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Effect of Seaweeds on Degradation of DDT in Soils

Simi Sudharshan · Megharaj Mallavarapu · Nanthi Bolan · Ravi Naidu

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Abstract Seaweed was investigated as an amendment to enhance remediation of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT)-contaminated soil. Under anaerobic conditions, the addition of seaweeds increased DDT degradation between 61 % and 88 % of the original concentration after 14 days of incubation whereas only 33 % of DDT was degraded in unamended soil. DDT was degraded to metabolites such as 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (DDE), and 1-chloro-2,2-bis (*p*-chlorophenyl) ethylene (DDMU). Seaweed-amended soils converted 35–56 % of DDT to DDD while the unamended soil formed only 15 % DDD. Seaweed amendments modified soil conditions which include soils' dissolved organic carbon (DOC), ionic strength, redox potential, and pH. These significant physicochemical changes influenced the increase in DDT bioavailability and transformation in seaweed-amended soils compared to the unamended soils. Multiple linear regression analysis also suggested that factors such as DOC, calcium, redox potential, and pH are involved against DDT degradation (p=0.02).

Keywords DDT · Seaweeds · Degradation

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S. Sudharshan · M. Mallavarapu · N. Bolan · R. Naidu (✉)
Centre for Environment Risk Assessment and Remediation (CERAR), University of South Australia,
Mawson Lakes, SA 5095, Australia
e-mail: ravi.naidu@unisa.edu.au

S. Sudharshan · M. Mallavarapu · N. Bolan · R. Naidu
Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), University of South Australia,
Mawson Lakes, SA 5095, Australia

1 Introduction

The addition of organic amendments to soil acts as additional energy sources that stimulate microbial activity and therefore has potential to increase rates of bioremediation processes. In addition to being energy sources, organic amendments directly influence soil chemical properties including dissolved organic carbon (DOC) content and redox reactions, which in turn influence degradation of organic contaminants. The addition of readily decomposable organic amendments under conditions of restricted oxygen diffusion results in reducing conditions at a faster rate than in soils without organic amendments. Numerous studies have demonstrated that reducing (low redox potential) conditions favour dechlorination of organochlorine compounds such as 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT; Glass 1972; Zoro et al. 1974; Sayles et al. 1997). Organic amendments such as alfalfa, rice straw, farmyard manure, and green manure have been used to enhance the bioremediation of persistent organic pesticides such as DDT in soils (Ko and Lockwood 1968; Sethunathan 1973; Rajaram and Sethunathan 1975; Farmer et al. 1974; Mitra and Raghu 1988).

Addition of seaweeds to soil not only causes bacteria to proliferate but also releases substances such as polyuronides (Stephenson 1968) that may influence DDT biodegradation. Alginic acid is a major component of brown seaweed and is known for its chelating properties. Chelation of metallic radicals present in soils can cause changes in soil properties and therefore biodegradation rates. This study compares a range of seven brown and green seaweed species for their potential to increase the biodegradation rate of DDT in freshly (aged for 3 weeks) spiked soil. This study also aimed to identify the key factors that contribute to seaweed-induced degradation processes.

2 Material and Methods

2.1 Soil and DDT Spiking

Uncontaminated soil was collected from the surface (0–20 cm) of a golf course. Soil was dried and passed through a 2 mm sieve. Soil was analysed for its pH and DOC at a soil to water ratio of 1:5 (*w*/*v*). Soil texture was determined using the hydrometer method (Gee et al. 1986). Soil organic carbon was analysed using the Walkley–Black method. Soil was analysed for inorganic ions concentration using inductively coupled plasma optical emission spectroscopy (ICP–OES). Physicochemical properties of the soil are shown in Table 1.

For degradation studies, 30 mg/kg DDT was added to the soil. Thirty milligram of DDT was dissolved in five milliliter of acetone and applied to 1 kg of sieved air-dried soil using an atomizer and thoroughly mixed. Spiked soil was stored in a tray under a fume hood for 1 day to remove acetone from the sample. After 3 weeks of incubation at room temperature, 96 % of the applied DDT concentration was extracted from the soil sample, showing that no degradation or other form of losses occurred under these conditions.

2.2 Seaweeds

Eight types of seaweeds were collected from Victor Harbour, South Australia. The seaweeds were washed three times with deionised water to remove soluble salts, epiphytes, and sand and then dried at room temperature (20±2°C) for more than 3 weeks. The dried seaweeds were powdered and sieved to pass a 0.25 mm mesh and stored in a sealed container. Seaweeds were analysed for inorganic total concentrations of Na^{+}, Al^{3+}, Ca^{2+}, Fe^{2+}, K^{+}, and Mg^{2+} after digestion in concentrated nitric acid using ICP–OES (Table 2).

2.3 Degradation Study

For the degradation study, 5 g samples of DDT-spiked soil were added to 40 mL glass tubes fitted with Teflon-lined caps. The soil was amended with seaweed powder at 0, 5, 10, and 15 % by weight. All the samples were prepared in duplicates. Twenty-five milliliter of milli-Q grade water was added and the soil samples were thoroughly homogenized and incubated at 37 °C (Kantachote et al. 2004) for up to 14 days. Soil samples prepared in duplicates were sampled at time (*t*) of incubation (0, 3, 7, and 14 days). Soil redox potential (*Eh*) was measured by dropping the electrode into the sample tube and the reading was recorded when the monitor indicated a constant value. The samples were then centrifuged at 7,000 rpm (17,228×*g*) for 15 min, and the supernatant was decanted and analysed for pH, DOC, and inorganic ions. The soil residues were analysed for DDT and its metabolites.

2.4 DDT Extraction and Analyses

The soil residues were extracted for DDT and its metabolites using hexane/dichloromethane (7:3 ratio) based on the method of Villa et al. (2006). A cleaning procedure was carried out to remove humic substances present in extracts. The extract (1 mL) was passed through a glass column containing 0.1 mg of sodium sulfate and 0.5 mg of florisil (200 mesh) and rinsed with 4 mL of hexane. The extracts were reduced to 1 mL before appropriate dilutions were carried out for the DDT and metabolites determination.

Identification of DDT and metabolites were carried out using Agilent Gas Chromatograph 6890 N equipped with electron capture detector and separated by a DB-5 J&W Scientific column (30 m×0.32 mm i.d., 0.50 μm film thickness). The GC program was set as follows: 190 °C initial temperature, ramped at 5 °C/min to 270 °C with a hold time of 5 min. A 1 μL splitless injection was used and the injection port was maintained at 250 °C. The carrier gas was helium and the make-up gas was nitrogen at 60 mL/min. The temperature of the detector was maintained at 325 °C. Standard solutions of DDT and its metabolites were

Table 1 Physicochemical properties of soil used in the study

Texture (%)			OC %	pH	EC ms/cm	DOC mg/L	Cations mg/kg of dry wt.				DDT mg/kg
Clay	Sand	Silt					Na^+	Ca^{2+}	Mg^{2+}	K^+	
25	45	30	3.7	7.3	1.3	62.5	107	556	640	811	30

prepared for 0.025, 0.05, 0.1, 0.5, 1, and 2 μg mL^{-1} in hexane for soil extracts, respectively. Standards (Sigma-Aldrich Chemical Co.) were prepared from the following solutions: 98 %, DDT; 1,1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane (DDD), 99 %; 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE), 99 %; and 1-chloro-2,2-bis(*p*-chlorophenyl) ethylene (DDMU). All the compounds were quantified using the Agilent Chemstation Software package.

3 Results and Discussion

3.1 Effect of Seaweeds on DDT Degradation in Soil

The effect of seaweed treatments at different percentages on DDT transformation was assessed after 3 days of incubation. Analyses of incubated soils showed a significant ($p<0.05$) decrease in DDT concentration across all seaweed treatments. However, there was no significant difference in the extent of DDT decrease amongst various levels of seaweed treatments (Fig. 1). Based on these results, it was decided that 5 % of seaweed addition is sufficient to carry out the degradation study. *Cystophora* sp.1 and *Ulva* sp. were the most effective seaweeds demonstrating maximum degradation of 88 and 86 %, respectively, with the lowest DDE concentration of 4.4–4.8 %.

3.2 DDT Degradation Products

DDT concentration in soils amended with 5 % (*w*/*w*) seaweed decreased with increasing duration of incubation relative to the untreated soil (Fig. 2a). At the end of 14 days of incubation, DDT concentrations in the unamended control sample decreased by 33 %. Seaweed-amended soil showed greater decreases in total DDT concentrations ranging from 61 to 88 % of the original spiked concentration (Fig. 2a). In the case of seaweed-amended soils, approximately 30–60 % of DDT was lost within the first 3 days of incubation. In contrast, less than 10 % decrease was observed in the samples that received no amendments after 3 days of incubation.

The concentrations of metabolites formed during incubation were low in comparison with the amount of DDT lost in both unamended and amended soils (Fig. 2a–c). Further incubation of the seaweed-treated soils up to 7 days showed significant increases in DDD, DDE, and DDMU metabolites (Fig. 2b,c) suggesting that the loss of DDT is attributed to its degradation to these daughter products. DDD was

Table 2 Characterisation of seaweeds used in the study

Seaweed	pH	EC (ms/cm)	Elemental composition mg/kg of dry wt							
			Mg^{2+}	Al^{3+}	P	K^+	Ca^{2+}	Mn^{2+}	Fe^{2+}	Na^+
Cystophora sp.1	4.2	6.1	712	5	91	3,639	1,619	0.6	10	1,567
Cystophora sp.2	4.8	9.0	675	13	156	4,719	1,845	0.9	19	1,784
Sargassum sp.2	5.7	3.1	794	53	66	7,665	1,813	0.7	51	1,333
Sacberia sp.	5.9	13.0	774	23	259	5,501	1,510	4.9	29	3,310
Ecklonia radiata	5.6	11.4	664	57	250	6,615	1,300	0.3	11	2,761
Sargassum sp.1	6.5	4.6	804	44	88	3,099	3,195	3.8	67	780
Ulva sp.	6.5	9.4	4,219	34	195	1,621	2,172	1.6	84	1,745
Homosira sp.	6.3	14.5	1,519	15	55	4,906	1,829	4.6	25	7,888

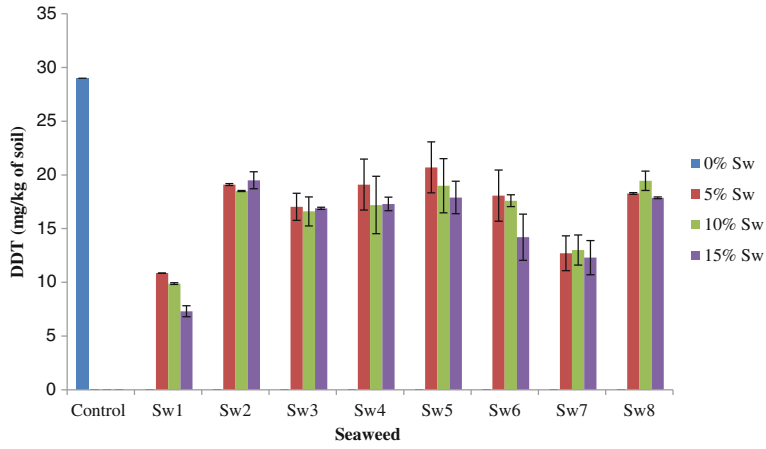

Fig. 1 Effect of seaweeds added at different percentage (*w*/*w*) on DDT concentration after 3 days of incubation. *Bars* represent±SE (*n*=2). *Sw* seaweed, *control* soil with no seaweed, *Sw1 Cystophora* sp.1, *Sw2 Cystophora* sp.2, *Sw3 Sargassum* sp.2, *Sw4 Scaberia* sp., *Sw5 Ecklonia radiata*, *Sw6 Sargassum* sp.1, *Sw7 Ulva* sp., *Sw8 Homosira* sp.

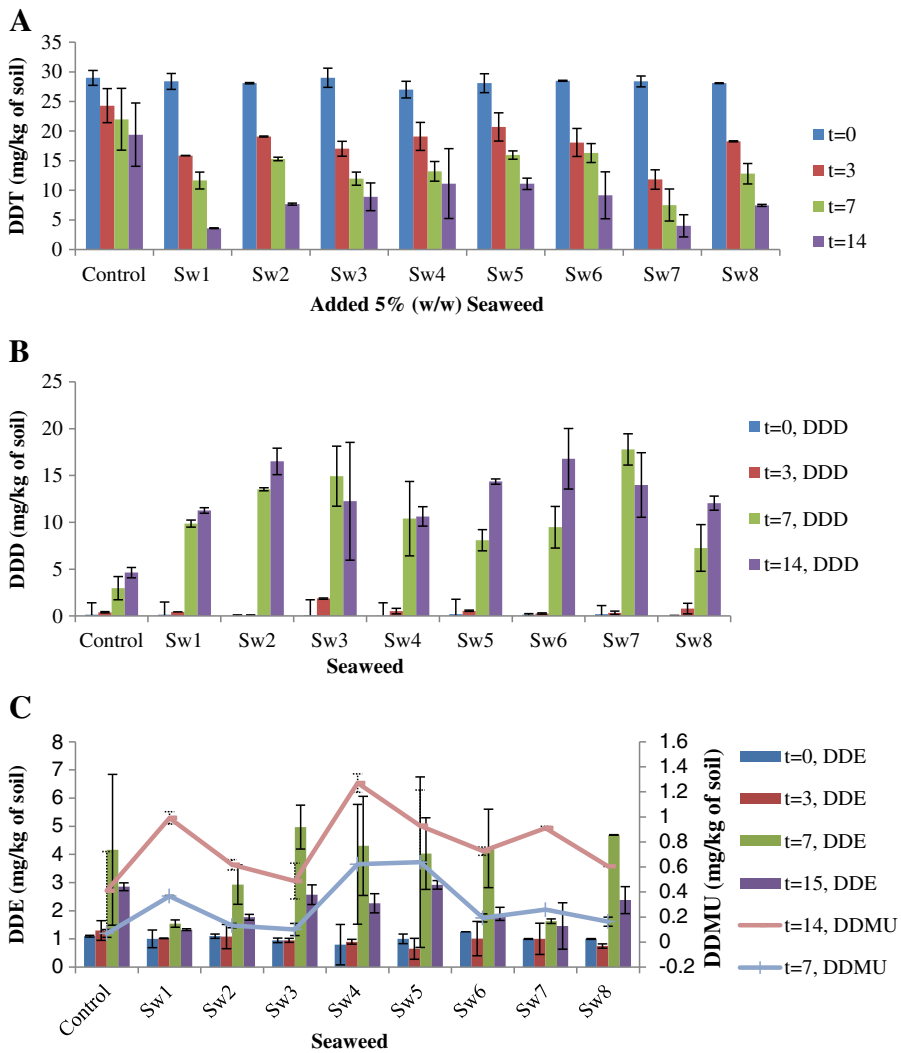

Fig. 2 Effect of 5 % (*w*/*w*) seaweed amendments on DDT (**a**) and formation of metabolites—DDD (**b**), DDE and DDMU (**c**) during the incubation period. *Bars* represent±SE (*n*=2). *Sw* seaweed, *control* soil with no seaweed, *Sw1 Cystophora* sp.1, *Sw2 Cystophora* sp.2, *Sw3 Sargassum* sp.2, *Sw4 Scaberia* sp., *Sw5 Ecklonia radiata*, *Sw6 Sargassum* sp.1, *Sw7 Ulva* sp., *Sw8 Homosira* sp.

found to be the major metabolite followed by DDE and DDMU. Incubation for 14 days not only increased the DDD and DDMU concentration but also showed a significant decrease in DDE concentration. Seaweed-amended soil converted approximately 35–56 % of DDT as DDD while the control formed only 15 % DDD. DDE formed was about 4–10 % with seaweed amendments while the control produced approximately 10 % DDE. There was a marginal increase of about 1–2 % DDMU in both the seaweed-amended soil and the unamended soils.

Among the eight seaweeds used in this study, soil amended with *Cystophora* sp.1 and *Ulva* sp. showed greater degradation of DDT. Both the seaweeds degraded more than 85 % of DDT within 14 days with least DDE and DDMU accumulation of less than 5 and 2 %, respectively.

Mass balance of metabolites at the end of the incubation period accounted for 60 and 71 % of the DDT lost in the case of *Cystophora* sp.1 and *Ulva* sp., respectively. The unaccounted mass could be due to further degradation of the metabolites to other products which were not identified in this study or sorption of DDT in restricted sites of organic matter. Seaweed addition increased DOC, EC, and decreased the *Eh* and pH of the suspensions (Table 3). These conditions enhance the formation of coiled compact humic acid and may influence the sorption of hydrophobic organic carbons such as DDT (Pan et al. 2008)

3.3 Effect of DOC

Seaweed additions increased the DOC levels of soil suspensions (Table 3). Soil amended with *Ulva* sp. released the highest concentration of DOC followed by *Cystophora* sp.1 and these were the two most effective seaweeds in enhancing DDT degradation. These results suggest that DOC played a significant role in the enhanced degradation in seaweed-amended soils.

Sorption of many pesticides by soils, especially the non-ionic pesticides such as DDT, is controlled by soil organic matter (Hamaker and Thompson 1972). Sorbed organic contaminants are retained either weakly and are readily desorbed or strongly sorbed in more restricted sites or diffusion-limited sites (Businelli 1997). Increased DOC due to seaweed addition may have affected the pesticide sorption or desorption. Seaweeds introduced both insoluble and soluble organic matter in soil. While insoluble organic matter enhances hydrocarbon adsorption (Hassett and Banwart 1989), the soluble or dissolved organic matter adsorb to active hydrophobic sites in soil. This displaces the weakly adsorbed DDT molecules resulting in enhanced degradation.

DOC concentrations resulting from seaweed addition increased up to 7 days of incubation after which the DOC levels began to drop (Fig. 3). The increase during the 7 days of incubation could be due to the slow release of soluble carbon. The decrease in the DOC levels at day 14 is likely due to the proliferation of soil microorganisms utilising the DOC for growth resulting in decreased concentration and enhanced biotransformation of DDT to DDD and further degradation products.

3.4 Effect of *Eh* Due to Seaweed Amendments to Soil

The *Eh* in seaweed-amended soil decreased significantly within 5 h of incubation. Further incubation for 7 days showed *Eh* of less than −100 mV, indicating highly reducing conditions, even in the unamended soil sample. At the end of the second week, seaweed-amended samples had an *Eh* of −170 to −260 mV while the control had an *Eh* of −210 mV (Fig. 4). Under anaerobic conditions, addition of carbon, metals, and minerals from seaweed-enhanced bacterial activity and resulted in low *Eh*. However, the control sample also showed low *Eh*, which could be due to organic matter present in the soil. The 33 % decrease of DDT in

Table 3 Effect of seaweeds on soil factors at t=0

Parameters	Control	Sw1	Sw2	Sw3	Sw4	Sw5	Sw6	Sw7	Sw8
pH	7.17	6.19	6.47	6.65	6.48	6.38	6.84	7.04	6.89
EC ms/cm	1.50	3.61	3.19	2.15	3.13	4.19	2.41	4.5	5.47
Eh (mV)	82	55	79	77.5	62	51	77	25	62
DOC mg/L	73	960	318	307	358	572	210	690	502

Control soil with no seaweed, *Sw1 Cystophora* sp.1, *Sw2 Cystophora* sp.2, *Sw3 Sargassum* sp.2, *Sw4 Scaberia* sp., *Sw5 Ecklonia radiata*, *Sw6 Sargassum* sp.1, *Sw7 Ulva* sp., *Sw8 Homosira* sp.

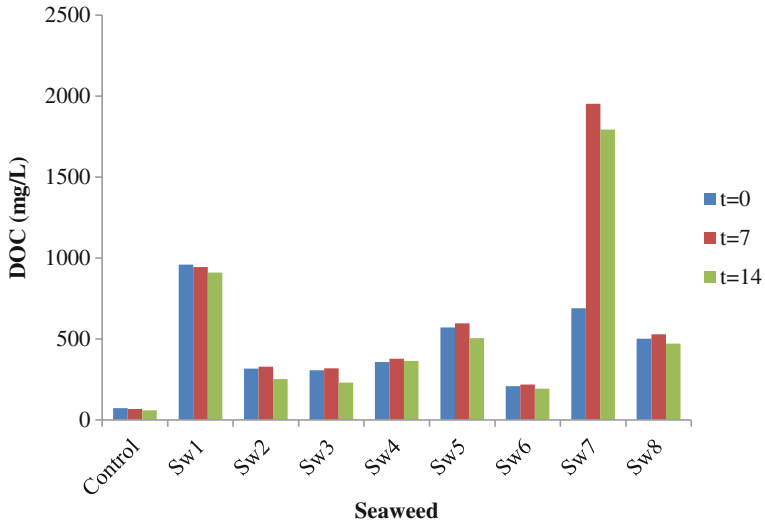

Fig. 3 DOC levels due to addition of different seaweeds during the incubation period. *Control* soil with no seaweed, *Sw1 Cystophora* sp.1, *Sw2 Cystophora* sp.2, *Sw3 Sargassum* sp.2, *Sw4 Scaberia* sp., *Sw5 Ecklonia radiata*, *Sw6 Sargassum* sp.1, *Sw7 Ulva* sp., *Sw8 Homosira* sp.

unamended soil samples is consistent with anaerobic degradation.

Redox potential decreased as DOC concentrations increased and reflect that DOC is a source of energy for anaerobic metabolism (Fig. 5). There was a significant negative correlation ($P<0.05$; $R=0.74$).

3.5 Evaluation of Factors Using Linear Regression Analysis

A stepwise multiple linear regression analysis was performed to identify the factors influencing the degradation process. The amount of DDT removed at the end of the incubation period was the dependent variable in the regression, whilst the independent factors (variables) considered were DOC, pH, *Eh*, EC, and the inorganic ions. Since a linear regression model was used, a logarithmic measure like pH was transformed into a linear measure to detect any possible relation to the dependent variable. A transformed pH (pH_t)=exp(7−pH) was applied to the pH value, which corresponds to a measure of available hydrogen ions relative to the neutral pH condition. The independent variables were fed in two blocks based on their possible colinearity. The first block contained inorganic ions (Al^{3+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and Fe^{2+}); DOC and EC underwent a stepwise regression. The factors—pH_t and *Eh*—were entered in the regression model as a separate, second block. Colinearity statistics are also used to eliminate factors that wield less significant influence on the dependent variable.

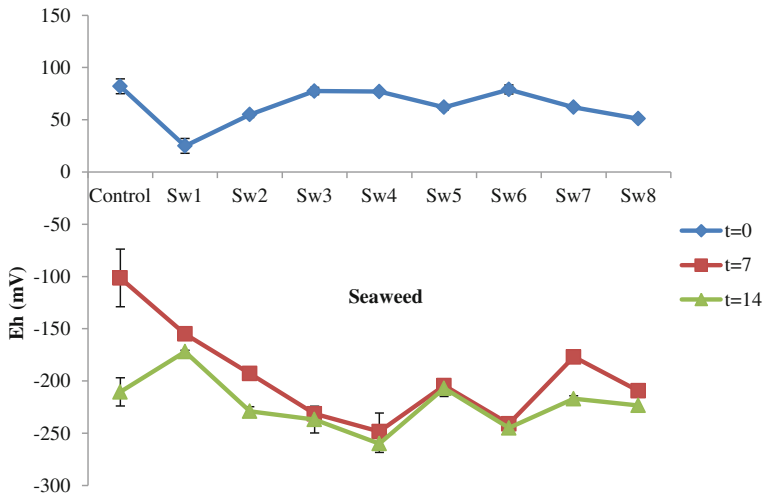

Fig. 4 Effect of seaweed amendments on redox potential during incubation

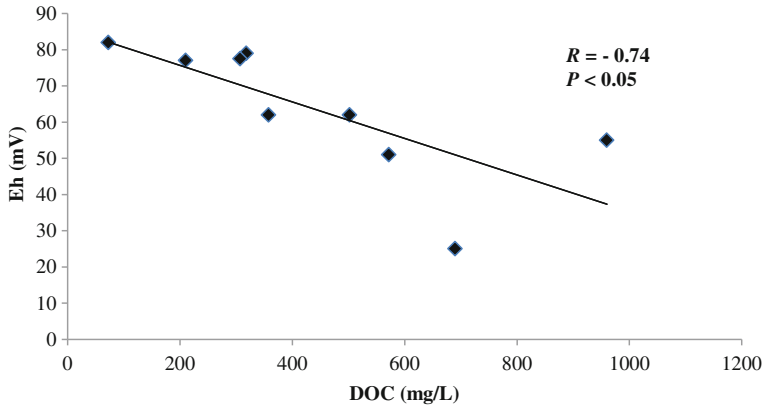

Fig. 5 Regression between DOC and *Eh* with seaweed amendments in soil (t=0)

The SPSS regression model was established so that any independent variables with a significance factor less than or equal to 0.05 ($f \leq 0.05$) were included in the model and any factor with $f \geq 0.1$ was removed from the model. The model summary output in Table 4 shows three models—one with DOC alone as the factor; the second with DOC and Ca as the factors; and the third with DOC, Ca, pH_t, and *Eh*. Adjusted R^2 value for each of these models is also shown in the table. The adjusted R^2 value for the second model with DOC and Ca is the highest (Table 4). However, the third model has an adjusted R^2 which is only marginally lower than the second model, but includes two additional factors pH_t and *Eh*. Numerous studies have reported the effect of pH and *Eh* on degradation of organic contaminants such as DDT (Murphy et al. 1994; Haarstad and Fresvig 2000; Glass 1972; Zoro et al. 1974; Sayles et al. 1997). Therefore, it is imperative that the third model is chosen as the model representing the factors that significantly influence reduction in DDT concentration. Thus, DOC, Ca, pH_t, and *Eh* were the major factors influencing the DDT degraded at the end of the incubation. The coefficient of each of these factors in the regression model had a significance of $P \leq 0.05$. Collinearity statistics was also used in the regression model to eliminate interdependent factors, a situation where high correlation is detected between two or more predictor variables. In the regression model, it was also found that the maximum multicollinearity, measured by the variation inflation factor, was 6.211, which is less than the allowed threshold (10.0).

The regression model arrived at from the statistical analysis is also consistent with our expected behaviour regarding the presence of multiple factors affecting DDT reduction. The model suggests that there is a significant interaction of Ca^{2+} with the DOC. From the seaweed composition (Table 2), we observe that high concentrations of the cations K^+, Ca^{2+}, Mg^{2+}, and Na^+ are added to the soil. Estimating the interaction between humic acid and the individual ions is therefore complicated. As far as Ca^{2+} is concerned, studies conducted by Laegdsmand et al. (2004) indicate that addition of Ca^{2+} increased the linearity and reversibility of the sorption process and produced a lower sorption capacity for pyrene. The study explained that this could be due to condensation and fixation of humic material by Ca^{2+}, which reduced the apparent sorption capacity by hindering diffusion into the interior of the soil organic matter. Although it is difficult to conclude from statistical analysis alone that Ca^{2+} is the only

Table 4 Model summary factors that influence DDT degradation

Dependent variable	Model	Independent variable	Adjusted R^2	Significance (p)	Variation inflation factor
DDT degraded	1	DOC	0.587	0.02	1.00
	2	DOC, Ca	0.857	0.001	1.03
	3	DOC, Ca, Eh, pH_t	0.826	0.02	6.21

Number of samples=9

interacting ion, it seems reasonable to conclude that condensation of the ions with DOC is one of the phenomena influencing DDT degradation.

4 Conclusion

The study showed seaweeds are an effective organic amendment for enhancing DDT degradation. Seaweed-amended soils degraded approximately 60–88 % of DDT over 14 days of incubation. The extent of DDT degradation varied amongst seaweeds. *Cystophora* sp.1 and *Ulva* sp. were the most effective, demonstrating maximum degradation of 88and 86 %, respectively, with the lowest DDE concentration of 4.4–4.8 %. The study demonstrated that seaweeds enhance degradation of DDT in soils and is related to the release of DOC possibly by affecting DDT sorption in soils. The presence of large concentrations of DDD in seaweed-amended soil suggests that seaweeds act as a biostimulant and increase the biotransformation process. The study also showed there was a decrease in DDE concentrations in soil at the end of the incubation period. However, lack of complete mineralisation of DDT suggests that future studies must be conducted over longer periods of incubation.

In summary, enhanced degradation of DDT by seaweed amendments could be due to physicochemical changes, which include DOC, ionic strength, pH, and biological due to biostimulation of soil bacterial community.

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