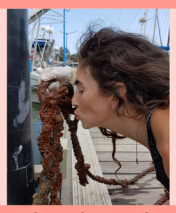
Ascidians as bio-indicators of micro-plastic and phthalates in marine environments

The George S. Wise Faculty of Life Sciences
Tel Aviv University

Gal Vered[1], Aviv Kaplan[2], Dror Avisar[2], Noa Shenkar[1,3]



E-mail : Galvered1@mail.tau.ac.il

Background

Micro-plastic are plastic particles of a size smaller than 5mm that are found nowadays in oceans all over the world. Ingested by different organisms, they can release toxic chemicals into the organism, bio accumulate, and enhance their toxic effects. One group of such chemicals are phthalate plasticizers, organic chemicals that are added to plastics during manufacture, and are known endocrine disruptors and ubiquitous global contaminants. However, these hazards are poorly examined in the marine environment as difficulties in monitoring of these pollutants conceal our ability to fully understand their eco-toxicological impact. Ascidians (aka sea squirts) are filter-feeding invertebrates, immobile and able to filter high volumes of water. Some ascidians are very successful invasive species and have a wide global distribution. As such, they make ideal candidates for biomonitoring phthalates and plastic particles suspended in the water column.

Study goals

Our main aim is to investigate the potential use of solitary ascidians as *in situ* biological indicators of micro-plastic and phthalates.

Phase A - Method development

Phthalate detection

Herdmania momus from Eilat marina, Red Sea and *Styela plicata* from Jaffa port, Mediterranean Sea were tested for the presences of phthalates. Internal organs of the tunicates underwent extraction and were analyzed with liquid chromatography - mass spectrometry.

Table 1. Concentration (ng/g dry weight) of commonly used phtalates found in *H. momus* and *S. plicata*.

	dibutyl phthalate (DBP)	di-2-ethylhexyl phthalate (DEHP)	di-n-octyl phthalate (DnOP)	didecyl phthalate (DDP)
H. momus	464.6	82.2	102.7	45.1
S. plicata	254.4	141.9	<LOQ	26.8

Micro-plastic detection

We compared digestion efficiency of three protocols: (1) potassium hydroxide (KOH) (Dehaut et al., 2016), (2) nitric acid (Claessens et al., 2013), (3) Proteinase-K (Cole et al., 2014). Unassimilated remains were filtered on GF/f filters and inspected under a stereomicroscope. The treatment with KOH was the most efficient protocol with the least remains observed on the filters.

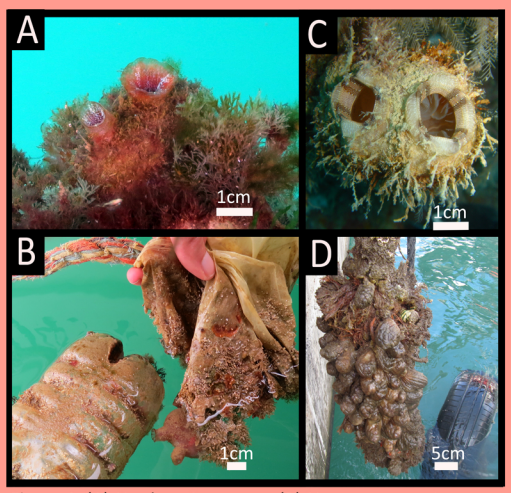


Figure 1. (A) *Herdmania momus*, (B) *H. momus* growing on plastic bag, (C) *Styela plicata*, (D) an aggregation of the invasive *S. plicata*.

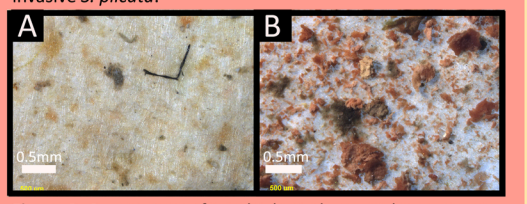


Figure 2. Comparison of non biological material remains on GF/f filters following digestion using (A) KOH, and (B) Proteinase-K.

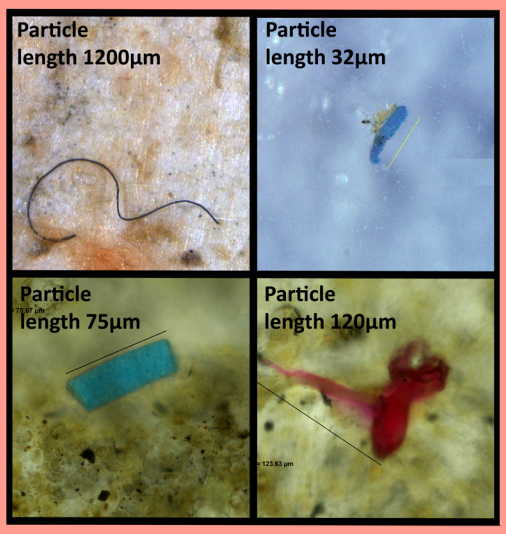


Figure 3: Non-biological particles found in solitary ascidians.

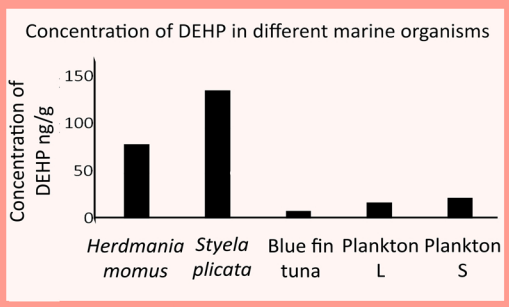


Figure 4. DEHP (ng/g dry weight) in plankton from the Ligurian Sea (L) and the Sardinian Sea (S) (Fossi et al. 2012), Blue fin tune from the Mediterranean Sea (Guerranti, et al. 2016), *H. momus* from the Red Sea, and *S. plicata* from the Mediterranean Sea.

Phase B - Biomonitoring micro-plastic and phthalates along the coasts of Israel

Based on the methods developed in phase A, samples collected from 9 sites along the Mediterranean and Red Sea coast of Israel will be analyzed. At each site we will investigate whether a correlation between quantities of micro-plastic and detected levels of phthalates exist, and whether there is a difference in contaminants level in different organs (tunic, body, hepatic gland, gonads).

Figure 5.
Study sites from north to south: Achziv, Haifa port, Shikmona, adera, Michmoret, Palmahim, Ashdod, Eilat marina and the Dolphin-reef beach.

Conclusions

Discovering bioaccumulation of phthalates and plastic particles in ascidians presents a new approach for biomonitoring the presences of contamination originating from plastic pollution. The global distribution of invasive ascidians offers a comparable method making use of the same species to assess micro-plastic and phthalates pollution in various locations around the world, and further ascertain their physiological effect.

Literature cited:
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[1]Department of Zoology, George S. Wise Faculty of Life Science, Tel-Aviv University, Tel Aviv, 69978, ISRAEL.
[2]The Hydro-Chemistry Laboratory, Department of Geosciences, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, 69978, ISRAEL.
[3]The Steinhardt Museum of Natural History and National Research Center, Tel-Aviv University, Tel Aviv, 69978, ISRAEL.
This research was supported by The Israel Science Foundation (grant #2182/15) to NS, and the Caroline von Humboldt Prize to NS.