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Sensitivity Assessment of Contaminant Pressures - Maerl – Evidence review

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1 Introduction

An evidence review of the effects of contaminants on Maerl was undertaken between February and March 2024. The evidence review followed the Rapid Evidence Assessment (REA) protocol developed previously (Tyler-Walters *et al.*, 2022).

The resultant 'Maerl Evidence Summary'[1] spreadsheet and 'evidence review' that follows benefited from improvements and resultant minor adjustments. The 'evidence summary' template was updated to improve data entry. The improvements included:

- the addition of both the reported and standardised values for the exposure concentrations of contaminants used (where available),
- the addition of both the reported and standardised values for the observed or effect concentrations of contaminants (where available), and
- use of 'common' or 'trivial' names for chemicals derived from the PubChem[2] database where possible, and
- the adoption of a standard 'summary narrative' writing style for consistency in reporting.

In addition, 'contaminant type' is recorded as the function of the chemical (e.g., herbicide, analgesic), rather than the structure of the chemical (e.g., organohalogen, organophosphate), if the information allows.

All the technical terms used in the 'Maerl Evidence Summary' and the report that follows are defined in Appendix 1.

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[1] https://www.marlin.ac.uk/sensitivity/contaminants
[2] https://pubchem.ncbi.nlm.nih.gov/

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2 Evidence review overview

The evidence review was conducted to inform the assessment of the sensitivity of maerl and maerl-dominated biotopes in UK waters. Preliminary searches revealed limited evidence on the effects of contaminants on UK maerl (*Phymatolithon calcareum and Lithothamnion* sp.,). Therefore, the literature review was expanded to include other coralline algae species (*Lithophyllum* sp., *Mesophyllum* sp. and *Corallina* sp.).

The initial searches (12 February 2024) resulted in 618 hits of which 190 were duplicates (Table 2.1) using the standard search strings developed previously (Tyler-Walters *et al.*, 2022). Only the Web of Science (WoS) science citation index and the ECOTOX[3] Knowledgebase (Olker *et al*., 2022) were used due to time constraints. The resultant references were screened for relevance based on the proposed REA protocol. Screening against the exclusion criteria reduced this number to nineteen articles[4], which were taken forward for detailed review. However, eight articles could not be accessed, even using inter-library loans, but another four articles on oil spills were added at the evidence review stage. Only articles written in English or with readily available English translations were included.

Table 2.1. Results of literature review for Maerl-forming and selected coralline red algal species.

Review stage	No. articles identified/retained	No. articles rejected/removed
Web of Science	618	
ECOTOX database	3	
Duplicates removed	431	190
Screening	30	393
Taken forward*	19	11
Not accessible	8	

* Does not include further articles identified from the articles reviewed, or alternative sources

[3] https://cfpub.epa.gov/ecotox

[4] The term 'article(s)' or 'study' are used for peer reviewed papers, reports, and other publications relevant to the review.

The detailed evidence extracted from fifteen articles is provided in the 'Maerl Evidence Summary' spreadsheet and the supporting evidence and sensitivity assessments are discussed below. In the 'Maerl Evidence Summary' spreadsheet, data was extracted from ECOTOX for one of the fifteen articles (Amiard, 1973), however the original source text was not accessible.

Another ten articles had no direct toxicological evidence suitable for 'mapping' in the evidence summary but relevant evidence from these articles was included below as they provided qualitative evidence and/or provided context on the sensitivity of coralline species.

The review provided 35 worst-case ranked mortalities (hereafter referred to as 'results'[5]) from fifteen articles. 'Mixtures' (five articles, 46% of results) were the most studied contaminant within the few reviewed articles, followed by 'Hydrocarbons (Petrochemical)' (three articles, 14% of results), 'Metals' (two articles, 17% of results), 'Pesticide/Biocide' (two articles, 11% of results), 'Inorganic chemicals' (one article, 6% of results), 'Personal Care Products (PCPs)' (one article, 3% of results) and 'Radionuclide' (one article, 3% of results).

Overall, the majority of articles reviewed reported no ('None') mortality (six articles, 43% of results) and sublethal effects (six articles, 37% of results). 'Significant' mortality was reported in one study of several species (14% of the results), but all of these results were reported in the effects of 'Mixtures' on the coralline algae species. 'Some' mortality was reported in one article (6% of the results) from one complex hydrocarbon (Diesel WAF[6]). The level of mortality or sublethal effect were 'Unspecified' in the remaining 3% of results.

Most of the studies examined *Corallina officinalis* (reported in eight articles, 20% of results) followed by *Mesophyllum lichenoides* (reported in five articles, 14% of results) and *Lithophyllum incrustans* (reported in four articles, 11% of results) (Table 2.2.). *Lithophyllum lichenoides, Jania rubens* and *Phymatolithon lenormandii* were each examined in two articles and each accounted for 6% of the results. The maerl forming species *Phymatolithon calcareum* and *Lithothamnion soriferum* were amongst the least studied species, examined in only one article each. However, *Phymatolithon calcareum* accounted for a high percentage (14%) of the worst-case results and *Lithothamnion soriferum* (6% of results). *Porolithon*

[5] Note a separate 'worst case' ranked mortality is given for the 'endpoints' and evidence from each combination of contaminant type and species reported in each article.

[6] WAF = Water Accommodated Fraction

onkodes (3% of results), *Rhodoliths* (3% of results), *Lithothamnion spp.* (3% of results), *Neogoniolithon fosliei* (9% of results) were amongst the least studied species, limited to one article each.

Table 2.2. The species name and 'worst-case' ranked mortality 'results' reported from articles reviewed.

Species name	Significant	Some	None	Sublethal	Unspecified	Total of worst-case' ranked mortality results
Corallina officinalis	1		5	1		7
Porolithon onkodes				2		2
Jania rubens			1	1		2
Lithophyllum incrustans	1		3			4
Lithophyllum lichenoides	1		1			2
Lithothamnion soriferum		1		1		2
Lithothamnion spp.			1			1
Mesophyllum lichenoides	1		4			5
Neogoniolithon fosliei				3		3
Phymatolithon calcareum				5		5
Phymatolithon lenormandii	1	1				2
Rhodoliths					1	1

3 Hydrocarbons

A total of five ‘worst-case’ ranked mortality results were obtained from three articles that studied the effects of hydrocarbons on Coralline algae species, of which four results examined the effects of oil spills and one result examined the effects of complex hydrocarbon, Diesel WAF. Three of the oil spill results reported no (‘None’) mortality and one result reported ‘Unspecified’ mortality. There was ‘Some’ mortality reported in the effects of the complex hydrocarbon. The effects of hydrocarbons were examined on *Phymatolithon lenormandii, Lithophyllum incrustans, Mesophyllum lichenoides* and *Corallina officinalis*.

3.1 Oil spills

The effect of an oil spill was reported in eight articles, of which quantitative evidence was available from only two articles (Diez *et al.,* 2009a and Felder *et al.,* 2014). The evidence is summarised below.

Bowman *et al.* (1978) reported the observed effects of the Dounreay Oil Spill, Northern Scotland (February 1977), where 17,000 gallons of 900 second Medium Furnace Fuel Oil discharged onto the shore and was treated by dispersant BP100X and barley straw. The study, a month after the spill, observed most or all of the 100% cover of Lithothamnia had bleached in the upper rock pools and observed rims of dead Lithothamnia in lower shore rock pools. Lithothamnia survived in some pools where the algae were shaded by grazing limpets or *Actinia*. A delayed bleaching effect and mortality of *Corallina* were also reported six months after the spill. However, the authors’ conclusions, suggested that oil and dispersant pollution did not cause the effects observed because similar symptoms were observed on other shores not impacted by pollution.

***Sea Empress* 1996 (crude oil). Crump *et al.* (1999)** examined a set of permanent quadrats in Manorbier and West Angle Bay, Pembrokeshire, before and after the *Sea Empress* oil spill. They observed bleaching on encrusting coralline algae, *Lithothamnion incrustans, Phymatolithon purpureum,* and *Corallina officinalis*, as a severe immediate effect caused by crude oil. However, the bleaching did recover quickly, which suggested the effects were restricted to surface layers. The authors stated that previous literature has shown oil and dispersants to have harmful effects on the pigmentation of red algae in experimental conditions.

Diez *et al.* (2009a) examined macroalgal assemblages in the Basque coast, following the single-hulled oil tank '*Prestige*' spill, in November 2002. The tanker was towed for five days, trailing an oil slick and then sank off the coast of northwestern Spain, discharging 63,700 tonnes of heavy fuel no.2-M100 into the ocean. This study focused on the Basque coast, which was the least affected area due to oceanography, and fishing fleet used to collect oil. However, the Basque coast was exposed to two major oil waves from the spill in 2003. The study did not quantify the direct damage of *Prestige* oil on intertidal vegetation due to the lack of pre-impact data but examined spatiotemporal changes and alterations of assemblages following the spill.

In the field, 12 sites were studied between in autumn and spring in 2004 and 2006. Six were identified as the most affected sites (moderate oil with patches measuring several tons per square metres along the intertidal zones) and six were identified as the least affected sites (small oil patches 5 to 50 cm in diameter and 1 to 3 mm thick, sparsely distributed at high tide level). No accessible or suitable control sites were found because the whole coast had been affected by some degree of oiling. Quadrats were used to collect samples of vegetation composition, species richness and percentage algae cover. Specifically, these samples were taken from macroalgal communities dominated by *Corallina elongata* in the highly diverse low intertidal zone, where the macroalgae was not oil coated but may have been affected by uptake of dissolved hydrocarbons. There were also seasonal differences recorded in this study.

Diez *et al.* (2009a) reported that *Corallina elongata* dominated the macroalgal assemblage, with an average cover of 59.2% ± 0.8 from 2004 to 2006. The main accompanying species with more than 2% cover included *Mesophyllum lichenoides* and *Lithophyllum incrustans. Lithophyllum incrustans* showed a significant difference in cover between seasons in each year studied. There were no significant differences in species richness and algal cover at moderate oil and small oil levels. The cover of *Corallina elongata* also showed no differences between oil levels and had a stable distribution overtime. Diversity, species richness and algal cover, particularly the *Corallina elongata* assemblage was significantly lower in the first year of study (2004), suggesting assemblage was recovering by the second year of study. However, the authors considered available data from 2002, pre-oil spill, from one of the moderately oiled sites and found the diversity and species richness of *Corallina elongata* was also low, suggesting the decrease in algal abundance in 2004 could have been natural.

The authors concluded that the calcareous macroalgae assemblage was considerably stable over time and there were no significant differences between the two oiling levels. It was suggested that *Corallina elongata* may have recovered from damage by the start of the study because evidence from another oil spills (*Sea Empress* and Panamanian coast spill) found that coralline algae recovered from initial detrimental effects after 12 months following the spill. In addition, the lack of effects on the assemblages could be explained by the fact that the Basque coast was the area least affected by the spill, and the intertidal vegetation was not smothered by oil. The coast is highly exposed to swell and wave action where oil tends to be washed away. Diez *et al.* (2009a) stated that calcareous red algae were considered to tolerate moderate levels of domestic pollution and in general coralline algae appear to have a rapid recovery after oil spills. They also suggested that the effects of the oil spill on the macroalgae assemblages would be similar along the Basque coast.

Felder *et al.* (2014) summarized the effects of the 2010 *Macondo* oil spill, in the northern Gulf of Mexico on seaweed communities on deep banks (Sackett bank and Ewing bank). The evidence before the spill showed the northwestern deep hard banks to have the highest known level of seaweed diversity in the northern gulf, where rhodoliths were major contributors of calcium carbonate. In the field, six post spill cruises were conducted from 2010 to 2013, and unconsolidated rubble and rhodoliths were dredged at depths of 55 to 75 m. They reported a dramatic decrease in seaweeds and diversity of species. The rubble and rhodoliths exhibited visual bleaching, lack of fleshy algae, or sparse cover by a crust-forming species e.g. *Lithothamnion* spp. Crustose corallines collected in the first post spill cruise were calcified (Felder *et al.,* 2014).

Dredged samples of bare or algal-denuded rubble or live rhodoliths were maintained in microcosm tanks to monitor their recovery in untreated tanks or ones sterilized with UV filter to reduce seeding of water column microorganisms (including potential algal spores). They observed a gradual regeneration of red, green and brown germlings from bare dead rhodoliths rubble after three weeks (but this result was not quantified), and the species composition resembled pre spill assemblages. The authors hypothesized that the resting stages of algae were repressed in the field during post spill sampling. The reasons for the decrease in macroalgae diversity were unknown but the authors hypothesised that it may be impacted by nutrient availability, microbial interactions and cascading effects from the loss of higher tropic levels and algal consumers (Felder *et al.,* 2014).

Fredericq *et al.* (2014) examined the seaweed diversity of rhodolith beds at 55-70 m in the Gulf of Mexico (Sackett bank and Ewing bank) associated with salt-domes or diapirs, after the 2010 *Deepwater Horizon* (DWH) oil spill. They recognised two forms of rhodolith; those formed by calcareous red algae or those formed by encrusting calcareous red algae growing on unconsolidated rubble formed from erosion of the salt-domes. Nine genera of calcareous algae were reported, *Lithophyllum* sp., *Lithothamnion* sp., *Mesophyllum* sp., and *Porolithon* sp. but the rhodoliths beds supported a one of the most diverse seaweed communities in the Gulf based on 2009 surveys. However, the rhodoliths looked 'chemically' bleached after the DWH spill or if the encrusting species were alive, they were devoid of fleshy algae (Fredericq *et al.,* 2014).

Jackson *et al.* (1989) reported the effects of a major oil spill in 1986 on the Caribbean coast of Panama on coastal populations and communities. The spill caused around 8 million litres of crude oil to be discarded from a ruptured storage tank into the sea. Results shortly after the spill found microalgae proliferated on intertidal reef flats at Puta Galeta. This area was previously dominated by perennial macroalgae, particularly crustose corallines (no species were specified) and other fleshy algae, which decreased in cover after the spill, to levels below those observed before the spill. However, within 12 to 18 months after the spill the abundance of the microalgae reduced and macroalgae recovered, regaining or exceeding typical abundance levels. This suggested that the crustose coralline algae can recover quickly.

Newey & Seed (1995) examined the effects of the oil tanker *Braer* oil spill, which ran aground in southern Shetland in January 1993, and 85,000 tonnes of crude oil and an unknown quality of bunker oil were discarded into the environment. In the field, 29 sites were surveyed to examine the composition and diversity of rocky intertidal communities between June and August 1993. In the mid-shore zone rockpools, in close vicinity to the wreck, results found extensive areas of bleached and dead coralline algae (no species were specified)

***Torrey Canyon* 1967 (crude oil). Smith (ed., 1968)** reviewed information on the effects of the *Torrey Canyon* oil spill in 1967. They reported various effects on coralline algae following exposure to detergent treatments. In deep pools in Newtrain Bay, *Corallina officinalis* and other algae were observed in apparent normal condition. However, in shallow rock pools, known to contain *Corallina* and *Lithophyllum* tufts, only contained tufts of *Corallina* and other small algae after exposure to the detergent treatments. *Lithophyllum* was considered dead and *Corallina* had begun to regrown in these pools. In addition, evidence found that the algae were more severely damaged by detergent at the higher levels on the shore. For example, *Corallina* and its associated epiphytes and encrusting calcareous algae in upper shore pools were killed but at the mid-tide, only the tips were bleached and killed, and near low water the *Corallina* appeared healthy. *Jania rubens* was also observed to be impacted by detergent and appeared to be either killed or visually unhealthy. Similar results were seen at Polpeor Cove, where *Corallina* algae survived despite extensive detergent exposure. In addition, Pendeen Watch, on the north coast, saw encrusting coralline algae killed in some pools and surviving in others; *Corallina officinalis* had bleached and disintegrated. Overall, the effects of detergents depended on duration and concentration of exposure, assuming lower shore populations were exposed for shorter periods than higher-shore populations as the tide returned.

Tuya *et al.* (2023) discussed the need to improve knowledge of rhodoliths beds (which includes maerl beds) and address conservation challenges. Tuya *et al.* (2023) stated that rhodolith beds face multiple anthropogenic threats and pressures, including land-based stressors (nutrient pollution, organic/inorganic pollution) and ocean-based stressors (pollution from offshore mariculture, mining, gas, and oil exploration). Rhodolith beds face threats from catastrophic events like the 2015 Doce River mining dam collapse in Southeast Brazil which significantly increased metal and sediment pollution. Negative impacts from fish and mussel farming have also been documented, with increased organic enrichment and sediment load leading to biofouling, reduced rhodolith performance, and burial. Extractive industries, particularly offshore oil and gas exploitation and direct rhodolith mining, further exacerbate the situation through the discharge of drill cuttings, sediment dislodgement, and direct habitat destruction. Furthermore, accidental oil spills, such as the BP Deepwater Horizon spill in the Gulf of Mexico in 2010 (Fredericq *et al.*, 2014) and the extensive spill on the Brazilian continental shelf in 2019/2020, pose additional threats to rhodolith beds and other marine ecosystems, including coral reefs.

3.2 Petroleum hydrocarbons – oils and dispersed oils

Bokn *et al.* (1993) examined the long-term effects of Water Accommodated Fractions (WAF) of diesel oil on the abundance of rocky shore populations, including red algae *Phymatolithon lenormandii* and *Chondrus crispus.* During the experiment, four mesocosms were supplied with a flow-through of seawater, and littoral populations taken from the Drøbak Sound, Norway were established for three years in the mesocosms. After the three years, two mesocosms were continuously supplied with doses of WAF of diesel oil and the other two mesocosms were controls. The higher dose mesocosm was supplied with 129.4 µg/l of WAF oil and the lower dose mesocosm was supplied with 30.1 µg/l of WAF diesel oil. The exposure period began in September 1982 and continued for two years until September 1984. Species abundance and cover at five tidal levels from supralittoral to sublittoral was collected every two to three months.

Bokn *et al.* (1993) reported that *Phymatolithon lenormandii* exhibited a negative change in algal cover in the highest dose mesocosm (129.4 µg/l) in the upper shore, but a two-to-four-fold increase in cover at the same level in the other three mesocosms (lower dose 30/1 µg/l and the two controls). They also found that the population of *Chondrus crispus* increased in all mesocosms and no significant differences in abundance were observed in the contaminated mesocosms compared to the controls. Bokn *et al.* (1993) concluded that there were significantly greater decreases in cover of seaweed species studied (*Phymatolithon lenormandii* and *Fucus evanescens*) in the high dose mesocosm in the upper shore. They recognised that there was spatial and temporal variability in rocky littoral populations, which influences the effects of oil. In addition, the lower shore level exposure of WAF diesel oil had limited direct impacts on other seaweed standing stocks, and more research was needed to improve knowledge on the effects of chronic oil exposure on seaweeds.

4 Transitional metals

Six results (ranked 'worst-case' mortalities) were obtained from two articles that examined the effects of transitional metals. Only sublethal effects of metals, which included Antimony (Sb), Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni) and Zinc (Zn), were recorded from the articles. *Corallina officinalis* and *Phymatolithon calcareum* were studied in these articles.

The data of one article (Amiard, 1973) that examined the effects of transitional metals was retrieved from ECOTOX but the original source text was not accessible. Therefore, there was only one article reviewed that examined the effects of metals, summarised below.

Wilson *et al.* (2004) studied the effects of heavy metal contamination on the photosynthetic capacity of *Phymatolithon calcareum* collected from Strangford Lough, Northern Ireland. In laboratory conditions, samples of *Phymatolithon calcareum* were exposed to a range of concentrations of heavy metals provided in a ratio 37:16:14:11:1, this included; 6.438 to 6,438 ppb of zinc (Zn), 2.784 to 2,784 ppb of lead (Pb), 2.436 to 2,436 ppb of nickel (Ni), 1.914 to 1,914 ppb of copper (Cu) and 0.174 to 174 ppb of cadmium (Cd) (Zn: Pb: Ni: Cu: Cd). Concentrations were given in one dose and measurements of the quantum yield of photosystem II were taken over seven days after exposure, to test photosynthesis capacity as a proxy for stress. Initial results after 24, 48 and 72 hours found a significant decrease in the photosynthetic capacity, the most marked decrease was at the highest metal concentration (120 ml^{-1}) compared to the controls. This suggested an initial negative effect of metal contamination on *Phymatolithon calcareum.* However, after seven days, the photosynthetic values were not significantly lower than the controls, and had returned to normal conditions, which suggested the algae had recovered. The authors suggested that recovery occurred due to the absorption of the heavy metals onto wall matric or non-metabolic components within the algae species. However, it was recognised that in the natural environment, algae may experience chronic exposure to metal pollution, which may have worse effects than a single dosage. In addition, the presence of other abiotic factors and threats may also worsen effects for the maerl species.

5 Synthetic compounds – including Pesticides and Pharmaceuticals

Five results (ranked 'worst-case' mortalities) were obtained from three articles, of which four results examined the effects of herbicides (Atrazine, Diuron and Hexazinone) and one result examined the effects of Ultraviolet (UV) filters from are Personal Care Products (PCPs). The PCPs result reported no ('None') mortality and the herbicide results all reported sublethal effects. The effects of these synthetic compounds were examined on *Lithothamnion* spp., *Neogoniolithon fosliei* and *Porolithon onkodes*.

5.1 Pesticides/biocides

A total of three articles reviewed the effects of herbicides on coralline algae species, however, evidence was extracted from only two articles (Harrington *et al.,* 2005 and Negri *et al.,* 2011) The relevant summaries are below.

Harrington *et al.* (2005) examined the effects of herbicide diuron (N'-(3,4-Dichlorophenyl)-N,N-dimethylurea) and sedimentation on the photosynthesis (photosynthetic efficiency expressed as effective photosystem II quantum yields) and survival of crustose coralline algae (CCA). In laboratory conditions, *Porolithon onkodes* from Davies Reef in the Great Barrier Reef (GBR) were exposed to 1, 3, 10 and 30 µg/L of diuron and fine estuarine sediment independently and in combination for 105 hours. The algae were examined in recovery for nine days in clean salt water after the exposure period. Diuron alone inhibited photosynthesis at 2.9 µg/l of diuron (LOEC) following 9 and 35 hours of exposure. However, after transfer into uncontaminated seawater, the effect reversed during the recovery period and the photosynthetic efficiency value reverted to control values. In addition, they observed a decrease in photosynthetic efficiency in the high exposure (27 µg/l of diuron) treatments throughout the exposure period.

Photosynthetic efficiency recovered to control values four days after transfer to the uncontaminated seawater. It was suggested that the significant reduction in photosynthetic efficiency was due to the reversible binding of diuron to the D1 protein in the chloroplast. Visible bleaching of *Porolithon onkodes* was observed at 29 µg/l of diuron, likely due to the destruction of chloroplasts and carotenoids that causes its colouration to lighten. In the high diuron concentrations from 11 to 27 µg/l the inhibition of photosynthetic efficiency is less pronounced in CCA than what is reported for corals and seagrasses.

In treatments that exposed *Porolithon onkodes* to estuarine sediments contaminated by diuron, the photosynthetic yields also decreased. Photosynthetic efficiency decreased to less than 30% of controls (from 100 to less than 40) in exposure for 35 hours to sediment contaminated with more than 0.79 µg/l of diuron. However, there were no significant differences between the sediment treatments with or without diuron at the end of the exposure period. They reported that 17% of CCA fragments had zero photosynthetic yield and were considered dead, and 59% of CCA fragments were partially bleached in treatments exposed to fine sediments or sediment contaminated by diuron, probably due to localised anoxia in their experimental tanks. The combined sediment / diuron exposure impacted the recovery of the CCA as they recovered less than 50% of the control photosynthetic efficiency value. The authors suggested the combination of sedimentation and diuron created more lethal effects. Harrington *et al.* (2005) concluded that diuron, like 50% of the other commercially available herbicides, inhibits photosynthesis in marine plants The combined exposure of sedimentation and herbicides is a likely scenario for nearshore marine organisms, particularly in the GBR, and could pose more significant effects.

McCoy & Kamenos (2015) reported that herbicides, particularly antifouling agent diuron, were shown to cause negative effects on photosynthesis in coralline algae. They noted that the use of diuron in coastal tropical sugar plantations decreased photosynthetic activity in coralline algae (Harrington *et al.* 2005, cited by McCoy & Kamenos, 2015).

Negri *et al.* (2011) examined the effects of temperature and three agricultural photosystem II (PSII) herbicides (Diuron, Atrazine and Hexazinone) on the crustose coralline algae (CCA) *Neogonionlithon fosliei* from Davies Reef in the Great Barrier Reef (GBR) and a coral species. The photosynthetic efficiency (as maximum quantum yield), photoinhibition (as the effective quantum yield) and bleaching of the CCA were estimated. After two weeks to acclimate in flow through tanks, *Neogoniolithon fosliei* was first examined in an acute herbicide experiment in static laboratory conditions and was exposed to 0.3, 1, 3, 10, 30 and 100 µg/l of diuron or 0.3, 1, 3, 10, 300 and 1,000 µg/l of atrazine or hexazinone for 24 hours. A long-term exposure test was then conducted on the combined effects of diuron and temperature, in which CCA were exposed to 0, 0.3, and 1 µg/l of diuron at four temperatures (26, 30, 31, 32°C) in flow-through climate-controlled tanks for seven days. The acute dose herbicide experiment found that CCA was more sensitive to diuron than the other herbicides. The assessment of herbicide concentrations inhibiting photosynthetic efficiency (half maximum inhibitory concentrations (IC50) and LOEC) revealed the order of toxicity as diuron > hexazione > atrazine. The results from the maximum quantum yield revealed chronic photoinhibition was less pronounced than photosynthetic inhibition. IC50s and LOEC were not reached. In the long-term experiment, high temperatures above 30°C significantly inhibited photosynthesis and caused chronic photoinhibition in the CCA. However, there was no significant effect of diuron concentrations, as up to 0.84 µg/l of diuron did not affect the photosynthetic efficiency or photoinhibition in *Neogonionlithon fosliei* and there was no interaction between temperature and diuron concentrations. In addition, they reported that that CCA was sensitive to thermal stress. *Neogonionlithon fosliei* was heavily bleached at 31°C, indicating a severe loss of photosynthetic capacity. The authors noted that even though the effects of herbicides on the photosynthetic efficiency of CCA were reversible, inhibition of photosynthesis at high temperatures (over 30°C) indicated chronic thermal damage, which may not be reversible. Negri *et al.* (2011) concluded that the CCA was more sensitive to diuron than other herbicides but less sensitive to diuron in short term exposures compared to coral species studied. The reasons for this were unclear but might be due to photosynthetic pigment and light-harvesting complex (antenna) organization in the CCA. The authors suggested that tests on respiration and production may be more appropriate to measure effects of herbicides on CCA.

5.2 Personal Care Products (PCPs)

One study examined the effects of an Ultraviolet (UV) filter. The evidence is detailed below.

MacVicar *et al.* (2022) examined the effects of oxybenzone (benzophenone-3; BP-3), a common UV filter in chemical sun cream, on the photosynthesis, respiration and photophysiology of *Lithothamnion* spp. *Lithothamnion* spp. collected offshore from Carabelle, Florida, were acclimatised for three weeks in flow-through seawater tanks, under changing light conditions mimicking conditions in the natural environment. The samples were exposed to one dose of 50 mg/l of BP-3 with 5 ug/l of solvent DMSO and DMSO alone at the start of the experiment. Testing occurred before exposure and 3 hours, 24 hours, 8 days and 15 days after exposure. They reported no differences in photosynthesis or respiration between the control, DMSO or BP-3 treatments during the study period. The photosynthetic yield increased in the BP-3 and DMSO-only treatments after 24 hours relative to the control, however, it was suggested that this is due to the solvent DMSO, rather than the contaminant BP-3 because there was no difference in those treatments. Therefore, the authors concluded there was no evidence of negative effects of UV filter BP-3 on respiration and photosynthesis in the coralline algae, suggesting they are resistant to BP-3. It was noted that the concentration levels may have degraded in the aquarium lights.

6 Radionuclide

One study examined the effects of plutonium.

Hernández *et al.* (2011) measured and extracted plutonium (Pu) from sediment but also analysed *Corallina elongata*, *Jania rubens* and other seaweed species that quickly metabolise and accumulate plutonium. Samples of various marine life were collected every couple of months over two years after an accident with the nuclear submarine '*Hartford*' in 2003 occurring close to a submarine base in Sardinia Island, Italy. They were analysed for alpha-emitting radioisotope concentrations, including 40 samples of green and red algae. They reported that *Corallina elongata* and *Jania rubens* specimens had a particularly high concentration of "hot spots" on their surface; defined by the author as alpha particle due to the decay of small alpha-emitter fragments, due to transuranics measured in the algae. Several micron-sized metal granules were identified at the centre of these hotspots composed of lead (Pb), thorium (Th), uranium (U), plutonium (Pu), antimony (Sb), cerium (Ce), lanthanum (La), and neodymium (Nd). The author reported no effects of these metal granules or "hot spots" on *Corallina elongata* or *Jania rubens*. However, the formation of granules provides evidence of metal detoxification within these species.

7 Other substances

‘Other substances’ include a range of chemicals that do not fit into the other categories of contaminant. Neither do they group conveniently. The majority of articles and 52% of the worst-case mortality results found fit into this category. Eighteen results were obtained from six articles, of which sixteen results examined the effects of unidentified ‘Mixtures’ and two results examined the effects of ‘Inorganic chemicals’. The ‘Mixtures’ results reported five ‘Significant’ mortalities and eleven no (‘None’) mortalities. Whereas, the ‘Inorganic chemicals’ results reported only sublethal effects.

The effects of ‘Mixtures’ were examined on *Corallina officinalis, Jania rubens, Lithophyllum incrustans, Lithophyllum lichenoides, Mesophyllum lichenoides* and *Phymatolithon lenormandii* and the effects of ‘Inorganics’ were examined on *Lithothamnion soriferum.*

7.1 Inorganic chemicals

One study examined the effects of hydrogen peroxide, a chemotherapeutant.

Legrand *et al.* (2022) examined the effects of hydrogen peroxide (H_20_2), a chemotherapeutant used as an antiparasitic treatment in Norwegian salmon farms, on the photosynthetic characteristics of maerl beds specifically coralline algae *Lithothamnion soriferum*. In laboratory conditions, *Lithothamnion soriferum* collected from Skårasund, Vestland County, Norway were acclimated in flow-through tanks for six weeks, before being exposed to measured concentrations; 0, 2, 20, 200 and 2000 mg/L of H_20_2 for one hour in dark conditions and then returned to flow through tanks for four weeks. The study measured the maximum quantum yield determined by the ratio of variable maximum chlorophyll fluorescence, to determine the maximum photochemical efficiency of energy transfer to the photosystem II (PSII). In addition, the authors studied the relative electron transfer rate of the PSII and the percentage of bleaching observed of sampled and dried-out branch tips.

The results observed a clear negative impact on the photosynthetic characteristics of coralline algae immediately after exposure, at concentrations 200 mg/l and above. The chlorophyll fluorescence ratio in *Lithothamnion soriferum* thalli was significantly affected by H_20_2, as 1-hour post-exposure, the ratio decreased by 20% when exposed to 200 mg/l of H_20_2 and 73% when exposed to 2,000 mg/l of H_20_2, compared to the control. The 1-hour EC50 for the chlorophyll fluorescence ratio was 881 mg/l (649 to 1113 mg/l) of H_20_2. Over the rest of the experiment, the ratio gradually increased, but thalli at 200 and 2,000 mg/L of H_20_2, 24 hours post-exposure and at 2000 mg/L of H_20_2. 14 days post-exposure remained significantly lower than the control.

In addition, results found the relative electron transport rates were significantly affected by H_20_2. The lowest rates were recorded at the highest exposure (2,000 mg/l) where values decreased significantly 1-hour post-exposure and remained significantly lower 48 hours and seven days post-exposure. No significant difference was found in relative electron transport rates for concentrations 2, 20 and 200 mg/l of H_20_2. The 1-hour EC50 based on the electron transport values in *Lithothamnion soriferum* were 268 mg/l (0 to 618 mg/l) of H_20_2 and 532 mg/l (11 to 1053 mg/l) of H_20_2. Therefore, exposure to H_20_2 of 200 mg/l and above impacts the algae ability to maintain photosynthesis, and reduced photosynthetic performance is likely to impact other metabolic processes, such as growth and calcification. After 28 days post-exposure, *Lithothamnion soriferum* exposed to 200 and 2,000 mg/L of H_20_2 showed significant bleaching, suggesting impacts to metabolic function and may weaken the structural integrity of the algae.

The authors suggested that as coralline algae exposed to H_20_2 were harmed and, as these laboratory experiments were indicative of processes in salmon fish farms, the coralline algae may be particularly vulnerable to exposure to this contaminant and other chemical effluents from salmon fish farms. Legrand *et al.* (2022) concluded that prolonged and repeated exposure may cause more severe impacts on coralline algae than those observed in the study, which may impact recovery. The effects will also be affected by the dispersal of the contaminant, which can be altered by local environmental conditions (depth, currents and wave exposure) and size of the fish farm.

7.2 Mixtures

Eight articles examined the effects of effluents, which were unidentified and therefore categorised as ‘Mixtures’. Evidence was extracted from only five articles (Diez *et al.*, 1999, Diez *et al.*, 2003, Diez *et al.*, 2009b, Gorostiga & Diez, 1996, Soltan *et al.*, 2001).

Bellgrove *et al.* (2017) reviewed benthic macroalgae as ecosystem engineers as case studies for rocky shore restoration efforts. The review recorded that in some studies, coralline algae, *Corallina officinalis* was abundant in the immediate vicinity of a sewage outfall whereas other studies have found the species absent from outfall sites yet form extensive turfs in intermediately polluted sites. The review also suggested that where macroalgal communities such as *Hormosira banksii* were weakened by varying levels of pollution, articulated coralline turfs could take over as the increasing intermediate level of pollution disrupts the dominance, creating a community shift. The coralline turfs prevented the recovery and recruitment of other macroalgal communities.

Blanfoume *et al.* (2016) studied the distribution of *Lithophyllum byssoides*, which form large bioconstructions called *Lithophyllum byssoides* rims, as an indicator of good ecological status in Vlora, Albania, an area mostly located within the Marine Protected Area (MPA) of Sazani Island and the Karaburuni Peninsula. The study mapped the distribution of communities, using aerial photographs, tested the ecological status of the water bodies, using the CARLIT method and assessed the water quality in relation to anthropogenic pressures.

They reported that *Lithophyllum byssoides* represented 3.6% of the coastline, which was significantly lower than percentages measured in western Mediterranean. At Sazani Island, particularly in the southern part, many *Lithophyllum byssoides* rims located at low tide level were in poor condition due to frequent wave exposure, resulting in extensive erosion and damage. These heavily damaged *Lithophyllum byssoides* structures were considered dead and were heavily colonized by perennial macroalgae *Corallina caespitosa*. On the other hand, structures in Karaburuni Peninsula and the north of Sazani had a continuous cover of live *Lithophyllum byssoides* rims. However, even these living structures were in poor condition, as they were deeply bored with holes, and network of blow-holes as found in dead structures.

The Bay of Vlora, is impacted by numerous pollutants from urbanisation, navy bases, maritime traffic, tourist pressure and sewage from a hydrochloric acid factory. The authors suggested that despite the multiple factors that could explain the regression of *Lithophyllum byssoides* rims, pollution, which may enhance the hole formations and the development of bioeroder organisms, could be the main factor. However, death could be wrongfully attributed to the decline in ecological quality (polluted waters) while actually being due to sea level rise.

Diez *et al.* (1999) examined the effects of a pollution gradient on *Corallina elongata*, *Lithophyllum incrustans, Lithophyllum lichenoides, Mesophyllum lichenoides*, *Phymatolithon lenormandii*, and other intertidal vegetation at the “Abra de Bilbao” (Basque coast, Northern Spain); an area impacted by industrial and domestic non-treated wastewater. The main pollutants in the discharged wastewater were reported in Gorostiga & Diez (1996, see below) and include metals (iron, cadmium, nickel, lead, arsenic and zinc) and nutrients. The study occurred during the summer of 1996 and eight study sites were established along the 17 km coastline and pollution gradient. Site 1, 2, 3 and 4 were subject to very turbid polluted waters, sites 5, 6 and 7 were frequently subject to polluted waters which may dilute through open sea water and lower turbidity, and site 8 was considered unpolluted. Following a transect along the coast, quadrats were used to estimate the percentage cover and frequency of algae species, and zonation patterns at each site.

They found that *Corallina elongata*, *Lithophyllum incrustans, Lithophyllum lichenoides, Mesophyllum lichenoides* and *Phymatolithon lenormandii* were absent from the most polluted sites 1 and 2. Overall the percentage cover and frequency of *Corallina elongata* increased from sites 3 to 8, moving away from the pollution source but it was the most abundant and dominant species in sites (site 3) subject to moderate pollution. which suggests it more tolerant than the other species examined. No clear trend in cover or frequency of *Lithophyllum incrustans, Lithophyllum lichenoides, Mesophyllum lichenoides* and *Phymatolithon lenormandi* was observed but the species were more abundant in sites further from the pollution source, and mainly recorded in sites 4 to 8. The authors did not discuss these species. Diez *et al.* (1999) concluded that algal species richness and algal cover decreased as pollution increases. However, it was unclear whether the effects were due to one or more of the contaminants components of the wastewater effluent or the resultant turbidity.

Diez *et al.* (2003) examined the effects of pollution and other abiotic conditions on the species composition, macroalgal cover and distribution of subtidal vegetation along the 108 km Bizkaia coastline, on the Spanish Basque coast. This coastline, particularly close to the Nervón river mouth, is impacted by the discharge of industrial and domestic wastewater. The wastewater includes nutrients and dissolved heavy metals (cadmium, mercury, copper, lead, zinc, and chromium). In the field, 21 transects were set up along the coastline and a degree of pollution was allocated for the transects on a gradient from unpolluted, slightly polluted and moderately polluted sites. Quadrats were used to assess the abundance and distribution of algae species including *Mesophyllum lichenoides*, *Jania rubens* and *Corallina officinalis*. Data collection involved measuring the percentage cover in each quadrat and the percentage frequency of occurrence of those algal species in the quadrats.

They found mean macroalgal cover and frequency of *Mesophyllum lichenoides* was high (29.03% and 83.86% respectively), and the algae was amongst the few species characterizing 80% of the overall algal cover. *Corallina officinalis* and *Jania rubens* had a lower percentage cover (3.73% and 0.23% respectively) but high frequency (43.03% and 12.96% respectively).

Multivariant analysis was used to examine the relationship between species distribution and distribution of environmental variables (sedimentation, wave exposure, substratum topography, depth and pollution). The analysis included canonical correspondence analysis to explain variation in vegetation in relation to environmental variables and species response curves to explain the probability of a species occurrence as a function of a measured environmental variable. The results from this analysis found that in the study area, pollution was the principal environmental variable explaining the variance in species data. The species response curve to pollution indicated that *Mesophyllum lichenoides* had a wide tolerance range to pollution, as it dominated the community in moderately polluted environments.

Overall, macroalgal cover, species richness and diversity remained constant from unpolluted to slightly polluted sites, but decreased under moderately polluted sites. Unpolluted sites were characterized by a perennial canopy of algae (including *Gelidium sesquipedale* or *Cystoseira baccata*) which grew over a basal layer of algae (including *Corallina officinalis* and *Pteroisiphonia complanta*) and over a crustose *Mesophyllum lichenoides* layer. When pollution was introduced, degradation of the perennial canopy was observed, particularly affecting *Cystosiera baccta*, this allowed the development of more pollution tolerant species such as *Pteroisiphonia complanta* to increase and dominant the community. As pollution persists, the perennial species of the basal layer may decrease. Therefore, when pollution increased and the canopy and basal layer decreased, there was an increase in the development of the crustose layer, which includes *Mesophyllum lichenoides*, suggesting the crustose species were more resistant to pollution. However, it was unclear whether the effects were due to one or more of the contaminants components of the wastewater effluent or the resultant turbidity.

Diez *et al.* (2009b) examined the recovery of phytobenthic marine assemblages in the 'Abra de Bilbao' area and its adjacent coastline, south-eastern corner of the Bay of Biscay. The Nervión river flows into the study area and this has been exposed to high loads of domestic and industrial wastewater, which is carried down the river into the estuary and to the coast. The wastewater includes industrial effluents from fertilizers, chemical manufacture, heavy metal processing acid and domestic waste. The amount of pollution may have decreased overtime due to the closure of polluting industries and a treatment plant that was set up in the 1990s. In the field, sampling surveys were conducted over two decades (from 1984 to 2006), at five sites allocated along a pollution gradient from the most polluted site (1) to the least polluted site (4); site 5 was the unpolluted control site. Quadrats were used to estimate the percentage cover of species in two intertidal levels, species richness and spatial and temporal trends of the community composition were also recorded.

Diez *et al.* (2009b) reported that the mean algal cover was generally higher in the control site in the high-level intertidal zones, although exceptions occurred for specific years and sites. Algal cover increased over time in all sites, with only the most polluted site (Site 1) showing a consistent upward trend. Conversely, in the low-level intertidal zone, only the most polluted sites (Site 1 and Site 2) exhibited significantly lower mean algal cover compared to the control site, with higher temporal variability and increased values by the end of the study. They suggested that overall algal cover increased in the most degraded sites over time. The species richness significantly increased over time, with greater variability observed in the polluted sites, particularly in the high-level intertidal zone. The spatial and temporal community composition changes revealed a pollution gradient and the community shifted towards less polluted conditions over time. *Corallina elongata* was the most abundant species in moderately polluted sites (42.82% cover in high-level intertidal zone and 41.36% cover in low-level intertidal zone) and slightly polluted sites (45.73% cover in high-level intertidal zone and 29.73% of the cover in low-level intertidal zone) in both intertidal levels. In addition, other species such as *Lithophyllum incrustans* became abundant alongside *Corallina elongata* in the low-level intertidal zone (11.16% cover in the slightly degraded sites and 3.70% cover moderately degraded sites) and in the high-level intertidal zone (4.49% cover in the slightly degraded sites). *Mesophyllum lichenoides* were also abundant in the moderately degraded sites in the low-level intertidal zone (6.39% cover).

Diez *et al.* (2009b) recognised that, while calcareous red algae including *Corallina* spp. were generally considered tolerant to domestic pollution, previous literature showed their partial replacement by *Gelidium pusillum* near outfalls. The authors stated it seemed likely that the differences in *Corallina* spp. and *Gelidium pusillum* abundances were related to the intensity and nature of pollution, with the latter being more tolerant to anthropogenic disturbance. The authors concluded that there was a partial recovery of phytobenthic marine assemblages following pollution reduction over the study period due to the treatment plant. However, it was unclear whether the effects were due to one or more of the contaminants components of the wastewater effluent.

Gorostiga & Diez (1996) studied the percentage cover of *Mesophyllum lichenoides* and *Corallina officinalis* among other sublittoral benthic macroalgae, in the summers of 1984 and 1992, in ‘Abra de Bilbao’ (Basque coastline in Northern Spain) an area impacted by industrial and domestic non-treated wastewater. The main pollutants in the wastewater included metals (iron, cadmium, nickel, lead, arsenic and zinc) and nutrients. Evidence suggested that the amount of discharge of wastewater had decreased due to a reduction in industrial activity and closure of mineral washeries, during the time period. The volume of industrial sewage discharge (which included metals) decreased from 67,454 m^3/day in 1984 to 40,374 m^3/day in 1992.

The main metal pollutants also decreased; iron decreased from 53,824 kg/day to 7, 977 kg/day, cadmium decreased from 39.1 kg/day to 0.9 kg/day, arsenic decreased from 1121 kg/day to 0 kg/day, copper decreased from 345 kg/day to 59 kg/day, nickel decreased from 34.3 kg/day to 17.3 kg/day, lead decreased from 189.6 kg/day to 58.6 kg/day and zinc decreased from 3520 kg/day to 238 kg/day.

The study established seven study sites along the 17 km coastline, split into four structural levels along a pollution gradient. Site 4 was regarded as level 1, closest to the pollution source, sites 2, 3 and 5 were level 2 as they were frequently affected by pollution, sites 6 and 7 were level 3 as they are often affected by pollution plumes due to coastal currents, and site 1 was a site not affected by the pollution plume. Following a transect along the coast, quadrats were used to estimate the percentage cover of algae species, overall and at each site. They reported that *Mesophyllum lichenoides* had the greatest percentage cover in both years, with an average percentage cover of 33.51% in 1984, which increased to 43.52% in 1992. It was common in all sites, except site 4 close to the pollution source which had an average cover of 0.01% in 1984 and 0.18% in 1992. The average cover overall and cover at sites 1, 4, 5 and 7 increased over the time period, which directly correlated with the decrease in wastewater pollution in the area and allowed the species to recover and restore cover. *Corallina officinalis* was not as common in all sites and had an average percentage cover 0.08% in 1984 and increased to 0.11% in 1992. In level 1, closest to the pollution source, results found the lowest percentage cover of the crustose layer which includes *Mesophyllum lichenoides* and *Corallina officinalis* in both years (0.1 % cover in 1984 and 0.2% cover in 1992). In the other levels, there was a significant increase in crustose layer cover between 1984 and 1992, *Mesophyllum lichenoides* developed due to an absence of canopy cover and weakened understory layer, and *Corallina officinalis* increased and replaced ceramiaceous algae, which is an indication of improved water quality.

Gorostiga & Diez (1996) concluded that the decrease in pollution between 1988 to 1992 led to a decrease in sedimentation, turbidity and toxicity of the waters in the study area, and a decrease in conditions that impacted algae development. However, it was unclear whether the effects were due to one or more of the contaminants components of the wastewater effluent.

Soltan *et al.* (2001) studied changes in macroalgal communities (included *Corallina elongata* assemblages) due to wastewater discharge from the urban area of Marseille, France into the sea directly on the coast at Coritou Cove. Samples were taken after a treatment plant for the sewage were set up (1995 to 1996) and was compared to results from a previous study (1972 to 1974). In 1978, the volume of effluent discharged (which included nutrients and heavy metals lead, cadmium, copper, zinc, mercury and iron) was recorded as 319,387 m^3 /day in the winter and 801,415 m^3 /day in the summer. After the treatment plant was set up, the volume of effluent decreased to 248,336 m^3 /day in 1995 and 261,850 m^3 /day in 1996. Sewage flowed from two small coastal rivers and was transported westwards by currents and wind. Samples were taken at five sites that occurred in a pollution gradient along the coastline. Sites 1 to 4 were at incremental distances from the outfall and site 5 was the control due to its lack of exposure to the pollution plume. Whole macroalgal assemblages were collected from 20 x 20 cm quadrats and sorted in laboratory conditions to examine taxa identification and percentage algae cover.

They reported that more than half of the taxa recorded in 1995 to 1996 were not observed in the earlier samples, suggesting the number of taxa had increased since the treatment plant had been set up. Amongst the macroalgal assemblages, *Corallina elongata* was recorded in all samples and had the highest percentage cover. In the earlier study, before the treatment plant, the percentage cover of *Corallina elongata* ranged from 50 to 100% in the polluted sites 1 to 4, but in unpolluted site 5, the percentage cover was ≤ 3%. In contrast, in the study period 1995 to 1996, after the treatment plant was set up, the percentage cover of *Corallina elongata* decreased to between 12 to 80% in sites 1, 2 and 4. Other algae species, *Lithophyllum incrustans* and *Lithophyllum lichenoides* were also present at the sites in a lower percentage cover that did increase in 1995 to 1996 study.

Soltan *et al.*'s (2001) evidence suggest that *Corallina elongata* are very tolerant and dominate polluted areas, developing close to outfalls. Since, the development of the treatment plant, the macroalgae communities became more diverse, and the former dominating *Corallina elongata* decreased in cover and was replaced by other turf building algae. The authors concluded that these changes can be linked to the decrease in effluent pollution in years after the treatment plant was set up.

Verlaque (2010) analysed the condition of *Lithophyllum byssoides* in the Mediterranean. In the field, the author identified that in the Northwestern Mediterranean, there was a general deterioration on the living surface of *Lithophyllum byssoides* rims because of exposure to increasing sea surface water pollution and the species was disappearing from areas exposed to discharge from urban outflows.

8 Sensitivity assessment

The 'worst-case' ranked mortalities are summarised in Table 8.1 below together with proposed species level resistances and sensitivities. The relevant resilience assessment are detailed below and the overall sensitivity assessments for maerl-forming species discussed in the following section.

Table 8.1 Summary of count of worst-case ranked mortalities to 'contaminants reported in the evidence review and resultant proposed sensitivity assessments for Coralline algae species.

Group/type/species name	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Hydrocarbons (Petrochemical)								
Diesel WAF								
Phymatolithon lenormandii		1			1	M	H[7]	L
Oil spill								
Corallina officinalis			1		1	H	H	NS
Lithophyllum incrustans			1		1	H	H	NS
Mesophyllum lichenoides			1		1	H	H	NS
Total		**1**	**3**		**4**	**H**	**H**	**NS**
Inorganic chemicals								
Hydrogen peroxide								
Lithothamnion soriferum		1		1	2	M	VL	M
Metals								
Antimony								
Corallina officinalis				1	1	H	H	NS
Cadmium (and its compounds)								
Phymatolithon calcareum				1	1	H	H	NS
Copper								
Phymatolithon calcareum				1	1	H	H	NS
Lead and organic lead compounds								
Phymatolithon calcareum				1	1	H	H	NS
Nickel (and its compounds)								
Phymatolithon calcareum				1	1	H	H	NS
Zinc								
Phymatolithon calcareum				1	1	H	H	NS
Total				**6**	**6**	**H**	**H**	**NS**
Mixtures								

[7] Resilience based on other encrusting species

Group/type/species name	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Wastewater discharge								
Corallina officinalis	1		4		5	L	M	M
Jania rubens			1		1	H	H	NS
Lithophyllum incrustans	1		2		3	L	M	M
Lithophyllum lichenoides	1		1		2	L	M	M
Mesophyllum lichenoides	1		3		4	L	M	M
Phymatolithon lenormandii	1				1	L	H	L
Total	**5**		**11**		**16**	**L**	**M**	**M**
Personal Care Product Chemicals (PPCPs)								
Benzophenone-3								
Lithothamnion spp.			1		1	H	H	NS
Pesticide/Biocide								
Atrazine								
Neogoniolithon fosliei				1	1	H	H	NS
Diuron								
Porolithon onkodes				2	2	H	H	NS
Neogoniolithon fosliei				1	1	H	H	NS
Hexazinone								
Neogoniolithon fosliei				1	1	H	H	NS
Radionuclide								
Plutonium								
Jania rubens				1	1	H	H	NS
Overall total	**5**	**1**	**15**	**14**	**35**			

8.1 Resilience and recoverability

Information on resilience (recovery rates) was taken from MarLIN.

8.1.1 Maerl-forming species

Maerl thalli grow very slowly (Adey & McKibbin, 1970; Potin *et al.,* 1990; Littler *et al.,* 1991; Hall-Spencer, 1994; Birkett *et al.,* 1998a; Hall-Spencer & Moore, 2000a, b) so that maerl deposits may take hundreds of years to develop, especially in high latitudes (BIOMAERL, 1998). Species of maerl are extremely slow growing. Growth rates of European maerl species range between tenths of a millimetre to 1 millimetre per annum (Bosence & Wilson, 2003). The growth rates of the three most abundant species of maerl in Europe (*Phymatolithon calcareum*, *Lithothamnion glaciale* and *Lithothamnion coralloides*) ranged between 0.5 to 1.5 mm per tip per year under a wide range of field and laboratory conditions (Blake & Maggs, 2003).

Individual maerl thalli may live for >100 years (Foster, 2001). Maerl beds off Brittany are over 5500 years old (Grall & Hall-Spencer, 2003) and the maerl bed at St Mawes Bank, Falmouth was estimated to have a maximum age of 4000 years (Bosence & Wilson, 2003) while carbon dating suggested that some established beds may be 4000 to 6000 years old (Birkett et al. (1998a). A maerl bed in the Sound of Iona is up to 4000 years old (Hall-Spencer *et al.,* 2003). Maerl is highly sensitive to damage from any source due to this very slow rate of growth (Hall-Spencer, 1998). Maerl is also very slow to recruit as it rarely produces reproductive spores. Maerl is considered to be a non-renewable resource due to its very slow growth rate and its inability to sustain direct exploitation (Barbera *et al.,* 2003; Wilson *et al.*, 2004).

Maerl species in the UK propagate mainly by fragmentation (Wilson *et al.,* 2004). Recruitment of *Phymatolithon calcareum* is mainly through vegetative propagation. Although spore bearing individuals of *Phymatolithon calcareum* thalli have been found in the British Isles, the crustose individuals that would result from sexual reproduction have yet to be recorded in the British Isles (Irvine & Chamberlain, 1994). Recruitment may occur from distant populations that exhibit sexual reproduction and have crustose individuals (e.g. Brittany). Hall-Spencer (pers. comm.) observed that colonization of new locations by maerl can be mediated by a 'rafting' process where maerl thalli are bound up with other sessile organisms that are displaced and carried by currents (e.g. *Saccharina latissima* holdfasts after storms). Cabioch (1969) suggested that *Phymatolithon calcareum* may have phasic reproduction with peaks every six years. This may account for observed changes in the relative proportions of live *Lithothamnion coralloides* and *Phymatolithon calcareum* in maerl beds. Dominance cycles with periods of about thirty years have been recorded on some of the maerl beds of northern Brittany. Adey & McKibbin (1970) undertook growth studies of *Phymatolithon calcareum* in the field and under laboratory conditions. Field studies in the Ria de Vigo, show that growth occurs predominantly in the summer and suggests an annual growth of about 0.55 mm/year for branch tips of *Phymatolithon calcareum* (Adey & McKibbin, 1970). Newly settled maerl thalli have never been found in the British Isles (Irvine and Chamberlain, 1994). Hall-Spencer (2009) wrote a maerl recovery report for the Port of Falmouth development initiative. Hall-Spencer (2009) suggested that a live maerl bed would take 1000’s of years to return to the site of navigation channel after planned capital dredging in the Fal estuary. He also suggested that it would take 100’s of years for live maerl to grow on a translocated bed, based on the growth and accumulation rates of maerl given by Blake *et al.* (2007) (Hall-Spencer, 2009).

Resilience assessment. The current evidence regarding the recovery of maerl suggests that if maerl is removed, fragmented or killed then it has almost no ability to recover. Therefore, resilience is assessed as **'Very low'** and probably far exceeds the minimum of 25 years for this category on the scale in cases where the resistance is Medium, Low or None.

8.1.2 Coralline algae

Coralline algae are red algae (Rhodophyta) characterized by a 'stony' thallus because of calcareous deposits contained within the cell walls. Although ubiquitous in marine coastal systems little is understood about the taxonomy, biology and ecology of these taxa (Littler & Littler, 2013). 'Coralline crust' is a generic term that, in UK biotopes, refers to non-geniculate (crustose) species from the family Corallinacea that could include *Lithophyllum incrustans,* which forms thick crusts in tidepools, especially in the south-west (Adey & Adey, 1973), *Lithothamnion* spp. and *Phymatolithon* spp. Due to the lack of evidence for species the assessments are generic, although species-specific information is presented where available. *Corallina officinalis* is a geniculate (articulated) species that occurs as a dense turf in this biotope. The fronds grow from a crustose base, similar to a coralline crust.

Recolonization of the coralline crust and the turf of *Corallina officinalis* will require either regrowth from surviving holdfast or basal crusts or recolonization by propagules. The crustose holdfast or base of *Corallina officinalis* is perennial and grows apically (continuous growth at tips), similar to encrusting corallines such as *Lithothamnia* sp. The basal crust may grow continuously until stimulated to produce fronds (Littler & Kauker 1984; Colhart & Johanssen, 1973). Littler & Kauker (1984) suggest that the crustose bases were adapted to resist grazing and desiccation whereas the fronds were adapted for higher primary productivity and reproduction. The basal crusts are tougher than the upright fronds (requiring a pressure of 94 g/mm^2 to penetrate compared to 43 g/mm^2). Regeneration of the basal crusts provides a more rapid route to recovery than recolonization. Experiments in the intertidal in southern California found that areas scraped back to crusts recovered four times more rapidly than sterilised plots where the crusts were removed (Littler & Kauker, 1984).

In culture, *Corallina officinalis* fronds exhibited an average growth rate of 2.2 mm/month at 12 and 18°C. The growth rate was only 0.2 mm/month at 6°C and no growth was observed at 25°C (Colhart & Johanssen, 1973). Similarly, Blake & Maggs (2003) observed much higher growth rates of 2 mm/month over six months starting from September in *Corallina officinalis* grown in Strangford Lough (Northern Ireland) at 5 and 10m depth, these rates are similar to those observed by Andrake & Johansen (1980) in winter in New Hampshire. The evidence for growth rate suggests that to achieve a height of 10 cm the turf would be at least four years old (probably older as higher temperatures appear to slow growth. A low-level turf of, for example 5 cm, could theoretically be achieved within two years.

Where the bases are removed, recovery will depend on recolonization. Areas that are cleared during the reproductive period have the potential to be rapidly colonized. *Corallina officinalis* was shown to settle on artificial substances within 1 week of their placement in the intertidal in New England summer (Harlin & Lindbergh, 1977). However, settlement plates laid out in the autumn were not recolonized until the next spring. In the lower rocky intertidal in southern California dominated by *Corallina officinalis* with foliose overstorey algae present, in this habitat, Littler & Kauker (1984) experimentally cleared plots and followed the recovery for 12 months. Some areas were scraped allowing the basal crusts to remain whereas others were completely sterilised (removal of all material and surfaces then scorched with a blow torch to remove bases). In scraped plots, up to 15% cover of *Corallina officinalis* fronds returned within 3 months after removal of fronds and all other epiflora/fauna while in sterilized plots (all basal crusts removed) appearance of articulated fronds occurred six months following clearance (Littler & Kauker, 1984). At the end of the 12-month observation period *Corallina officinalis* cover had increased to approximately 18% in plots where basal crusts remained and to approximately 10% in sterilised plots. Similarly, Bamber & Irving (1993) reported that new plants grew back in scraped transects within 12 months, although the resistant crustose bases were probably not removed.

Once established turfs of *Corallina* spp. can persist for a long time. Surveys of rocky intertidal ledges at Hinkley point, Somerset in England found that the patches mapped in the 1980s (Bamber & Irving, 1993) had not changed position when resurveyed 18 years later (Burdon *et al*., 2009). It has been speculated but not definitively demonstrated that turf-forming algae and canopy-forming algae may represent alternate stable states on temperate rocky shores and a shift in balance to the alternate state may prevent recovery. For example, Lubchenco (1980) found that the removal of *Chondrus crispus* turf allowed the establishment of *Fucus* spp. on shores in New England. Removal of grazers and the turf allowed *Fucus* spp. to establish 100% cover, highlighting the significance of grazers in structuring the biotope. Some potential mechanisms for inhibition of canopy-forming species are space pre-emption by turfs that prevent recruitment of taller algae (Perkol-Finkel & Airoldi, 2010, Kennelly, 1987) due to the coverage of suitable rock surfaces and the presence of sediments within the turf (Airoldi, 2003).

A number of papers by Edyvean & Ford (1984a & b; 1986;1987) describe aspects of reproduction and growth of encrusting coralline, *Lithophyllum incrustans*. Studies by Edyvean & Ford (1987) in populations of *Lithophyllum incrustans* in Pembroke south-west Wales suggest that reproduction occurs on average early in the third year. Reproduction may be sexual or asexual. Populations release spores throughout the year but abundance varies seasonally, with the populations studied in Cullercoats Bay, and Lannacombe Bay (North East and South West England, respectively) producing fewer spores in the summer. Spore release is initiated by changes in temperature or salinity (see relevant pressure information) at low tide so that spore dispersal is restricted to within the tide pool enhancing local recruitment. Spore survival is extremely low with only a tiny proportion of spores eventually recruiting to the adult population (Edyvean & Ford, 1986). The spores are released from structures on the surface called conceptacles, these are formed annually and subsequently buried by the new layer of growth. Plants can be aged by counting the number of layers of conceptacles. Edyvean & Ford (1984a) found that the age structure of populations sampled from Orkney (Scotland) Berwick (northern England) and Devon (England) were similar, mortality seemed highest in younger year classes with surviving individuals after the age of 10 years appear relatively long-lived (up to 30 years). In St Mary's Northumberland, the population was dominated by the age 6 to 7-year classes (Edyvean & Ford, 1984a). Growth rates were highest in young plants measured at Pembroke (south-west Wales) with an approximate increase in diameter of plants of 24 mm in year class 0 and 155 mm in year 1 and slowing towards an annual average horizontal growth rate of 3 mm/year (Edyvean & Ford, 1987).

Some repair of damaged encrusting coralline occurs through vegetative growth. Chamberlain (1997) observed that, although *Lithophyllum incrustans* was quickly affected by oil during the *Sea Empress* oil spill, recovery occurred within about a year. The oil was found to have destroyed about one third of the thallus thickness but regeneration occurred from thallus filaments below the damaged area. Recolonization by propagules is an important recovery mechanism. Airoldi (2000) observed that encrusting coralline algae recruited rapidly on to experimentally cleared subtidal rock surfaces in the Mediterranean Sea, reaching up to 68% cover in two months. As encrusting corallines are sensitive to desiccation (Dethier, 1994) it should be noted that these subtidal habitats are probably more favourable for recruitment, growth and survival than intertidal rock pools.

Resilience assessment. The resilience of coralline algae is assessed as **'High'** where resistance is 'High' (no significant impact) or 'Medium' (where <25 % of *Corallina officinalis* fronds or encrusting corallines are removed and coralline crusts remain), based on regrowth from the basal crusts and vegetative growth from surrounding turfs or crusts. Where resistance is assessed as 'Low' or 'None' then resilience is assessed as **'Medium'** (2-10 years) for the *Corallina officinalis* turf and encrusting corallines.

8.2 Sensitivity assessment - Hydrocarbons (Petrochemical)

The effect of petrochemical hydrocarbons on encrusting coralline algae was only reported by a single paper using intertidal field mesocosms (Bokn *et al.*, 1993). They reported that the encrusting coralline *Phymatolithon lenormandii* exhibited a significant decrease in cover in the upper shore mesocosm after exposure to 129.4 µg/l Diesel WAF for two years but an increase in cover in the mid and lower shore mesocosms. This suggests that diesel WAF only affect the species in the more physiologically demanding upper shore. However, the resultant worst-case resistance of *Phymatolithon lenormandii* could be assessed as **'Medium'**. No information on its recovery was available. If it is similar to other encrusting corallines then its resilience is probably '**High**' and its sensitivity **'Low'**. However, it is difficult the extrapolate to maerl-forming species with any confidence, especially based on a single study.

The effect of oil spills on coralline species was reported in several papers but no evidence on the effects on maerl-forming species was found. Diaz *et al.* (2009a) examined changes in macroalgal abundance (inc. *Corallina elongata* and *Lithophyllum incrustans*) along the Basque coast after the *Prestige* oil spill but did not find any significant differences between oiled and non-oiled sites. However, Bowman *et al.* (1978) reported that 100% of cover of Lithothamnia was bleached and dead rims of Lithothamnia in lower shore rock pools after the Dounreay oil spill and treatment with BP100X. Similarly, Newey & Seed (1995) reported bleached and dead coralline algae (no species were specified) in mid-shore rockpools close to the wreck of the *Braer* oil tanker. Jackson *et al.* (1989) also reported that crustose corallines (no species were specified) and other fleshy algae decreased in cover after the Panamanian oil spill, to levels below those observed before the spill. Crump *et al.,* (1999) reported that encrusting coralline algae, *Lithothamnion incrustans, Phymatolithon purpureum,* and *Corallina officinalis* were bleached in West Angle Bay immediately after the *Sea Empress* oil spill but recovered quickly, which suggested only the surface layers were affected rather than individuals were killed. Crump *et al.* (1999) also stated that previous literature has shown oil and dispersants to have harmful effects on the pigmentation of red algae in experimental conditions.

Smith *et al.* (1968) reported that the dispersants used to treat the *Torrey Canyon* oil spill killed *Corallina* and *Lithophyllum* in shallow rock pools, while those in deep pools survived, and that *Corallina* was killed in high shore pools but appeared healthy in mid shore pools. Overall, the effects of detergents depended on duration and concentration of exposure, assuming lower shore populations were exposed for shorter periods than higher-shore populations as the tide returned.

Felder *et al.* (2014) and Fredericq *et al.* (2014) both examined the effects of the DWH oil spill on deep (50-77 m) 'rhodolith' beds in the Gulf of Mexico. They both reported that the 'rubble' and 'rhodoliths' were visibly bleached and where the encrusting calcareous algae were alive, the diverse seaweed community was lost. They suggested that the rhodolith and encrusting species included *Lithophyllum* sp., *Lithothamnion* sp., *Mesophyllum* sp., and *Porolithon* sp.

The above evidence suggests that exposure to oil spills and/or their dispersants can result in bleaching or death of calcareous coralline algae, especially encrusting corallines, depending on the length of exposure, shore height, and type of oil. Therefore, the **resistance of encrusting corallines or *Corallina* sp. to exposure to oil spills and dispersants is assessed as 'Low'** based on the worst-case scenario reported by Bowman *et al.* (1978). Hence, resilience is assessed as '**Medium'** and sensitivity as **'Medium'**. Confidence in the assessment is 'Medium' due to the variation in the effect between studies.

No direct evidence of the effect of petrochemical hydrocarbons on maerl-forming species or their beds was found. However, the evidence of effects on similar species, e.g. *Phymatolithon* spp. and *Lithothamnion* spp. above suggests that maerl-forming species and their beds may experience similar bleaching and possible death depending on exposure to oil spills. As maerl beds are sublittoral they may be protected from direct exposure, although the evidence from the DWH spill at 50 to 77 metres suggests they are not immune. The potential for smothering by oil was not discussed. However, Tuya *et al*. (2023) suggested oil spills were a potential threat to 'rhodoliths' beds worldwide. Therefore, **the resistance of maerl-forming species and their beds to exposure to oil spills and/or dispersants is assessed as 'Low' as a precaution**, albeit with **'Low' confidence** due to the lack of direct evidence. Hence, resilience is assessed as '**Very low'** and sensitivity as **'High'**.

8.3 Sensitivity assessment – Transitional metals

Only sublethal effects were reported after exposure of calcareous red algae to heavy metals (Amiard, 1973; Wilson *et al*., 2004). In particular, Wilson *et al.* (2004) exposed the maerl-forming species *Phymatolithon calcareum* to a single dose of a mixture of heavy metals in the ratio 37:16:14:11:1, Zn: Pb: Ni: Cu: Cd, where the Cd concentration of ranges from 0.174 to 174 ppb and 1.74 ppb represented standard industrial effluent. *Phymatolithon calcareum* experienced a significant reduction in photosynthetic capacity depending on concentration but recovered quickly. However, the authors noted that longer term exposure to heavy metals may have chronic effects. Nevertheless, only **sublethal effects** were reported. **'Insufficient evidence' is recorded as it is imprudent to suggest that all maerl-forming species are 'Not' sensitive' without further evidence.**

8.4 Sensitivity assessment – Synthetic compounds

The herbicides Diuron, Atrazine and Hexazinone were found to inhibit photosynthesis in crustose coralline algae (Harrington *et al.* 2005; Negri *et al.,* 2011; McCoy & Kamenos, 2015). Negri *et al.* (2011) reported that diuron was the most toxic after 24 hours, then hexazione, and then atrazine in *Neogoniolithon fosliei.* Harrington *et al.* (2005) reported that diuron was also toxic to *Porolithon onkodes* when applied alone. Visible bleaching of *Porolithon onkodes* was observed at 29 µg/l of diuron, likely due to the destruction of chloroplasts and carotenoids that causes its colouration to lighten. However, when exposure to diuron was combined with sedimentation, the toxicity of diuron was greater and the time taken to recover in clean water was increased. However, MacVicar *et al.* (2022) reported no effects of exposure of *Lithothamnion* spp. to a high concentration of the UV filter oxybenzone (benzophenone-3; BP-3) after 15 days.

A reduction in photosynthesis is likely to reduce growth rates and increase an individual's susceptibility to other stresses, such as sedimentation. Nevertheless, only **sublethal effects** were reported based on short-term experiments and no evidence of long-term effects were found. **'Insufficient evidence' is recorded as it is imprudent to suggest that all coralline algae and maerl-forming species are 'Not' sensitive' to exposure to herbicides without further evidence.**

8.5 Sensitivity assessment – radionuclides

Hernández *et al.* (2011) reported that *Corallina elongata* and *Jania rubens* accumulated plutonium (Pu) in granules but did not report any adverse effects on the species. Hence, the evidence does not support an assessment of resistance or sensitivity.

8.6 Sensitivity assessment – Inorganics

Legrand *et al.* (2022) examined the effects of exposure to hydrogen peroxide (H_20_2), used as an antiparasitic treatment in Norwegian salmon farms, on photosynthesis in the maerl-forming species *Lithothamnion soriferum*. Photosynthesis was significantly reduced at concentration >=200 mg/l H_20_2 after 1-hour exposure but recovered after 48 hours or 28 days. However, they also showed significant bleaching (28% at 200 mg/l or 63% at 2,000 mg/l) after 28 days recovery. McCoy & Kamenos (2015) noted that bleaching could indicate death in coralline red algae and could result in structural damage but was also reversible, and the long-term effects of bleaching required further study. The authors went on to suggest that prolonged or repeated exposure may have greater impact. The authors also noted that the crustacean fauna of maerl beds may be particularly sensitive to H_20_2 so the diversity of affect maerl beds may be threatened, although further study was required (Legrand *et al.*, 2022).

Overall, the evidence suggests that the maerl-forming species *Lithothamnion soriferum* is adversely affected by hydrogen peroxide exposure >=200 mg/l, resulting in reduced photosynthesis, reduced growth, and significant bleaching. Therefore, **resistance is assessed as 'Medium' as a precaution** but with 'Low' confidence as mortality was not reported directly. Hence, resilience is assed as **'Very low'** and sensitivity as **'Medium'**.

8.7 Sensitivity assessment – Mixtures

Eight articles examined the effects of mixed effluents and wastewater discharges on *Corallina officinalis, Jania rubens, Lithophyllum incrustans, Lithophyllum lichenoides, Mesophyllum lichenoides* and *Phymatolithon lenormandii* (Bellgrove *et al.*, 2017; Diez *et al.*, 1999, Diez *et al.*, 2003, Diez *et al.*, 2009b, Gorostiga & Diez, 1996, Soltan *et al.*, 2001). The wastewaters were reported to result in 'significant' mortality in all of the species studied, except *Jania rubens*. However, it was not possible to distinguish between the effects of any one component of the effluent and it is probable that sedimentation, turbidity and nutrient enrichment were responsible for the effects reported, rather than contaminants alone. Therefore, **no sensitivity assessment** is attempted.

Verlaque (2010) and Blanfoume *et al.* (2016) attributed a reduction the distribution and condition of large bioconstructions called *Lithophyllum byssoides* rims to urban wastewater and a reduction in water quality. However, no single type of contaminant was identified. Therefore, **no sensitivity assessment** is attempted.

9 Conclusions

The evidence on the effects on contaminants on maerl-forming species of red coralline algae was limited to only two articles and, hence, two types of contaminants, hydrogen peroxide and heavy metals. Expanding the scope of the literature review to include other coralline red algae increased the coverage of the review to only 15 articles in total after screening for relevance.

Only one paper reported significant bleaching in the maerl-forming species maerl-forming species *Lithothamnion soriferum,* while oil-spills were reported to result in bleaching and death of coralline algae in some instances, depending on location, shore height, and exposure. All other effects reported in the evidence collated were sublethal.

Therefore, there was ‘insufficient evidence’ on which to base a sensitivity assessment for most of the contaminant types reported in the evidence. Where sensitivity assessment were suggested (oil spills and hydrogen peroxide), they are inferred from other coralline algae or precautionary and made with ‘Low’ confidence. They should be interpreted with caution.

In conclusion, the evidence on the effects of contaminants on coralline red algae and especially, maerl-forming species is extremely limited. Further evidence is required to inform sensitivity assessment. A more extended study of red algae (Phylum Rhodophyta) as a group may provide a better evidence base on the likely effects of contaminants in maerl-forming species.

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11 Appendix 1

The evidence extracted (or mapped) is limited to fields likely to be relevant to sensitivity assessment or to categorise the 'level of effect' recorded in each article. The extensive systematic map suggested by Randall *et al.* (2015) was felt to be too onerous.

The field names and standard terms used within the 'Evidence summaries' were developed during Phase 2 and 3, based on terms used by the US EPA ECOTOX database or MarLIN glossary, or adapted from the literature review, wherever possible or relevant. Not reported (NR) is used wherever the relevant data/evidence is not reported or specified in the evidence. The field names and relevant standard terms follow.

Short citation

Standard short form of citation for article/paper/book/ report etc.

Study type

Outline of the type of study adapted from ECOTOX definitions:

Term	Definition
Field (obs.)	Observation in the field e.g., effect of spills, physical disturbance
Field (expt.)	Field based study, e.g., in situ mesocosm, field based experimental design exposed and control plots/quadrats/transects
Laboratory	Experimental or observational study conducted under laboratory conditions
Mesocosm	Experimental or laboratory studies conducted within mesocosms either based in the laboratory or the field
Review	Review article (paper/report). Reviews used as sources of evidence and only novel data in reviews included, originals articles examined for detail
Survey	Survey of multiple site presence/absence/abundance etc. of chemical or species

Note –chemical analysis requires access to a laboratory but is not included within the study type.

Chemical names and groups

'Contaminants group', 'contaminant type', 'contaminant name' and 'CAS number' from the agreed 'Contaminant Chemicals Groups' March 2022' spreadsheet. Two versions of 'contaminant name' are listed:

- 'Contaminant name' reported by the article cited, and
- 'Contaminant synonym' used by ECOTOX or others, if available and different from 'contaminant name'.

Species name

The name of the species studied as reported in the original article. Relevant synonyms, based on WoRMS, are used in the report text.

Life stage studied

Terms defined in MarLIN glossary

- Adult
- Juvenile
- Larvae
- Embryo
- Egg
- Sporophyte
- Gametophyte
- Multiple

Exposure concentration

The experimental concentrations the samples were exposed to, where available, and expressed in reported units and µg/l where possible.

Exposure type

Definitions of the type or route of exposure to the contaminant, adapted from ECOTOX.

Term	Definition
Environmental	Field and incidental exposures, includes via the water column or sediment
Environmental (sediment)	Optional where sediment concentration are paramount (e.g. sedimentary communities)
Flow-through	Continuous or frequent flow through test chamber with no recycling
Food	Introduced via food
Lentic	Static water without measurable flow e.g., lakes, ponds, lagoons
Pulse	Intermittent or fluctuating dosing
Renewal	Without continuous flow of solution, but with occasional renewal of test solutions after prolonged periods, e.g., 24 hours
Spill	Incidental spills
Static	Toxicity tests with aquatic organisms in which no flow of test solution occurs; solutions may remain unchanged throughout the duration of the test.
Tidal	Affected by tides

Study duration

The length of the study and reported by article in hours, days, months or years etc.

Exposure Duration (ECOTOX definition)

The Exposure Duration is the time of actual exposure to the chemical and is expressed as ‘days’. In cases where the observation time is the only duration reported, it is assumed that the Exposure Duration is equivalent to the longest observation time (field: Observed Duration).

For most field studies the ‘Exposure’ and ‘Study Duration’ are identical because it is difficult to determine when the exposure ends. For lab studies the ‘Exposure’ and ‘Study Duration’ may be different, such as when effect measurements were reported from a post-exposure period. For lab studies with injection, topical, or dietary (e.g., intraperitoneally or by gavage) exposure, ‘Exposure and Study Duration’ are typically the same.

For a fluctuating or intermittent dosing experiment, the total exposure time is recorded. In some instances, a biological, or qualitative, time is used, such as an exposure time reported as "until hatch", "growing season" or "after the nth egg has been laid".

Effect group (definitions from ECOTOX)

Term	Definition
Accumulation	Measurements and endpoints that characterize the process by which chemicals are taken into and stored in plants or animals; includes lethal body burden
Behaviour/Avoidance,	Activity of an organism represented by three effect groups - avoidance, general behaviour, and feeding behaviour
Biochemical (inc. enzyme(s), hormone(s))	Measurement of biotransformation or metabolism of chemical compounds, modes of toxic action, and biochemical responses in plants and animals; includes three effect groups - biochemical, enzyme and hormone effects
Cellular/ Histology/ Genetic	Measurements and endpoints regarding changes in structure and chemical composition of cells and tissues of plants or animals as related to their functions; includes three effect groups -cellular, genetic and histological effects
Ecosystem process	Measurements and endpoints to track the effects of toxicants on ecosystem processes; includes microbial processes
Growth/ Development/ Morphology	Category encompasses measures of weight and length, and includes effects on development, growth, and morphology
Mortality	Measurements and endpoints where the cause of death is by direct action of the chemical
Multiple	Measurements related to multiple or undefined effect.
No Effect	The author reported an end point but not a specific effect
Physiology/ Immunological/ Injury/ Intoxication	Measurements and endpoints regarding basic activity in cells and tissues of plants or animals; includes four effect groups - injury, immunity, intoxication, and general physiological response
Population	Measurements and endpoints relating to a group of organisms or plants of the same species occupying the same area at a given time

Term	Definition
Reproduction	Measurements and endpoints to track the effect of toxicants on the reproductive cycle; includes behavioural and physiological measurements

Effect measurement

A description of the effect measured. These are likely to vary between different taxonomic groups. The ECOTOX database includes many more categories than listed below for some of the ‘effect groups’; the numbers are given in brackets. Examples of standard ‘effect measurement’ terms, organized by ‘effect group’, include:

- Accumulation
  - Body burden
  - BCF
- Behaviour/Avoidance
  - Chemical avoidance
  - Substratum avoidance
- Biochemical (ECOTOX =1,641 entries)
  - Acyl-CoA oxidase activity
  - Acetylcholinesterase (AchE) activity
  - Acid phosphatase
  - Catalase (CAT)
  - Cytochrome P450 activity
  - Gamma-Glutamyl Transpeptidase
  - Glutathione disulphide
  - Glutathione peroxidase (GPX),
  - Glutathione reductase (GR),
  - Heat shock proteins
  - Lactate dehydrogenase
  - Lipid peroxidation,
  - Metallothioniens
  - MFO (BPH, CYP-dependent monoxygenase)
  - Multixenotoxicity resistance
  - NADPH-Neo tetrazolium Reductase activity

    - NF-E2-related factor 2 (Nrf2),
    - Superoxide dismutase (SOD)
- Cellular (ECOTOX has 143 entries)
    - DNA damage/Micronuclei/Adduct formation
    - Genotoxicity
    - Haemocyte counts population
    - Phagocytosis
    - Lysosomal membrane stability
    - Ovarian and spermatic follicles
    - Transmembrane sodium energy gradient
    - Transcriptomics
- Ecosystem processes
    - General
    - Reduced/Increased productivity (primary/secondary)
    - Community
- Growth/Development/Morphology
    - Abnormal development/larvae
    - Growth rate
    - Leaf/shoot/rhizome/root elongation
    - Leaf shape/morphology
    - Mortality (adult/larval)
    - Adult survival
    - Larval survival
- Physiology/Immunological/Injury/Intoxication
    - Byssal thread production
    - Clearance/filtration rate
    - Excretion rate
    - Larval swimming velocity/ability
    - Respiration rate
    - Condition indices
    - Photosynthetic efficiency
    - PSII function/damage
    - Scope for growth (SFG)
    - Valve gape
    - Population

    - Abundance/biomass
    - Condition
    - Cover/canopy
    - Distribution/extent
    - Diversity
    - Population decline (general)
- Reproduction
    - Fecundity
    - Gametogenesis reduction
    - Gonad index
    - Fertilization success/failure
    - Recruitment success
    - Settlement
    - Sexual maturity (rate/age)
    - Sex ratios
    - Imposex

Response site

The part (or type) of the organism where the effect (response) is measured (or observed). ECOTOX has 594 entries, which vary between taxonomic groups. We should expect to add terms as we tackle more taxonomic groups but use ECOTOX definitions where possible. For example:

- Community
- Digestive gland
- Embryo
- Gametes (oocytes and sperm)
- Gonad
- Haemocytes
- Larva
- Leaf/shoot
- Lysosomes
- Muscle tissue
- Rhizomes/roots
- Population

- Seedling
- Soft tissues
- Whole organism (assumes adult)

End points

List of observed end points reported by the articles examined, used for consistency with ECOTOX data, but also includes population level effects due to environmental exposure, spills etc. For example:

- BCFD - Bioconcentration factor calculated using dry weight tissue concentration
- ECXX– Effect concentration at XX percentile
- ICXX - Inhibition concentration at XX percentile
- IDXX - Inhibition dose at XX percentile
- LCXX– Lethal concentration at XX percentile
- LDXX – Lethal dose at XX percentile
- LTXX – Lethal time at XX percentile
- LOEC/L – Lowest Observable-Effect-Concentration/Level: lowest dose (concentration) producing effects that were significantly different (as reported by authors) from responses of controls (LOEAL/LOEC)
- NOEC/L – No Observable-Effect-Concentration/Level: highest dose (concentration) producing effects not significantly different from responses of controls according to author's reported statistical test (NOEAL/NOEC)
- Mortality (e.g., after spills)
- NR-LETH – 100% Mortality
- NR-ZERO – 0% Mortality
- Population loss
- Population decline
- Recruitment failure

Endpoint concentrations

ECOTOX provides a single concentration or range (with or without confidence intervals) for each Endpoint. ECOTOX lists the confidence intervals as a range (min, max). In the 'Evidence summary' different End point concentrations (or ranges) are listed separately. Lethal (100%) is included where papers give a concentration resulting in 100% mortality, which is one endpoint recorded by ECOTOX.

Concentrations are expressed as mg/l (ECOTOX) and/or µg/l.

Mortality (%) reported

The percentage mortality reported in the articles examined, where available.

Ranked mortality

The mortality reported in the articles examined is 'ranked' according to the MarESA resistance scale. For example:

Ranked mortality	Resistance
Severe (>75%)	None
Significant (25-75%)	Low
Some (<25%)	Medium
None (reported)	High
Sublethal	High
Unspecified	Unspecified

Unspecified = mortality is reported but not quantified or no detail provided

Quality/Applicability of Evidence – based on MarESA scales

Summary of evidence

The relevant evidence from the articles is summarized in narrative form, using the standard MarESA format description of evidence.

'Worst-case' ranked mortality

The reported 'end points' and evidence from each article is expressed as a 'worst-case' ranked mortality for each contaminant examined in each article. For example, where the specimens are exposed to a range of concentrations of one chemical and several 'end points' (e.g., EC_{50}, LC_{50}) determined, the 'worst-case' or greatest mortality is reported.

Please note, many papers examined several different combinations of contaminant type and seagrass species. Therefore, the 'worst case' mortality is recorded for each unique species vs. contaminant combination within each paper but not for every experimental permutation. For example, if a paper studied three metals and one herbicide, then we would report the four 'worst case' mortalities rather than every mortality or effect from every concentration tested.

However, if the papers examined the same combination on three distinct species (e.g., in seagrasses) then we would record twelve separate ‘worst-case’ mortalities.

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