Bulletin
Marine and Freshwater Sciences

Vol. 48, 2023, 79–85

Research Article

Acute and subacute toxicity of naphthalene in a filter-feeding species Styela plicata (Chordata, Ascidiacea)

Tahani EL AYARI [1], **Raja BEN AHMED** [*2], **Samir GHANNEM** [1]

& Najoua TRIGUI EL MENIF [1]

[1]University of Carthage. Faculty of Sciences of Bizerte. Laboratory of Environment Bio-monitoring (LBE). Group of Fundamental and Applied Malacology (MAF), Bizerte, Tunisia.
[2]University of Tunis El Manar. Faculty of Sciences of Tunis. Department of Biology, Ecology, Biology and physiology of Aquatic Organisms Laboratory (LR18ES41), Tunis, Tunisia

*__Correspondence:__ raja_benahmed@yahoo.fr

Received: 15/04/2023; **Accepted**: 01/11/2023; **Published**: 29/11/2023

Abstract: The aim of the current study was the assessment of a Polycyclic aromatic hydrocarbon (PAHs) toxicity on an ascidian species, *Styela plicata*. Acute and subacute toxicity of naphthalene (NAP) has been determined using five increasing concentrations (0.5, 1, 1.5, 2.5 and 5 mg/L) during 96 h of exposure. The half lethal concentration (LC50, 96h) value of naphthalene for *S. Plicata* was 2.41 mg/L. Furthermore, the filtration activity decreased with the five tested concentrations of NAP; a significant decrease by 69 % and 91.8 % was observed after 1h in the groups exposed to C1 and C5, respectively. A similar pattern was noted after 2h of filtration; the decrease was estimated to 39 % with C1 and 72.5 % with C5. The results from this bioassay confirmed the toxicity of NAP on *S. plicata*; we also provided information about the use of *S. plicata* as a potential bioindicator species for the assessment of PAHs toxicity.

Keywords: *Styela plicata*; Ascidiacea; acute toxicity; filtration; naphthalene; aromatic hydrocarbon; Ghar El Melh lagoon; Tunisia; Mediterranean.

1. Introduction

Styela plicata (Leseur 1823) is a solitary ascidian mostly found in harbours and lagoon near aquaculture farms (Lambert 2002; Chebbi et al., 2010). It is a filter-feeding organism, commonly reported in the Mediterranean Sea, Pacific, Indian, and Atlantic Ocean (Pineda et al., 2011; Barbosa et al., 2018). Recent studies on ecotoxicology have proved the suitable use of *S. plicata* for the biomonitoring of aquatic pollution (Cima et al., 1996; Parrinello et al., 2017). Indeed, it was used to investigate the effects of heavy metal, Polychlorinated biphenyl (PCB), diesel oil and organic mercury (Maruyama et al., 1983; Aydın-Önen 2016; Parrinello et al., 2017; Barbosa et al., 2018). Moreover, the embryotoxicity of tributyltin (TBT) and triphenyltin (TPT) has been

assessed using this species (Cima et al., 1996).
In Tunisia, *Styela plicata* is recognized as a non-native species found in Bizerte lagoon and Gulf of Hammamet (Chebbi et al., 2010 a and b).

The industrial revolution caused a hydrocarbonpollution, which has affected negatively the aquatic life (Almeida et al., 2012; Abha and Singh 2012; Nikitha et al., 2017). Among the most identified compounds in the aquatic environment, the Polycyclic aromatic hydrocarbon (Wu et al., 2011; Barhoumi et al., 2014; Mdaini et al., 2023). PAHs were 16 compounds, those toxicities were established on a number of aquatic species (Barhoumi et al., 2016; DeMiguel-Jiménez et al., 2022; Mdaini et al., 2023).

In 1979, PAHs have been listed by the United State Environmental Protection Agency (US EPA) as priority pollutants (Wise et al., 2015). The toxicity caused by PAHs in aquatic organisms still occur till now, especially that caused by naphthalene, pyrene, anthracene and phenanthrene (Vieira and Guilhermino 2012; Vasanth et al., 2012; Wang et al., 2023).

The current bioassay aimed to determine the toxicity of naphthalene in an ascidian species, *Styela plicata*. In this aim, five increasing concentrations of NAP have been used. Furthermore, the filtration rate was determined during 1 and 2h of activity.

2. Materials and Methods

2.1. *Sampling site*

Specimens of *Styela plicata*, body length (40± 0.5 mm) were handly collected in 2023 from a station situated in Ghar El Melh lagoon, Northern Tunisia (Figure. 1).

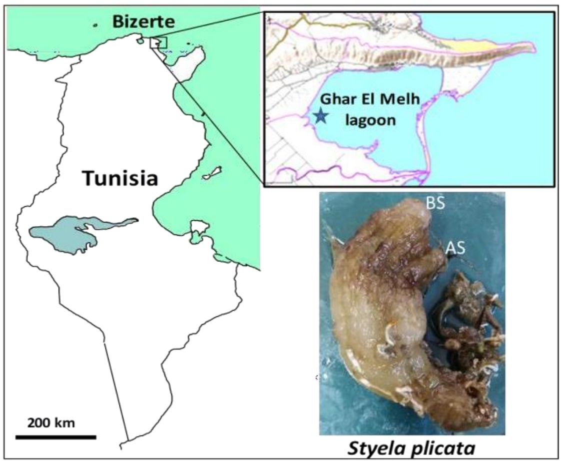

Figure 1. Location of the sampling station (the star indicated the site of collection in the lagoon). AS: atrial siphon; BS: branchial siphon.

2.2. *Acute toxicity test*

Specimens of *Styela plicata* (N = 100) were first acclimated to the condition of the laboratory. The temperature was maintained to near 19°C, pH = 7.80 ± 0.10, the photoperiod (12 h light: 12 h dark), and the salinity was 34‰. Filtered seawater was daily renewed, 10 specimens were placed in aquarium of 10L. Naphthalene (purity ≥ 99%) was purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).Acute and subacute toxicity of NAP were assessed using five increasing concentrations 0.5, 1, 1.5, 2.5 and 5 mg/L of NAP selected based on results from previous studies 0.5, 1, 1.5, 2.5 and 5 mg/L of NAP (Hansen et al., 2008; Jing et al., 2020; Nayak and Patnaik 2023). The oral siphon was stimulated 1 h before the administration of the contaminant and then every 24h during 96h. The absence of response to mechanical stimulation is an indication of mortality (Barbosa et al., 2018).

2.3. *The Filtration rate (FR)*

After 96h of exposure to naphthalene, three specimens of *Styela plicata* were placed in 100 ml of neutral red solution (1 g/L). Just before the beginning of the filtration activity, the concentration (C0) of the neutral red was measured at 550 nm using UV-spectrophotometer (Multiskan GO). After 1 and 2h of filtration at dark room, the neural red concentrations (Ct) were measured. The neural red concentrations were extrapolated from a standard curve. The neural red retention was calculated following this equation:

The Filtration rate (FR) = [M/nt] log (C0/Ct)(Coughlan 1969), where M is the volume of the test solution (ml), n is the number of ascidia, t is the time (hours), and C0 and Ct are the concentrations of the red neutral at the beginning and after 1 and 2 h, respectively.

2.4. Statistical analysis

The sublethal concentration (LC50) was calculated with GraphPad Prism 5.The dose response relationship was tested with Pearson correlation test (*p < 0.01*). The difference in the filtration rate was tested by mean of Chi-square test in the software IBM SPSS Statistics v. 27. Significant differences were considered for (*p < 0.05*).

3. Results

3.1. Acute toxicity

The findings from the acute toxicity test showed that ascidia mortality increased with the increase of NAP concentration, confirming a dose response relationship (Figure 2).This relationship was verified by Pearson's correlation (r = 0.93; *p < 0.01*). The sublethal concentration (LC50) estimated for 96 h of exposure to NAPH is 2.41± 0.35 mg/L (Figure 2).

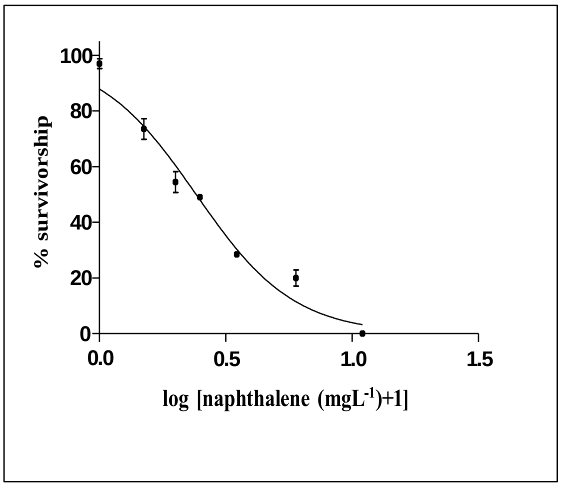

Figure 2. Curve of dose–response obtained from the exposure of *Styela plicata* to naphthalene.

3.2. Filtration rate

The results from the filtration activity calculated in *Styela plicata* exposed to five concentrations of NAP after 1h and 2h is given in Figure 3. After 1hof filtration, the activity decreased by 69%, 50%, 49.6%, 46.5%, and 94.8% with C1, C2, C3, C4 and C5, respectively. After one additional hour of filtration activity, the still decrease sill occurred 39% (C1), 15.1% (C2), 20.8% (C3), 14% (C4), and 72.5% (C5). The most important decrease in the FR 69% (1h), 39% (2h), and 91.8% (1h), 72.5% (2h) was noted with C1 and C5, respectively (Figure 3).

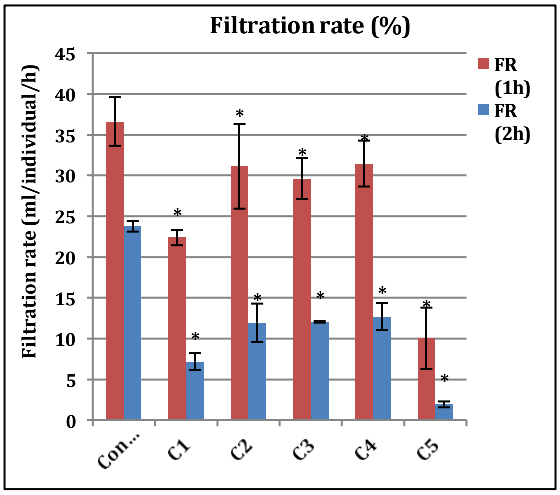

Figure 3. Filtration rate in *Styela plicata* exposed to naphthalene during 96h.

4. Discussion

Polycyclic aromatic hydrocarbon toxicity on the aquatic lifewas demonstrated owing to their bioavaolabity, and to the difficulty of biodegradation (Haritash and Kaushik 2009; Barhoumi et al., 2014). Toxicity caused by naphthalene was studied on microalgae (*Chlorella vulgaris*), crustaceans (*Calanus finmarchicus* and *Artemia franciscana*) and actinopterygians (*Oncorhynchus mykiss*, *Pimephales promelas* and *Anabas testudineus*) (DeGraeve et al., 1982; Hansen et al., 2008; Kong et al., 2010; Sogbanmu et al., 2018; Albarano et al., 2022). Nevertheless, none study was conducted on ascidian species.

The sublethal concentration (LC50, 96h) calculated for *S. plicata* exposed to NAP was 2.41mg/L. This value is superior than values 1.6 mg/L by DeGraeve et al., (1982) in *O. mykiss*. The values calculated in *Clarias gariepinus* and *P. promelas* were however much higher than the value calculated for *S. plicata* 7.21 mg/l and 7.9 mg/L, respectively (DeGraeve et al., 1982; Sogbanmu et al., 2018).

Filter-feeding as sponges, some polychaetes, bivalves, and ascidians filter water in order to nutritional elements and for gas-exchange (Stabili et al., 2016). In ascidians, the filtration activity is nowadays proposed in water remediation (Stabili et al., 2016). It is also used as an endpoint in ecotoxicological studies (Kraak et al., 1994; Ray et al., 2020; Elyousfi et al., 2021). *Sytela plicata* exhibited a better capacity for water filtration than other ascidians species due to the anatomy of its branchial basket (Stabili et al., 2016). Our results showed that the filtration activity of *S. plicata* is significantly reduced due to naphthalene exposure. A similar response was noted in bivalves, the filtration activity decreased in contaminated specimens in order to reduce the bioaccumulation of xenobiotics (Kraak et al., 1994; Elyousfi et al., 2021).

5. Conclusion

The results from the present study reported the usefulness of the ascidian species (*Styela plicata*) as bioindicator in ecotoxicology. This non-native species is ubiquitous in various coastal ecosystems. Also, the selection of *S. plicata* is promoted by the intrinsic biological and ecological criteria of this species. Among which the filtration rate which could be used as an endpoint for ecotoxicological studies using this species. All the tested concentrations of naphthalene were found toxic on *S. plicata*; highest concentration (5 mg/l) was the most toxic. Information from this bioassay could serve in the future for the risk assessment of PAHs in the aquatic system using *S. plicata* as a model.

References

1. Abha, S., & Singh, C.S., 2012. Hydrocarbon pollution: effects on living organisms, remediation of contaminated environments, and effects of heavy metals co-contamination on bioremediation. In: Romero-Zern, L. (Ed.). Introduction to Enhanced Oil Recovery (EOR) Processes and Bioremediation of Oil-Contaminated Sites. InTech. https://doi.org/10.5772/48014
2. Albarano, L., Serafini, S., Toscanesi, M., Trifuoggi, M., Zupo, V., Costantini, M., Vignati, D.A., Guida, M., & Libralato, G. (2022). Genotoxicity Set Up in Artemia franciscana Nauplii and Adults Exposed to Phenanthrene, Naphthalene, Fluoranthene, and Benzo (k) fluoranthene. *Water*, 14(10), 1594. https://doi.org/10.3390/w14101594
3. Almeida, J.R., Gravato, C., & Guilhermino, L. (2012). Challenges in

assessing the toxic effects of polycyclic aromatic hydrocarbons to marine organisms: A case study on the acute toxicity of pyrene to the European seabass (Dicentrarchus labrax L.). *Chemosphere*, 86(9), 926-937. https://doi.org/10.1016/j.chemosphere.2011.10.059

4. Aydın-Önen, S. (2016). Styela plicata: a new promising bioindicator of heavy metal pollution for eastern Aegean Sea coastal waters. *Environmental Science and Pollution Research*, 23, 21536-21553. https://doi.org/10.1007/s11356-016-7298-5
5. Barbosa, D.B., Mello, A.d.A., Allodi, S., & de Barros, C.M. (2018). Acute exposure to water-soluble fractions of marine diesel oil: Evaluation of apoptosis and oxidative stress in an ascidian. Chemosphere, 211:308-315. https://doi.org/10.1016/j.chemosphere.2018.07.138
6. Barhoumi, B., El Megdiche, Y., Clérandeau, C., Ben Ameur, W., Mekni, S., Bouabdallah, S., Derouiche, A., Touil, S., Cachot, J., & Driss, M.R. (2016). Occurrence of polycyclic aromatic hydrocarbons (PAHs) in mussel (Mytilus galloprovincialis) and eel (Anguilla anguilla) from Bizerte lagoon, Tunisia, and associated human health risk assessment. *Continental Shelf Research*, 124, 104-116. https://doi.org/10.1016/j.csr.2016.05.012
7. Barhoumi, B., LeMenach, K., Devier, M.H., Ben Ameur, W., Etcheber, H., Budzinski, H., Cachot, J., & Driss, M.R. (2014). Polycyclic aromatic hydrocarbons (PAHs) in surface sediments from the Bizerte Lagoon, Tunisia: levels, sources, and toxicological significance. Environmental Monitoring and Assessment, 186(5), 2653-2669. https://doi.org/10.1007/s10661-013-3569-5
8. Chebbi, N., Mastrottotaro, F. & Missaoui, H. (2010a). Ascidio-Ascidia fauna from the gulf of Hammamet (Mediterranean sea, Tunisia). Bulletin INSTM, 37, 51-56. https://www.instm-bulletin.tn/index.php/bulletin/article/view/551
9. Chebbi, N., Mastrototaro, F. & Missaoui, H. (2010b). Spatial distribution of ascidians in two Tunisian lagoons of the Mediterranean Sea. Cahiers de Biologie Marine, 51(2), 117-127 https://dx.doi.org/10.21411/CBM.A.E590FC4D
10. Cima, F., Ballarin, L., Bressa, G., Martinucci, G., & Burighel, P. (1996). Toxicity of organotin compounds on embryos of a marine invertebrate (Styela plicata; Tunicata). *Ecotoxicology and Environmental Safety*, 35(2), 174-182. https://doi.org/10.1006/eesa.1996.0097
11. Coughlan, J. (1969). The estimation of filtering rate from the clearance of suspensions. Marine Biology, 2(4), 356-358. https://doi.org/10.1007/BF00355716
12. DeGraeve, G.M., Elder, R.G., Woods, D.C. & Bergman, H.L. (1982). Effects of naphthalene and benzene on fathead minnows and rainbow trout. *Archives of Environmental Contamination and Toxicology*, 11(4), 487-490. https://doi.org/10.1007/BF01056076
13. DeMiguel-Jiménez, L., Etxebarria, N., Reinardy, H.C., Lekube, X., Marigómez, I., & Izagirre, U. (2022). Toxicity to sea urchin embryos of crude and bunker oils weathered under ice alone and mixed with dispersant. *Marine Pollution Bulletin*, 175,113345. https://doi.org/10.1016/j.marpolbul.2022.113345
14. Elyousfi, S., Dellali, M., Mezni, A., Ben Ali, M., Hedfi, A., Almalki, M., Mezni, A., Rohal-Lupher, M., Dervishi, A. & Boufahja, F. (2021). Toxicity of silver nanoparticles on the clam Ruditapes decussatus assessed through biomarkers and clearance rate. *Materials Research Express*, 8 (10), 105005.

https://doi.org/10.1088/2053-1591/ac2c2f

15. Hansen, B.H., Altin, D., Vang, S.-H., Nordtug, T. & Olsen, A.J. (2008). Effects of naphthalene on gene transcription in Calanus finmarchicus (Crustacea: Copepoda). *Aquatic Toxicology*, 86(2), 157-165. https://doi.org/10.1016/j.aquatox.2007.10.009
16. Haritash, A.K., & Kaushik, C.P. (2009). Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. Journal of Hazardous Materials, 169(1-3), 1-15. https://doi.org/10.1016/j.jhazmat.2009.03.137
17. Jing, M., Han, G., Wan, J., Zhang, S., Yang, J., Zong, W., Niu, Q., & Liu, R. (2020). Catalase and superoxide dismutase response and the underlying molecular mechanism for naphthalene. *Science of The Total Environment*, 736, 139567. https://doi.org/10.1016/j.scitotenv.2020.139567
18. Kong, Q., Zhu, L., & Shen, X. (2010). The toxicity of naphthalene to marine Chlorella vulgaris under different nutrient conditions. *Journal of Hazardous Materials*, 178(1-3), 282-286. https://doi.org/10.1016/j.jhazmat.2010.01.074
19. Kraak, M.H.S., Toussaint, M., Lavy, D. & Davids, C. (1994). Short-term effects of metals on the filtration rate of the zebra mussel Dreissena polymorpha. *Environmental Pollution*, 84(2), 139-143. https://doi.org/10.1016/0269-7491(94)90096-5
20. Lambert, G. (2002). Nonindigenous ascidians in tropical waters. Pacific Science, 56(3), 291-298. https://doi.org/10.1353/psc.2002.0026
21. Maruyama, K., Sahrul, M., Tanabe, S. & Tatsukawa, R. (1983). Polychlorinated biphenyl pollution from shipbuilding in Nagasaki Bay, Japan. *Ecotoxicology and Environmental Safety*, 7(5), 514-520. https://doi.org/10.1016/0147-6513(83)90089-1
22. Mdaini, Z., Telahigue, K., Hajji, T., Rabeh, I., Pharand, P., El Cafsi, M., Tremblay, R., & Gagné, J.P. (2023). Spatio-temporal distribution and sources of polycyclic aromatic hydrocarbons in Tunis Lagoon: Concentrations in sediments and Marphysa sanguinea body and excrement. *Marine Pollution Bulletin*, 189, 114769. https://doi.org/10.1016/j.marpolbul.2023.114769
23. Nayak, S., & Patnaik, L. (2023). Histopathological and Biochemical Changes in the Gills of Anabas testudineus on Exposure to Polycyclic Aromatic Hydrocarbon Naphthalene. *Applied Biochemistry and Biotechnology*, 195(4), 2414-2431. https://doi.org/10.1007/s12010-022-04214-x
24. Nikitha, T., Satyaprakash, M., Vani, S.S., Sadhana, B., & Padal, S. (2017). A review on polycyclic aromatic hydrocarbons: their transport, fate and biodegradation in the environment. *International Journal of Current Microbiology and Applied Sciences*, 6(4), 1627-1639. https://doi.org/10.20546/ijcmas.2017.604.199
25. Parrinello, D., Bellante, A., Parisi, M., Sanfratello, M., Indelicato, S., Piazzese, D. & Cammarata, M. (2017). The ascidian Styela plicata hemocytes as a potential biomarker of marine pollution: In vitro effects of seawater and organic mercury. *Ecotoxicology and Environmental Safety*, 136, 126-134. https://doi.org/10.1016/j.ecoenv.2016.11.001
26. Pineda, M.C., Lopez-Legentil, S., & Turon, X. (2011). The whereabouts of an ancient wanderer: global phylogeography of the solitary ascidian Styela plicata. *PLOS ONE*, 6(9), e25495. https://doi.org/10.1371/journal.pone.0025495
27. Ray, A., Gautam, A., Das, S., Pal, K., Das, S., Karmakar, P., Ray, M., &

Ray, S. (2020). Effects of copper oxide nanoparticle on gill filtration rate, respiration rate, hemocyte associated immune parameters and oxidative status of an Indian freshwater mussel. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 237, 108855. https://doi.org/10.1016/j.cbpc.2020.108855

28. Sogbanmu, T.O., Osibona, A.O., Oguntunde, O.A., & Otitoloju, A.A. (2018). Biomarkers of toxicity in Clarias gariepinus exposed to sublethal concentrations of polycyclic aromatic hydrocarbons. *African Journal of Aquatic Science*, 43(3), 281-292. https://doi.org/10.2989/16085914.2018.1491825
29. Stabili, L., Licciano, M., Gravina, M.F., & Giangrande, A. (2016). Filtering activity on a pure culture of Vibrio alginolyticus by the solitary ascidian Styela plicata and the colonial ascidian Polyandrocarpa zorritensis: a potential service to improve microbiological seawater quality economically. *Science of The Total Environment*, 573, 11-18. https://doi.org/10.1016/j.scitotenv.2016.07.216
30. Vasanth, S., Ganesh, A., Vijayakumar, T.S., Karthikeyeni, S., Manimegalai, M., & Subramanian, P. (2012). Assessment of anthracene on hepatic and antioxidant enzyme activities in Labeo rohita (Hamilton, 1822). International Journal of Pharmacy & Life Sciences, 3(5), 1696-1704
31. Vieira, L.R., & Guilhermino, L. (2012). Multiple stress effects on marine planktonic organisms: Influence of temperature on the toxicity of polycyclic aromatic hydrocarbons to Tetraselmis chuii. *Journal of Sea Research*, 72, 94-98. https://doi.org/10.1016/j.seares.2012.02.004
32. Wang, Y., Zhang, M., Ding, G., Shi, H., Cong, Y., Li, Z., & Wang, J. (2023). Polystyrene microplastics alleviate adverse effects of benzo [a] pyrene on tissues and cells of the marine mussel, Mytilus galloprovincialis. *Aquatic Toxicology*, 256, 106430. https://doi.org/10.1016/j.aquatox.2023.106430
33. Wise, S.A., Sander, L.C., & Schantz, M.M. (2015). Analytical Methods for Determination of Polycyclic Aromatic Hydrocarbons (PAHs) — A Historical Perspective on the 16 U.S. EPA Priority Pollutant PAHs. *Polycyclic Aromatic Compounds*, 35(2-4), 187-247. https://doi.org/10.1080/10406638.2014.970291
34. Wu, B., Zhang, R., Cheng, S.-P., Ford, T., Li, A.-M., & Zhang, X.-X. (2011). Risk assessment of polycyclic aromatic hydrocarbons in aquatic ecosystems. Ecotoxicology, 20:1124-1130. https://doi.org/10.1007/s10646-011-0653-x

© By the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).