



## Evaluation of Organochlorine pesticides in edible seaweed *Ulva lactuca* Linnaeus, 1753 in the Gulf of Mannar, Southern India

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The level of residual organochlorine pesticide (OCP) toxicity in green seaweed, *Ulva lactuca* was examined in the present study. Samples were collected from Hare Island (site 1), Erwadi (site 2), Seeniyappa Dhargah (site 3), Mandapam (site 4) and from Rameswaram (site 5) of Gulf of Mannar coast during North-East Monsoon and the beginning of post-monsoon and were analyzed by the standard protocol of AOAC 2007.01 QuEChERS using GC-MS. The analysis revealed that the total OCPs were ranged from 49.61 ng g<sup>-1</sup> to 123.68 ng g<sup>-1</sup>, respectively in sites 5 and site 1. The persistence of Hexachlorocyclohexane, endosulfan, endrin, and DDT was observed in this study. This study inferred that green seaweeds were found to contain high OCP residues immediately after the rainy season.

[**Keywords:** OCPs, Pesticides, Safety, Seaweed, *Ulva lactuca*]

### Introduction

Seaweeds are non-flowering aquatic plants that grow abundantly in the littoral zone of coastal waters<sup>1</sup>. Total world production of seaweeds is estimated to be 30.4 million tonnes in 2015, with cultivation and capture sectors responsible for production of 29.4 and 1.1 million tonnes, respectively<sup>2</sup>. Seaweeds are beneficial as they have good nutritive value and high dietary fibre that helps to prevent constipation, colon cancer, cardiovascular disease, and obesity<sup>3,4</sup>. They are also the indicators of chemical contaminants such as heavy metals, pesticides, and polyaromatic hydrocarbons in the marine environment<sup>5,6</sup>. Chemical contamination is an emerging issue in the marine environment. Chemicals often contaminate seaweeds through agriculture, aquaculture and allied activity runoffs that enters the marine environment<sup>7,8</sup>.

Pesticides are the major synthetic chemicals used by farmers to protect their crops from moulds, insects, microbes, and rodents. Based on the chemical group, there are different classes of pesticides such as organochlorine (OCPs), organophosphorus (OPPs), pyrethroids, and carbamates. OCPs are one of the group of pesticides used for pest control in agriculture. DDT (Dichloro diphenyl trichloro

ethane), aldrin, methoxychlor, mirex, dieldrin, benzohexochloride, lindane, endosulfan, taxopane, endrin, and heptachlor, despite their ban in several countries, are illegally used worldwide. OCPs are very persistent, as they resist biodegradation<sup>9-12</sup>. This nature of OCPs results in them getting biomagnified in living organisms and can pose severe threat and risk to the ecosystem and ultimately to human health. The OCP compounds are also known to affect the hormones in organisms, disrupting their reproductive cycles<sup>13</sup>. These pesticide residues can cause immunological, teratogenic, carcinogenic, reproductive, and neurological problems in organisms<sup>14</sup>.

It is reported that around three million people are been poisoned, and 2 million people die each year worldwide due to pesticide poisoning<sup>15,16</sup>. Due to their low cost and versatility, OCPs are still being used in industries and agriculture in some developing countries<sup>17</sup>. In India, DDT and HCH (Hexachlorocyclohexane) are used extensively both for agricultural and sanitary purposes<sup>18,19</sup>. Approximately 25,000 million tones of chlorinated pesticides were used annually. In this, DDT accounts for over 40 % in India<sup>20</sup>. In the marine ecosystem, pesticide poisoning occurs may be due to their

bioaccumulation and biodegradation characteristics in tropical conditions.

Global research on OCP residues in edible seaweeds has been carried out by very few authors. DDT metabolites have been recorded in *Dictyota* sp.<sup>2</sup> and twelve other seaweeds along the East Coast of Sicily<sup>21</sup>. Pesticide level in *Gracilaria*, *Fucus* and *Cystoseira* species was more up to 5 ng g<sup>-1</sup>(ref. 23). Gulf of Mannar in the southeast coast of India stretches from Kanyakumari to Rameswaram. It includes a protected marine national park which includes a chain of 21 islands from Mandapam to Thoothukudi to a distance of 140 km along the coast<sup>24</sup>. The Gulf of Mannar supports various ecosystems including the rich diversity of seaweeds. The coastal area along the Gulf of Mannar is dominated by agricultural farming and industrial activities<sup>25</sup>. Studies on OCP residues in seaweeds is scarce and hence, it is necessary to determine the pesticide contamination by runoff as well as to assess the edibility of seaweeds of this area. Green seaweed, *Ulva lactuca* is widely available and is consumed as food or food ingredient by the local people of this region. Therefore, the present study was undertaken to study contamination of OCP in *U. lactuca* along the Gulf of Mannar coast, Tamil Nadu.

## Materials and Methods

### Study area

Gulf of Mannar is located from the Rameswaram Island to Kanyakumari in Southern India (8°47' to 9°15' N; 78°12' to 79°14' E). Thamirabarani and Vaipar rivers in South India and Malvathu Oya (Malvathu river) in Sri Lanka drain into the Gulf of Mannar<sup>26</sup>. These rivers drain a significant quantity of rainwater, urban sewage and rural pollutants round the year<sup>27</sup>. Five sites *viz.*, Hare Island (site 1), Erwadi (site 2), Seeniyappa Dhargah (site 3), Mandapam (site 4), and Rameswaram (site 5), were selected for the study along the Gulf of Mannar and their geographical positions are shown in Table 1.

Table 1 — Sampling sites and their geographical location

Sites	Name of the location	Latitude and Longitude
1	Hare Island	8.78° N, 78.19° E
2	Erwadi	9.20° N, 78.71° E
3	Seeniyappa Dhargah	9.26° N, 70.06° E
4	Mandapam	9.27° N, 79.21° E
5	Rameswaram	9.25° N, 79.32° E

### Sample collection

Samples (*Ulva lactuca* -Green seaweed) were collected for a period of 4 months, from October 2017 to January 2018, based on seasonal availability. Seaweeds were collected by handpicking during low tide. The representative samples (100 g) were collected at six places in each sampling site and pooled to form the incremental samples to minimize the sampling error. The collected seaweed samples were transferred to polythene bags with seawater to prevent dehydration. They were washed in the lab to remove dirt and other epiphytic substances. Washed seaweeds were dried under shade for 4-5 days, powdered, stored in containers wrapped in aluminium foil, and placed at room temperature prior to analysis.

### Chemicals and reagents

A standard OCP mix containing Isomers of HCH, heptachlor, aldrin, endrin, endosulfan, DDT metabolites, and methoxychlor was purchased from Ultra International, Bangalore, India. Chemicals such as primary secondary amines (PSA), graphitized carbon black (GCB), carbon -18 (C18), calcium chloride (CaCl<sub>2</sub>), magnesium sulphate (MgSO<sub>4</sub>), and sodium acetate (NaOAc) and solvents such as dichloromethane, ethyl acetate, hexane, acetone nitrile, acetic acid and methanol of HPLC grade were obtained from Sigma Aldrich, India Pvt. Ltd., Bangalore.

### Extraction of OCPs from seaweeds using the QuEChERS method

5 g of dried and powdered sample was mixed with 15 ml of acetic acid in acetonitrile and was vortexed for 1 min. The fat portion was extracted using 2 ml of hexane as the extractant. To this, 6 g of MgSO<sub>4</sub> and 1.5 g of NaOAc were added, vortexed vigorously for 1 min, and centrifuged in a refrigerated centrifuge at 5000 rpm, for 5 min. This results in solvent partitioning facilitating recovery of the pesticides. Fat deposition was carried out by centrifuging 5 ml of supernatant in centrifuge tube, kept at -20 °C for 15 min. The upper layer (1.5 ml) was transferred to a kit containing 150 mg MgSO<sub>4</sub> and 100 mg CaCl<sub>2</sub>, vortexed well for 1 min, and centrifuged again at 5000 rpm for 5 min. PSA-25 mg, C18-25 mg, GCB-7 mg, and MgSO<sub>4</sub>-150 mg were added to the QuEChERS cleanup kit with 1 ml of supernatant. The mixture was vigorously shaken and centrifuged for 5 min at 9000 rpm. Finally, 1 ml of supernatant was taken up for GC-MS analysis (AOAC 2007.01)<sup>28,29</sup>.

**Gas Chromatography-Mass Spectrometry (GC-MS) analysis**

Gas Chromatography-Mass Spectrometry, with column TG-5MS was used for analysis of OCPs. The injector temperature was maintained at 290 °C. A gradient programming was followed by setting an initial oven temperature at 90 °C for 0.5 min, which was then increased to 280 °C at 8 °C min<sup>-1</sup> and again to 300 °C at 15 °C min<sup>-1</sup> for 2.5 min. Split flow and carrier gas flow was 50 ml/min, respectively. MS transfer line and ion source temperature were set at 290 and 250 °C, respectively. Helium (purity 99.995 %) was used as a carrier gas. The compounds were detected by selected ion monitoring (SIM) mode and retention time (Table 2). For quantification purposes, six concentrations of each standard pesticide mix (1, 5, 10, 50, 100, and 250 ng g<sup>-1</sup>) were calibrated.

Table 2 — Retention time (RT) and target ions (m/z) of OCPs

OCPs	RT [min]	Target/qualifier ions [m/z]
α – HCH	13.65	181,183,219,254
β – HCH	14.35	109,181,183,219
γ – HCH	14.52	109,181,183,219
δ – HCH	15.12	109,181,183,219
Heptachlor	16.37	100,237,272,337
Heptachlor epoxide	18.21	81,263,353,388
Endosulfan I	19.08	195,241,265,339
Endosulfan II	20.42	195,207,241,237
Endosulfan sulfate	21.41	229,237,272,387
pp' DDE	19.61	105,176,246,318
pp' DDD	20.6	75,165,199,235
pp' DDT	21.46	165,212,235,246
Dieldrin	19.7	79,81,108,263
Aldrin	17.23	66,263,293,329
Endrin	20.27	81,263,281,245
Endrin aldehyde	20.86	67,173,250,345
Methoxychlor	22.74	196,212,227,238

**Recovery, linearity, and limit of detection**

To check the accuracy of the QuEChERS method, the seaweeds were spiked with 100 ng g<sup>-1</sup> OCP standard mix and were analyzed as described above<sup>30</sup>. The linearity of the method was determined by plotting a six-point calibration curve for each pesticide (1, 5, 10, 50, 100, and 250 ng g<sup>-1</sup>). The calibration curve's lowest point is called the detection limit (LOD). The following equation was used to calculate the accuracy in percentage recovery

Recovery (%)

$$= \frac{\text{Concentration of analyte recovered}}{\text{Concentration of analyte spiked}} \times 100$$

**Results and Discussion****Recovery, linearity, and limit of detection**

The data obtained on the recovery (%), linearity, and limit of detection for the QuEChERS method used for the analysis of OCPs from the seaweed matrix is presented in Table 3. The recovery percentage ranged from 80 to 108 % for all the OCPs. For the pesticides, the acceptable recovery limit for routine analysis as recommended by the Codex Alimentarius Commission Guidelines (CAC, 2017) and EU Commission Guidelines is 70 to 120 %<sup>(ref. 30)</sup>. For all compounds except δ-HCH ( $r^2 = 0.89$ ), endosulfan II ( $r^2 = 0.90$ ) and endosulphan sulphate ( $r^2 = 0.96$ ), the correlation coefficient of linearity ( $r^2$ ) achieved during calibration was  $r^2 = 0.98$  (Table 3). The QuEChERS method LOD is 1 ng g<sup>-1</sup>.

Table 3 — Recovery and linearity of OCPs

Sl. No.	Compound Name	Spiking Concentration (ng g <sup>-1</sup> )	Calculated Amount (ng g <sup>-1</sup> )	Recovery (%)	Linearity ( $r^2$ )
1	Alpha-BHC	100	100.27	100.27	0.9960
2	Beta-BHC	100	96.38	96.38	0.9985
3	Gamma-BHC	100	99.31	99.31	0.9950
4	Delta-BHC	100	88.82	88.82	0.8975
5	Heptachlor	100	101.16	101.16	0.9961
6	Heptachlor-epoxide	100	108.62	108.62	0.9853
7	Aldrin	100	102.71	102.71	0.9987
8	Dieldrin	100	100.51	100.51	0.9956
9	Endrin	100	81.99	81.99	0.9925
10	Endrin-aldehyde	100	100.29	100.29	0.9605
11	Endosulfan I	100	100.62	100.62	0.9969
12	Endosulfan II	100	95.01	95.01	0.9094
13	Endosulfan-sulphate	100	96.91	96.91	0.9653
14	PP'-DDT	100	93.21	93.21	0.9885
15	PP'-DDE	100	99.01	99.01	0.9873
16	PP'-DDD	100	97.22	97.22	0.9862
17	Methoxychlor	100	100.30	100.3	0.9969

**Description of OCP residues concentration**

In this study, the presence of 17 OCPs, consisting of HCH, heptachlor, aldrin, endosulfan, endrin, DDT, and methoxychlor, and their derivatives were analyzed. The concentration of OCP residues in *U. lactuca* from the five sampling sites of the Gulf of Mannar from October 2017 to January 2018 is given in Table 4. The total OCPs concentration ranged from 0.12 to 123.68 ng g<sup>-1</sup> at site 1, 0.06 to 80.09 ng g<sup>-1</sup> site 2, 9.02 to 83.81 ng g<sup>-1</sup> at site 3, 0.12 to 49.61 ng g<sup>-1</sup> site 4 and 0.08 to 58.03 ng g<sup>-1</sup> at site 5 (Fig. 1a). The maximum concentration was recorded at site 1, which is 2.5 times higher than site 4. With respect to the sampling months, the total concentration of OCP residues ranged from 0.12 to 15.87 ng g<sup>-1</sup> in October, 0.06 to 9.02 ng g<sup>-1</sup> in November, 0.17 to 12.35 ng g<sup>-1</sup> in December and 49.61 to 123.68 ng g<sup>-1</sup> in January. OCPs were detected during all months at site 3. In the month of January, the concentration of total OCPs accumulation by *U. lactuca* was very high, irrespective of the sampling sites. The maximum concentration recorded in January was 8 to 12 times higher than those recorded in other months. HCH, endosulfan, endrin, and DDT are the predominant OCPs present in *U. lactuca*.

Scientists noted that maximum accumulation of chemical pollutants especially OCPs and PCBs occurs during the seaweed fruiting period<sup>31</sup>. The extensive growth period of *Ulva lactuca* was at the end of December in the Gulf of Mannar<sup>32</sup>, which could have been the reason for the presence of greater amounts of OCPs in *Ulva lactuca* in January. Maroli *et al.*<sup>22</sup> have reported that the concentration of PCBs and DDTs were about 1.5 times higher in old fronts than in young fronts of *Ulva lactuca*<sup>31</sup>. The formation of spores by the seaweed had subsequently aided the maximum absorption of OCPs through their expanded thallus portion<sup>31</sup>.

Lindane ( $\gamma$ - HCH) or  $\gamma$ - BHC, is widely used in agriculture as an insecticide<sup>33</sup>. There are eight isomers in HCH but only  $\alpha$ -, $\beta$ -, $\gamma$ - and  $\delta$ - HCH are of commercial significance. The concentration of  $\Sigma$ HCH in *U. lactuca* collected from the different sites during the sampling period is given in Figure 1b. In October, the  $\Sigma$ HCH is only 0.03 ng g<sup>-1</sup> in sites 1, 2, and 4 but at site 3 and 5 it was recorded as 1.95 ng g<sup>-1</sup> and 1.78 ng g<sup>-1</sup>, respectively. In November and December, the  $\Sigma$ HCH was found more only at site 3 at the concentration of 1.88 ng g<sup>-1</sup> and 1.72 ng g<sup>-1</sup>, respectively, which in the other sites, the mean

Table 4 — OCP residues (ng g<sup>-1</sup>) in *U. lactuca* wet weight basis

	Site 1					Site 2					Site 3					Site 4					Site 5				
	OCT	NOV	DEC	JAN	Avg	OCT	NOV	DEC	JAN	Avg	OCT	NOV	DEC	JAN	Avg	OCT	NOV	DEC	JAN	Avg	OCT	NOV	DEC	JAN	Avg
alpha-HCH	0.001	0.000	0.001	0.007	0.002	0.001	0.000	0.000	0.019	0.005	0.025	0.029	0.013	0.111	0.045	0.001	0.001	0.000	0.022	0.006	0.027	0.000	0.001	0.027	0.014
beta-HCH	0.028	0.023	0.030	8.803	2.221	0.032	0.011	0.047	7.986	2.019	1.550	1.581	1.421	0.084	1.159	0.039	0.057	0.055	6.588	1.685	1.604	0.014	0.025	1.604	0.812
gamma-HCH	0.001	0.000	0.001	0.052	0.014	0.001	0.000	0.001	0.031	0.008	0.280	0.235	0.222	7.659	2.099	0.001	0.002	0.001	0.012	0.004	0.153	0.000	0.001	0.153	0.077
delta-HCH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.038	0.063	0.030	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$\Sigma$ -HCH	0.030	0.024	0.031	8.863	2.237	0.034	0.012	0.049	8.036	2.033	1.952	1.883	1.718	7.885	3.360	0.040	0.059	0.056	6.622	1.694	1.783	0.014	0.027	1.783	0.902
Heptachlor	0.001	0.006	0.000	0.483	0.123	0.003	0.000	0.003	3.153	0.790	0.015	0.005	0.008	0.125	0.038	0.008	0.002	0.000	0.030	0.010	0.179	0.000	0.002	0.179	0.090
Heptachlor-epoxide	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.249	0.237	0.190	0.000	0.169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$\Sigma$ -Heptachlor	0.001	0.006	0.000	0.483	0.123	0.003	0.000	0.003	3.153	0.790	0.264	0.242	0.198	0.125	0.207	0.008	0.002	0.000	0.030	0.010	0.179	0.000	0.002	0.179	0.090
Aldrin	0.004	0.009	0.003	0.835	0.213	0.006	0.002	0.003	0.922	0.233	0.445	0.394	0.361	2.277	0.869	0.006	0.008	0.003	0.949	0.242	0.795	0.002	0.006	0.795	0.399
Dieldrin	0.000	0.000	0.000	0.249	0.062	0.000	0.000	0.000	0.000	0.000	0.238	0.168	0.036	0.148	0.147	0.000	0.005	0.000	0.520	0.131	0.000	0.000	0.000	0.000	0.000
$\Sigma$ -Aldrin	0.004	0.009	0.003	1.084	0.275	0.006	0.002	0.003	0.922	0.233	0.683	0.562	0.397	2.425	1.017	0.006	0.013	0.003	1.469	0.373	0.795	0.002	0.006	0.795	0.399
Endosulfan I	0.000	0.060	0.027	78.493	19.645	0.052	0.023	0.020	39.138	9.808	3.528	3.275	2.244	44.461	13.377	0.007	0.073	0.027	20.594	5.175	3.274	0.022	0.047	33.096	9.110
Endosulfan II	0.070	0.073	0.093	9.327	2.391	0.067	0.020	0.130	16.837	4.264	5.467	1.417	17.028	17.028	7.621	0.045	0.039	0.056	14.797	3.734	6.344	0.024	0.054	11.632	4.513
Endosulfan-sulphate	0.003	0.000	0.000	0.506	0.127	0.000	0.000	0.000	0.000	0.000	0.343	0.219	0.249	0.000	0.203	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$\Sigma$ -Endosulfan	0.074	0.133	0.121	88.326	22.164	0.119	0.043	0.150	55.976	14.072	9.337	4.911	9.067	61.489	21.201	0.052	0.112	0.084	35.391	8.910	9.619	0.046	0.101	44.728	13.623
Endrin	0.007	0.005	0.005	2.538	0.639	0.007	0.002	0.007	0.136	0.038	0.126	0.048	0.096	0.144	0.103	0.009	0.020	0.010	0.974	0.253	0.114	0.005	0.021	0.114	0.064
Endrin-aldehyde	0.008	0.014	0.052	1.642	0.429	0.000	0.004	0.011	2.443	0.614	0.873	0.938	0.495	2.391	1.174	0.000	0.039	0.013	1.505	0.389	1.668	0.004	0.000	1.668	0.855
$\Sigma$ -endrin	0.016	0.019	0.057	4.180	1.068	0.007	0.006	0.018	2.580	0.653	0.999	0.986	0.591	2.535	1.278	0.009	0.059	0.023	2.479	0.643	1.782	0.009	0.021	1.782	0.899
PP-DDD	0.000	0.002	0.002	0.041	0.011	0.001	0.000	0.003	0.022	0.007	0.072	0.058	0.033	0.011	0.043	0.000	0.000	0.000	0.004	0.001	0.010	0.000	0.008	0.010	0.007
PP-DDE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.249	0.342	0.294	0.000	0.221	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PP-DDT	0.004	0.007	0.001	20.635	5.162	0.002	0.001	0.006	8.547	2.139	0.025	0.016	0.037	9.167	2.311	0.004	0.004	0.001	3.553	0.890	1.384	0.009	0.002	8.434	2.458
$\Sigma$ -DDT	0.004	0.009	0.003	20.676	5.173	0.003	0.001	0.009	8.569	2.146	0.346	0.417	0.364	9.178	2.576	0.004	0.004	0.003	3.557	0.892	1.394	0.009	0.010	8.444	2.464
Methoxychlor	0.000	0.000	0.000	0.073	0.019	0.002	0.000	0.002	0.857	0.215	0.010	0.017	0.013	0.172	0.053	0.003	0.000	0.001	0.059	0.016	0.325	0.000	0.014	0.325	0.166
Total	0.129	0.202	0.215	123.685	31.058	0.176	0.065	0.234	80.092	20.142	13.592	9.018	12.349	83.809	29.692	0.122	0.250	0.169	49.607	12.537	15.877	0.081	0.181	58.036	18.544

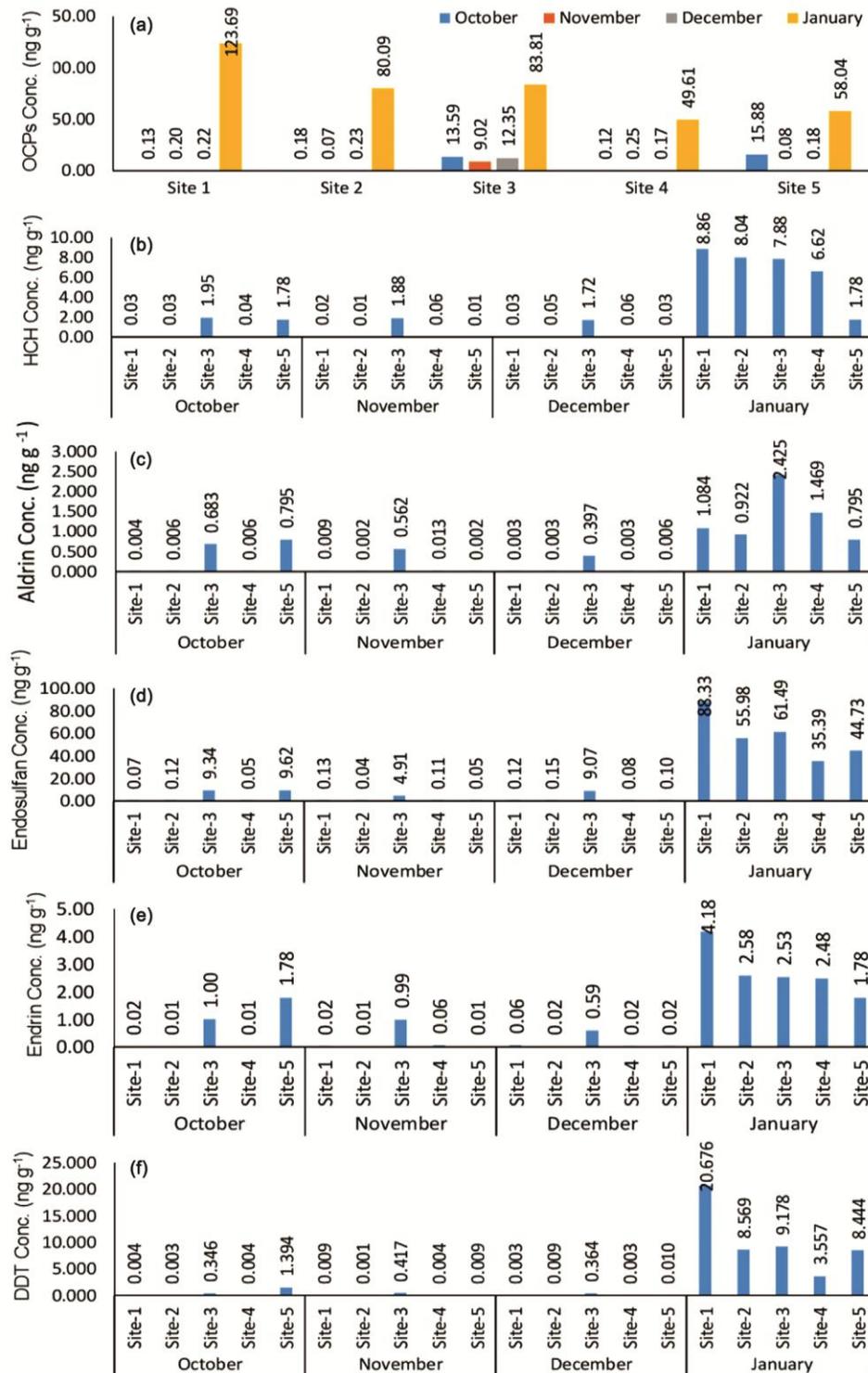


Fig. 1 — Concentration of: a) total OCPs residues, b)  $\sum$  HCH, c)  $\sum$  aldrin, d)  $\sum$  endosulfan, e)  $\sum$  endrin, and f)  $\sum$  DDT; in *Ulva lactuca*

concentration was  $0.03 \text{ ng g}^{-1}$ . In January, the  $\sum$ HCH was high in all the sites with concentration ranging from  $1.78$  to  $8.86 \text{ ng g}^{-1}$ . Particularly, at sites 1 to 4 had the  $\sum$ HCH above  $6.5 \text{ ng g}^{-1}$ . Therefore, the

concentration of  $\sum$ HCH was more in January at all the sites.  $\beta$ -HCH was the major isomer detected in *U. lactuca* collected from all the sites which give an indication that persistence of HCH, probably as

deposits in marine sediments and getting accumulated by seaweeds. At site 3,  $\gamma$ -HCH was recorded at a concentration of  $7.66 \text{ ng g}^{-1}$  in January.  $\beta$ -HCH has been banned for agricultural use due to its carcinogenic effect and persistent nature<sup>34</sup>. About 12 to 30 % of the HCH used in agriculture is volatilized into the atmosphere which is deposited by rainfall and leaching into the seawater and hence bioaccumulation in the food chain<sup>35</sup>. On further investigation, it was found that more than 90 % of the HCH accumulated by seaweeds occurs as  $\beta$ -HCH isomers. The  $\gamma$ -HCH applied in agriculture is converted into  $\alpha$  and  $\beta$ -isomers, which are slightly more toxic than  $\alpha$ -isomers<sup>36</sup>. The sites 3, 4 and 5 are located in the northernmost part of the Gulf of Mannar region. The mouth of the rivers, Palar and Vaigai shed their agriculture runoff from the mainland in the region, particularly during monsoon (October to January). There are backwaters from Thirupullani to Mandapam which receives water only during monsoon and discharge through small creeks into the Gulf of Mannar. Thirupullani region has around 6500 ha of farmland, mainly growing cotton and sugarcane in addition to paddy.

The presence of aldrin residues in seaweeds is not very significant as the concentration ranged between  $0.002$  to  $2.42 \text{ ng g}^{-1}$  (Fig. 1c), which is below the prescribed MRL of  $10 \text{ ng g}^{-1}$  for aldrin derivatives in green leafy plants<sup>37</sup>. They are lipophilic with low water solubility ( $0.027 \text{ mg l}^{-1}$ ). Most of the residues were absorbed as aldrin by the seaweeds. Aldrin derivatives were detected in low concentration in the month of October at site 3 ( $0.68 \text{ ng g}^{-1}$ ) and at site 5 ( $0.80 \text{ ng g}^{-1}$ ); while in November and December, it was  $0.56 \text{ ng g}^{-1}$  and  $3.97 \text{ ng g}^{-1}$ , respectively at site 3. In January, the  $\sum$  aldrin residues were slightly high varying from  $0.79$  to  $2.42 \text{ ng g}^{-1}$  and the maximum at site 3. *U. lactuca* accumulates mainly aldrin derivatives but at site 3 it has accumulated dieldrin derivatives in appreciable quantities. It has observed that they accumulate OCP residues more after rainy season but this was not recorded earlier.

The occurrence of endosulfan residues in *U. lactuca* collected from different sampling sites is presented in Figure 1d. Endosulfan is a two-isomeric form insecticide and acaricide viz. endosulfan I ( $\alpha$  isomer) and II ( $\beta$  isomer). Endosulfan sulphate is another oxidation product. The coefficient of  $\alpha$  isomers was low at  $4.74$  and  $\beta$  isomers at  $4.79$ <sup>(ref. 38)</sup>, which shows low water affinity and hence absorb

more organic matter. In *U. lactuca* the concentration of endosulfan was more than other OCPs. The concentration was more at site 3 ( $9.34 \text{ ng g}^{-1}$ ) and site 5 ( $9.62 \text{ ng g}^{-1}$ ) during October and at site 3 during November ( $4.91 \text{ ng g}^{-1}$ ) and December ( $9.07 \text{ ng g}^{-1}$ ). During January the concentration was very high in all five sites and was ranged from  $35.39$  to  $88.33 \text{ ng g}^{-1}$  which was 5 to 10 folds higher than the other months. Endosulfan which is commonly used in India for cultivation and may be the source of pesticides at sites 3, 4 & 5 of Gulf of Mannar. Site 5 is located between the Gulf of Mannar and Palk Bay; and hence, receives discharges from both the regions. The influx of discharge from river Vaigai contributes more as it lies in the Palk Bay region. The significant amounts of endosulfan recorded in January correlated with the fruiting season of the seaweed and Northeast monsoon which brings lot of pesticide rich agriculture runoffs. The continuous presence of endosulfan at sites 3, 4 and 5 might be from the adjacent backwaters or rivers, Palar and Vaigai. It is true as India is still extensively using Endosulfan. Even then, the Honorable Supreme Court of India has issued an order dated 13.05.2011 banning the production and sale of endosulfan in India. The huge accumulation of endosulfan in the samples gives a clear conception on its continuous usage along the Gulf of Mannar region, for which suitable conservative measures need to be taken up by the regulatory authorities to save the edible seaweeds from OCP contamination.

Endrin is an OCP that is mainly used as an insecticide and rodenticide; presently banned in many countries<sup>39</sup>. Endrin exists in the environment as endrin aldehyde or endrin ketone, primarily in water bodies and bottom sediments<sup>40</sup>. Endrin residue concentrations were high in January, varying from  $1.78$  to  $4.18 \text{ ng g}^{-1}$ . The maximum concentration of endrin in site 1 was observed in January (Fig. 1e).

DDT was banned worldwide for agricultural use as formalized in 2004 by the Stockholm Convention POPs<sup>41</sup>. DDT is highly hydrophobic and almost water-insoluble, but it is soluble in organic solvents, fats and oils. Commercial DDT is a 75 % pp' isomer mix, 15 % op' isomer and the remaining are DDE and DDD, the major DDT metabolites<sup>42</sup>. pp' DDE and pp' DDD have similar properties as pp' DDT. In 2015, IARC classified DDT as "probably human-carcinogenic" group 2A<sup>43</sup>. India is still the only manufacturer and world's largest consumer with about 2786 tonnes being used for disease control<sup>44</sup>. Accumulation of DDT by *U.*

*lactuca* during the study period is given in Figure 1f. In general, the  $\sum$  DDT residues were minimum during October and December, except at site 5 in October, wherein the  $\sum$  DDT was 1.39 ng g<sup>-1</sup>. DDT accumulation was maximum only in January (2.54 to 4.18 ng g<sup>-1</sup>). At site 1, *U. lactuca* contains more  $\sum$  DDT, mainly in pp'-DDT form. Most of the DDT residues detected in *U. lactuca* appeared as pp'-DDT, when the  $\sum$  DDT was above 1 ng g<sup>-1</sup>. The pp'-DDE isomers were also detected at site 3 from October to November, but at lower concentration. The physico-chemical characteristics of *U. lactuca* can change the hydrophobic and lipophilic nature of the DDT, which decides the absorption of pesticides by seaweeds. The half-life of DDT in the aquatic environment is reported as 150 years.

### Conclusion

The present study gave a baseline data on the extent of OCP residues in *U. lactuca* of Gulf of Mannar. The presence of HCH, endosulfan, endrin and DDT were observed in *U. lactuca* during the entire study period. *U. lactuca* is abundant from October to January, particularly in January after the rainy season. The concentration of OCPs was maximum during the fruiting period (January), which indicates that heavy rain lashes agricultural runoff and sewage into the ocean. The study has to be extended further to examine the presence of OCPs available in this region throughout the year. To ascertain the safety of *U. lactuca* for edible purposes, there are no recommended guidelines on the MRLs of each OCP in seaweeds. Therefore, it is necessary to establish such guidelines as seaweed consumption is in increasing trend owing to their high nutritional and medicinal values.

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### Conflict of Interest

No conflict of interest.

### Author Contributions

SS - Writing - original draft, review & editing; RJS - Conceptualization, Investigation; GJ - Investigation, Supervision; RS - Resources, review & editing; and SA - Review & editing.

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