Assessment of IMAP Common Indicator 18: Level of pollution effects of key contaminants where a cause and effect relationship has been established

Geographical scale of the assessment	The Sub-regions within the Mediterranean region by using scientific literature sources
Contributing countries	Countries in alphabetical order: Algeria, Egypt, Italy, Spain, Tunisia, Türkiye based on scientific literature sources
Mid-Term Strategy (MTS) Core Theme	Enabling Programme 6: Towards Monitoring, Assessment, Knowledge and Vision of the Mediterranean Sea and Coast for Informed Decision-Making
Ecological Objective	EO9. Contaminants cause no significant impact on coastal and marine ecosystems and human health
IMAP Common Indicator	CI18. Level of pollution effects of key contaminants where a cause and effect relationship has been established
GES Definition (UNEP/MED WG473/7) (2019)	Concentrations of contaminants are not giving rise to acute pollution events
GES Targets (UNEP/MED WG473/7) (2019)	• Contaminants effects below threshold • Decreasing trend in the operational releases of oil and other contaminants from coastal, maritime and off-shore activities.
GES Operational Objective (UNEP/MED WG473/7) (2019)	Effects of released contaminants are minimized.

Available data

570. The list of bibliographic studies on biomarkers used for the preparation of the 2023 MED QSR is sorted alphabetically by country as shown in Table 3.1.5.1.

571. Based on the literature search results it can be concluded that a comparison among the studies is hard or mostly impossible. This is due to the use of different biomarkers, with different biota species, using different tissues, and different methodologies. Moreover, as found in the 2017 QSR, there are confounding factors that hinders environmental status assessment such as species, gender, maturation status, season and temperature. In addition, an inherent bias exists in publications towards studies showing an effect. Authors and journals do not usually publish studies showing lack of effect or response. Italy submitted national data for CI 18 following the Meeting of CorMon Pollution that took place in Athens, 1-2 March 2023[103].

[103] Data included biomarkers (Acetylcholinesterase activity, Lysosomal membrane stability on cryostat sections, Micronuclei frequency, Metallothioneins, EROD-microsomal, EROD-S9, Fulton's Condition Factor, Gonadosomatic Index and Hepatosomatic Index) were measured in the fish M. barbatus sampled in 2019 and 2020. Data were not uploaded in the IMAP-Info System because they were found not compliant given the lack of data related to the 'maturation key' and of the 'tissue weight', which are considered mandatory. The national data could not be integrated into the CI 18 assessment as the 2023 MED QSR for CI18 was based on the use of regional scientific literature sources, using the evaluation provided by the authors. The newly submitted data of Italy were all for M. barbatus, for which no criteria were adopted yet, by the CPs. The assessment criteria for the biological effects on *M. barbatus* might be set in the future conditional to optimal data reporting by the CPs. Moreover, no conclusions were also set in the scientific literature.

Table 3.1.5.1: Studies on biomarkers in the Mediterranean Sea since 2016 reviewed in present assessment of CI 18. The list is sorted alphabetically by country.

Reference	Country	Sub-region	Sampling year	Taxa	Species	Organ/tissue	Stressor	Biomarker
Kaddour et al. 2021	Algeria	WMS	2019-2020	Fish	*Mullus barbatus*	blood	non specific	MN, NRRT
Amamra et al. 2019	Algeria	WMS	2016	mollusc	*Donax trunculus*	gonad, mantle, digestive gland	non specific	AChE, GST, MDA
Benaissa et al. 2020	Algeria	WMS	2016	mollusc	*Patella rustica*	Soft tissue	desalination brine	AChE, CAT, SOD, GR, GPx, GST, LPO, Genotox
Laouati et al. 2021	Algeria	WMS	2017	mollusc	*Perna perna*	digestive gland and gills	non specific, TM	AChE, CAT, GSH, GST, MDA
Gabr et al. 2020	Egypt	AEL	2018-2019	mollusc	*Ruditapes decussatus*	soft tissue	TM	AChE, SOD, GPx, MDA
Salvaggio et al. 2019	Italy	FAO Area 37	not reported	Fish	*Lepidopus caudatus*	liver, gonads	Microplastic, TM	VTG, MT
Frapiccini et al. 2021	Italy	ADR	2019	Fish	*Mullus barbatus*	muscle	PAH	CAT,SOD,GST,LPO
Chenet et al. 2021	Italy	CEN	2018	fish	*Trachurus trachurus*	liver	plastic	VTG, MT
Morroni et al. 2020	Italy	WMS	2017	Fish	*Diplodus vulgaris*	various	PAH, TM	AChE, MT, MN, LMS, EROD
Morroni et al. 2020	Italy	WMS	2017	Fish	*Mullus barbatus*	various	PAH, TM	AChE, MT, MN, LMS, EROD
Morroni et al. 2020	Italy	WMS	2017	Fish	*Pagellus erythrinus*	various	PAH, TM	AChE, MT, MN, LMS, EROD
Parrino et al. 2020	Italy	WMS	not reported	Fish	*Parablennius Sanguinolentus*	Brain and blood	pesticides	AChE, BChE
Morroni et al. 2020	Italy	WMS	2017	mollusc	*Mytilus galloprovincialis*	various	PAH, TM	AChE, MT, MN, LMS, EROD
Capo et al. 2022	Spain	WMS	2019	Fish	*Sparus aurata*	blood, plasma, liver	microplastic, plasticizers	CAT,SOD,GRd,GPx, MPO, GST, MDA, EROD, BFCOD, CE
Solomando et al. 2022	Spain	WMS	2020	Fish	*S. dumerili*	liver	microplastic	CAT,SOD,GST, EROD, MDA
Rios-Fuster et al. 2022	Spain	WMS	2019	mollusc	*Mytilus galloprovincialis*	Soft tissue	Anthrop. Particles, bisphenols, phthalate	CAT,SOD,GRd,GPx, GST, TES, GLY, CE, LPO, CARB, GSH
Capo et al 2021	Spain	WMS	not reported	mollusc	*Mytilus galloprovincialis*	gills	microplastic	CAT,SOD,GRd,GPx, GST,MDA, ROS
Rodríguez-Romeu et al., 2022	Spain	WMS	2019	Fish	*Engraulis encrasicolus*	Muscle and liver	Anthopogenic items ingestion	AChE, LDH, CS, CE, CAT, GST, EROD
Mansour et al. 2021	Tunisia	CEN	2016	mollusc	*Ruditapes decussatus*	Soft tissue	hydrocarbons	CAT,SOD,GRd,MDA, AChE

Reference	Country	Sub-region	Sampling year	Taxa	Species	Organ/tissue	Stressor	Biomarker
Zaidi et al. 2022	Tunisia	CEN	2018	mollusc	*Patella caerulea*	soft tissue	TM	CAT,SOD,GPx,GST,MDA
Ghribi et al. 2020	Tunisia	CEN	2017 mesocosm	mollusc	*Mytillus spp*	hemolymph, gills, and digestive gland	non specific PAH, TM	CAT, GPx, GST, AChE
Missawi et al. 2020	Tunisia#	CEN	2018	Seaworm	*Hediste diversicolor*	whole (gut cleaned)	Microplastic	CAT,GST,MDA, AChE
Zitouni et al. 2020	Tunisia*	WMS	2018	Fish	*Serranus scriba*	gastrointestinal tract	Microplastic	CAT,GST,MDA, AChE,MT
Telahigue et al. 2022	Tunisia	WMS	2020-2021	mollusc	*Flexopecten glaber*	gills, digestive gland	TM	CAT,SOD,GPx,GSH, MT, MDA
Bouhedi et al 2021	Tunisia	WMS	not reported	polychaete	*Perinereis cultrifera*	whole body	TM	CAT,GST, AChE, MT, GSH, TBARS
Uluturhan et al. 2019	Türkiye	AEL	2015	mollusc	*Mytilus galloprovincialis*	Hepatopancreas	TM, Pesticides	CAT,SOD,GPx, AChE
Uluturhan et al. 2019	Türkiye	AEL	2015	mollusc	*Tapes decussatus*	Hepatopancreas	TM, Pesticides	CAT,SOD,GPx,AChE
Dogan et al, 2022	Türkiye	AEL	2021	Fish	*Mullus barbatus*	muscle, liver	TM	CAT, MDA
Dogan et al, 2022	Türkiye	AEL	2021	Fish	*Boops boops*	muscle, liver	TM	CAT, MDA
Dogan et al, 2022	Türkiye	AEL	2021	Fish	*Trachurus trachurus*	muscle, liver	TM	CAT, MDA

#data related to the WMS as well; * data related to the CEN as well.

Biomarkers Abbreviations: AChE-Acetylcholinesterase, BChE-Butyrylcholinesterase, BFCOD-7-benzyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase, CAT-Catalase, CE-Carboxylesterase, CS-Citrate synthase,EROD-Ethoxyresorufin-O21 deethylase, ETS-Electron Transport System, GLY-Glycogen, GPx-Glutathione peroxidase, GRd-Glutathione reductase, GSH- Glutathione, GST-Glutathione-S-transferase, LDH-Lactate dehydrogenase, LMS-Lysosomal Membrane Stability, LPO-Lipid peroxidation, MDA-Malondialdehyde, MN-Micronucleus Assay, MT-Metallothionein, NRTT-Neutral red retention time, SOD-Superoxide dismutase, SoS-Stress on Stress,VTG-Vitellogenin

Results of the IMAP Environmental Assessment of CI 18 in the Mediterranean region.

Due to absence of any data reporting by the CPs, data for present assessment were retrieved from the scientific literature. The studies surveyed do not include the parameters assessed in the 2017 MED QSR in mussel. The only exception is Morroni et al., 2020 that measured LMS, AChE and MN in *M. galloprovincialis* but not in the same organs except for MN that was measured in haemocytes with a value of 0.3 permil in reference area and a maximal value of 1.3 permil. The maximal value is slightly higher than 1 permil, the MED BAC adopted in Decision IG.23/6. Ghribi et al., 2020 and Uluturhan et al, 2019 reported AChE in haemolymph and hepatopancreas, respectively and not in gills.

572. Given GES assessment was not possible for CI 18 within the preparation of the 2023 MED QSR, the regional overall assessment findings were provided for the Mediterranean as presented here-below. Instead of providing GES /non-GES classification, the assessment for IMAP CI 18 was based on the determination of biomarkers that were affected by contamination.
573. A summary of reviewed studies is sorted by sub-regions and countries. The biomarkers that were affected by contamination are marked in red, those that were not affected are marked in green, while inconclusive results are marked in blue. Moreover, the biomarkers included in the DDs and DSs are highlighted in yellow, but with no differentiation among species or tissues studied.

a) AEL sub-region (Egypt, Türkiye)

574. Egypt. One study was reviewed. The effect of TM was studied in the mussel *Ruditapes decussatus* collected from Alexandrian Port and Port Said (Gabr et al. 2020). The concentrations of metals were higher in samples from the Alexandrian Port (Site I). Malondialdehyde (MDA) and SOD were higher in samples from Site I while GPx, Total protein and AChE were lower. The reported values in this study are considered as basic data to monitor of the anthropogenic influence on the coastal environment.

575. Türkiye**.** Two studies were reviewed for Türkiye: one from 2015 and one from 2022[104]. The effect of TM and pesticides was studied on the molluscs *Mytilus galloprovincialis* and *T. decussatus* collected from Homa Lagoon (Aegean Sea). The study showed marked differences on the biomarkers (CAT, SOD, GPx, and AChE) but the differences were mainly attributed to seasonal variations and to differences among the two species (Uluturhan et al. 2019). The effect of TM was also studied in the fish *M. barbatus, B. boops and T. trachurus* collected along the coast of Türkiye in the Levantine and the Aegean Seas. Correlations were found between CAT and MDA and some of the trace metals measured in the fish specimens.

b) ADR sub-region (Italy)

576. Italy. One study reported the effect of PAHs in the fish *Mullus barbatus* collected in the northern Adriatic (Frapiccini et al. 2020). The expressions of CAT and GST in *M. barbatus* were dependent on the season, lower in the winter and higher in the summer. SOD expression did not depend on the season. LPO was higher in the winter. CAT showed a significant negative correlation with total

[104] Submitted to Research Square, not peer reviewed by a scientific journal

PAH concentrations, especially total LMW-PAH, in individuals collected during winter. Both GST and SOD did not show any significant correlation with PAH levels.

c) CEN sub-region (Tunisia, Italy)

577. Seven studies were reviewed for Tunisia: 2 from the WMS , 3 from the CEN and 2 with data from both the WMS and the CEN. In the CEN, one mesocosm experiment was performed in *Mytilus spp*. exposed to sediment contaminated by PAH and TM collected from the Zarzis area (Ghribi et al. 2020), while the effects of hydrocarbons were studied in the mollusc *Ruditapes decussatus* collected from the southern Lagoon of Tunis (Mansour et al. 2021). The effect of TM on the mollusc *Patella caerulea* was studied in specimens collected from 4 sites in the CEN (Zaidi et al. 2022).

578. *Mytilus spp* exposed to contaminated sediments in a mesocosm experiment presented the highest values of the tested oxidative stress biomarkers (CAT, GST, GPx) and a significant inhibition of AChE activity in comparison with the unpolluted reference site.

579. Hydrocarbons were found to affect the biomarkers CAT, GR, SOD, MDA and AChE activities in *Ruditapes decussatus.*

580. SOD and GPx activities measured in *P. caerulea* were different among sites (higher in more affected stations), while CAT was similar on all four stations. MDA was inducted but no differences were found among the sites.

581. Italy. In the CEN, the effect of plastic ingestion was studies in the fish *Trachurus trachurus* collected for the Sicily straits (Chenet et al. 2021).

582. Vitellogenin was highly expressed in *T. trachurus* females as expected, there is also a significant expression of the VTG gene in 60% of the males analyzed, from both sampling sites. Moreover, females in Lampedusa island showed a lower expression of vitellogenin than in Mazara del Vallo (with one female sample, TT54, not expressing VTG at all). The endocrine disruption represented by the alteration of VTG expression in specimens observed in this work can be caused by microplastic ingestion, as well as by the interactions between the marine organisms and the wide variety of endocrine-disrupting chemicals possibly present in seawater.

d) WMS sub-region (Algeria, Spain, Tunisia, Italy)

583. Algeria. Four studies reviewed for Algeria studied the effects of non-specific stressor in the mollusc *Donax trunculus* from Annaba Bay (Amamra et al. 2019) , in the fish *Mullus barbatus* along the Algerian west coast (Kristel, Oran, Ghazaouet) (Kaddour et al. 2021), on the mollusc *Perna perna* transplanted to the Gulf of Annaba initianorth-eastern coast) (Laouati et al. 2021) and on the mollusc *Patella rustica* affected by the brine of the Bousfer desalination plant in Oran Bay (Benaissa et al. 2020).

584. *Donax trunculus* specimens showed a significant inhibition of AChE and induction of GST and MDA in individuals of Sidi Salem and Echatt as compared to El Battah with significant effects of both site and season. The effects were more pronounced during summer and spring compared to the other seasons. In addition, the comparison between tissues revealed a more marked response in gonad than mantle and digestive gland.

585. In *M. barbatus*, a significant increase in the frequency of micronuclei (MN) occurrence in the summer period correlated with significantly shorter NRRT. In addition, the erythrocytes of *M. barbatus populations* from polluted areas presented statistically higher MN frequencies and shorter NRRT than those of the reference site.

586. GSH decreased in the gills and digestive glands of *P. perna* specimens transplanted to two of the sites affected by anthropogenic input while GST and CAT activities showed no significant variation. The MDA content in the mussel digestive glands, but not in the gills, increased significantly after the deployment period in the three caging sites, and were significantly different among the 3 sites. AChE activity was significantly inhibited registered in the gills of mussels from the 3 sites and in the digestive glands from one site.

587. A multibiomarker approach (oxidative stress, biotransformation enzyme, lipid peroxidation, neurotoxicity and genotoxicity) were applied in the soft tissue of *P. rustica* . This biomonitoring confirmed the negative impact of brine discharges of the desalination plant, with samples collected close to the outfall more affected. by all the environmental disturbances than ones from the other sites. CAT, TGPx, GR, GST, CSP-3like activities were increased in samples from the outfall. AChE was lower however not significantly different from samples collected from the reference site. Genotoxic effect revealed by ADN and lipid damages.

588. Spain. Five studies were reviewed for Spain: four studies studied the effect of microplastic ingestion and of plasticizers on the biomarker responses, while one studied the effect of anthropogenic items ingestion. Three studies were conducted in the Integrated Multi-Trophic Aquaculture cages in Palma de Majorca, where specimens of the mussel *Mytilus galloprovincialis* and of the fish *Sparus aurata* were transplanted to and analyzed at time 0, after 60 days (T_{60}) and after 120 days (T_{120}) of exposure (Capó et al. 2022, Capo et al. 2021, Rios-Fuster et al. 2022). One study was performed with *S. dumerili* collected around the Balearic Islands (Solomando et al. 2022). Anthropogenic items ingestion was studied in *E. encrasicolus* collected off Catalunia (Rodríguez-Romeu et al. 2022).

589. No effects of time were observed in CAT, SOD, and GRd activities *M. galloprovincialis*, but they were significantly higher in specimens sampled from the cages than in specimens from the controls. GST activity did not change with time, and it increased significantly only in samples for the cages at T_{60}. In T_{120} activity was higher in the cages only if compared to one of the control sites. GPx activity was modulated by both sampling site and time: higher activities in specimens from the cages at T_{120}. MDA was higher in samples from the cages compared to the controls at T60. In a different study with *M. galloprovincialis* higher expressions were observed in the biomarkers CAT, SOD, GPx and LPO in specimens from the aquaculture cages. Those could be triggered by the presence of bisphenol but also by other possible contaminant inputs from the aquaculture.

590. MDA increased throughout the study both in liver and blood cells of *S. aurata* but with a progressive decrease in plasma. EROD, BFCOD and CE, showed a comparable decrease at T_{60} with a slight recovery at T_{120}. In contrast, GST activity was significantly enhanced at T_{60} compared to the other sampling stages.

591. SOD, CAT, and GST activity were significantly higher in *S. dumerili* with higher microplastic (MP) load, while no significant differences were observed for MDA, and EROD enzyme activity.

592. AChE, CAT and GST were lower in *E. encrasicolus* collected off Barcelona, compared to specimens collected Blanes and Tarragona; Terragona LDH, CE and EROD were higher in Terragona

than in the other two locations; Blanes CS was higher than in Tarragona. These differences could not be correlated with any potential stressors nor with fish size Catalunia (Rodríguez-Romeu et al. 2022).

593. Italy. Five studies were reviewed for Italy: 2 from the WMS, 1 from FAO zone 37 (not further specified), 1 from the CEN, 1 from the ADR . In the WMS, the effect of pesticides were studied in the fish *Parablennius sanguinolentus* from the port of Bagnara (western Calabria) (Parrino et al. 2020), and the effect of TM and PAHs on mollusc (*Mytilus galloprovincialis*) and fish (*Mullus barbatus, Pagellus erythrinus* and *Diplodus vulgaris)*from the bay of Pozzuoli (Naples)(Morroni et al. 2020). Microplastics and TM effects were studied on the fish *Lepidopus caudatus* collected from FAO area 37 (area not further specified) (Salvaggio et al. 2019).

594. AChE activity in the brain and BChE activity in blood were significantly inhibited in specimens of *P. sanguinolentus* from the affected port area, by 23.5 and 72.0%, respectively. The esterase inhibition was primarily due to carbamate and organophosphorus insecticides presence.

595. In the Bay of Pozzuoli, the effect of pollution varied by species and biomarkers. In *M. galloprovincialis*, there was a decreased LMS and increased MN at two sites compared to organisms from other areas while no variations were observed for the AChE in haemolymph, nor for MT in digestive gland of mussels from various sites. AChE activity was not affected in *M. barbatus* sampled in the industrial area while a decrease of this biomarker AChE was observed in *P. erythrinus* and *D. vulgaris*. The EROD enzymatic activity was significantly induced in *M. barbatus* and *P. erythrinus* sampled in the industrial area compared to specimens from the reference site, while the cytochrome P450 biotransformation pathway was unaffected in *D. vulgaris*. At the same time, all the fish species exhibited higher levels of aromatic metabolites, particularly B[a]P-like and pyrene-like, in organisms sampled in the industrial compared to reference area. MN increased in gills of *M. barbatus* from the industrial area.

596. Immunohistochemical analysis for anti-metallothionein 1 antibody in *L. caudatus* showed a strong positivity of liver cells, both in females and males, showing a strong stress that activated a cell detoxification system. The immunohistochemical analysis for the anti-vitellogenin antibody showed in females a strong positivity both in the liver cells, and in the gonads, as expected. The analysis of the liver and gonadal preparations of the male specimens was found to be always negative except for one specimen.

597. Tunisia. Seven studies were reviewed for Tunisia: 2 from the WMS, 3 from the CEN and 2 with data from both the WMS and the CEN. In the WMS, the effect of TM was studied in the mollusc *Flexopecten glaber* collected from the Bizerte Lagoon (Telahigue et al. 2022) and on the polychaete *Perinereis cultrifera* collected from the port of Tades and the Punic port of Carthage (Bouhedi et al. 2021). The following 2 studies have data from the two sub-regions: WMS and CEN. The effect of microplastic ingestion was studied in the fish *Serranus scriba* collected from 6 sites along the Tunisian coast (Zitouni et al. 2020) and on the seaworm *Hediste diversicolor* collected from 8 sites along the Tunisian coast (Missawi et al. 2020).

598. The distribution of most analyzed metals in *F. glaber* tissues varied significantly between sites, seasons, and organs. The highest levels were recorded at the polluted site during the warm period. Moreover, the digestive gland was found to accumulate greater concentrations of TM than the gills. The biomarkers (MDA, GSH, GPx, SOD, CAT) in gills were higher in the polluted site while MT was not affected. In the digestive gland, only CAT and MDA showed an increase activity in the polluted site.

599. Higher level of thiobarbituric acid were found in *P. cultrifera specimens* from polluted site. In addition, CAT, GST, SOD, glutathione and MT were enhanced and AChE activities decreased in *specimens from* the contaminated site compared to those from the reference (or less contaminated site).

600. Biomarkers of oxidative stress (MT, CAT, GST, MDA) and neurotoxicity (AChE) responses in *S. scriba* were dependent on site and on the size of the microplastic. High content of microplastic in the gastrointestinal track increased MT levels and GST activity. CAT activity and MDA accumulation were positively related with the medium size class MP A significant negative correlation was found between AChE activity and the small size class of microplastic (MP). The study could not rule out some influence of other pollutants that may be present in some of the sites on biomarker response.

601. In the seaworm *Hediste diversicolor,* responses increased with increased microplastic tissue concentration, in particular CAT but also MDA. A decrease of GST activity was reported in the same sites. AChE was significantly inhibited indicating neurotoxicity.

602. ***Figures 3.1.5.1 and 3.1.5.2*** depict the sampling areas. Figure 3.1.5.1 shows the whole Mediterranean Sea, while Figure 3.1.5.2 shows in detail the study areas off eastern Algeria and Tunisia, where many of the reviewed studies were performed.

Figure 3.1.5.1. Areas of study for biomarkers, reviewed in the recent (since 2016) scientific literature for the Mediterranean Sea. When no coordinates were presented in the papers, the general area was marked in the map.

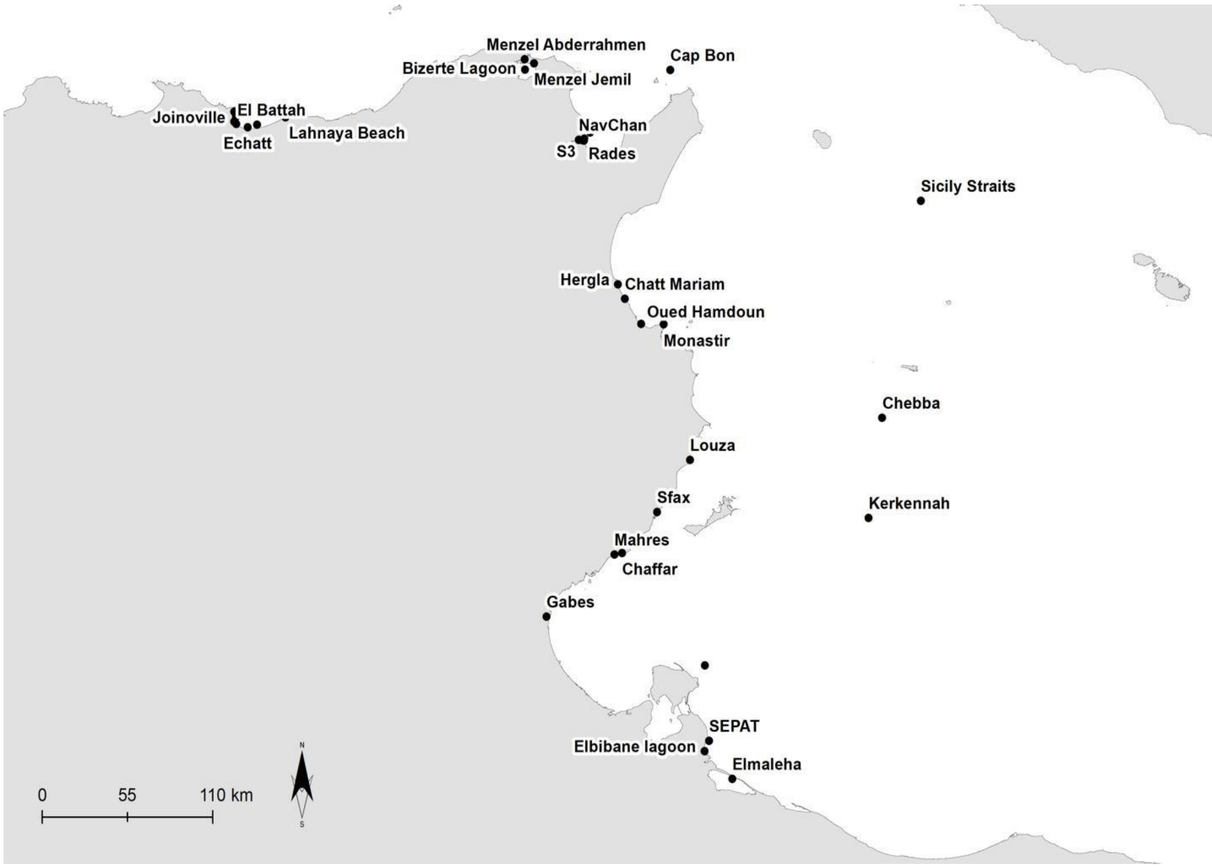

Figure 3.1.5.2. Detailed map of the study areas for biomarkers reviewed in the recent (since 2016) scientific literature for eastern Algeria and Tunisia coasts. Many stations were occupied in this area of the Mediterranean Sea.

603. Further to the above results based on a review of the studies by sub-regions and countries, it can be concluded that twenty-four studies were retrieved from the scientific literature as follows: 4 studies from Algeria (WMS), 1 from Egypt (AEL), 5 from Italy (2 from WMS, 1 from ADR, 1 from CEN and one from FAO zone 37), 5 from Spain (WMS), 7 from Tunisia (2 from WMS, 2 from CEN and 3 with data from both the WMS and CEN), and 2 from Türkiye (AEL).

604. The sub-region most represented is the WMS, followed by the CEN. In the CEN all studies except one were performed in Tunisia. There was one study from the ADR and three in the AEL.

605. The monitoring species, M. galloprovincialis and M. barbatus, appeared in 5 and 4 studies, respectively. In addition, 10 fish species, 6 mollusc species and 2 polychaeta species were also studied.

606. Of the mandatory biomarkers as defined in in the DDs and DSs for IMAP CI-18, AChE appeared in 13 studies, MT in 5 studies (2 with molluscs, 2 with fish and one with a polychaete species), MN in 2 and LMS-NRTT in 1 study.

607. Data from studies cannot be compared to BAC and EACs values as agreed by Decisions IG.22/7 and IG.23/6 (COP 19 and COP 20) because they were not measured in the specific tissue of M. galloprovincialis.

608. The most common additional biomarkers measured in the reviewed studies were: CAT (15 studies), MDA (12 studies), GST (11 studies), SOD (9 studies), and GPx (8 studies).

609. The anthropogenic stressors identified were: Trace metals (10), Plastic/microplastic (8), non-specific (4), PAHs (3), Pesticides (2), hydrocarbons (1), anthropogenic items, and one study with desalination brine as a source.

610. Drivers and pressures reported in the studies, encompassed the whole range of them: domestic and industrial discharges, agricultural and riverine runoff, fisheries, harbor and marina utilization, maritime activities, tourism. Most of the studies described the environmental conditions at the sampling areas. The exemption was for microplastics, where the source was not determined, and microplastics were considered ubiquitous in the environment.

611. Most biomarkers studied showed a response to anthropogenic stressor. In the case of microplastics, the size of the microplastic also influenced the response.

612. Studies demonstrated that, in addition to anthropogenic stressors, biomarker responses were influenced also by seasonality, tissue analyzed, spawning status, and on species identity.

Assessment of IMAP Common Indicator 19: Occurrence, origin (where possible), extent of acute pollution events (e.g. slicks from oil, oil products and hazardous substances), and their impact on biota affected by this pollution

Geographical scale of the assessment	Sub-regions within the Mediterranean region based on integration of the assessments at Sub-divisions level
Contributing countries	Data from MEDGIS-MAR, Lloyd List Intelligence Seasearcher, CleanSeaNet Service
Mid-Term Strategy (MTS) Core Theme	1-Land and Sea Based Pollution
Ecological Objective	EO9. Contaminants cause no significant impact on coastal and marine ecosystems and human health
IMAP Common Indicator	CI19. Common Indicator 19: Occurrence, origin (where possible), extent of acute pollution events (e.g. slicks from oil, oil products and hazardous substances), and their impact on biota affected by this pollution
GES Definition (REMPEC/WG.51/9/1)	Occurrence of acute pollution events are reduced to the minimum.
GES Targets (REMPEC/WG.51/9/1)	1. Decreasing trend in the occurrence of acute pollution events
GES Operational Objective (REMPEC/WG.51/9/1)	Acute pollution events are prevented, and their impacts are minimized

Available data

613. Three major datasets are available to extract data on oil and HNS spills at the Mediterranean scale: MEDGIS-MAR, Lloyd List Intelligence Seasearcher (hereafter Lloyd), CleanSeaNet Service.

614. The Mediterranean Integrated Geographical Information System on Marine Pollution Risk Assessment and Response (MEDGIS-MAR) is a database managed by REMPEC containing national data about response equipment, accidents, oil and gas installations, and oil handling facilities. Data on

accidents are collected in MEDGIS-MAR since 1977. For this assessment, MEDGIS-MAR data were filtered considering the events causing pollution (“Pollution” = YES) and located into the sea or within a 1 km inland buffer (to include events in any case occurring close to the sea, as for example in port areas).

615. The Lloyd List Intelligence Seasearcher, privately managed, gathers several data on shipping, including ship incidents, recorded since the 70s. The exportable tables do not include information about the spilled substances and volumes. Several incidents registered in the Lloyd database are also included in MEDGIS-MAR. For this assessment, Lloyd data were filtered considering the events causing pollution (“Pollution indicator = YES”) and located in the Mediterranean Sea (thus, excluding those in the Black Sea).

616. CleanSeaNet is a European satellite-based service for oil spills and vessel detections managed by the European Maritime Safety Agency (EMSA). The full access to CleanSeaNet database is granted to Member States National Competent Authorities, while the open access website provides access to the so-called yearly “Detection and Feedback data”, for the period 2015-2021. These pdf documents have been used for this assessment and include the parameters of interest for the assessment. The available dataset does not include information enabling to distinguish the spilled substance. For the assessment Class A events (high confidence of detection) were considered.

617. The above databases are based on the two different approaches: MEDGIS-MAR and Lloyd are populated with incident reports provided by ships or countries. CleanSeaNet includes satellite observations of possible spills. The number of events reported in each database is therefore very different: MEDGIS-MAR and Lloyd register tens of events per year in the Mediterranean while CleanSeaNet registers hundreds of events per year in the sea basin. CleanSeaNet detections can be caused by mineral oil and other pollutants, but may also indicate naturally occurring features (e.g. algae blooms, areas of upwelling, etc.). CleanSeaNet includes observations spills of different sizes, including also very small ones, not only related to incidents but also to accidental or illicit discharges. In addition to that, it should be observed that spills recorded by CleanSeaNet can derive from offshore (O&G prospections and extractions) or coastal activities, not linked to maritime transport. The datasets extracted from the three databases provide different and complementary information and were therefore assessed separately.

618. With reference to MEDGIS-MAR and Lloyd, the two databases show some overlaps (this means that some incidents are present in both databases). For recent data, integration between the two datasets has been carried out by REMPEC. Despite this, several differences between the two databases still remain and need to be considered by the Contracting Parties and others. A full integration of the two datasets remains outside the scope of this assessment.

619. CleanSeaNet data are considered in the study in order to accomplish for operational pollution events. Such events refer to voluntary or accidental release of oil or other substances. They can result from human decision, error or technical failure. In the Mediterranean any discharge into the sea of oil or oily mixture from the cargo area of an oil tanker is prohibited, according to Annex I of the International Convention for the Prevention of Pollution from Ships (MARPOL). Notwithstanding this, operational pollution and, particularly, illicit discharges, is recognized as a major problem in the region. With the worldwide and regional decrease in the number of big spills caused by important ship accidents, the issue of small but very numerous spills has become an important element to be considered when assessing the state of this indicator in the Mediterranean (REMPEC, 2022).

620. When considering CleanSeaNet dataset, uncertainty related with oil spill detection should be considered. Percentage of correctly detected slicks is known to vary with sensor type, data processing and slick recognition methods, as well as their temporal evolution. Such a percentage is reported to generally rank above 80% (e.g. Carvalho et al., 2021; Shaban et al., 2021; Huang et al., 2022). A fixed correction factor cannot be applied to the entire Mediterranean and to the whole temporal range considered, because this percentage not only depend on above elements but may vary also in relation with several local conditions. Thus, for the purpose of the present study, all reported CleanSeaNet Class A records (observations) have been considered in the assessment. In addition, CleanSeaNet datasets might be biased by increasing monitoring effort from 2015 to the present. Within present assessment of CI 19, it was possible to obtain information on this aspect. Based on these considerations, it is recognised that the adopted methodological approach can lead to an overestimation of the number of oil spills events detected by CleanSeaNet and of their extension. To cope with this possible overestimation, CleanSeaNet data have been used in relative terms (as detailed further below), to identify the areas with the highest spill occurrence and to calculate differences between time periods. In addition to that, in the integrated evaluation of the three datasets and formulation of the final assessment, CleanSeaNet data have been considered with a lower weight than data reported by MEDGIS-MAR and Lloyd. This approach is considered to be in line with the precautionary principle and with the need to account for small spills and illicit discharges.

The integrated assessment of datasets related to CI 19

621. For the purpose of the present assessment of CI 19, the four main sub-regions and related sub-divisions have been established namely: the Western Mediterranean Sea (including the Alboran Sea characterized by the exchange of the Mediterranean waters with the Atlantic Ocean), the Adriatic Sea (which is a double semi-enclosed area by itself and the Mediterranean Sea), the Central Mediterranean (acting as the nexus for the eco-regions and located in the centre of the basin with a low anthropogenic influence), and the Aegean and Levantine Sea in the Eastern Mediterranean part.

622. The application of the environmental assessment methodology for CI 19, is based on the integration of evidences from all the three analyzed datasets.

The assessment for CI 19 in the period 2018-2021 jointly considers: (1) the information on the frequency of spill occurrence i.e., yearly average number of spills/10000 km^2 and yearly average extension of areas interested by pollution/10000 km^2, and (2) the information on the trend of such frequency i.e., increasing, decreasing, stable with no spill, represented by the variation in % in comparison with the previous assessment period (2013-2017). This element (variation of spill density) is based on a CHASE-like approach and capitalizes some elements of the methodology adopted by HELCOM for the assessment of oil spill in the Baltic Sea (HELCOM 2018). The spatial component of the analysis was detailed: the 2023 MED considers the sub-regions and the relative sub-divisions identified in the Mediterranean Sea.
For each of three datasets, the assessment was based on the following steps:

i. Quantification of the average number of oil spills per year in the period 2018-2021 for the entire Mediterranean Sea and its sub-divisions.
ii. The average number of oil spills was standardised on the extension of each sub-division, thus enabling to calculate the average number of spills per 10000 km2 in the assessment period for the entire Mediterranean and its sub-divisions.
iii. The three sub-divisions characterised by higher values of the indicator calculated in step 2 were highlighted in dark red/red/orange to remark the three highest oil spill occurrences.
iv. Percentage of variation (2018-2021 vs. 2013-2017) of average yearly spill occurrence was then calculated for the entire Mediterranean and for each sub-division.
v. Based on the computed percentage variation, the following colour-based classes were defined for variation in percentage: blue = no spills recorded in the sub-division, in the period of assessment (2018-2021) nor in the previous reference period (2013-2017); green = decreased frequency of spill occurrence in the sub-division; yellow = increased frequency of spill occurrence ≤ 100% in the sub-division; red = increased frequency of spill occurrence > 100% in the sub-division.

In the case of CleanSeaNet dataset, the same assessment above described was implemented also for the extension of areas interested by pollution due to oil spills, still comparing 2018-2021 with the previous 2015-2017 period. MEDGIS-MAR enabled to implement the same assessment also on the number of spills of substances other than oil: Hazardous and Noxious Substances (HNS), other substances (non-HNS) and Unknown substances.
This integrated assessment of the evidences from the three data sets was based on the following three criteria:

a) Occurrence of spills reported through MEDGIS-MAR and Lloyds, which are mainly linked to relatively large pollution events and to incidents. Occurrence of reported events is considered as a “negative” factor in the overall assessment of the quality status of a given sub-division, while the absence of reported events is considered as “positive”. As additional element to the sub-divisions ranked among the first three for frequency of occurrence of spills, an additional “negative” factor was considered.
b) CleanSeaNet data are used as an indicator of relatively smaller spills, related to minor incidents or illicit discharges. This second criterion has been weighted less than the previous one, to take into consideration the possibility of overestimation of the number and extension of spills reported in this dataset. Thus, a negative contribution to the overall status was considered for the sub-divisions ranking among the first three in terms of average extension of areas affected by oil pollution.
c) The temporal variation of the average number of spills (for all the three datasets) and their extension (for CleanSeaNet) between the assessment period (2018-2021) and the previous reference period (2013-2017 for MEDGIS-MAR and Lloyds; 2015-2017 for CleanSeaNet) was considered. An increasing trend was considered as negative for the overall assessment of the quality status, while a decreasing trend provided a positive indication.

Results of the IMAP Environmental Assessment of CI 19 in the Mediterranean region

623. Table 3.1.6.1. provides an overview of the assessment results based on synthetic data extracted from datasets and used for the assessment. Considering the spills reported by the ships and countries regarding the incidents, MEDGIS-MAR and Lloyd List data indicate for the entire Mediterranean in the assessment period an average occurrence frequency of 0.033 and 0.051 n/y/10000 km2, respectively. The most affected sea is the Aegean Sea, followed by the Ionian Sea, according to MEDGIS-MAR (no incidents reported by Lloyd List, instead) and the Alboran Sea according to Lloyd List (no incidents reported by MEDGIS-MAR, instead). The Northern Adriatic Sea ranks third for occurrence of incidents, according to the Lloyd List (no incidents reported by MEDGIS-MAR, instead). These results are in accordance with the relative intensity of vessel traffic (hours/km), that indicates the Aegean Sea, the Alboran Sean and the Northern Adriatic as the most trafficked areas of the Mediterranean.

624. Focusing on the spills detected by satellite monitoring (CleanSeaNet data), the Adriatic Sea is the area with the highest standardised (per 10000 km2) frequency of spill occurrence and the area where the largest extension of polluted areas is detected. This could be explained by the fact that satellite monitoring enables to detect also small spills, (including small, non-reported incidents, illicit discharges, spills due to other offshore activities. These are particularly numerous in the Adriatic where, beside significant traffic density due to cargos, tankers and passenger vessels, other type of vessels are present in large number,including fishing vessels.

625. The temporal variations in spill occurrence computed from the three different databases are very different. According to MEDGIS-MAR a general improvement of the status can be observed for this indicator, with Alboran Sea, Tyrrhenian Sea and the whole Adriatic Sea reporting no spills both in the considered and in the previous assessment period. Considering Lloyd, a general worsening of the status of the indicator can be observed in the Alboran Sea, Western Mediterranean, the Tyrrhenian Sea, the Northern Adriatic the Aegean Sea showing increased spill occurrence. These findings mostly agree with the ones from CleanSeaNet which additionally highlight an increase of spill occurrence also for the Central Mediterranean, the Middle Adriatic Sea, the Ionian Sea and the Levantine Sea.

626. It is worth noting that CleanSeaNet datasets might be biased by increasing monitoring effort from 2015 to the present. Within present assessment of CI 19, it was possible to obtain information on this aspect.

627. MEDGIS-MAR is the only datasets among the three considered in this assessment allowing to describe the trend in the number of spills of substances other than oil. In MEDGIS-MAR, such substances are categorized as Hazardous and Noxious Substances (HNS), other substances (non-HNS) and Unknown substances. Decrease in number of events with respect to the previous period, or no events recorded, was observed in the last four year in all sub-divisions, with the exception of Ionian Sea and the Aegean Sea. The Levantine sea scores third in number of events, even if with a decreasing trend. iLarge (above 700t) and medium size spills (7-700t) have not been reported since 2018. The last four years are characterised only by small spill events, although several events with unknow size (4 in 2019) have been registered.

Table 3.1.6.1.: CI 19 assessment. (1) average number of oil spills in the assessment period (2018-2021) per 10000 km^2 for the three datasets; (2) average extension of areas interested by oil pollution in the assessment period (2018-2021) per 10000 km^2 (from CleanSeaNet) - the three highest values only are highlighted; (3) average number of other substances spills in the assessment period (2018-2021) per 10000 km^2 (from MEDGIS-MAR); (4) % of variation compared to the previous period of the above indicators for oil spills; (5) % of variation compared to the previous period of the above indicator on other substance spills. Colour code for spill frequency and variation in the extension of the area affected by pollution: dark red = highest value; red = second highest; orange = third highest. Colour code for % variations: blue = no spills recorded, in the assessment period, nor in the previous period; green = decreased frequency of spill occurrence; yellow = increased frequency of spill occurrence <= 100%; red = increased frequency of spill occurrence > 100%. Data sources: MEDGIS-MAR, Lloyd List Intelligence Seasearcher, CleanSeaNet.

Frequency of spills / total polluted area (average values in the period 2018-2021, per 10000 km^2)											
	TOT MED	**ALBS**	**WMS**	**TYRS**	**CEN**	**NADR**	**MADR**	**SADR**	**IONS**	**AEGS**	**LEVS**
Oil											
(1) MEDGIS-MAR	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.089**	**0.334**	0.000
(1) LLOYD	0.051	**0.178**	0.039	0.012	0.000	**0.075**	0.000	0.000	0.000	**0.371**	0.028
(1) CleanSeaNet (n)	9.3	11.3	9.0	6.8	5.9	**16.5**	**15.4**	**15.6**	9.6	10.9	11.3
(2) CleanSeaNet (km^2)	68.2	57.5	76.6	44.6	62.8	**104.7**	**130.5**	**120.3**	54.4	39.6	75.9
Other substances											
(3) MEDGIS-MAR	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.104**	**0.284**	**0.004**
Summary of variation %											
	TOT MED	**ALBS**	**WMS**	**TYRS**	**CEN**	**NADR**	**MADR**	**SADR**	**IONS**	**AEGS**	**LEVS**
Oil											
(4) MEDGIS-MAR	-57	-	-100	-	-100	-	-	-	25	-56	-100
(4) LLOYD	12	67	41	25	-100	-	-	-100	-100	34	-27
(4) CleanSeaNet (n)	85	32	62	22	139	207	100	79	137	60	108
(4) CleanSeaNet (km^2)	103	64	106	24	244	197	48	87	141	12	99
Other substances											
(5) MEDGIS-MAR	-14	-100	-100	-	-100	-	-100	-	192	31	-89

628. The combined application of the three assessment criteria defined above (a, b, c) led to the classification of the quality status of CI 19 in the Mediterranean sub-divisions in five classes: bad (red), poor (brown), moderate (yellow), good (green), high (blue). As provided in Table 3.1.6.2, and mapped in Figure 3.1.6.1, according to the adopted methodology, four sub-divisions are classified as bad or poor, five as moderate, one as good and none as high.

629. It is worth noting that the methodology applied is subjected to uncertainty, mostly linked to the heterogeneity of the datasets it is based on. The results from the assessment should be interpreted as best knowledge-based indications on the status of CI 19, aiming at providing a relative indication of priority areas for future monitoring, assessment and, most importantly, pollution prevention measures.

Table 3.1.6.2: Assessment of the marine environment status for CI 19 for sub-divisions of the Mediterranean Sea

Sub-division	Considerations for the assessment	Status of CI 19
ALBS	Spills reported, second highest | Increase (in most of the datasets)	POOR
WMS	Spill reported | Increase (in most of the datasets)	MODERATE
TYRS	Spills reported | Increase (in most of the datasets)	MODERATE
CEN	No spills reported | Increase (only CSN)	GOOD
NADR	Spills reported, third highest | Third ranked for satellite observation (area extension) | Increase (in most of the datasets)	POOR
MADR	No spills reported | First ranked for satellite observation (area extension) | Increase (only CSN)	MODERATE
SADR	No spills reported | Second ranked for satellite observation (area extension) | Increase (only CSN)	MODERATE
IONS	Spills reported, second highest | Increase (for most of the datasets)	POOR
AEGS	Spills reported, first highest in two datasets | Increase (for most of the datasets)	BAD
LEVS	Spills reported | Increase (only CSN)	MODERATE

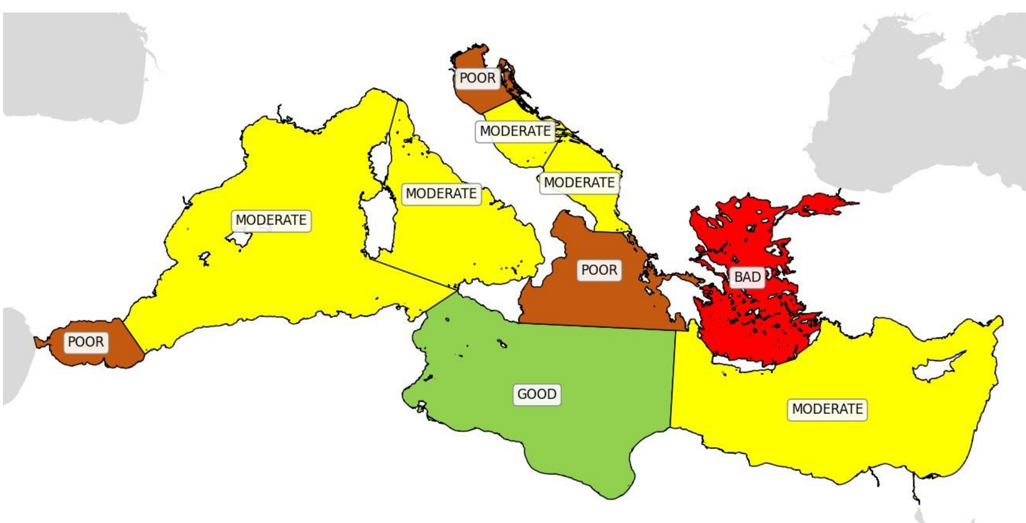

Figure 3.1.6.1. Map of the assessment of the marine environment status for CI 19 for sub-divisions of the Mediterranean Sea