi lake and the Arabian sea. Therefore Site I is comparatively less saline than Site II and extreme saline variations have been observed. Hence the two sites were selected accordingly. Both these regions were also found to be polluted (Nagaraj Sitaram, 2014).

Etroplus suratensis, a brackish water euryhaline cichlid is found inhabiting both these regions and is the most sought after food fish. IUCN has now categorised this fish as declining. The capacity to process variations in osmotic pressure will also be impacted by shifting environmental conditions. The reasons may be varied but whether pollution is one of the reasons is the least studied. Information pertaining to the impact of toxicants on the osmoregulatory activity of a cichlid *Etroplus suratensis*, is scanty. Hence to comprehend the effects of pesticides and heavy metals on the osmotic response of this fish, wild fishes from the two extremely different saline areas were exposed to sub-lethal concentrations of the toxicants under laboratory acclimatized saline conditions and the NKA enzyme as well as serum Na^+ and K^+ ions was assayed.

Materials and Methods

Chemicals

The three heavy metals chosen for the investigation were lead nitrate (98.5%), mercuric chloride (99%), and cadmium chloride (99%). Sevin, an organophosphorus pesticide (Dichlorvos, EC 76%), Nuvan (Dichlorvos, EC 76%), and Lindane (1.3% DD) are the insecticides chosen for the study. The toxicants were diluted in double-distilled water to prepare the stock solutions.

Experimental Set-up

From two locations in Ashtamudi lake, live *E. suratensis* specimens weighing 13.5 g to 15.0 g and measuring 8.0 to 9.5 cm in length were collected. In fibre tanks with water from the specific locations, fish were maintained. The photoperiod was roughly a 12 hour light/12 hour dark cycle. The fish were starved for 24 hours before the experiments commenced. Six fish were exposed to sub-lethal concentrations of the chosen toxicants for 96 hours while retaining the optimum salinity determined for the two sites, namely 10 ppt and 20 ppt for Site I and Site II, respectively. Each treatment consisted of two replicates and a control that contained only saline water. The fish were fed *ad libitum* with commercial feed during the experiment.

Experimental Analysis

Fish that had been exposed to pesticides and heavy metals at levels below lethal levels were sacrificed after 96 hours. The gills were immediately removed, put on ice, and subjected to differential centrifugation in order to prepare them for an NKA enzyme assay. Blood was taken into 2 ml capillary tubes in order to measure the plasma sodium and potassium levels before the gills were removed. The serum was then extracted from the blood and used for biochemical analysis after being centrifuged for five minutes at 2000 rpm in a tabletop centrifuge. Following the theory of Maruna (1958) and Trinder (1951), the Mono Test Colorimetric Method was used to determine the Na^+ and K^+ ion concentration. Using the sodium and potassium reagent kit, both ions' concentrations were estimated (BEACON).

Statistical Analysis

Branchial Na^+- K^+/ATPase (NKA) and serum Na^+ and K^+ levels obtained from experimental analysis are expressed as mean SD. The data collected following the successful conclusion of the experiment were subjected to One-way Analysis of Variance (ANOVA), and Tukey's Multiple Range Test was used to identify any significant changes in mean values. Origin Software was used for the analysis (2021b).

Results and Discussion

Branchial Na^+ /K^+ -ATPase (NKA) Activity in *E.suratensis* exposed to heavy metals and pesticides

The enzyme's activity was stimulated in *E.suratensis* from Site I in response to mercuric chloride and lead nitrate. Cadmium chloride exposure caused the strongest reaction. In Site II, *E.suratensis* experienced substantially enhanced responses to cadmium chloride, mercuric chloride, and lead nitrate, respectively, on all three heavy metals. Fish from Site II recorded the highest rate as compared to those from Site I upon comparing the site-by-site response of enzyme activity being analyzed (Fig. 1).

Pesticide exposure in *E.suratensis* at sub-lethal levels also induced a stimulatory response in the enzyme's activity. As compared to control fish, all three pesticides exhibited identical behaviour in terms of fish from both sites' osmotic reaction. On exposure to pesticides, fish from Site II exhibited a comparable stimulatory pattern in enzyme activity. Fish from Site II seem to be more responsive compared to those from Site I, and the stimulatory activity of the enzyme in *E.suratensis* subjected to pesticides exhibited a similar pattern to that of exposure to heavy metals (Fig. 2).

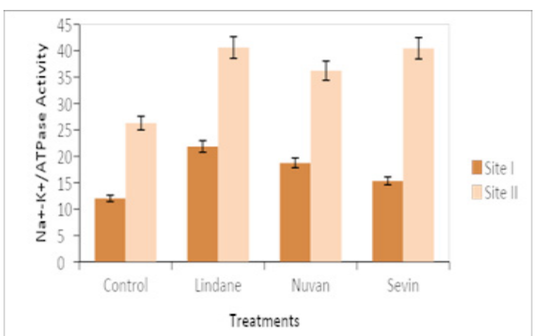

Fig. 1. Branchial NKA Activity in *E.suratensis* from Site I and II on exposure to heavy metals

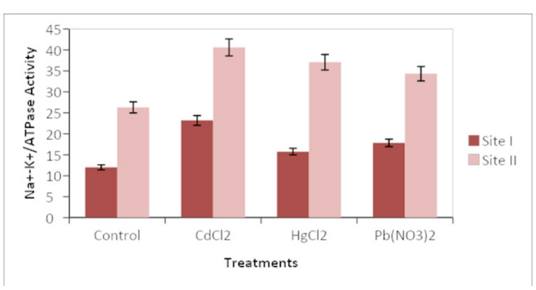

Fig. 2. Branchial NKA activity in *E. suratensis* from Site I and II on exposure to pesticides

Plasma Sodium Content in *E.suratensis* on exposure to heavy metals and pesticides

In contrast to Site II, where all three heavy metals created an extremely heightened response in *E.suratensis* when compared to the control, it was observed that none of the heavy metals produced any excitatory responses in the fish of Site I. (Fig.3).

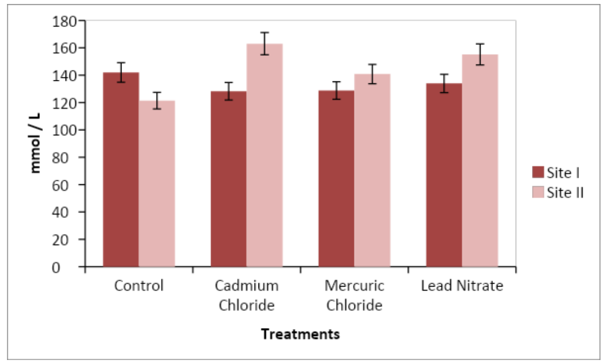

Fig. 3. Plasma Na^+ Content in *E. suratensis* from Site I and II on exposure to heavy metals

When exposed to pesticides, *E.suratensis* from Site I blood serum sodium levels decreased in sevin and nuvan and slightly increased in lindane, but these differences were statistically insignificant when compared to the control group. Fish from Site II exhibited an increase in lindane and nuvan compared to the control; however it was not statistically significant. As against the other pesticides, sevin appeared to be significantly higher (Fig. 4).

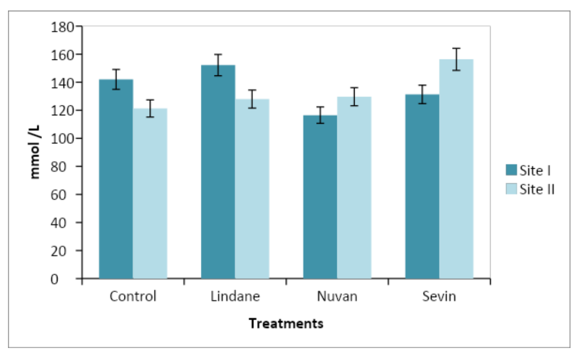

Fig. 4. Plasma Na^+ Content in *E. suratensis* from Site I and II on exposure to pesticides

Plasma Potassium Content in *E.suratensis* on exposure to heavy metals and pesticides

When compared to the control, all three treatments for *E. suratensis* at Sites I and II increased the amount of potassium in the fish (Fig. 5).

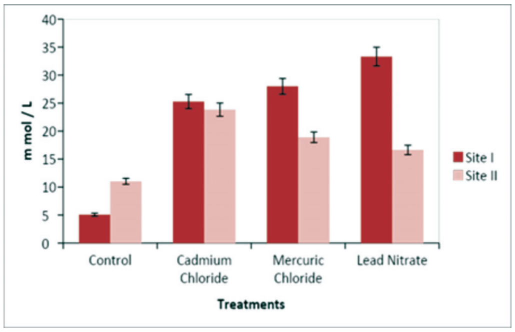

Fig. 5. Plasma K^+ Content in *E. suratensis* from Site I & II on exposure to heavy metals

When compared to the control, exposure to pesticides indicated an increase in serum potassium concentration in all three treatments at Sites I and II (Fig.6).

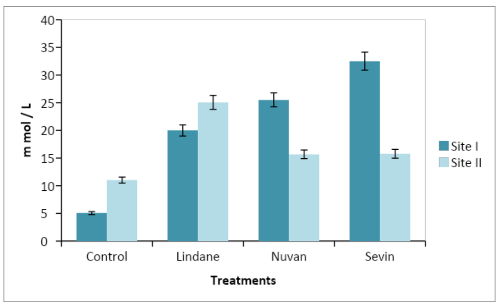

Fig. 6. Plasma K $^+$Content in *E. suratensis* from Site I & II on exposure to pesticides

Heavy metal and pesticide exposure to *E. suratensis* from Sites I and II resulted in an increase in Na^+/K^+ -ATPase activity. In terms of serum sodium concentration, opposing outcomes were seen. With the exception of lindane, all treatments at Site I exhibited a drop in serum salt content, whereas in Site II, the fish displayed an increasing tendency. In contrast, both Sites had an increase in serum potassium levels after exposure to all of the present study's chosen toxicants. In the presence of metals, the Na^+/K^+-ATPase (NKA) enzyme is suppressed, changing the internal ion concentrations and significantly impairing fish osmoregulation. Metals alter the concentration of different plasma ions, so the body has to make up for these ionic disruptions. One method to restore normal ion concentrations is by increased ion uptake, and another is through the development of a new cell population specifically designed for this function (Perry, 1997). Therefore, ionic fluxes in plasma sodium and potassium ions as well as increased NKA activity were found when *E.suratensis* was exposed to heavy metals. Fish bodies can absorb heavy metals in three different ways: through the gills, the digestive system, or the skin. The gills are considered to be a significant site for direct uptake of metals from the water (Romeo *et al.*, 1999), despite the fact that the body surface is normally believed to have a minimal role in heavy metal absorption in fish (Selda and Nur°ah, 2012).. There have been reports of the presence of lead, mercury, and cadmium in several locations of Ashtamudi Lake (Razeena and Sherly Williams, 2015; Santhosh *et al*., 2018; Lekshmi Priyaand Sherly Williams, 2019). *E.suratensis* exposed to toxicants in controlled saline conditions in the lab demonstrated a greater rate of expression of the osmoregulatory enzyme Na^+/K^+ - ATPase than untreated fish. Fish from two different Ashtamudi lake locations were exposed to sublethal concentrations of pesticides like lindane, nuvan, and sevin, as well as heavy metals like cadmium chloride, mercuric chloride, and lead nitrate. Fish from Site II revealed a greater rate of enzyme activity than fish from Site I upon comparison of the osmotic responses of fish from both sites.

The application of insecticides in farm fields to get rid of pest species is another concern for aquatic organisms, including fish. These pollutants interfere with the fish's osmotic reaction, which causes stress to the organism. In the current study, when *E. suratensis* was exposed to organic pesticides, it enhanced the activity of the osmoregulatory enzyme, Na^+/K^+ -ATPase, which is attributed to the fish's compensatory mechanism in response to the stress imposed by the pesticides. The secretion of cortisol, which controls ion absorption and energy balance and which mostly occurs in the presence of a stressor, is triggered by stressful situations, according to earlier study on osmoregulation in freshwater fish. Fish increase the number of chloride cells in their gills in response to cortisol, which helps them to enhance their osmoregulatory mechanisms and bring their plasma ions levels back to normal (Mancera and McCormick, 2007; Ramesh *et al.,* 2009). These theories concur with the current study's findings that *Etroplus*is stressed as a result of toxicant exposure and that branchial osmotic enzyme activity is increased to counteract the stress-induced condition and maintain normal osmolarity. It has been demonstrated that stress and pollution reduce water out flux and net ion influx while increasing net ion outflux and surface permeability. The Na^+ /K $^+$ -

ATPase activity rises in stressed animals, and this rise in activity may represent a compensatory mechanism for ionic regulatory impairments, such as an elevation in surface permeability (Lin *et al*., 1993), as seen in the current study. The proportion of these ions in plasma remained similar with earlier studies even though the amount of sodium and potassium ions in plasma changed depending on the toxicants the fish from both sites were exposed to (Renzis and Bornancin, 1984). The decrease in ion influx and increase in permeability are both clearly caused by structural lesions of the ion-transporting epithelia. In ionocytes, the development of vacuoles and subcuticular gaps may be associated with a reduction in water outflux. When the architecture of the gill epithelium is disrupted, alterations in blood ionic levels, gill Na^+/K^+-activated ATPase, and ionic fluxes may take place. (Evans, 1987). The capability of the fish's osmoregulatory system is thought to be reflected by the activity of the enzyme Na^+/K^+ -ATPase and the density of chloride cells (Bonga and Rock, 1992). The loss of gills, on the other hand, can inhibit growth because it affects osmoregulation, respiration, and swimming. Oxygen deprivation can also reduce a fish's ability to swim. A toxicity buildup may be a sign of a physiological issue, such as a detoxification or osmoregulation issue. Due to irregular excretory and diffusional ions losses, the clearance of metabolic waste products, and other factors, chronic gill disruption will have a negative impact on fish development and reproduction (Bonga and Rock, 1992). On the other hand, branchial reactions lower the rate of excretion through the gills while also preventing the entrance of new toxicants (Ruiz-Picos and Lopez-Lopez, 2012). This finding is consistent across both sites of Ashtamudi Lake based on our analyses. Freshwater fish are extremely sensitive to the presence of toxicants in their environment, even in tiny amounts. They control their main osmotic mechanism, the branchial apparatus, to resist the situation.

- They regulate the branchial Na^+/K^+-ATPase, a crucial osmotic enzyme, to ensure that the ions in their bodily fluids are kept at the right concentration.
- In this work, *E. suratensis* from two separate Ashtamudi wetland sites were tested for toxicity under laboratory circumstances that replicated their natural habitat's salinity.
- According to the observation, *E. suratensis* from both sites reacted to toxicant concentrations below the lethal threshold by activating the enzyme Na^+/K^+-ATPase.
- The fish's stimulatory osmotic response to the toxins showed a noticeable variation in the rate of enzyme activity at the relevant locations.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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