

## Original Research Article

# DEGRADATION OF EMAMECTIN BENZOATE IN THE AQUACULTURE POND ENVIRONMENT UNDER TROPICAL CLIMATIC CONDITION

### ABSTRACT

**Aims:** To investigate the influence of abiotic factors (sunlight exposure, pH, salinity and soil texture) on the degradation of emamectin benzoate (EMB) in aquaculture pond water and sediment.

**Study Design:** Experimental study examining EMB degradation under various controlled environmental conditions.

**Methodology:** EMB degradation was studied in aquaculture pond water and sediment under various abiotic conditions sunlight, pH (5, 7 & 8.5), salinity (0.5, 15 & 25ppt) and soil texture (clay & sandy). The experiment was conducted based on OECD guidelines and placed under sunlight. The light intensity and photoperiod was calculated every day. The samples were analysed and kinetics was fitted and its half-life was calculated.

**Results:** EMB degradation in both water and sediment followed first-order kinetics. The half-life (days) of EMB was 5.6 in water and 12.4 in soil under the exposure of sunlight.

Higher degradation was observed under alkaline conditions (pH 8.5) and at lower salinity (0.5ppt). Under exposure to sunlight, EMB degrades more rapidly in low-saline environments with alkaline pH. EMB degradation is accelerated in light-textured sandy soil when exposed to sunlight.

**Conclusion:** In countries with ample year-round sunlight, like India, EMB degradation in aquaculture pond environments occurs rapidly, reducing the risk of accumulation in water or sediment. This study provides insights for developing effective strategies to optimize EMB usage under different abiotic conditions in aquaculture settings.

**Keywords:** Abiotic factors, Aquaculture, Degradation, Emamectin benzoate, Kinetics

### 1. INTRODUCTION

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Seafood plays a significant role in the global food system and is undoubtedly one of the world's most valuable commodities in the global context. The nutritional value, health benefits and food security and sustainability of seafood has been well recognized (1). The exponential growth of the human population and the increasing per capita consumption of seafood have led to a rising demand for fishery products. Presently, over seven billion people depend on fish as a source of over 15 % of their animal protein intake (Reference?). In economically disadvantaged coastal regions, this dependency can soar to as high as 90 % (2,3). Aquaculture is one of India's fastest-growing food production sectors with an export of 1.73 million MT, contributing significantly to foreign exchange revenue to the tune of US\$ 8.1 billion in the year 2022–23 (4)

Effective control of diseases in fish is an important aspect for increasing the fish culture to meet the ever-rising demand of aquatic products. There are many parasites in the aquatic ecosystem which attach to the fish and feed on the mucus, blood and skin, therefore causing damage to the fish (Reference?). Ectoparasitic copepods (Copepoda: *Caligidae* and *Lernaeidae*), isopods (Isopoda: *Cymothoidae*) and brachiurans (Brachiura: *Argulidae*) particularly parasites, pose a substantial threat to the health and productivity of the global aquaculture industry. These parasites are known to cause considerable economic losses, with an estimated annual impact exceeding 1.05 billion to 9.58 billion US\$ (5). EMB is a semi-synthetic derivative of avermectins, a group of macrocyclic lactones derived from the soil bacterium *Streptomyces avermitilis* (6). It is primarily used in aquaculture to control sea lice and other parasites in farmed fish, particularly salmonids. The drug is typically administered orally as feed top dressing and is known for its broad-spectrum efficacy, long-lasting effect and was identified and developed as an anti-parasiticide for both marine and freshwater-reared fish species. It has been recommended by Food and Drug Administration (FDA) and European Medical Agency (EMA) for the standard treatment of 50 µg of EMB kg<sup>-1</sup> of fish body weight (BW) d<sup>-1</sup> for seven consecutive days (7,8).

As EMB is widely used as an anti-parasitic agent in aquaculture, it is crucial to understand its fate and degradation in environment. It is estimated that about 75% of antibiotics/therapeutics/ drugs which are induced in the feed eventually reach the pond environment (9,10,11).

The degradation of EMB in aquaculture environments is reportedly influenced by various abiotic factors such as water salinity, temperature, pH, sunlight exposure and the presence of other organisms (12,13). Hydrolysis, photolysis and biodegradation are considered as a main degradation pathways involved in the breakdown. Hydrolysis, facilitated by water and influenced by pH, leads to the cleavage of ester bonds in EMB, resulting in the formation of

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**Table 1. Soil characteristics of different soil textures.**

primary and secondary degradation products (14). Photolysis, driven by sunlight can contribute significantly to the degradation of EMB as the compound is susceptible to degradation when exposed to UV light (15).

The complete degradation of EMB from sediment is a challenging process. Studies (16,17) have shown that EMB undergoes photolysis, which is influenced by soil parameters. The breakdown of EMB in soil largely depends on the aerobic and anaerobic conditions, as well as the soil's characteristics. Studies on environmental degradation of EMB in tropical climate like India are limited. Hence, the present study reports the influence of abiotic factors on the degradation of EMB in tropical aquatic environments. The understanding gained by the study will help in the development of strategies for the efficient use of EMB and reduce the effect on the pond environment.

## **2. MATERIAL AND METHODS**

### **2.1 Chemicals and sample preparation**

Eamectin benzoate, 99.3% pure (Analytical standard), powder ((4''R)-4''-Deoxy-4''-(methylamino) avermectin B1 benzoate) with molecular formula of  $C_{56}H_{81}NO_{15}$  and molecular weight of 1008.2 g/mol was obtained from Sigma- Aldrich (Milwaukee, WI, USA). All organic solvents (HPLC grade) were purchased from Sigma-Aldrich and Hi-Media and ultrapure water was used in the preparation of the reagents and purity was maintained. Aqueous EMB solution (1000ppm) was prepared by dissolving 50mg of EMB powder in 50ml of methanol. The stock solution was further diluted into 100ppm using ultrapure water and used for the experiments.

The water (fresh and sea water) and soils from aquaculture ponds used in the experiment was characterized and checked for any possible contamination with EMB before the start of the experiment. (Tables 1,2).

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**Comment [Ma10]:** The manuscript does not specify the number of replications for each treatment. The experiment was conducted without any replication, which raises concerns about the reliability and robustness of the results. Consequently, these findings may not be considered effective or applicable for the aquaculture industry.

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Soil texture	Loamy sand	Clay
pH	8.9	8.46
Electrical Conductivity (mS/cm)	3.24	4.15
Organic carbon (%)	0.02	0.24
Available nitrogen (ppm)	38.65	49.1
Available phosphorus (ppm)	23.75	26.5
Sodium (ppm)	2030	430
Potassium (ppm)	179	136
Pore space volume (ml)	5	7
Pore space %	38.46	41.17
Bulk density (g/cc)	1.53	1.17
Particle density	2.5	2
Calcium (ppm)	27.2	40.7
Magnesium (ppm)	46.3	41.1

**Table 2. Water quality parameters at different pH and salinity levels. (0.5, 15, 25 indicates salinities); (A, B, C indicates pH 5, 7, 8.5 respectively)**

	0.5A	0.5B	0.5C	15A	15B	15C	25A	25B	25C
<b>pH</b>	5.05	7.01	8.59	5.03	7.05	8.50	5.07	7	8.60
<b>Salinity (ppt)</b>	0.5	0.6	0.5	14	15	15	25	24	25
<b>Carbonate (ppm as CaCO<sub>3</sub>)</b>	0	0	61.2	0	0	0	0	0	122.4
<b>Bicarbonate (ppm as CaCO<sub>3</sub>)</b>	62.22	273.8	286.2	37.3	124.4	24.888	74.7	223.9	323.5
<b>Total alkalinity (ppm as CaCO<sub>3</sub>)</b>	51	224.4	285.6	30.6	102	81.6	61.2	183.6	265.2
<b>Calcium (ppm)</b>	29.73	29.73	33.98	127.45	212.42	212.42	254.90	339.87	254.90
<b>Magnesium (ppm)</b>	15.61	23.42	15.61	624.53	598.51	572.49	1014.87	910.78	936.80
<b>Total hardness (ppm as CaCO<sub>3</sub>)</b>	137.6	169.6	148.4	2862	2968	2862	4770	4558	4452

## 2.2 Photochemical experiments

To study the photodegradation of EMB in water, experiments were conducted in three different saline waters (0.5, 15 and 25ppt), three different pH (5,7 and 8.5) and under sunlight and dark conditions. In 250 ml **bottles**, a final concentration of 1000 ppb of EMB solution was prepared and the **containers** were kept in sunlight for a photolytic degradation

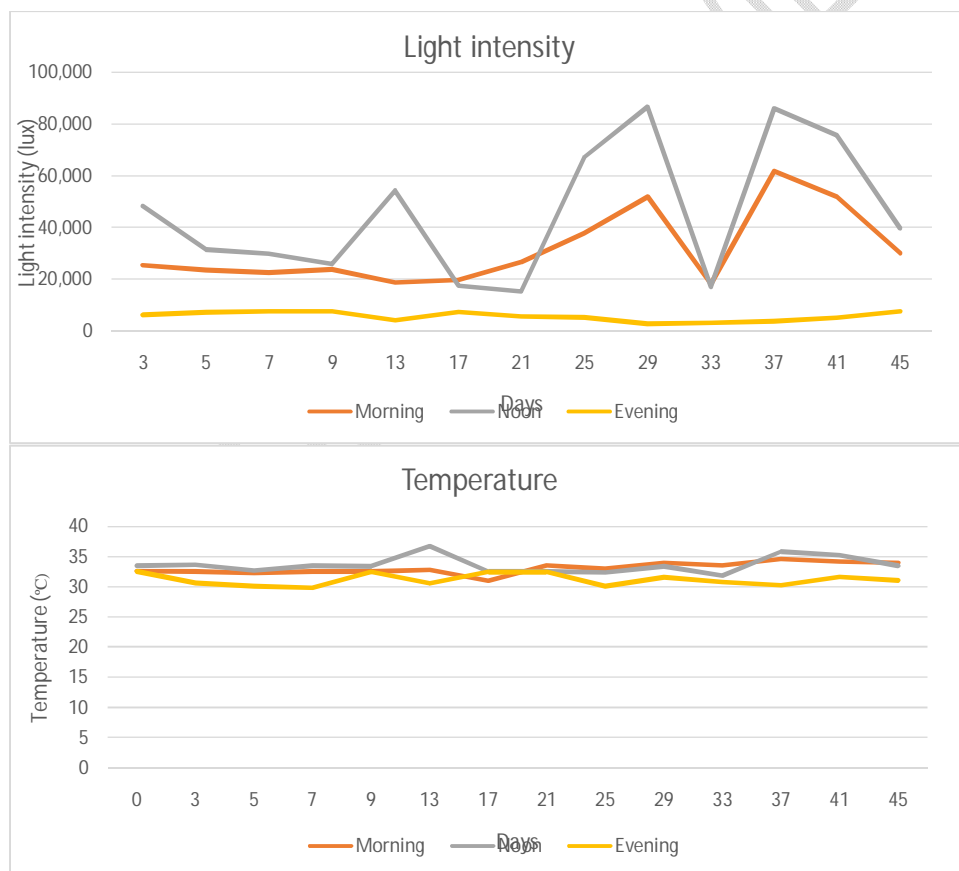
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study. A similar setup was kept in dark environment with constant room temperature at  $25 \pm 1$  °C.

In another experiment, two different textured soils (loamy sand and clay) were used and water holding capacity was determined to know the amount of EMB aqueous solution needed to get a final wet weight concentration of 1000ppb. Accordingly, EMB solution was added in 25 g of soil and EMB were mixed using a vortex to get a uniform spread. The soil was kept in sunlight and in the dark to study the degradation of EMB. The water holding capacity was maintained throughout the experiment.

During the experimental period, data on photoperiod, light intensity (lux) and atmospheric temperature (°C) were recorded (Figure 1). Water and soil samples were collected at regular time intervals and analyzed for levels of EMB.



**Fig.1.** Variation in the light intensity and temperature during the experimental period

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## 2.3 Analytical determination

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The quantification of EMB in the water samples was conducted using Liquid Chromatography Mass Spectrometry (LC-MS/MS). A 10 ml water sample was transferred into a centrifuge tube, followed by the addition of 10 ml of acetonitrile. After vortexing the mixture, 10 g of sodium sulphate was introduced and the solution was subjected to centrifugation (7500 g). From the solution, 1 ml was taken and diluted to 10 ml with acetonitrile and injected. For soil, 5g of sample was weighed and 10ml water and 10ml acetonitrile ratio of water and acetonitrile (1:1) was added and mixed well. To this mixture, 10g of sodium sulphate was added, vortexed and centrifuged, 1ml of supernatant was taken and made up to 10ml using acetonitrile and injected in LC-MS/MS (Agilent, LC 6470, USA). For water samples, the Limit of Detection (LOD) was set at 2 µg/L and the Limit of Quantification (LOQ) was determined to be 5 µg/L. In the case of sediment samples, the LOD and LOQ were respectively defined as 5 µg/kg and 10 µg/kg. These thresholds ensure the reliability and precision of EMB quantification in both water and sediment matrices.

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The data were analysed and checked for the best fit while rate constant (k) and half-life ( $t_{1/2}$ ) period for degradation were determined using Computer Assisted Kinetic Evaluation (CAKE) software.

## 3 RESULTS

### 3.1 Photodegradation of emamectin benzoate in water

The degradation data for EMB were evaluated using the kinetic equations of both first (1a) and second order (2b).

For the first-order reaction:

$$\text{Reaction rate} = d[C]/dt = -k [C]$$

$$\text{Linear form of first - order kinetics: } C = C_0 \cdot e^{-kt} \quad (1a)$$

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The reaction rate is in molar/time, and 'k' is the reaction rate coefficient ( $\text{time}^{-1}$ ). When you plot  $\ln [C]$  against time for a first-order reaction, you get a straight line. The slope of this line corresponds to the rate constant (k) for the reaction.

The half-life ( $t_{1/2}$ ) was calculated using the formula:  $t_{1/2} = 0.693/k$ , 'k' was obtained from the slope of the  $\ln (C)$  vs time graph.

Second-order reaction equation:

$$\text{Reaction rate} = d[C]/dt = -k [C]^2$$

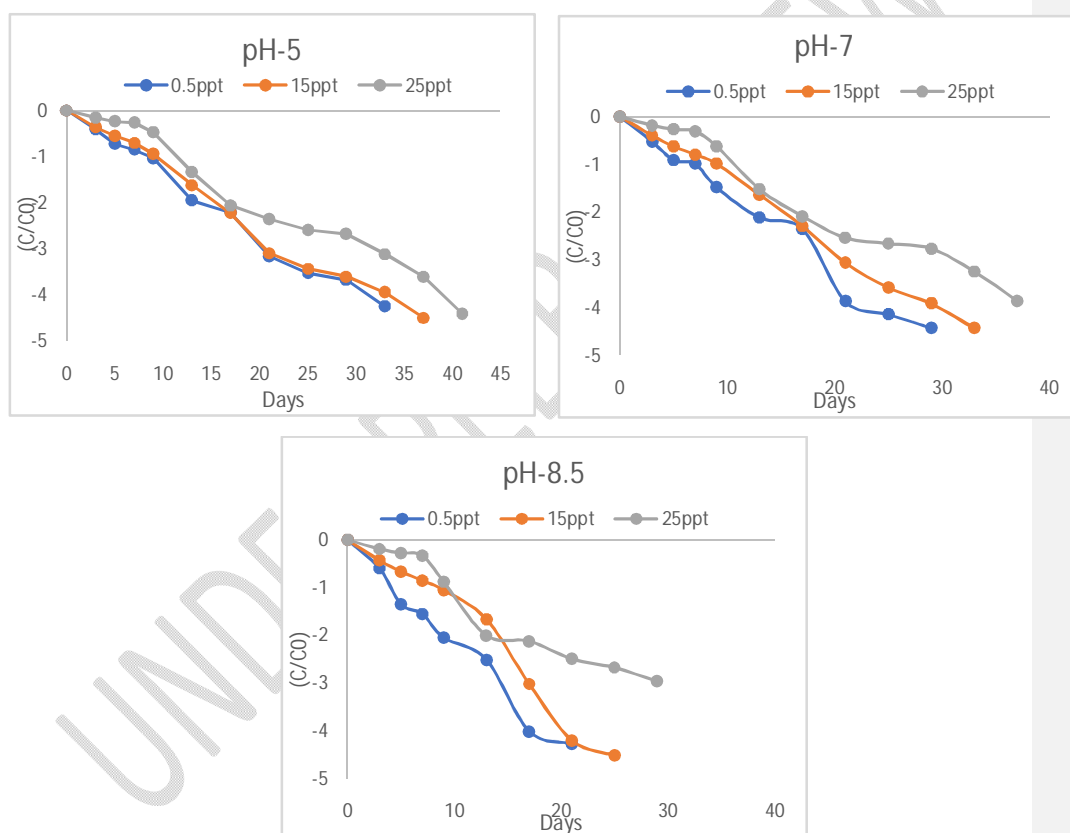
$$\text{Linear form of second-order kinetics: } 1/C = 1/C_0 + kt \quad (2b)$$

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In these equations, ' $C_0$ ' represents the initial EMB concentration, ' $C$ ' is the residual EMB concentration at time ' $t$ ', and ' $k$ ' is the rate constant.

The photodegradation of EMB was studied under natural sunlight with an average sunlight intensity of 35,894 lux, a temperature of 33°C and a photoperiod of 12 hrs 30 mins. The average light intensity showed notable variations based on weather conditions. The highest intensity occurred at approximately 2:00 PM during sunny weather. Meanwhile, a constant dark condition was maintained in a separate room with temperature set at 25±1 °C. The data confirmed exponential decay, indicating that the reaction was first order. The aqueous solution of EMB was highly stable at pH 5 and it was essentially stable in the dark.

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**Fig. 2. The effect of pH on the photodegradation of emamectin benzoate under varying salinities**

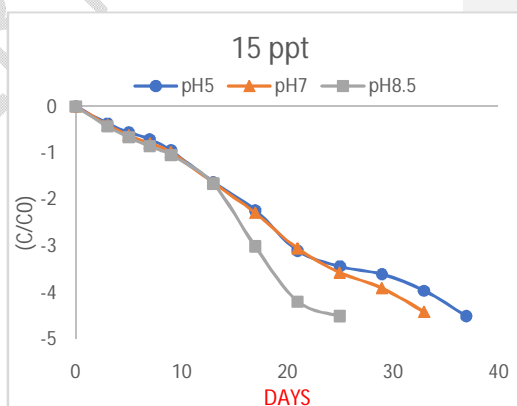
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In all the saline water (0.5, 15 and 25 ppt), it was observed that at pH 8.5 tends to degrade rapidly in both sunlight and dark conditions. The drug in freshwater when exposed to sunlight showed a half-life of 3.03, 4.33 and 5.23 days at pH levels of 8.5, 7 and 5 respectively.

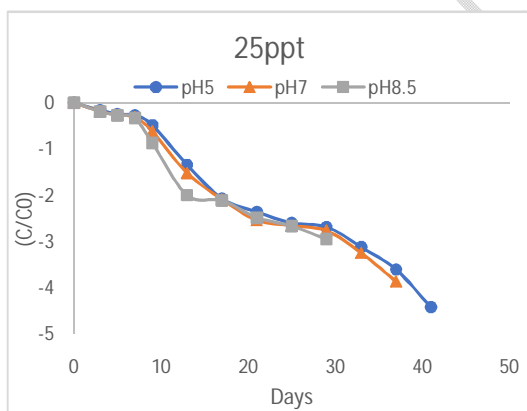
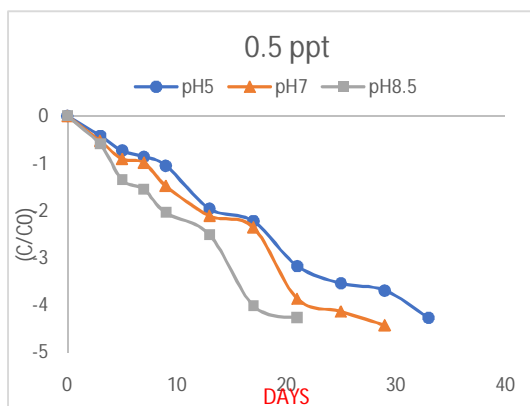
In contrast, degradation under 25ppt followed a little slower degradation under different pH with a half-life period of 6.75, 7.37 and 7.43 days at pH 8.5, 7 and 5. In comparison with dark conditions, it was higher in all pH with a half-life of 34.3, 54.8 and 55.8 days at pH 8.5, 7 and 5 in low salinity. The results (Table 3) indicates that EMB undergoes photodegradation,

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especially at high pH (alkaline) and low saline (0.5ppt) showed to have faster degradation.



**Fig. 3.** The effects of salinities on the photodegradation of emamectin benzoate under varying pH

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**Table 3 . Rate constant (k), half-life (D50) and 90% degradation (D90) of emamectinbenzoate under natural sunlight in aqueous solutions**

	0.5ppt			15ppt			25ppt		
	pH5	pH7	pH8.5	pH5	pH7	pH8.5	pH5	pH7	pH8.5
<b>k</b>	0.1325	0.1602	0.2286	0.1174	0.1229	0.1331	0.0932	0.0940	0.1027
<b>D50 (days)</b>	5.23	4.33	3.03	5.9	5.64	5.21	7.43	7.37	6.75
<b>D90 (days)</b>	17.4	14.4	10.1	19.6	18.7	17.3	24.7	24.5	22.4

The degradation rate was lower in lower pH (5) and increased drastically in higher pH (8.5), irrespective of the salinity.

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At higher salinity the degradation was less compared with low saline water. Similarly, in pH 8.5 the half-life period was 3.03 and 6.75 days for freshwater and high saline (25ppt) waters respectively, while it was 5.23 and 7.43 days at pH 5.

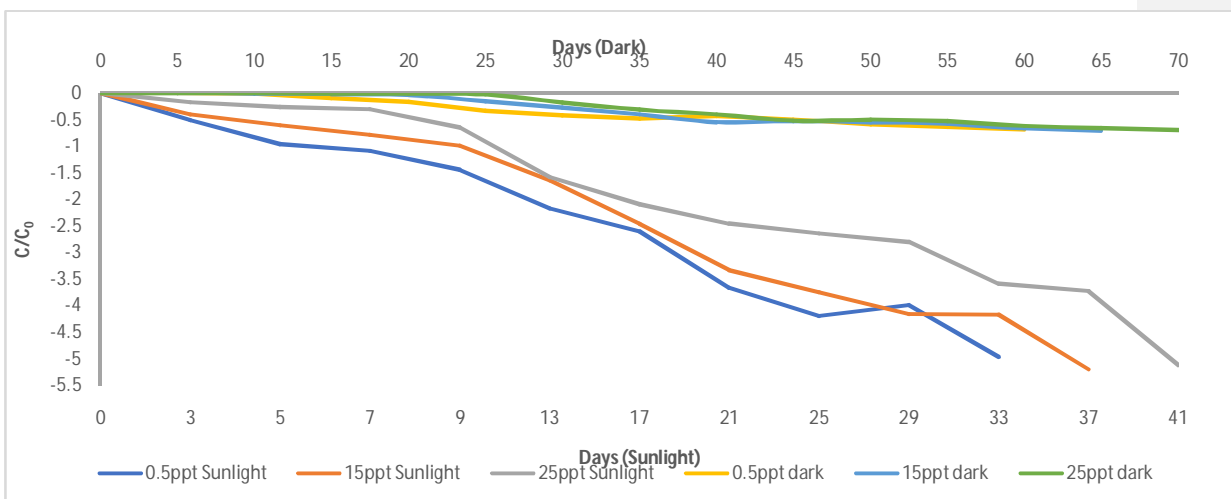
### 3.2 Photodegradation of emamectin benzoate in soil

**Table 4 .Rate constant (k), half-life (D50) and 90% degradation (D90) of emamectinbenzoate under natural sunlight and dark in soils**

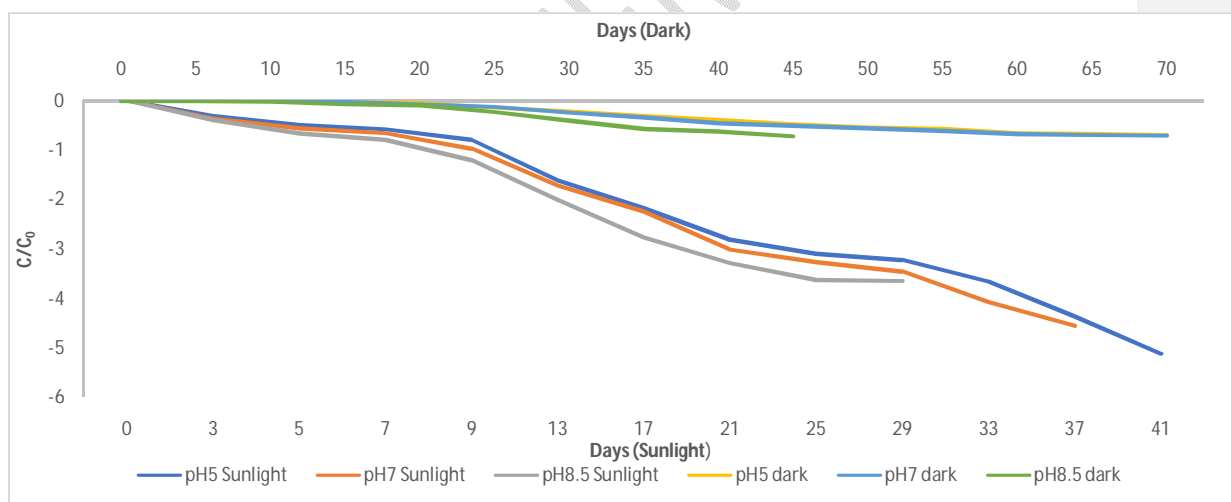
	Sunlight		Dark	
	Loamy sand	Clay	Loamy sand	Clay
<b>k</b>	0.0559	0.03037	0.02752	0.01564
<b>D50 (days)</b>	12.4	22.8	25.2	44.3
<b>D90 (days)</b>	41.2	75.8	83.7	147

Based on EMB concentration in the soil at periodical intervals under different pH and texture as well as under sunlight and dark, the degradation rate and half-life period was calculated (Table 4). In natural sunlight, the half-life was 12.4 and 22.8 days in loamy sand and clay soil with a rate constant of 0.0559 and 0.0303 k. Whereas in the dark condition, the half-life

was about 25.2 and 44.3 days with a rate constant of 0.0275 and 0.0156 in loamy sand and clay soil respectively. The data fit into first-order kinetics.



**Fig. 4. Comparative degradation of EMB in sunlight vs dark under various salinities**



**Fig. 5. Comparative degradation of EMB in sunlight vs dark under various pH**

#### 4. DISCUSSION

Emamectin benzoate has been successfully used in farmed fish worldwide as an effective antiparasitic compound. Data on the application and degradation of the compound in tropical

climates is scanty and therefore, it is essential to generate data for better environmental practices and safety. In this study, the fate and degradation of EMB in water and sediment under Indian tropical conditions were evaluated. Hydrolysis, photolysis and biodegradation are the main degradation pathways involved in the breakdown of EMB (18). The photolysis of the aqueous EMB solution resulted in a notable degradation of its active ingredients, in contrast to the stability observed under dark conditions. The chemical bonds found within the drug, including ether and ester linkages, exhibit susceptibility to photodegradation when exposed to UV light. This photodegradation process can generate numerous by-products, as documented by earlier studies (19,20,21,22). Hydrolysis, facilitated by water and influenced by pH, leads to the cleavage of ester bonds in EMB, resulting in the formation of primary and secondary degradation products (14)

#### **4.1 EMB degradation in water**

In this study, the photolytic degradation rate was more than 10 times as compared with the dark. Earlier studies reported faster photolysis of EMB under natural sunlight compared to artificial light sources (15) as sunlight contains ultraviolet (UV) radiation, particularly in the UVA and UVB ranges, which can initiate photolysis reactions. Photolysis involves the breaking of chemical bonds in EMB by absorbing UV light energy (23). Some substances present in the environment, such as certain organic matter or metals, can act as sensitizers, which can absorb light energy and transfer to EMB thereby enhancing the degradation process (15). Photolysis and sensitization reactions can additionally stimulate the production of highly reactive oxygen species, including singlet oxygen, hydroxyl radicals, and superoxide radicals. These reactive species play a pivotal role in driving the degradation of the compound (24).

EMB has varied water solubility under different salinities and is very poor in seawater with only 5.5 mg/l solubility. In this experiment, the degradation was slower with increasing salinity of water with a half-life of 4.19, 5.61 and 7.18 days under 0.5, 15 and 25 ppt salinity respectively. This may be due to the higher concentration of minerals like calcium, magnesium, sodium and potassium in high-saline water. The presence of salts in seawater acts as stabilizers, interacting with the chemical structure of the compound, making it more resistant to degradation by various abiotic factors.

The solubility of EMB changes significantly with the pH of water with a solubility rate of  $320 \pm 30$  mg/L under pH 5,  $24 \pm 2$  mg/L under pH 7 and only  $0.1 \pm 0.1$  mg/L under pH 9. The current study showed that the EMB decay rate is faster in higher pH, it follows as pH  $8.5 > 7 > 5$  in both photolytic and dark conditions. In a similar study (25), photodegradation of EMB in solution exhibited varying half-life periods. Specifically, EMB had a half-life of 22 days

in a pH-buffered solution with a pH of 7, while in natural pond water (0 ppt). It was reported that EMB was stable at pH 5.2, 6.2, 7.2 and 8.0 at 25°C, whereas at pH 9 the compound breaks down with a half-life of 19.5 weeks under sterile buffered aqueous solution (26). Therefore, the study shows that as the salinity of the water increases the degradation rate increases with respect to its pH or photolytic conditions. EMB undergoes photolysis and experiences degradation within the water column at depths where light can reach (Mushtaq *et al.*, 1998).

#### 4.2 EMB degradation in soil

Sunlight was more effective in the degradation of EMB irrespective of the soil texture. The rate of degradation was faster (doubled) under sunlight than in dark conditions. The drug reportedly shows almost no degradation in soil under dark conditions (16). Between the soil textures, the drug tended to degrade slower in clay soil than in loamy sand under both sunlight and dark. It may be due to large surface area, high cation exchange capacity and overall negative charge, which draws and holds positively charged molecules through electrostatic interaction. Higher organic matter in the clay soil contains various substances, including humic and fulvic acids, which can be complex with EMB forming a stable complex. In addition to this, temperature, moisture and aerobic and anaerobic conditions influence the degradation rate in soil (17).

#### 5. CONCLUSION

In brackishwater and freshwater fish farming, there is a high risk of ectoparasite and endoparasite infections. Emamectinbenzoate is frequently used to treat and prevent these infections. It's crucial to assess how EMB degrades in various abiotic conditions within the pond environment. The degradation rate of anti-parasitic agent, EMB in both water and soil followed the first-order kinetics and the degradation was faster in low saline water with alkaline pH when exposed to sunlight. Similarly, the degradation was faster under sunlight in the light textured soil than heavy texture soil. The study revealed that under tropical conditions, the risk of EMB accumulation in water and soil is minimal.

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