Hue University Journal of Science: Natural Science
Vol. 134, No. 1S-2, 117–125, 2025 (Special Issue: CBA 2025)

pISSN 1859-1388
eISSN 2615-9678

Locomotor behaviour of *Moina macrocopa* exposed to permethrin: Potential for AI-based biological early warning systems

Phan Thi Thao Linh[1], Tran Nguyen Quynh Anh[2], Le Thi Nguyet Nga[2], Vo Van Minh[2], Trinh Dang Mau[1,2]*

[1] Environment & Biological Resource (DN-EBR), The University of Danang - University of Science and Education, 459 Ton Duc Thang St., Danang, Vietnam
[2] The University of Danang - University of Science and Education, 459 Ton Duc Thang St., Danang, Vietnam

* Correspondence to Trinh Dang Mau <tdmau@ued.udn.vn>

(*Received: 15 June 2025; Revised: 15 August 2025; Accepted: 17 August 2025*)

Abstract. Permethrin, a widely used pyrethroid pesticide, has emerged as a significant contaminant in aquatic ecosystems, with particularly high concentrations in Vietnam's urban waterways. This study aims to develop a novel early warning system by assessing the sublethal behavioural effects of permethrin on the cladoceran *Moina macrocopa* and integrating these responses into an artificial neural network (ANN) model. A total of 21 individuals of *M. macrocopa*were exposed to seven permethrin concentrations (0–2.5 μg/L). Their swimming behaviour was video-recorded and analysed by using the motion-tracking software to extract a suite of locomotor parameters, which were then used to train a multi-layer ANN model. The results reveal a distinct biphasic (hormetic) dose-response: low concentrations (≤0.5 μg/L) stimulated swimming activity, whereas higher concentrations caused inhibition. In contrast, parameters such as turning angle and sinuosity increased proportionally with permethrin levels. The developed ANN model demonstrates high efficacy, achieving an overall accuracy of 94.05% on an independent test set for classifying four pollution levels. Notably, the model perfectly distinguished between non-polluted and highly polluted samples, confirming its reliability for detecting high-risk events. This study successfully establishes that integrating sublethal behavioural responses with artificial intelligence provides a sensitive, rapid, and automated tool for environmental monitoring. This approach holds significant potential for proactive water quality protection, especially in regions such as Vietnam, where automated biological monitoring systems are still underdeveloped. This system, enabling early detection of ecological stress through behavioural cues, can support local authorities in making timely, evidence-based decisions to mitigate pollution impacts and protect aquatic biodiversity.

Keywords: permethrin, behavioural biomonitoring, artificial neural network, early warning system

1 Introduction

The contamination of aquatic environments with pesticides has become a severe global issue, with Vietnam being no exception [1]. The widespread use of pyrethroid compounds, such as permethrin, in agriculture and public health has led to their accumulation in aquatic ecosystems. The severity of this issue in Vietnam is highlighted by Duong [2], who found average permethrin concentrations in Hanoi's urban river sediments as high as 1,876 ng/g (dry weight). As a synthetic pyrethroid, permethrin is known for its high toxicity to non-target aquatic life. Its acute effects are well-documented; for instance, the 48-hour LC50 for the crustacean *Daphnia magna* is only 0.6 μg/L [3]. Additionally, research shows that even at trace concentrations in the parts-per-billion (ppb) range, permethrin exposure can be lethal to various aquatic species [4].

Zooplankton play a crucial role in aquatic food webs and are highly sensitive to changes in water quality. Among these organisms, the cladoceran *Moina macrocopa* is commonly used as a bioindicator because of its short life cycle, ease of culture, and rapid response to toxic substances [5]. Alterations in zooplankton swimming behaviour, in particular, are considered early biological indicators of the presence of toxic substances in aquatic environments [6]. Research on *Daphnia* demonstrated that behavioural metrics, such as velocity and turning frequency, could signify toxicological stress well ahead of observable mortality or reproductive impairment. Therefore, monitoring the locomotor behaviour of *M. macrocopa* presents a promising approach for the early detection of pesticide pollution.

Biological Early Warning Systems (BEWS) utilise the responses of organisms to detect environmental changes; however, their effectiveness is often limited by the challenges of manually interpreting signals and the reliability of warnings provided by humans [7]. To overcome these issues, recent research has explored the use of machine learning and artificial neural networks (ANNs) to analyse behavioural data. For instance, Jeong [7] demonstrated that employing a machine learning approach to analyse *Daphnia* swimming data improved warning precision and recall by approximately 29.5% and 43.4%, respectively, compared with traditional indices.

Therefore, this study aims to: (1) evaluate the sublethal effects of permethrin on the locomotor behaviour of *M. macrocopa*, and (2) develop an ANN model using these behavioural data for the early warning of permethrin contamination. This research will help establish the behavioural alterations of this species as a sensitive and accurate tool for rapid environmental monitoring.

2 Material and method

M. macrocopa individuals were obtained from the laboratory of the Faculty of Biology, Agriculture, and Environment at The University of Science and Education, the University of Danang. The stock cultures were maintained at 25 ± 1 °C under a 16:8 h light/dark photoperiod and fed daily with *Chlorella vulgaris* at a density of 4.5×10^6 cells/mL.

Behavioural assays were conducted with a control (0 μg/L) and six different permethrin concentrations: 0.25, 0.5, 0.75, 1, 1.5, and 2.5 μg/L. For each treatment level, six replicates were performed. Each replicate consisted of a single neonate (<24 hours old) placed in the test solution. Immediately following exposure, the swimming behaviour of each individual was continuously recorded for 5 minutes.

The videos were then analysed by using the motion-tracking software (Rotitracker) to extract the movement trajectories of the organisms. From the trajectory data, key locomotor behavioural parameters were calculated, including:

i. Distance, D (mm): The total distance travelled by each individual over the observation period.

$D_i = \sqrt{(X_i - X_{i-1})^2 + (Y_i - Y_{i-1})^2}$ (Eq. 1)

where X_i and Y_i are the coordinates of the object at time i; X_{i-1} and Y_{i-1} are the coordinates of the object at time i–1.

ii. Velocity, V (mm·s⁻¹), is calculated as the total distance travelled divided by the actual swimming time.

$V = \frac{D_i}{\Delta t}$ (Eq. 2)

where Dt (s) is the time interval between two frames (frame rate); D (mm) is the distance travelled between two successive time points.

iii. Angle (A_i - degrees): Calculated based on three displacement points recorded in three

pISSN 1859-1388
eISSN 2615-9678

different video frames; the approach angle is measured regardless of left-right direction (<180°).

$$A_i = \tan^{-1}\left(\frac{Y_i - Y_{i-1}}{X_i - X_{i-1}}\right) \quad \text{(Eq. 3)}$$

iv. Sinuosity (SI): Calculated as the ratio of the actual distance travelled to the straight-line distance between the start and end points of the path [8].

$$SI = 2\left[p\left(\frac{1+c}{1-c} + b^2\right)\right]^{-0.5} \quad \text{(Eq. 4)}$$

where p is the mean step length;
c is the mean cosine of turning angles;
b is the coefficient of variation of step length.

v. Straightness (ST): Calculated as the ratio of the straight-line distance (net displacement) between the start and end points to the actual distance travelled along the path [9].

$$ST = \frac{dE}{L} \quad \text{(Eq. 5)}$$

where dE (mm) is the Euclidean distance between the beginning and the end of the path; L (mm) is the total path length.

vi. Moving time (s): The proportion of time an individual spends in active swimming relative to the total observation period (the remaining time is considered as resting or drifting).

Statistical analyses were conducted to assess differences in locomotor behavioural parameters among the permethrin treatments. Specifically, one-way analysis of variance (ANOVA) was performed for each parameter, followed by Tukey's post hoc test with a significance level of $p < 0.05$.

Based on the acquired behavioural dataset, we developed an artificial neural network (ANN) model to classify permethrin contamination levels (Fig. 1). The model's input features comprised a set of behavioural parameters (distance travelled, mean velocity, turning angle, activity time, sinuosity, and straightness) for each replicate at the observation time point. The ANN architecture consisted of three consecutive hidden layers: the first hidden layer included eight nodes; the second comprised four nodes; the third contained two nodes. Each hidden layer employed the LeakyReLU activation function, applied L2 regularisation with a coefficient of 0.02, a dropout rate of 0.4 to reduce overfitting, and batch normalisation to enhance training stability. The output layer consisted of four nodes corresponding to four classification levels: non-polluted (control), low pollution (0–0.25 μg/L), moderate pollution (0.25–1.5 μg/L), and high pollution (≥1.5 μg/L). All layers were fully connected. The model was trained by using the Adam optimisation algorithm with categorical cross-entropy loss [10]. The dataset was randomly divided into a training set (70%), a validation set (20%), and a test set (10%). Early stopping was implemented based on the performance on the validation set to prevent overfitting. After training, the model was evaluated based on classification accuracy on the test set and the confusion matrix among classes.

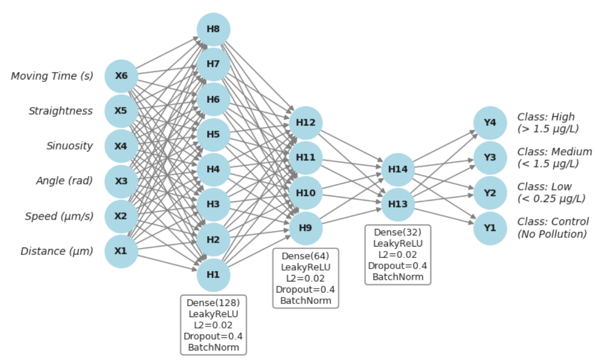

Fig. 1. Schematic diagram of the neural network architecture used for classification of permethrin exposure levels

3 Results and discussion

3.1 Effect of permethrin on locomotor behaviour of *Moina macrocopa*

The locomotor behaviour of *M. macrocopa* was significantly affected by permethrin exposure. A biphasic dose-response relationship was observed and characterised with stimulation at low concentrations and inhibition at higher concentrations (Fig. 2). At lower concentrations (0.25 and 0.5 µg/L), swimming activity was stimulated compared with the control group (Fig. 3). Specifically, the mean of velocity increased by approximately 9.7–59.4% relative to the control (6.20 ± 0.36 mm/s), reaching 8.94 ± 2.80 mm/s at 0.25 µg/L and peaking at 9.80 ± 1.65 mm/s at 0.5 µg/L (one-way ANOVA, Tukey's HSD, $p < 0.05$). Similarly, both total distances travelled and swimming time increased within this concentration range, reflecting a state of neural excitation.

However, as the permethrin concentrations increased further from 0.75 to 2.5 µg/L, this stimulatory effect was reversed, and a clear inhibitory phase began. All measured parameters progressively decreased, eventually falling below the baseline levels observed in the control group. For example, the total travelled distance fell from 1,584.13 to 1,258.47 mm; mean velocity dropped from 5.28 to 4.19 mm/s, and swimming time decreased from 93.04 to 88.03 s. This trend indicates that following the initial excitatory phase, the increasing toxicity of permethrin began to exert strong inhibitory effects on the locomotor functions of *M. macrocopa*.

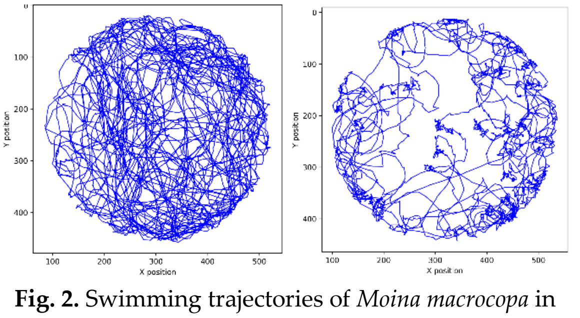

Fig. 2. Swimming trajectories of *Moina macrocopa* in control solution (left) and at 2.5 µg/L permethrin (right), recorded over 5 minutes of observation

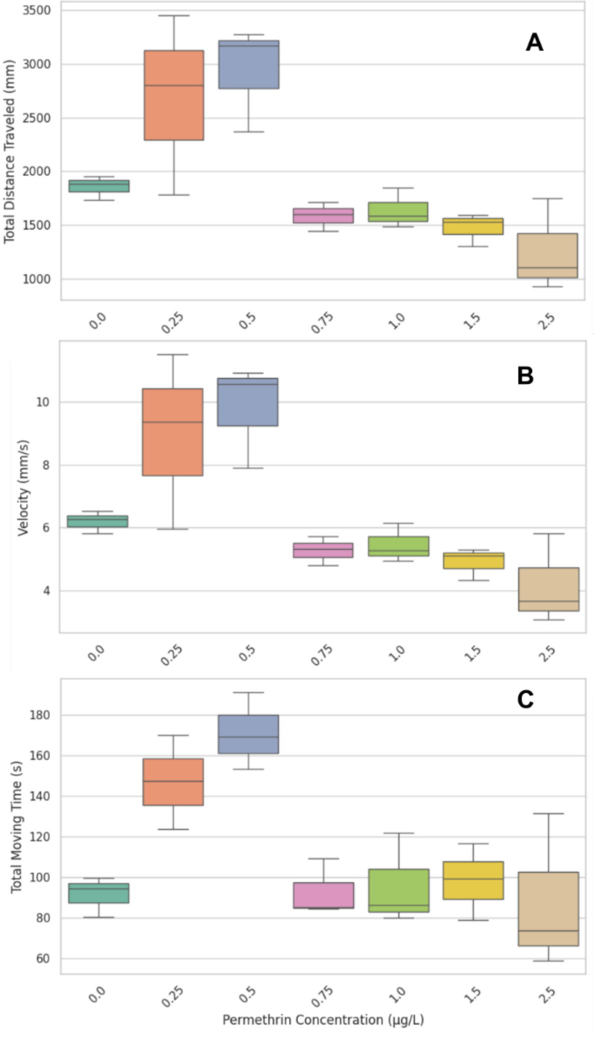

Fig. 3. Boxplots showing the effects of permethrin concentration on (a) total distance travelled; (b) velocity; (c) total moving time of *M. macrocopa*

In contrast to other behavioural parameters, both turning angle and sinuosity show a statistically significant increasing trend with rising permethrin concentrations (one-way ANOVA, $p < 0.05$) (Fig. 4, Table 1). Specifically, the mean turning angle increased from 47.43 ± 7.99 degrees in the control group to 76.58 ± 6.84 degrees at the highest permethrin concentration (2.5 µg/L), indicating that the organisms swam in a more erratic, zigzag manner. Simultaneously, sinuosity rose by approximately 1.53 times, from 0.36 ± 0.02 to 0.55 ± 0.07, suggesting that the swimming paths became significantly more convoluted under permethrin-induced stress.

Hue University Journal of Science: Natural Science
Vol. 134, No. 1S-2, 117–125, 2025 (Special Issue: CBA 2025)

pISSN 1859-1388
eISSN 2615-9678

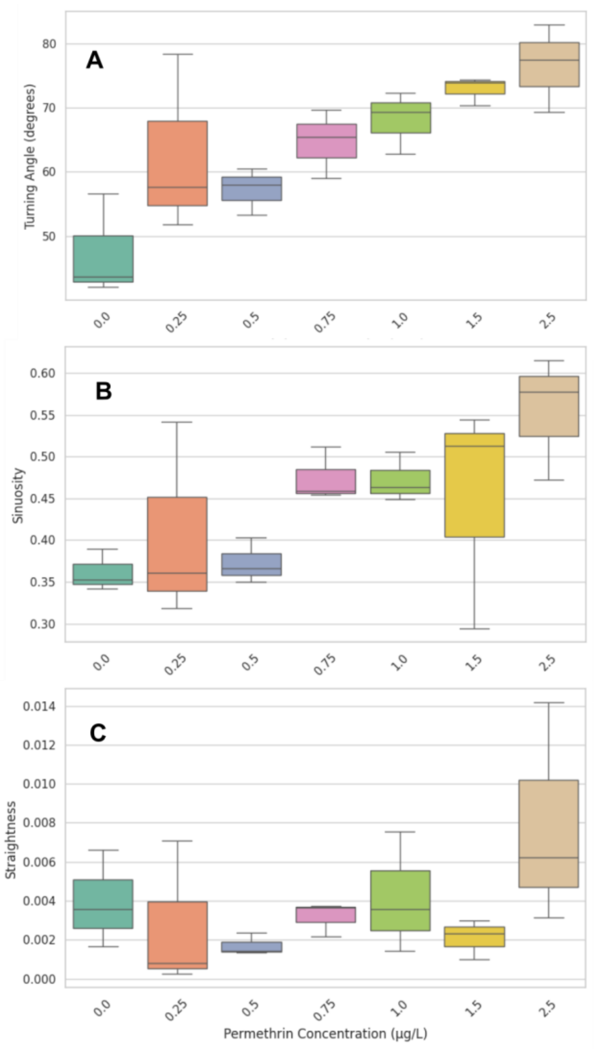

Fig. 4. Boxplots showing the effects of permethrin concentration on (a) turning angle; (b) sinuosity; (c) straightness of *M. macrocopa*

These results confirm the hypothesis that permethrin induces a hormetic effect on *Moina macrocopa*, where low doses stimulate neuromotor activity while high doses cause behavioural disruption and inhibit locomotion [11]. This biphasic response—an initial increase in velocity followed by a sharp decline at higher concentrations—points to the well-established neurotoxic mechanism of pyrethroids, which act by overstimulating the nervous system [4, 10]. At the molecular level, permethrin binds to sodium (Na^+) channels on neuronal membranes, delaying their closure and causing neurons to fire repetitively [13, 14]. At low doses, this hyperexcitation can temporarily boost activity, explaining why *M. macrocopa* swims faster upon initial exposure. This stimulatory effect is consistent with observations in other aquatic crustaceans; these observations can be increased swimming activity in *Pandalus borealis* exposed to deltamethrin [15] and in *Daphnia magna* exposed to low-dose λ-cyhalothrin [16]. However, at higher doses, this neuronal disruption surpasses the organism's compensatory threshold, leading to loss of motor coordination and paralysis. This manifests as the pronounced reduction in swimming activity observed in the high-dose groups, a symptom also described for *Pandalus borealis* and *Daphnia magna* [15, 16].

Table 1. Locomotor behavioural characteristics of *Moina macrocopa* at different permethrin concentrations

Concentration (μg/L)	Distance (mm)	Speed (mm/s)	Angle (deg)	Sinuosity	Straightness	Moving time (s)
0.0	1857.4 ± 112.4	6.2 ± 0.4	47.4 ± 7.9	0.4± 0.0	4.0 ± 2.5	91.5 ± 10.0
0.25	2681.3± 840.7	8.9 ± 2.8	62.6 ± 13.9	0.4 ± 0.1	2.7 ± 3.8	147.15± 23.2
0.5	2938.4± 494.1	9.8 ± 1.6	57.3 ± 3.7	0.4 ± 0.0	1.7 ± 0.6	171.3 ± 19.1
0.75	1584.1± 136.4	5.3 ± 0.5	64.7 ± 5.4	0.5 ± 0.0	3.2 ± 0.9	93.0 ± 14.2
1.0	1639.2± 186.3	5.5 ± 0.6	68.2 ± 4.8	0.5± 0.0	4.2 ± 3.1	96.0± 22.5

Concentration (μg/L)	Distance (mm)	Speed (mm/s)	Angle (deg)	Sinuosity	Straightness	Moving time (s)
1.5	1474.6± 152.4	4.9 ± 0.5	72.9± 2.2	0.4 ± 0.1	2.1 ± 1.0	98.3 ± 18.8
2.5	1258.5± 430.4	4.2 ± 1.4	76.6 ± 6.9	0.6± 0.1	7.9 ± 5.7	88.0 ± 38.4

From an ecological perspective, these permethrin-induced behavioural changes can trigger cascading effects throughout aquatic communities. These impacts are particularly significant given that *M. macrocopa* occupies a key position on the food web, serving as both a primary consumer of phytoplankton and a crucial food source for higher trophic levels, such as fish larvae [17]. The hyperactivity caused by low-dose exposure could increase the organism's conspicuousness, elevating its risk of predation. Conversely, in areas with high permethrin contamination, impaired mobility and eventual mortality are likely to cause severe population declines. The depletion of a vital filter-feeder like *M. macrocopa* could, in turn, degrade water quality through uncontrolled algal blooms and diminish the food supply for fish. Ultimately, these sublethal behavioural alterations, by disrupting critical life-sustaining activities such as foraging and reproduction, pose a significant threat to the long-term viability of zooplankton populations and the stability of the entire aquatic ecosystem.

3.2 Early warning model performance and application prospects

To classify permethrin pollution levels from behavioural data, we constructed and trained a multi-layer artificial neural network. Before training, input features were normalised via StandardScaler, and target labels were converted by using one-hot encoding. The model's architecture consisted of three sequential hidden layers with 8, 4, and 2 nodes. For ensuring robustness and preventing overfitting, a suite of strong regularisation techniques was employed, including Dropout at a rate of 0.4, Batch Normalisation after each hidden layer, and L2 regularisation. The training process was further optimised by using the LeakyReLU activation function and an EarlyStopping callback to halt training when performance ceased to improve.

The model's stability was confirmed by its learning curves, which show no signs of overfitting; the validation and training accuracy tracked each other closely while loss values for both sets decreased steadily throughout the training epochs (Fig. 5). Upon completion of training, the model's generalisation capability was assessed on an independent test set of 84 samples. It achieved an excellent overall accuracy of 94.05% (Fig. 6), with corresponding weighted averages for precision, recall, and F1-score all reaching 0.94 (Table 2).

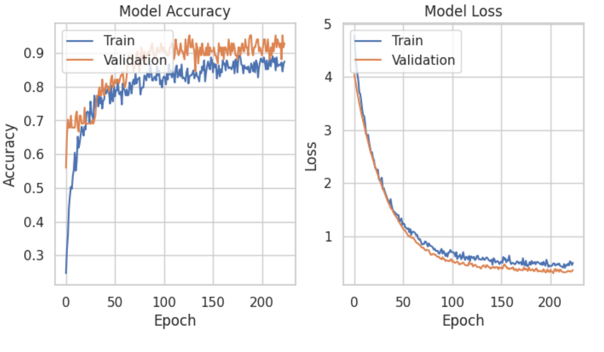

Fig. 5. Learning curves showing the changes in accuracy (left) and loss (right) on the training and validation datasets over epochs

A detailed class-wise analysis underscored the model's reliability. It perfectly classified the non-polluted control group (Class 0) with precision, recall, and F1-scores of 1. Performance remained high for the low pollution group (Class

pISSN 1859-1388
eISSN 2615-9678

1: 0–0.25 μg/L; precision = 1.00; recall = 0.92), the moderate pollution group (Class 2: 0.25–1.5 μg/L; precision = 0.96; recall = 0.94), and the high pollution group (Class 3: ≥1.5 μg/L; precision = 0.85; recall = 0.92). Critically, the confusion matrix verified that no high-pollution samples were misclassified as non-polluted. The few misclassifications that did occur were limited to adjacent classes, a biologically reasonable outcome reflecting the subtle transitions in behavioural responses at concentration boundaries.

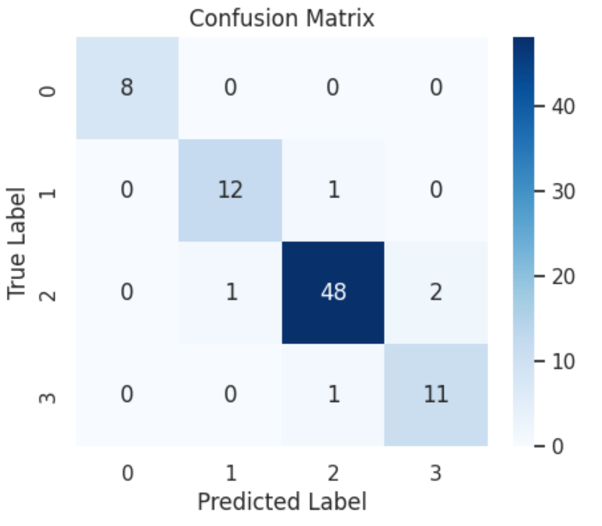

Fig. 6. Confusion matrix illustrating the performance of the classification model on the test dataset

Table 2. Classification report for the classification model

Classes	precision	recall	F1-score	support
0	1.00	1.00	1.00	8
1	0.92	0.92	0.92	13
2	0.96	0.94	0.95	51
3	0.85	0.92	0.88	12
accuracy			0.94	84
macro avg	0.93	0.95	0.94	84
weighted avg	0.94	0.94	0.94	84

From a practical perspective, this study powerfully demonstrates the potential of developing automated water pollution warning systems on the basis of the behavioural responses of indicator organisms. The ANN model developed here enabled accurate, near-real-time classification of permethrin pollution levels by using the locomotor features of *M. macrocopa*. The primary advantage of this approach is its early detection capability, as behavioural changes occur almost immediately after exposure, allowing for significant alerts before the toxic substances reach lethal concentrations. Furthermore, such machine learning models can process multiple, complex behavioural signals simultaneously, far outperforming the capacity of manual observation. Our results align with global trends in environmental monitoring, where integrating AI and machine learning into biological systems significantly enhances the reliability of early warnings [7]. For example, Jeong [7] reported that applying a LightGBM model to behavioural data from *Daphnia* markedly improved warning precision and recall compared with traditional indices. Notably, they also identified velocity and velocity patterns as key predictive parameters, which corroborates our findings.

Despite its promise, the practical deployment of early warning systems based on *M. macrocopa* still requires further research. Commercial systems, such as the *Daphnia* Toximeter and other automated behavioural sensors, are already available for integration into water monitoring stations [18]. The integration of robust and highly sensitive machine learning algorithms, such as the one developed in this study, could further enhance the capabilities of these systems, reduce false alarms, and move the field closer to truly intelligent, autonomous water quality monitoring tools.

4 Conclusion

This study establishes *Moina macrocopa* as a highly promising bioindicator for permethrin pollution through a quantitative analysis of its behavioural changes. The characteristic biphasic response—stimulation of swimming activity at low doses and inhibition at high doses—reflects not only the organism's toxicological stress but also provides a robust foundation for developing early warning models. By integrating biology with artificial intelligence, such behaviour-based systems can enable the rapid detection of pollution events before severe ecological consequences occur, thereby empowering environmental managers with tools for timely intervention. Although the current model is designed and adjusted under laboratory conditions, the expansion and testing in a complex natural environment with diversified stress factors require further necessary steps. In the future, the expansion of training data and simulating multi-agent scenarios will help increase the application and reliability of the system in practice. Given the complex and ongoing challenge of pesticide contamination in Vietnam and the wider region, coupled with a lack of automated biological monitoring systems [1], this research direction offers significant potential for the proactive and effective protection of aquatic ecosystems.

Acknowledgement

This research was funded by the Ministry of Education and Training, Vietnam, under project number B2023.DNA.20. Besides, we would like to thank the Faculty of Biology, Agriculture and Environment Sciences, University of Science and Education, The University of Da Nang, for providing research facilities.

References

1. Li H, Cheng F, Wei Y, Lydy MJ, You J. Global occurrence of pyrethroid insecticides in sediment and the associated toxicological effects on benthic invertebrates: an overview. J Hazard Mater. 2017; 324:258-71.
2. Duong HT, Kadokami K, Pan S, Matsuura N, Nguyen TQ. Screening and analysis of 940 organic micro-pollutants in river sediments in Vietnam using an automated identification and quantification database system for GC–MS. Chemosphere. 2014;107:462-72.
3. Tomlin CD. The pesticide manual: a world compendium. [Internet]. 2009 [cited 2025 Jun 15]. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/20103110198
4. Cox C. Permethrin. Journal of Pesticide Reform [Internet]. 1993 [cited 2025 Jun 15]. Available from: https://www.eap.mcgill.ca/MagRack/JPR/JPR_19.htm
5. Janakiraman A, Thangaraj S, Gokula V, Stalin A. Report on high density population of a Cladoceran, Moina macrocopa and their gut microbes in anoxic

pISSN 1859-1388
eISSN 2615-9678

sewage water: a potential bio-indicator of aquatic pollution. Aquat Ecol. 2024;58(4):1255-65.

6. Bownik A. Daphnia swimming behaviour as a biomarker in toxicity assessment: A review. Sci Total Environ. 2017;601:194-205.
7. Jeong H, Park S, Choi B, Yu CS, Hong JY, Jeong TY, et al. Machine learning-based water quality prediction using octennial in-situ Daphnia magna biological early warning system data. J Hazard Mater. 2024;465:133196.
8. Benhamou S. How to reliably estimate the tortuosity of an animal's path:: straightness, sinuosity, or fractal dimension?. Journal of theoretical biology. 2004;229(2):209-220.
9. Batschelet E. Circular statistics in Biology. New York: Academic Press; 1981.
10. Ghosh J, Gupta S, editors. ADAM Optimizer and CATEGORICAL CROSSENTROPY Loss Function-Based CNN Method for Diagnosing Colorectal Cancer. 2023 International Conference on Computational Intelligence and Sustainable Engineering Solutions (CISES); 2023 28-30 April 2023.
11. Stanley JK, Perkins EJ, Habib T, Sims JG, Chappell P, Escalon BL, et al. The Good, the Bad, and the Toxic: Approaching Hormesis in *Daphnia magna* Exposed to an Energetic Compound. Environ Sci Technol. 2013;47(16):9424-33.
12. Soderlund DM. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Arch Toxicol. 2012;86(2):165-81.
13. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, et al. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology. 2002;171(1):3-59.
14. Meacham CA, Brodfuehrer PD, Watkins JA, Shafer TJ. Developmentally-regulated sodium channel subunits are differentially sensitive to α-cyano containing pyrethroids. Toxicol Appl Pharmacol. 2008;231(3):273-81.
15. Bamber S, Rundberget JT, Kringstad A, Bechmann RK. Effects of simulated environmental discharges of the salmon lice pesticides deltamethrin and azamethiphos on the swimming behaviour and survival of adult Northern shrimp (Pandalus borealis). Aquat Toxicol. 2021;240:105966.
16. Bownik A, Kowalczyk M, Bańczerowski J. Lambda-cyhalothrin affects swimming activity and physiological responses of Daphnia magna. Chemosphere. 2019;216:805-11.
17. Kang CK, Park HY, Kim MC, Lee WJ. Use of marine yeasts as an available diet for mass cultures of Moina macrocopa. Aquac Res. 2006;37(12):1227-37.
18. Lechelt M, Blohm W, Kirschneit B, Pfeiffer M, Gresens E, Liley J, et al. Monitoring of surface water by ultrasensitiveDaphnia toximeter. Environ Toxicol. 2000;15(5):390-400.