Indian J. Applied & Pure Bio. Vol. 38(2), 785-792 (2023). **ISSN: 0970-2091**
A web of Science Journal

Microplastic potentiates Chlorpyrifos toxicity to the fresh water Cladoceran, *Macrothrix rosea* (Jurine, 1820)

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Abstract

Microplastics (MP) are ubiquitous pollutants of environmental concern partly due to plastics ability to sorb and transport chemical pollutants from their surrounding environment. The impact of this "vector effect is raising concern as to their role in the movement of these pollutants through the food chain is required to be adequately addressed. In this study, effects of soaked polyethylene microplastic (PEMP) in chlorpyriphos (a commonly used pesticide in agricultural) were assessed on the fresh water cladoceran *Macrothrix rosea* (Jurine, 1820). The animals were exposed to individual MP concentrations (25, 100, 200 and 1000 particle ml^{-1}), chlorpyrifos concentrations (0.001 to 1 μgL^{-1}), and soaked MPs (100 particle ml^{-1}) in chlorpyrifos at similar concentrations. Results showed MP had no mortality, however, significant reduction in population growth rate at higher concentration (1000 MP ml^{-1}) were observed in *M. rosea*. The organism exposed in soaked MP had higher mortality then in chlorpyrifos concentrations after 48 hr. These findings indicated that MP potentiates the toxicity of chlorpyriphos in *M. rosea*. This investigation provided evidence toward the interaction between plastics and Persistant Organic Pollutants (POPs) when addressing the environmental importance of the vector effect in areas with high concentrations of microplastics and pesticides.

Key word : Soaked Microplastic, Vector effect, Toxicity, Chlorpyrifos.

Microplastic (MPs; <5 mm) are ubiquitous pollutants and a growing concern found throughout the world in oceans[1,2], estuary[12], river[17] and wastewater[11,26]. Sources of MPs are plastic fibre generated from clothing in washing machine[23], fragmentation of larger plastics and plastic beads used in industry as abrasives for sandblasting. These MPs enter the river system via the sewage system and finally transported to the marine

environment which had an average daily discharge of 60 billion pieces of plastic particles[9]. Most MPs are smaller than 2mm (small MP), a range which could make them easily ingestible by small aquatic organisms[7]. Recently, ingested MP by aquatic organism is a commonly noted problem, and MP have been consumed by a variety of creatures, including fish, sea birds, zooplankton, coral, and marine mammals, resulting in trophic transfer[22]. MPs has also cause in the death of aquatic organisms through entanglement and blockage of the organism's digestive system.

Concerns have been raised about persistent organic pollutants (POPs) that sorbs to plastics ability to function as a vector for hydrophobic organic contaminants (HOC)[21, 33]. The contamination of aquatic ecosystems with POPs is well known. POPs are characterised by environmental persistence, long-range environmental movement, bioaccumulation and toxicity. Due to hydrophobic property of microplastics' surface, the adsorption of POPs to MPs has been widely reported[19], include polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs)[28], organo-chlorine pesticides, polyaromatic hydrocarbons (PAHs)[24] and perfluorinated surfactants (PFCs)[20]. These chemicals were tending to accumulate on plastics with elevated concentration. Ingestion of such contaminated MPs and bioaccumulation of sorbed chemicals has been documented in Lugworms[4,5], amphipods[6], fish[30] and zooplankton[31]. The pollution of aquatic ecosystems through agricultural areas is of growing concern owing to the extensive use of pesticides.

Chlorpyrifos (CP, O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate insecticide and acaricide, which is widely used for the control of a variety of pests in paddy fields and animal farming in India. Chlorpyrifos is low solubility in water (1.39 mg L^{-1} at 25°C)[10], and thus tend to stay within the organic fraction of sediments. Because of its intensive use, aquatic ecosystems may be contaminated with chlorpyrifos[34], and has been observed sorbed to plastic particles[21]. Recent studies have reported toxicities of chlorpyrifos to aquatic organisms[32] such as in fish[14], *Oncorhynchus mykiss*[16], marine copepod (*Acartia tonsa*)[3], and microalgae (*Isochrysis galbana*)[8]. Polyethene and polystyrene (PE and PS) microplastics increased the toxicity of the chlorpyrifos insecticide on *A. tonsa* and *O. mykiss*, respectively[3, 16], while polyethene microplastics decreased the toxicity of chlorpyrifos on *I. galbana*[8]. The role of microplastics as pollutant carriers for marine organisms was confirmed from the above study.

In order to address this issue, this study examines the toxicity test of a medium-size cladoceran, *Macrothrix rosea* (Jurine, 1820), is a cosmopolitan species and is often found in ponds or lakes that are rich in organic matter[13]. In this study, the soaked microplastic was used to investigate the acute toxicity of MP as a vector for chlorpyrifos, to the fresh water cladoceran. These species are the primary consumers in the freshwater environment and may contribute to the bioaccumulation and biomagnification of chlorpyrifos via the ingestion of MPs. It is therefore of interest to investigate the accumulation of chlorpyrifos via contaminated MPs as a result of environmental exposure.

Microplastics preparation:

Used plastics were collected from the trash and crushed into tiny pieces using a grinder and screening them via a differential sieving machine (Fisher brand test sieve) to get microplastics (<63µm in diameter and irregularly shaped). Microplastics were collected and used for the experiment. Chemical composition of the microplastics were identified by using Fourier Transform Infrared Spectroscopy (FTIR) (JASCO FT/ IR-4600) and the spectrum obtained at 0.7/ cm between 4000 and 649/cm. FTIR Spectra were matched with inbuilt library data of FTIR for the confirmation of the polymer type.

Stock solution preparation :

Chlorpyrifos insecticide (10%) were purchased from local market, was dissolved in distilled water to make 1 mg L^{-1} stock solution. The prepared MPs were suspended in a 1000 MP/ 250 ml of different concentration of chlorpyrifos, for 24 hr prior to experimental initiation.

Experimental organism :

The benthic detritivorous freshwater cladocera, *Macrothrix rosea* (Jurine, 1820) was collected from sampled from southern bank of the Ganga River (25.622231 N; 85.17208 E) at NIT Ghat, Patna, Bihar and brought to the Ecosystem Ecology Laboratory, Central University of South Bihar (Gaya, Bihar, India) for establishing the laboratory culture. *M. rosea* were culture in aquaria at 24 °C with filtered autoclaved aerated aquarium water (FAAAW), using as food the green alga *Chlorella* sp. For experimental purpose large number of ovigerous female were acclimatized the resulting neonates were collected.

Experimental setup :

Experiment I: Impact of Microplastic on population of *Macrothrix rosea.*

Experimental protocol consisted of control (without microplastic) and four treatment (25, 200, 400 and 1000 particle ml^{-1}), each with 3 replicates. Ten individual species were placed in each of 15 replicates in 50ml glass vial containing 25 ml FAAAW in a temperature-controlled (24 °C) plankton wheel at 6-8 RPM to ensure dispersion of the MP. The FAAAW and microplastics were changed on each day. Observations were taken daily until the population reached asymptote. The population growth rate (r) was calculated from the exponential phase of population growth using the formula:

$$r = \frac{\ln N_t - \ln N_0}{t}$$

where N_0= initial population density, and N_t = population density after time t.

We used an average of r values derived using different time intervals over a period of 10–15 days, during which the stable age distribution was presumed to have been reached, where the population growth curve displayed crests and troughs.

Experiment II: Impact of Socked Microplastic (<63µm and >153µm) in different concentration of chlorpyrifos on Population of *Macrothrix rosea*

Following the experiment 1 protocol, ten individual species were placed in 14 concentration (between 0.001µg L^{-1} to 100µg L^{-1}) of

chlorpyrifos and in soaked MPs (25 particle ml^{-1} each) of different concentration. To check the amount of chlorpyrifos soaked by MP, again ten individuals in each concentration were placed in the chlorpyrifos solution in which MPs were kept for soaked. After 48 h of exposure, organism counted under a sterio zoom microscope (Magnus). The above experiment was performed with Socked microplastic of size >153µm following the same protocol.

Experiment I :

Mortality in *M. rosea* by MP (<63µm) alone were tested in four concentrations 25, 100, 200 and 1000 MP ml^{-1} had no observed mortality at any concentrations in 48hr. So, the population dynamic was conducted (Figure 1A), however a significant reduction in population growth rate at higher concentration (1000 MP ml^{-1}) were observed (Figure 1B). The population began to increase exponentially (0-14 days) and growth rate (r) was >0.18 in 40, 200 and 400 particle ml^{-1} concentration of MP while r >0.18 in 1000 particle ml^{-1} concentration of MP and before reaching asymptote at each concentration at the population size significantly lower than the control ($p < 0.05$; Mann-Whitney U test).

Experiment II:

Macrothrix rosea recorded significantly higher survival in control than in treatments (chlorpyrifos and soaked MP) (Figure 2). The mortality in treatment was a direct function of chlorpyrifos concentrations. We recorded 7%, 47%, and 100% mortalities, respectively in 0.01, 0.02 and 0.04 μgL^{-1} concentrations of chlorpyrifos in 48 h of exposure treatment (Figure 2). However, 17%, 44%, 57%, 87% and 100% mortality recorded with microplastic soaked in respectively in 0.001, 0.002, 0.004, 0.006 and 0.008μgL^{-1} concentrations of chlorpyrifos after 48 h of exposure. In the chlorpyrifos solution in which microplastic were kept for soaked, there was a remarkable decrease observe in mortality and 100% mortality recoded at 10µg chlorpyrifos L^{-1} in which microplastic were soaked (Figure 2).

LC_{50} was calculated with the help of probit regression model as 0.021 ± 0.01 μgL^{-1} (mean ± SE) in the chlorpyrifos. Addition of soaked 100 MP/ml in chlorpyrifos concentrations resulted in an increased toxicity, by the LC50 of the mixture which was markedly lower than that of chlorpyrifos 0.002 ± 0.001 μgL^{-1} (mean ± SE) (Figure 3).

It has been well demonstrated by various researchers that microplastics may act as a carrier of chemical pollutants that are transferred to aquatic organisms[29,30]. The individual MP of size <63µm and >153 µm used in present study had no mortality in *M. rosea* even at higher concentration (1000 particles ml^{-1}), however there is decrease in population growth rate was observed (Figure 1). Individual microplastics did not significantly affect the biomarker response of living organisms, according to recent investigations carried out under laboratory conditions[8,15,27,25]. Present findings are similar with previous studies of zebrafish larvae (*Danio rerio*) exposed to low density polyethene (LDPE) microplastics where no significant impact on biomarker responses was observed[15].

Hydrophobicity, pollutant concentrations, and the physical characteristics and appearance of microplastic particles all have a role in the adsorption of chemical pollutants on surfaces of microplastics[18]. Present study showed that

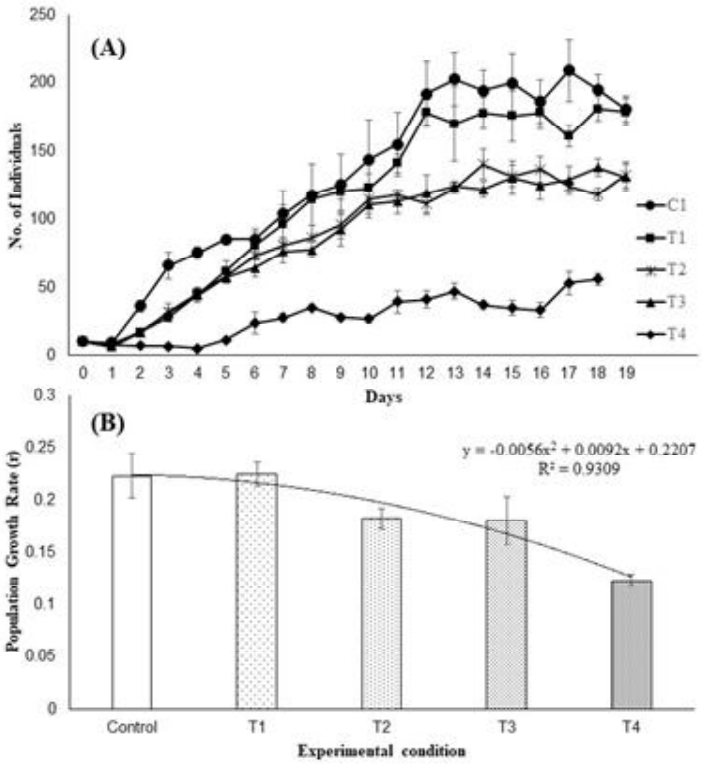

Figure 1: Population growth trajectories **(A)** and population growth rate (r) **(B)** of *Macrothrix rosea* in control and in four concentrations of treatment (40,200,400 and 1000 particle ml^{-1}) of microplastic.

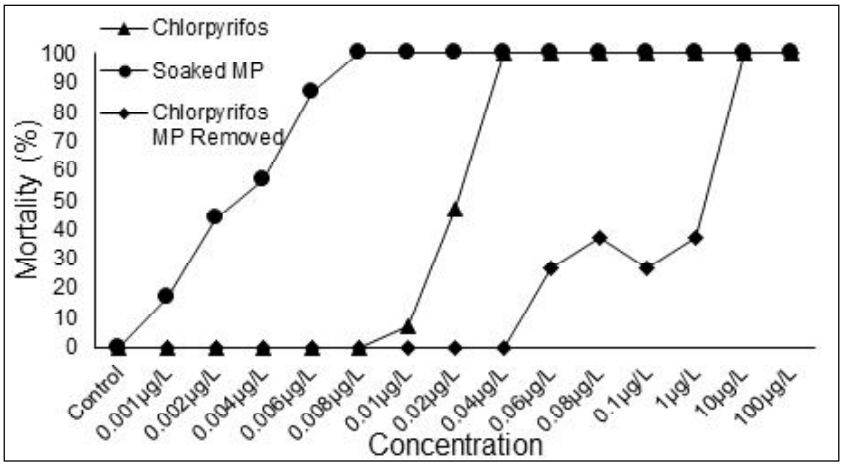

Figure 2: Percent mortality in *Macrothrix rosea* exposed to different concentration of chlorpyrifos, soaked microplastic (100 MPs mL^{-1}) and after microplastic treated chlorpyrifos concentration.

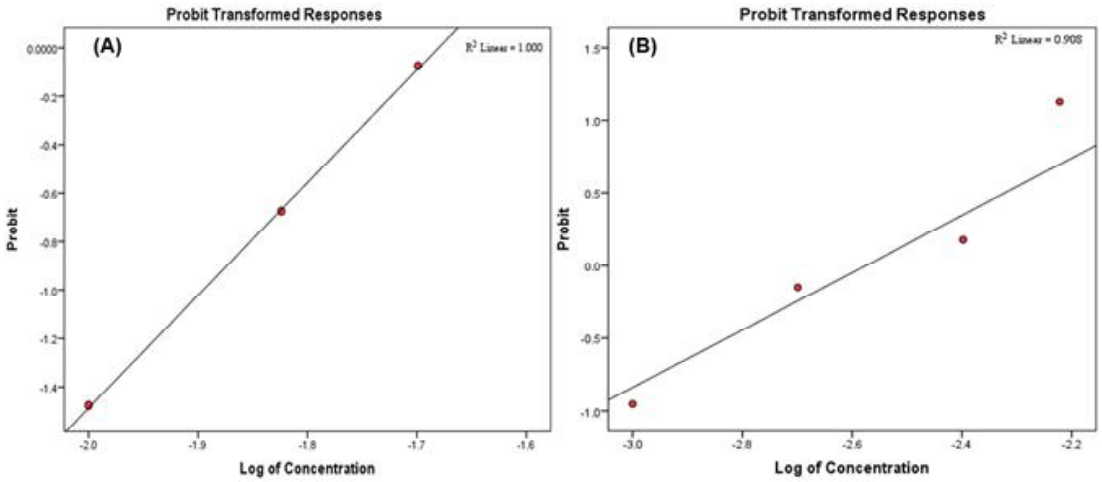

Figure 3: Probit regression model for *Macrothrix rosea* exposed to different concentration of **(A)** chlorpyrifos and **(B)** soaked microplastic in chlorpyrifos concentration.

chlorpyrifos insecticide was adsorbed onto microplastic surfaces, which is more toxic than chlorpyrifos alone and act as a vector. This indicates that MP have the potential to potentiate chlorpyrifos mediated toxicity, where potentiation is defined as a mixture scenario where a non-hazardous agent increases the effect of another agent. Potentiation is thus a distinct mixture effect from additivity. Many vector studies describe the influence of MP on the toxicity or bioavailability of POPs. At the same time as our results based on typical vector studies, show how the toxicity of the POPs is mediated in the presence of MP, it was possible to assess this effect as potentiation by recognizing that MP is a potential toxicant within the mixture that interacts with POPs. In keeping with the vector effect, adsorption of chlorpyrifos to MP, and subsequent change in an exposure pathway, may explain our results. It is a possible hypothesis that the change in exposure from soluble chlorpyrifos to uptake via gut alters uptake or toxicity to *M. rosea*. When soaked MP of size >153 µm was used no mortality was observe. This may due to this size particle cannot be in taken by the animal. The use of the soaked MP in the present study demonstrated that soaked MP (<63 µm) in chlorpyrifos is more toxic than chlorpyrifos alone. This is likely not due to any additive toxicity of the MP, but rather a potentiation of chlorpyrifos induced toxicity. Data indicate the importance of recognizing vector scenarios as mixtures in order to elucidate and classify such interactions.

References :

1. Andrady, A. L. (2011). *Marine pollution bulletin, 62*(8): 1596-1605.
2. Avio, C. G., S. Gorbi, and F. Regoli (2017). *Marine environmental research, 128*: 2-11.
3. Bellas, J. and I. Gil (2020). *Environmental Pollution, 260*: 114059.
4. Besseling, E., A. Wegner, E. M. Foekema, M. J. van den Heuvel-Greve, and A. A. Koelmans, (2012) *Environ Sci Technol,*

47(1): 593-600.

5. Browne, Mark A., J. Niven, Stewart, S. Galloway, Tamara, J. Rowland, Steve, and C. Thompson, Richard, (2013) *Curr Biol, 23*(23): 2388-2392.
6. Chua, E. M., J. Shimeta, D. Nugegoda, P. D. Morrison, and B. O. Clarke (2014) *Environ Sci Technol, 48*(14): 8127-8134.
7. Egbeocha, C.O., S. Malek, C. U. Emenike and P. Milow (2018). *Aquatic Biology, 27*: 93-106.
8. Garrido, S., M. Linares, J. A. Campillo, and M. Albentosa, (2019). *Ecotoxicology and environmental safety*, *173*: 103-109.
9. GESAMP, (2015). Sources fate and effects of microplastics in the marine environment: a global assessment, ed. PJ Kershaw (No. 90, p. 96). IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), Rep. Stud.–GESAMP. Retrieved from. (http://www.gesamp.org/site/assets/files/1275/sources-fate-and-effects-of-microplastics-in-the-marine-environment-part-2-of-a-global-assessment-en.pdf) (accessed on 26 August 2022).
10. Giesy, J. P., K. R. Solomon, J. R. Coats, K. R. Dixon, J. M. Giddings, and E. E. Kenaga (1999). Chlorpyrifos: ecological risk assessment in North American aquatic environments (pp. 1-129). Springer New York.
11. Habib, R.Z., T. Thiemann and R. Al Kendi (2020). *Journal of Water Resource and Protection, 12*(01): 1.
12. Hitchcock, J.N. and S.M. Mitrovic (2019). *Environmental pollution, 247*: 457-466.
13. Huang, X., S.H.I. Xinlu, X. U. Sen, L.I.U. Guijie, M. A. Limin, and S. U. N. Zhiqiang (2011). *Journal of Limnology, 70*(2): 248.
14. Ismail M., R. Ali, M. Shahid, M.A. Khan, M. Zubair, T. Ali, and Q. Mahmood Khan (2018) *Drug Chem Toxicol 41:* 22–26.
15. Karami, A., A. Golieskardi, C. K. Choo, N. Romano, Y. B. Ho, and B. Salamatinia, (2017). *Science of the total environment, 578*: 485-494.
16. Karbalaei, S., P. Hanachi, G. Rafiee, and P. Seifori, (2021). *Journal of Hazardous Materials, 403:* 123980.
17. Kibria, G., D. Nugegoda, and A.Y. Haroon, (2022). *Environmental Occurrence and Treatment Technologies,* 133-166.
18. Koelmans, A.A. (2015). *Marine anthropogenic litter,* 309-324.
19. León, V. M., I. García, E. González, R. Samper, V. Fernández-González, and S. Muniategui-Lorenzo (2018). *Environmental pollution, 236*: 442-453.
20. Llorca, M., M. Farré, H. K. Karapanagioti and D. Barceló, (2014). *Marine pollution bulletin, 87*(1-2): 286-291.
21. Ma, L., K. Li, C. Wang, B. Liu, H. Peng, Y. Mei, and P. Ning, (2020). *Environmental Science and Pollution Research, 27*: 7309-7317.
22. Madhav, S., A. Ahamad, A. K. Singh, J. Kushawaha, J. S. Chauhan, S. Sharma, and P. Singh, (2020). *Sensors in Water Pollutants Monitoring: Role of Material,* 43-62.
23. Napper, I. E., and R. C. Thompson (2016). *Marine pollution bulletin, 112*(1-2): 39-45.
24. Ogata, Y., H. Takada, K. Mizukawa, H. Hirai, S. Iwasa, S. Endo, Y. Mato, M. Saha, K. Okuda, A. Nakashima, M. Murakami, N. Zurcher, R. Booyatumanondo, M. P. Zakaria, L.Q. Dung, M. Gordon, C. Miguez,

S. Suzuki, C. Moore, H.K. Karapanagioti, S. Weerts, T. McClurg, E. Burres, W. Smith, M. V. Velkenburg, J.S. Lang, R.C. Lang, D. Laursen, B. Danner, N. Stewardson and R. C. Thompson (2009) *Mar Poll Bull 58*(10): 1437-1446.

25. Oliveira, M., A. Ribeiro, K. Hylland, and L. Guilhermino, (2013). *Ecological indicators, 34,* 641-647.
26. Prata, J. C. (2018). *Marine pollution bulletin, 129*(1): 262-265.
27. Rainieri, S., N. Conlledo, B. K. Larsen, K. Granby, and A. Barranco, (2018). *Environmental Research, 162:* 135-143.
28. Rios, L. M., C. Moore, and P. R. Jones, (2007) *Mar Poll Bull 54*(8): 1230-1237.
29. Rivera-Hernández, J. R., B. Fernández, J. Santos-Echeandia, S. Garrido, M. Morante, P. Santos and M. Albentosa (2019). *Science of the total environment, 674:* 412-423.
30. Rochman, C.M., E. Hoh, T. Kurobe, and S. J. Teh, (2013) *Scientific Reports, 3 :* 1-7.
31. Syberg, K., A. Nielsen, F. R. Khan, G. T. Banta, A. Palmqvist, and P. M. Jepsen, (2017). *Journal of Toxicology and Environmental Health, Part A, 80*(23-24): 1369-1371.
32. Zhang J., L. Liu, L. Ren, W. Feng, P. Lv, W. Wu, and Y. Yan (2017) *J Hazard Mater 334:* 121–131.
33. Zhang, M., and L. Xu, (2022). *Critical Reviews in Environmental Science and Technology, 52*(5): 810-846.
34. Zhao J., and B. Chen (2016) *Ecotoxicol Environ Saf 125:* 161–169.