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Assessment of Industrial Agrochemical Leachate Toxicity and Neurobehavioral Toxicity Thresholds in *Channa punctatus*

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ABSTRACT
Uncontrolled discharge and environmental percolation of industrial agrochemical waste generate complex toxic leachates that degrade aquatic ecosystems. This investigation utilized the hardy air-breathing teleost *Channa punctatus* as a bio-indicator to evaluate the acute toxicity and neurobehavioral impact of pesticide manufacturing waste leachate. Utilizing static acute bioassay methods (APHA/USEPA), fish were subjected to exposure concentrations of 5.0%, 7.5%, 10.0%, 12.5%, 15.0%, 20.0%, and 25.0% alongside parallel controls over 96 hours. The calculated 96-hour LC50 was determined to be 12.5%. Qualitative behavioural profiling revealed progressive neurotoxic stress, manifested by hyperventilation (increased opercular frequency), heightened surfacing activity to gulp air, hyperexcitability, erratic swimming, loss of motor control, and body discoloration. Complete mortality (100%) was recorded within 96 hours at concentrations $\geq$ 20%. These biological outcomes highlight the vulnerability of native teleost species to landfill leachates and underscore the imperative for stringent environmental compliance regarding hazardous waste disposal.

KEYWORDS: Industrial waste, Leachate bioassay, Bio-indicator, Neurotoxicity, Median lethal concentration, Bio-monitoring

1. INTRODUCTION

In modern agricultural management, intensive pesticide application remains a primary strategy to prevent yield loss caused by pests and to meet global food demand (Ahmad et al., 2023; Ismail et al., 2004). However, the vast production and excessive application of active pesticide ingredients worldwide generate substantial chemical waste, creating persistent environmental hazards (USEPA, 2001; Ibrahim et al., 2025). On a global scale, agrochemicals and industrial pesticide residues redistribute throughout surrounding environments via surface runoff, atmospheric transport, deposition processes, and subsurface leaching (Bhat & Kumar, 2025). Heavy rainfall and agricultural drainage frequently induce bypass flow—both overland and vertically through soil macropores—facilitating the rapid transport of persistent pesticide chemicals into surface freshwater bodies and vulnerable groundwater aquifers (Foster et al., 1991; Williams et al., 1991). When water percolates through industrial solid waste disposal sites and unlined landfills containing toxic effluents, complex leachates are generated, concentrating hazardous chemicals (Steel et al., 2025).

The contamination of aquatic ecosystems by industrial effluents and agrochemical waste leachates has emerged as a major ecotoxicological concern in recent decades (Eaton, 1970; Johnson, 1965; Jacob et al., 1982). Non-target aquatic organisms, particularly fish, are continuously exposed to complex chemical mixtures that disrupt cellular, physiological, and metabolic processes (Rathore & Singh, 2023). Chronic exposure to sub-lethal concentrations often induces oxidative stress, neurotoxicity, endocrine disruption, histopathological tissue damage, and immunotoxicity (IARC, 1990, 1993; Ibrahim et al., 2025). Furthermore, hydrophobic pesticide formulations tend to bioaccumulate in aquatic biota, leading to biomagnification across higher trophic levels in the aquatic food web and posing severe indirect health risks to human consumers (Bhat & Kumar, 2025). Consequently, evaluating the toxicological impacts of hazardous industrial pollutants is vital for establishing regulatory discharge thresholds and formulating strategies to safeguard freshwater biodiversity (Ahmad et al., 2023).

Behavioral and physiological responses in teleost fish serve as sensitive bio-indicators for detecting immediate chemical stress and environmental degradation (Steel et al., 2025). The spotted snakehead, *Channa punctatus* (Bloch), an air-breathing freshwater teleost belonging to the family Channidae, is widely distributed across tropical and subtropical Asian freshwater habitats (Rathore & Singh, 2023). *C. punctatus* possesses accessory respiratory organs that allow it to survive in oxygen-depleted and polluted waters, making it an ideal model species for aquatic toxicity assays due to its hardiness, ease of laboratory acclimation, and year-round availability (Ahmad et al., 2023).

The present study evaluated the acute aquatic toxicity and neurobehavioral impacts of industrial pesticide waste leachate on *Channa punctatus* using a static bioassay system. The primary objectives were to establish the 96-hour median lethal concentration (LC50) of the waste leachate and to characterize dose- and time-dependent behavioral and morphological alterations resulting from acute toxic exposure.

2. MATERIALS AND METHODS

2.1 Leachate Collection and Preparation

Solid pesticide waste for the preparation of the test leachate was collected from a freshly deposited dumping site near an industrial facility manufacturing lindane (Rai & Singh, 2015). Samples were placed in airtight bags and transported immediately to the laboratory to prevent contamination or volatile loss (Rai & Singh, 2015). The Toxicity Characteristic Leaching Procedure (TCLP) leaching test (USEPA, 1990) was employed to extract leachate from the solid waste. An acetic acid solution was mixed at a liquid-to-solid ratio of 20:1 (20 parts acetic acid solution to 1 part solid waste by weight) and agitated for 18 hours to simulate environmental leaching dynamics (USEPA, 1990; Rai & Singh, 2015).

2.2 Test Organism and Acclimation Procedures

Healthy specimens of the air-breathing teleost *Channa punctatus* (order Channiformes, family Channidae) were procured from local, unpolluted aquatic bodies (Rai & Singh, 2015). To prevent dermal fungal or bacterial infections, specimens were subjected to a prophylactic bath in 0.05% potassium permanganate (KMnO) solution for 2 minutes (Rai & Singh, 2015; Rathore & Singh, 2023). The fish were then transferred to static glass aquaria systems 10 days prior to exposure for acclimation under standard laboratory conditions (Rai & Singh, 2015). Fish were fed commercial fish food daily during acclimation, and no mortality occurred during this period (Rai & Singh, 2015). The average wet weight and average total length (SD) of the test specimens were 35 ± 3 g and 12.5 ± 2 cm, respectively (Rai & Singh, 2015).

2.3 Experimental Setup and Bioassay Design

Preliminary exploratory range-finding experiments were conducted to determine the range of chemical concentrations required for definitive testing, following standard ecotoxicological procedures (APHA/AWWA/WPCF, 1998; Rai & Singh, 2015). Definitive 96-hour static acute toxicity bioassays were performed by exposing fish to distinct concentrations of pesticide waste leachate: Control (0%), 5%, 7.5%, 10%, 12.5%, 15%, 20%, and 25% (Rai & Singh, 2015).

Test media were prepared by diluting calculated aliquots of stock leachate in dechlorinated water 3–4 hours prior to introducing the fish (Rai & Singh, 2015). A set of 10 fish specimens was randomly assigned to each test container, and all concentrations were triplicated under identical experimental conditions (Rai & Singh, 2015). Individual fish were used only once across all bioassays (Rai & Singh, 2015). To prevent metabolic waste interference with water quality, feeding was terminated 24 hours prior to test initiation, and fish were starved throughout the 96-hour bioassay, adhering to standard protocols (Ward & Parrish, 1982; Reish & Oshida, 1987; Rai & Singh, 2015). Continuous aeration was provided to maintain adequate dissolved oxygen levels across all beakers, and test solutions (both control and treatment media) were renewed daily (Rai & Singh, 2015).

2.4 Mortality Determination and Statistical Analysis

Mortality was recorded at 24-, 48-, 72-, and 96-hours post-exposure (Rai & Singh, 2015). The endpoint for mortality was defined as the total absence of physical response after mechanical prodding with a glass rod, and dead fish were removed immediately to avoid fouling the test solution (Rai & Singh, 2015). Behavioral and morphological alterations were systematically recorded using qualitative observation techniques relative to parallel control groups (Rai & Singh, 2015; Steel et al., 2025). Median lethal concentration (LC50) values were calculated from the 96-hour acute toxicity data using Finney's (1971) probit analysis method (Finney, 1971; Rai & Singh, 2015).

3. RESULTS

3.1 Behavioral and Morphological Alterations

Control fish displayed normal aquatic behavior during the entire 96-hour experimental period: swimming smoothly, maintaining cohesive group dynamics, resting periodically near the vessel bottom, and surfacing at regular intervals to gulp atmospheric air via their accessory respiratory organs (Rai & Singh, 2015).

In contrast, fish exposed to industrial pesticide waste leachate displayed progressive, dose- and time-dependent behavioral and morphological abnormalities (Rai & Singh, 2015). Immediately after initial exposure, fish exhibited heightened alertness, briefly becoming motionless in response to environmental alteration (Rai & Singh, 2015). This initial response was followed by clear avoidance behaviors, including rapid, erratic swimming patterns and frequent attempts to jump out of the test vessels (Rai & Singh, 2015). Rapid opercular movement and an increased frequency of surfacing to gulp air were observed across all treatment concentrations (Rai & Singh, 2015; Rathore & Singh, 2023).

At higher exposure concentrations (12.5%), severe signs of neurotoxicity were recorded, including violent jerky swimming movements, extreme hyperexcitability, and total loss of equilibrium (Rai & Singh, 2015; Steel et al., 2025). Morphologically, fish subjected to elevated leachate levels showed noticeable body paleness due to a loss of dermal pigmentation (Rai & Singh, 2015). Prior to death, severely affected specimens lost muscular control, became completely lethargic, and hung vertically near the water surface with their terminal mouths protruding upward to draw air (Rai & Singh, 2015). Exhausted specimens ultimately sank to the bottom of the vessel, where complete mortality occurred (Rai & Singh, 2015).

3.2 Quantitative Evaluation and Toxicity Metrics

Table 1: Behavioural Responses of *Channa punctatus* Exposed to Pesticide Waste Leachate (96 Hours) (Rai & Singh, 2015)

Behavioural Parameter	**Control (0%)**	**5.0%**	**7.5%**	**10.0%**	**12.5%**	**15.0%**	**20.0%**	**25.0%**
Linear Movement	+	+	+	++	+++	+++	Nil	Nil
Staying Period	++	+	+	+	++	++	Nil	Nil
Eye Movement	+++	+++	++	++	++	++	Nil	Nil
Opercular Movement	-	-	-	+	++	++	Nil	Nil

Behavioural Parameter	Control (0%)	5.0%	7.5%	10.0%	12.5%	15.0%	20.0%	25.0%
Equilibrium Status	+++	+++	+++	++	++	+	Nil	Nil
Distance Traveled	+	+	++	++	+++	+++	Nil	Nil
Jumping Movement	+++	+++	++	+++	++	++	Nil	Nil

Note: "+" indicates relative increase/presence of parameter intensity; "-" indicates no detection/baseline; "Nil" indicates total absence of activity due to complete lethality or severe prostration (Rai & Singh, 2015).

Table 2: Acute Mortality Data and Calculated 96-Hour LC50 Value for *C. punctatus* (Rai & Singh, 2015)

Sl. No.	Leachate Concentration (%)	Number of Fish Exposed	Number of Dead Fish	Mortality (%)
1	Control (0%)	10	0	0%
2	5.0%	10	1	10%
3	7.5%	10	2	20%
4	10.0%	10	3	30%
5	12.5%	10	5	50%
6	15.0%	10	8	80%

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Sl. No.	Leachate Concentration (%)	Number of Fish Exposed	Number of Dead Fish	Mortality (%)
7	20.0%	10	10	100%
8	25.0%	10	10	100%

- **Calculated 96-hr LC50 Value: 12.5% leachate concentration** (determined via probit analysis; Finney, 1971; Rai & Singh, 2015).
- **Control Group:** 0% mortality recorded throughout the 96-hour exposure duration (Rai & Singh, 2015).

4. DISCUSSION

Acute bioassays remain an essential ecotoxicological tool utilized by regulatory agencies to evaluate the immediate biological hazards posed by complex chemical effluents and industrial leachates (Sprague, 1969; Calow, 1992; Rai & Singh, 2015). In the present study, exposing *Channa punctatus* to industrial pesticide waste leachate resulted in significant, dose- and time-dependent mortality alongside severe behavioural and physiological disruptions (Rai & Singh, 2015).

The initial hyperresponsiveness, accelerated opercular beat rates, and increased surfacing frequency observed in exposed fish represent primary homeostatic adaptations to physiological stress and environmental hypoxia (Agrawal, 1991; Rai & Singh, 2015). As an air-breathing teleost, *C. punctatus* relies heavily on its accessory respiratory organs when branchial gas exchange is compromised by toxic chemical exposure (Agrawal, 1991; Rai & Singh, 2015). Similar abnormal swimming behaviors, rapid surfacing, and loss of equilibrium have been reported in teleost models exposed to heavy metals and organophosphate insecticides, where neuromuscular disruption was attributed to severe loss of skeletal minerals and cellular enzyme inhibition (Gupta et al., 1981; Agrawal, 1991; Mishra et al., 2005). Furthermore, chemical constituents present in pesticide formulations—such as organophosphates, pyrethroids, and organochlorines—are known to inhibit membrane-bound acetylcholinesterase (AChE) and branchial Ca^{2+}-ATPase activity, directly disrupting ionic regulation and neuromuscular coordination (Desaiah & Koch, 1975a, 1975b; Bansal et al., 1985; Rathore & Singh, 2023).

The calculated 96-hour LC50 value of 12.5% leachate concentration reflects the high toxic potency of industrial runoff mixtures (Rai & Singh, 2015). Direct comparison of numerical toxic thresholds across different studies is inherently difficult due to variations in experimental conditions, ambient water chemistry (such as pH, hardness, temperature, and dissolved oxygen), organism size, age, and the variable chemical composition of complex leachates (Sprague, 1969; Verma et al., 1991; Rai & Singh, 2015). Nevertheless, the 100% mortality observed at concentration thresholds of 20% and 25% underscores the severe ecological hazards posed by untreated industrial dumping into surrounding aquatic ecosystems (Rai & Singh, 2015; Steel et al., 2025).

References

1. Agrawal, S. K. (1991). Bioassay evaluation of acute toxicity levels of mercuric chloride to an air breathing fish *Channa punctatus* (Bloch): mortality and behaviour study. *Journal of Environmental Biology*, 12: 99–106.
2. Ahmad, A., & Farooq, M. (2023). Pesticide exposure and its physiological consequences in freshwater fish Channa punctatus. International Journal of Innovative Research in Technology, 10(2): 112–118.
3. APHA/AWWA/WPCF (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th Ed. American Public Health Association, New York.
4. Bansal, S. K., Murthy, R. C., & Chandra, V. S. (1985). The effect of some divalent metals on cardiac and branchial Ca^{2+}-ATPase in a fresh water fish *Saccobranchus fossilis*. *Ecotoxicology and Environmental Safety*, 9: 373–377.
5. Bhat, A. R., & Kumar, S. (2025). Pesticide toxicity in some fish species: A review on physiological, histopathological, and behavioral alterations. *International Journal of Fisheries and Aquatic Studies*, 13(2): 189–194.
6. Calow, P. (1992). The three criteria of ecotoxicology. *Functional Ecology*, 6: 617–619.
7. Desaiah, D., & Koch, R. B. (1975a). Inhibition of ATPase activity in Channel catfish brain by ketone and its reduction product. *Bulletin of Environmental Contamination and Toxicology*, 13: 153–158.
8. Desaiah, D., & Koch, R. B. (1975b). Toxaphene inhibition on ATPase activity in catfish, *Ictalurus punctatus*, tissues. *Bulletin of Environmental Contamination and Toxicology*, 13: 238–244.
9. Eaton, J. E. (1970). Chronic malathion toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). *Water Research*, 4: 673–684.
10. Finney, D. J. (1971). *Probit Analysis*. 3rd Ed. Cambridge University Press, Cambridge, UK, pp. 333.
11. Foster, S. S. D., Chilton, P. J., & Stuart, M. E. (1991). Mechanisms of groundwater pollution by pesticides. *Journal of the Institution of Water and Environmental Management*, 5: 186–193.
12. Gupta, P. K., Khangroot, B. S., & Durve, V. S. (1981). The temperature dependence of the acute toxicity of copper to a freshwater pond snail *Viviparus bengalensis* L. *Hydrobiologia*, 83: 461–464.
13. IARC (1990). *Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 49: Nickel and Nickel Compounds. IARC, Lyon.
14. IARC (1993). *Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 58: Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry. IARC, Lyon.
15. Ibrahim, M., et al. (2025). Mechanisms of pesticide toxicity in fish: Insights into the ameliorative role of plant-derived compounds—A review. *Ecotoxicology and Environmental Safety*, 289: 117420.
16. Ismail, B. S., Ngan, C. K., Cheah, U. B., & Abdullah, W. Y. (2004). Leaching potential of pesticides in a vegetable farm in the Cameron Highlands, Malaysia. *Bulletin of Environmental Contamination and Toxicology*, 72: 836–843.
17. Jacob, S. S., Nair, N. B., & Balasubramanian, N. K. (1982). Toxicity of certain pesticides found in the habitat to the larvivorous fishes *Aplocheilus lineatus* (Cuv. and Val.) and *Macropodus cupanus* (Cuv. and Val.). *Proceedings of the Indian Academy of Sciences (Animal Sciences)*, 91: 323–328.
18. Johnson, D. W. (1965). Pesticides and fishes: a review of selected literature. *Transactions of the American Fisheries Society*, 97: 398–424.
19. Mishra, D., Srivastava, S. K., & Srivastav, A. K. (2005). Acute toxicity and behavioural anomalies of a fresh water catfish, *Heteropneustes fossilis*, to an organophosphorus insecticide methyl-parathion. *Pollution Research*, 24(2): 307–310.

20. Rai, S., & Singh, D. (2015). Effect of pesticide waste leachate on the behaviours and morphology of air breathing fish *Channa punctatus* (Bloch). *Proceedings of the International Seminar on SPEEDS-2015*, pp. 326–332.
21. Rathore, S., & Singh, R. (2023). Acute toxicity and behavioral alterations in *Channa punctatus* after exposure to organophosphate pesticides. *Pollution Research*, 42(3): 45–51.
22. Reish, D. L., & Oshida, P. S. (1987). *Manual of Methods in Aquatic Environment Research, Part 6: Short-term bioassays*. FAO Fisheries Technical Paper 247: 1–62.
23. Sprague, J. B. (1969). Measurement of pollutant toxicity to fish: I. Bioassay methods for acute toxicity. *Water Research*, 3: 793–821.
24. Steel, A., et al. (2025). Aqueous exposure to pyrethroid pesticides results in behavioral effects in early life stage fish. *Conservation Physiology*, 13(1): coaf055.
25. USEPA (1990). *Toxicity Characteristic Leaching Procedure (TCLP)*. Method 1311, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA Publication SW-846. Washington, D.C.
26. USEPA (2001). *Pesticide Industry Sales and Usage: 1996 and 1997 Market Estimates*. U.S. Environmental Protection Agency, Washington, D.C.
27. Verma, Y., Bhatt, H. V., & Saiyed, S. M. (1991). New dimensions in ecotoxicology and a case study of aquatic ecosystems. *Advances in Biosciences*, 10: 61–66.
28. Ward, G. S., & Parrish, P. R. (1982). *Manual of Methods in Aquatic Environment Research, Part 6: Toxicity tests*. FAO Fisheries Technical Paper 185: 1–23.
29. Williams, R. J., Bird, S. C., & Clare, R. W. (1991). Simazine concentration in a stream draining an agricultural catchment. *Journal of the Institution of Water and Environmental Management*, 5: 80–84.