UNITED
NATIONS

EP

UNEP (DEPI)/MED WG.444/Inf.13

UNITED NATIONS
ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN

3 August 2017
Original: English

6th Meeting of the Ecosystem Approach Coordination Group

Athens, Greece, 11 September 2017

Agenda item 4: Review of Quality Status Report (QSR)

Review of Proposed Background Concentrations (BC)/Background Assessment Concentrations (BACs)/Environmental Assessment Criteria (EACs) for Contaminants and Biomarkers at Mediterranean and Sub Regional Scale

UNEP/MAP
Athens, 2017

UNITED
NATIONS

EP

UNEP(DEPI)/MED WG.427/Inf.3

UNITED NATIONS
ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN

5 October 2016
Original: English

Meeting of the Ecosystem Approach Correspondence Group on Pollution Monitoring

Marseille, France, 19-21 October 2016

Agenda item 5. Review of Proposed Background Concentrations (BC)/Background Assessment Concentrations (BACs)/Environmental Assessment Criteria (EACs) for Contaminants and Biomarkers at Mediterranean and Sub Regional Scales

Background to the Assessment Criteria for Hazardous Substances and Biological Markers in the Mediterranean Sea Basin and its Regional Scales

UNEP/MAP
Athens, 2016

PREAMBLE

This document presents a revision[1] to further establish Mediterranean background assessment criteria (Med BACs) and environmental assessment criteria (Med EACs) for hazardous chemical substances and biological markers within the Ecological Objective 9 of the Integrated Monitoring and Assessment Program (IMAP). The UNEP/MAP-MEDPOL Database and the Reference Areas/Stations datasets submitted by the informal online expert group on contaminants through INFO/RAC, conforms the basis of this revision at different geographical scales. New assessment criteria are provided for the Mediterranean Sea and its eco-regions, as well as indicative background concentrations at sub-regional seas scales. The document has been prepared by Dr. Carlos Guitart (Marine Environment Consultant, Spain) in collaboration with Dr. Juan Miguel Marín (Department of Statistics, University Carlos III of Madrid, Spain), under the supervision of UNEP/MAP-MEDPOL.

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[1] 5th Meeting of the Ecosystem Approach Coordination Group. Rome, Italy, 14-15 September 2015. UNEP(DEPI)/MED WG.420/Inf.10

INDEX

LIST OF ACRONYMS AND KEY DEFINITIONS

GES	Good Environmental Status
BC	Background Concentration
BAC	Background Assessment Criteria
EAC	Environmental Assessment Criteria
Med BAC	Mediterranean BAC (MEDPOL datasets)
Med BC	Mediterranean BC (MEDPOL datasets)
WMS	Western Mediterranean Sea eco-region
ADR	Adriatic Sea eco-region
CEN	Central Mediterranean Sea
AEL	Aegean and Levantine Seas
N	Number of individual data
Mean	Arithmetic mean as central tendency estimator
Median	Midpoint value (50th percentile) of the datasets
IQR	Interquartile range as a mesure of the data dispersion (non-parametric distributions)
Percentile(s)	Indicative value(s) below a given % of the ordered datasets can be found
Cd	Total cadmium
Hg/HgT	Total mercury
Pb	Total lead
PAHs	Polycyclic Aromatic Hydrocarbons (group of petroleum hydrocarbons compounds)
OCs	Organochlorinated Compounds (group of compounds including PCBs and Pesticides)
AChE	Acetylcholinesterase biomarker
MT	Metallothioneins biomarker
MN	Micronuclei Frequency
LMS	Lysosomal Membrane Stability
SoS	Stress on Stress

1. BACKGROUND

The application of the Ecosystem Approach (EcAp) for the management of human activities in the Mediterranean Sea adopted by the Contracting Parties of the Barcelona Convention, as part of the Mediterranean Action Plan (UNEP/MAP), requires scientific-based environmental criteria for the assessment of the state of the marine environment. In line with the preparation of the Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) (UNEP(DEPI)/MED WG.421/Inf.9) and the recent COP19 decisions with regard the IMAP (UNEP(DEPI)/MED IG.22/7 Decision), eleven ecological objectives (EO1-EO11) have been defined, including the EO9 (Contaminants cause no significant impact on coastal and marine ecosystems and human health). For each EO, operational objectives and their associated common indicators (and targets) are being developed and refined by different working groups coordinated by the UNEP/MAP.

In this sense, for the EO9 on pollution (related to the MEDPOL Program), it is necessary to establish threshold values for key common indicator assessments, such as for contamination by hazardous chemical substances, to allow distinguish between acceptable and un-acceptable environmental risks to the marine ecosystems, as well as in order to provide a tool to measure the progress made towards the achievement of a Good Environmental Status (GES) and its targets. In the Mediterranean Sea basin and its eco-regions, specific assessment criteria within the EO9 for some major hazardous contaminants and biomarkers still need to be defined or further revised.

Therefore, the UNEP/MAP-MEDPOL work in this direction has resulted in different working documents and reports in relation to background information, with data provided from the national monitoring networks to the MEDPOL Database, for the definition of pollution assessment criteria for the Mediterranean Sea. The first estimates of background concentrations (BCs) and background and environmental assessment criteria (BACs and EACs) were made for trace metals in sediments and biota and PAHs in sediments in 2011, following the OSPAR Convention methodology approach (UNEP(DEPI)/MED WG.365/Inf.8). In December 2014, an informal online expert group on contaminants was established nominated by the Contracting Parties to further develop the Mediterranean assessment criteria related to the IMAP EO9. The group delivered its first report in March 2015 and was further discussed during 2015 (UNEP(DEPI)/MED WG.417/Inf. 15). The informal online group made a preliminary proposal regarding the Mediterranean BACs for major chemical pollutants (in sediment and biota) and biomarkers and further recommended as a first step the use of the assessment criteria both adopted by OSPAR and developed by scientific studies in the Mediterranean Sea. The informal online group pointed out the need to undertake a further analysis taking into account the additional data from Reference Areas/Stations provided by Contracting Parties (through INFO/RAC) to adjust as appropriate the current OSPAR BACs and the developed assessment criteria for the Mediterranean Sea region.

This report is within the present framework of the EcAp/IMAP implementation phase (2016-2017) and the11 EOs defined for the Mediterranean Sea, presents a revision required to objectivise the environmental monitoring information against scientific-based ecological criteria to be able to assess pressures, impacts and state of the environment. Further, within the Ecological Objective 9, there is a need to continue developing assessment criteria [Background Concentrations (BCs), Background Assessment Criteria (BACs) and Environmental Assessment Criteria (EACs)], for hazardous chemical substances and biological markers in a specific manner for the Mediterranean Sea and its regional scales.

2. OBJECTIVES AND SCOPE

The objective of this report is the revision of the Background Concentrations (BCs), Background Assessment Criteria (BACs) and Environmental Assessment Criteria (EACs)determination for selected substances (hazardous chemical contaminants and biological markers), in the light of new available datasets for Reference Areas/Stations in the Mediterranean Sea submitted by Contracting Parties (through the informal online expert group on contaminants), altogether with Reference Areas/Stations datasets from the MEDPOL Database.

Therefore, the scope of this report is to recommend assessment criteria based on Reference Areas/Stations data at different geographical scales in order to assess the Mediterranean Sea and its eco-regions with regard to hazardous chemical substances and their biological effects. This report is a major building block within the Ecological Objective 9 of the IMAP implementation to revise national monitoring strategies and the database quality assurance, as well as a starting point in the preparation of a Quality Status Reports for the Mediterranean Sea by UNEP/MAP Secretariat in 2017.

3. CONCEPTUAL FRAMEWORK

3.1. Development of environmental criteria in the Mediterranean Sea region

There are some conceptual approaches to determine environmental assessment criteria for the protection and sustainability of the marine environment, namely, the OSPAR Convention approach, the NOAA/USEPA approach and the Canadian methodologies. In a similar way, these approaches involves the categorization of the available environmental and toxicological data to establish two thresholds to define three 3 categories, where the transitions relates to a state of the marine environment which range from acceptable to unacceptable ecosystem risks, according the concentrations of hazardous substances in the marine environment and their toxicological effects. These thresholds could be connected, for example, to the Environmental Quality Standards (EQSs) and Good Environmental Status (GES) definitions under both the European Union Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD), respectively, which are currently being applied to hazardous substances in the marine environment.

The NOAA/EPA approach uses benchmarks, based upon a database primarily of synoptic marine sediment chemistry and sediment toxicity bioassay data. For a given contaminant, the samples which were categorized as toxic by the original data generator are selected, and that subset is then ranked by increasing contaminant concentration and the 10th (Effect Range-Low, ERL) and 50th (Effect Range-Medium, ERM) percentiles determined. The ERL is calculated as the lowest 10th percentile concentration of the available data at which biological effects were empirically observed. The NOAA ERL is at the low ranges of levels at which effects were empirically observed and it represents the value at which toxicity may begin to be observed in sensitive species. Another approach is presented in the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life based on a similar database compilation but using different calculations, Threshold Effect Levels (TELs, as the geometric mean of the 15th percentile) and Probable Effects Levels (PELs, as the geometric mean of the 50th impacted samples and the 85th of the non-impacted).

The earlier UNEP/MAP work (UNEP/DEPI/WG365/Inf.8), based the approach to determine BACs and EACs in the developments achieved by working groups within the OSPAR Convention. Therefore, similarly for the Mediterranean Sea, there are two hazardous chemical “concentration thresholds” to be defined: T_0 and T_1. T_0 will be defined in sediments and biota, as the concentration of a contaminant at a “pristine” or “remote” site, where no deterioration of the environment can be expected. On the other hand, T_1 is the concentration above which significant adverse effects to the environment or to human health are most likely to occur (the green/red transition point). Between T_0 and T_1, the contaminant levels do not pose significant risk to the environment or to human health (Figure 1).

These thresholds are considered to be the Background Assessment Concentrations (BACs) and Environmental Assessment Criteria (EACs), for hazardous chemical substances (T_0 and T_1, respectively), and were originated within the OSPAR CEMP long-term monitoring program (Coordinated Environmental Monitoring Program) and from toxicological studies, respectively. Further, the BACs were derived from Background Concentrations (BCs) depending on the parameter, whilst taking into account the long-term monitoring program variances.

Following this approach for the Mediterranean Sea, the definition of T_0 thresholds requires specific statistical analysis of the MEDPOL Database and additional information. The definition of T_1 requires toxicological information for the key species. The outcome of the definition of these threshold assessment criteria would be described by a “traffic light” for the Mediterranean Sea basin and its eco-regions (as shown in Figure 1) in a similar manner.

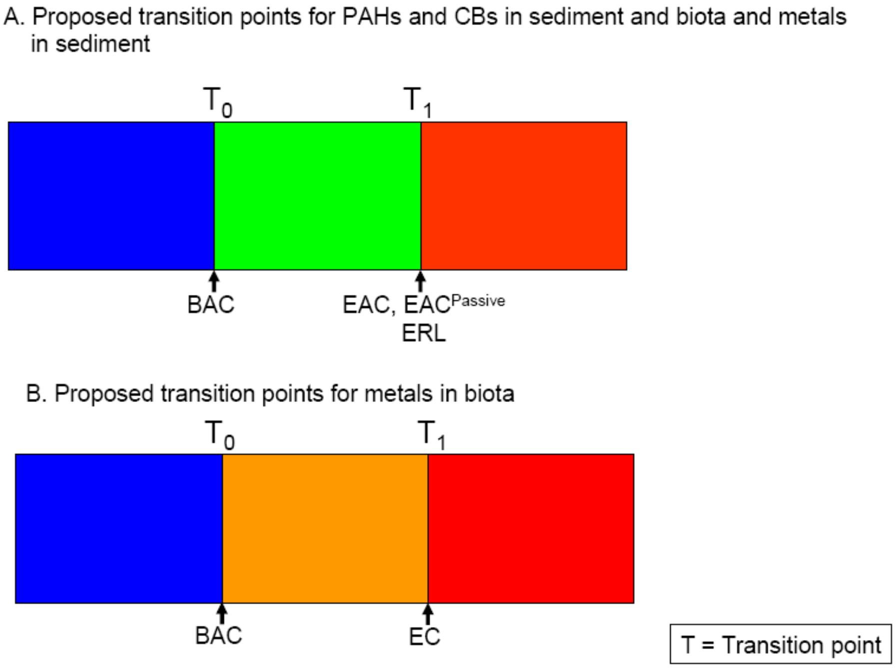
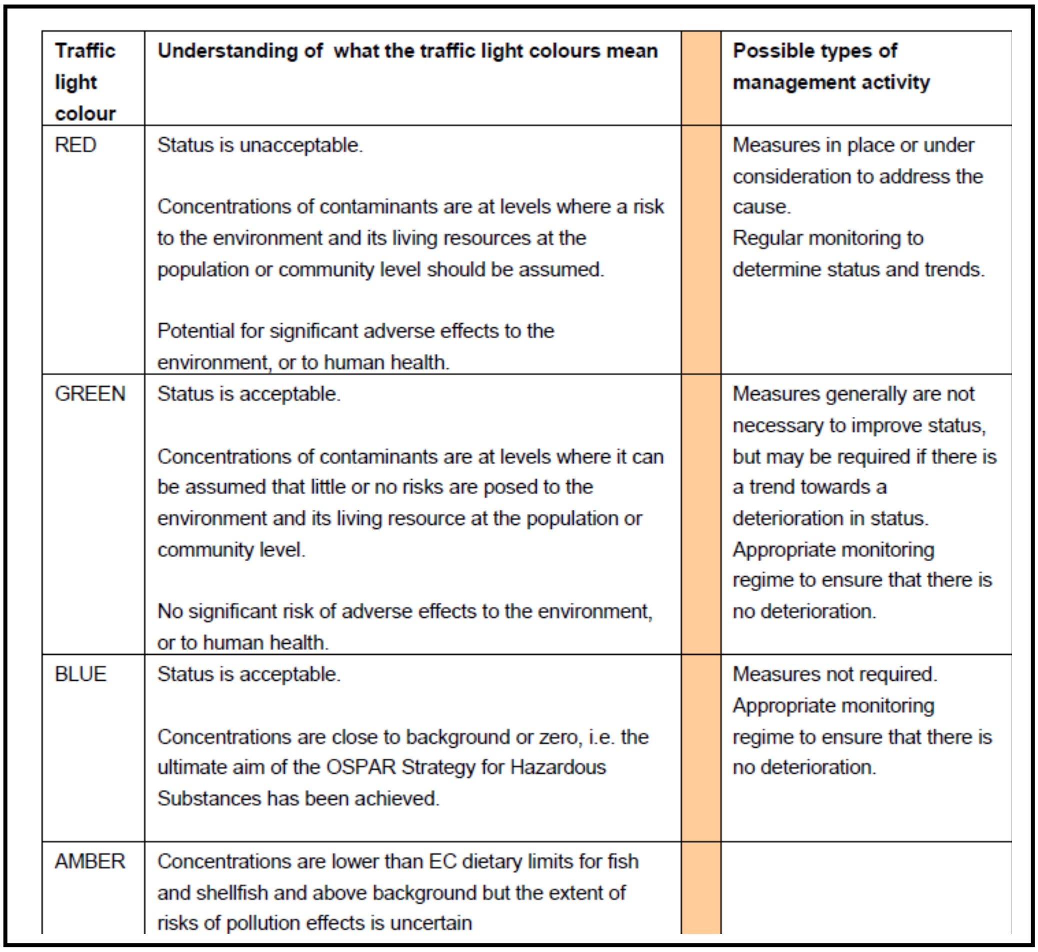


Figure 1.Illustration of the proposed “traffic light” system by OSPAR and the relevant transition point criteria for: (A) PAHs and CBs in sediment and biota and metals in sediments and (B) metals in biota. Source: OSPAR Commission, 2009.

In the following Dialog Box the interpretation of the proposed blue/green/red or blue/amber/red schemes in Figure 1, in relation to hazardous substances, is explained in the context of the contaminant concentrations and the type of management activity which may be undertaken.

DIALOG BOX: Understanding Traffic Light Colors



Traffic light colour	Understanding of what the traffic light colours mean		Possible types of management activity
RED	Status is unacceptable. Concentrations of contaminants are at levels where a risk to the environment and its living resources at the population or community level should be assumed. Potential for significant adverse effects to the environment, or to human health.		Measures in place or under consideration to address the cause. Regular monitoring to determine status and trends.
GREEN	Status is acceptable. Concentrations of contaminants are at levels where it can be assumed that little or no risks are posed to the environment and its living resource at the population or community level. No significant risk of adverse effects to the environment, or to human health.		Measures generally are not necessary to improve status, but may be required if there is a trend towards a deterioration in status. Appropriate monitoring regime to ensure that there is no deterioration.
BLUE	Status is acceptable. Concentrations are close to background or zero, i.e. the ultimate aim of the OSPAR Strategy for Hazardous Substances has been achieved.		Measures not required. Appropriate monitoring regime to ensure that there is no deterioration.
AMBER	Concentrations are lower than EC dietary limits for fish and shellfish and above background but the extent of risks of pollution effects is uncertain		

Source: OSPAR Commission, 2009

3.2. Definitions of Background Concentrations [BCs], Background Assessment Criteria [BACs] and Environmental Assessment Criteria [EACs]

In line with the definitions within the OSPAR approach, Background Concentrations (BCs) are derived hazardous chemical concentrations intended to represent the concentrations of certain substances that would be expected in “pristine” or “remote” sites, based on the available monitoring data (such as Reference Areas/Stations). The BCs for man-made substances (e.g. chlorinated pesticides) should be regarded as zero, and therefore, the so called low concentrations (LCs) might be used instead to derive assessment criteria. The latter, could be derived from reliable datasets of analytical variability information reported from either certified reference materials (CRMs) or independent proficiency testing (PTs) scheme databases, such as:

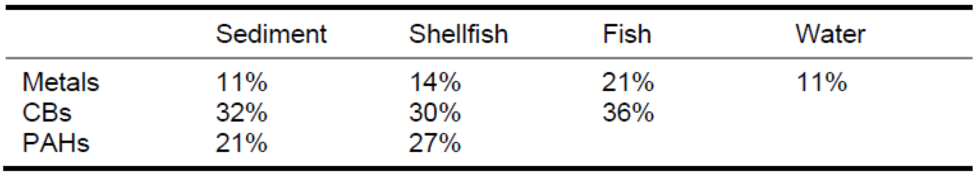
- QUASIMEME Database
- IAEA Database (MEDPOL)

It is recognised that natural processes such as geological variability or upwelling of oceanic waters may lead to significant variations in background concentrations of chemical substances, for example, trace metals. The natural variability within the Mediterranean Sea eco-regions of background concentrations (BCs) should be taken into account within the development and interpretation of assessment criteria, as it will be shown later in this document.

The Background Assessment Criteria (or Concentrations[2]) (BACs) are statistical tools defined in relation to background concentrations (BCs) and monitoring variances, which enable statistical testing of whether, observed concentrations can be considered to be near background concentrations. The observed concentrations are said to be ‘near background’ if the mean concentration is statistically significant below the corresponding BAC (OSPAR Commission 2008/379 CEMP Assessment Manual). More, the outcome of this method is that, on the basis of what is known about variability in observations, there is a 90% probability (power) that the observed mean concentration will be below the BAC when the true mean concentration is at the BC. BACs are calculated according to the method set out in Section 4 of the CEMP Assessment Manual and summarized below.

The determined BAC threshold values are required to be accommodated above the monitoring data variability (sampling and analytical combined). Within OSPAR, monitoring datasets were assessed to evaluate the precision (*ca*. uncertainty) of the monitoring program (OSPAR Commission, 2008/379). This was considered by CEMP using the temporal monitoring data from the UK National Marine Monitoring Program (Table 1 gives the precision by contaminant group and matrix). Then, provisional BACs were set to conclude using statistical hypothesis testing that measured concentrations would be below BACs (for each given monitoring BC probability distribution).

Table1. Precision of the OSPAR Monitoring Programme. Source: OSPAR Commission, 2008.



	Sediment	Shellfish	Fish	Water
Metals	11%	14%	21%	11%
CBs	32%	30%	36%	
PAHs	21%	27%		

The OSPAR developments, calculated also the corresponding statistical power of concluding that concentrations are near background as the BAC increases relative to the BC (Figure 2). Therefore, for trace metals, setting the BAC to be between 1.5 and 2 the BC (depending on the matrix) would give at least 90% power of concluding that concentrations are near background when the true mean concentration is at the BC (thus, below BAC). Similarly, setting to 2.5 and 3.5 for polycyclic aromatic hydrocarbons (PAHs) and organochlorinated compounds (OCs), respectively, should give the same statistical power. Different multipliers could be used for contaminant group/matrix combination (or single contaminant/matrix combination), as appropriate.

At present, a statistical assessment as described above for the MEDPOL monitoring program database could not be produced. Therefore, in this report, we have used the OSPAR monitoring program variability and their relationships between BC and BAC for hazardous chemical substances to determine the BACs for the Mediterranean Sea, as similar monitoring strategies and analytical capabilities exists. In this way, we have chosen the Mediterranean BACs for trace metals in sediments and shellfish to be BAC=1.5xBC and for fish BAC=2xBC, and for PAHs the relationship between BAC and BC is chosen to be 2.5.

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[2] The term Concentration or Criteria has been used indifferently, although the term Criteria would be preferred for BACs definition, thus includes an statistical-derived multiplier

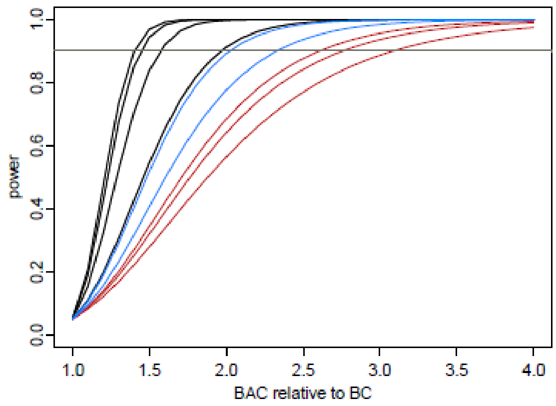

Figure 2. The power of concluding that concentrations are near background as the BAC increases relative to the BC based on UK National Marine Monitoring Program datasets. Running from left to right, the black lines are for metals in sediment, water, shellfish and fish; the blue lines are for PAHs in sediment and shellfish; and the red lines are for CBs in shellfish, sediment and fish. Source: OSPAR Commission, 2008/379.

On the other hand, Environmental Assessment Criteria (EAC) are defined as assessment tools intended to represent the threshold contaminant concentrations in sediment and biota below which no chronic effects are expected to occur in marine species (the green/red transition point). As starting point, an ideal approach for the derivation of EACs for any given substance based on dose-response relationships is used. The immediate consequence of the EAC definition is that a relation between exposure and its associated biological effect must be known. Also, the EAC concept relies on the assumption that not only a relation between dose (contaminant concentrations) and response (the biological effect) exists, but that this relation is strictly monotone. The development of Mediterranean EACs is a more difficult task because it requires together with chemical contaminant concentrations, ecotoxicological data for autochthonous marine species which is largely lacking. Further, the defined EAC criteria does not take into account specific long-term biological effects such as carcinogenicity, genotoxicity and reproductive disruption and do not include synergistic toxicological effects or the assessment of confounding factors within ecotoxicological studies (González-Fernández et al., 2015). The determination of EACs, as well as other environmental threshold criteria, such as ERLs or PELs developments for use in data assessments is a continuous process in the light of new scientific research.

Within the Mediterranean Sea (UNEP/MAP), as mentioned, a similar approach was set to be followed (for both “threshold” criteria T_0 and T_1 or BAC and EAC, respectively) to assess the levels and biological effects of hazardous chemicals for sediments and biota. Currently, within the context of the IMAPEO9, a revised calculation of BCs from the MEDPOL Database is required to set accurate BACs using Reference Areas/Stations information data. For EACs, the adopted criteria by OSPAR and NOAA/USEPA (ERL values) are further suggested in this report in both sediment and biota samples for the Mediterranean Sea (see Tables in Section 5). It should be stressed, that BACs and EACs should be used as an assessment tool specifically for the interpretation of monitoring data and the development of monitoring strategies. Therefore, caution should be exercised when using generic environmental assessment criteria in specific situations or local scales to undertake environmental management actions. Their use does not preclude the use of common sense and expert judgement when assessing environmental effects and/or the potential for them. These environmental threshold criteria should not be used as a trigger for source directed action without further evaluation and consensus.

3.3. Data sources

The MEDPOL database contains datasets from almost all the countries validated up to 2012, although further datasets for hazardous chemical substances and biomarkers have been received and are in process of validation. Despite the database content is highly variable and the weight of each component and country is unequal, it constitutes a relevant and unique source of marine pollution information in the Mediterranean Sea.

This document uses the evaluated datasets for the MEDPOL Reference Areas/Stations updated until 2012, as well as the datasets submitted by Contracting Parties through the informal online expert group on contaminants during the period 2014-2015 (INFO/RAC). The later, constitutes a selection of reference stations undertaken by national experts that has made available more recent data (including 2014) from national monitoring networks. These data was provided in the majority of cases for trace metals, petroleum hydrocarbons and biological markers (in the MEDPOL database format) for different biota species (fish and bivalves) and marine sediments all over the Mediterranean coastal environments. However, these datasets were not representative of Reference Areas/Stations for all the countries datasets submitted and both historical datasets and from coastal and polluted areas were also submitted which would influence the results if computed straight. Therefore, before starting the statistical analysis a data selection process was found to be mandatory.

As a result, adding the two sources of datasets (MEDPOL Database and selected Reference Areas/Stations datasets), new substantial information is available for the calculation and revision of theEO9 assessment criteria. Particularly, the datasets from countries that contributed to the informal online expert group on contaminants provided a refinement of the T_0 thresholds (BACs) for hazardous chemical contaminants and biomarkers for the Mediterranean Sea and allowed to develop BCs for its eco-regions.

3.4. Data selection

The definition of a reference station implies that the two following premises are fulfilled:

1- It should be a location considered to be “pristine” or “remote”, thus not influenced by anthropogenic activities, were natural levels of contaminants are expected to be determined and;
2- No temporal trends should be observed, either upward or downward trends, and then it can be assumed that there are no inputs or losses and any changes would occur at the natural time scales.

It is recognised that the main concentrations of anthropogenic contaminants, such as Pb and Hg, have decreased in the past two decades, particularly in the Western Mediterranean Sea and the national networks of monitoring stations implemented in the coastal areas within the MEDPOL Program have detected these decreasing temporal trends (UNEP/DEPI/WG365/Inf.4). Therefore, this fact should be considered during the data analysis in this reportto avoid bias when calculating background concentrations (BCs) and background assessment criteria (BACs). It would be incorrect to determine Mediterranean BCs and BACs including historical datasets with higher values than the recently measured in the coastal environment to establish the background assessment criteria for EO9.

Therefore, a dataset selection process was performed, as depicted in Figure 3, and described below.

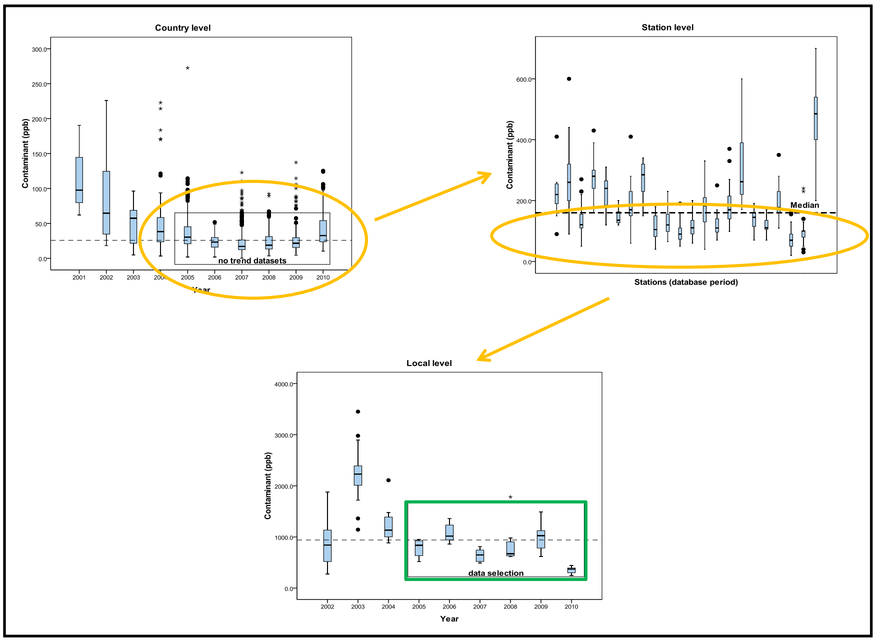

Figure 3. Datasets exploration, cleaning, selection process.

Initially, at a country level, each database parameter was grouped by years and explored to detect temporal trends and solely the most recent datasets without temporal trends (at least 6 consecutive years, if possible) were selected (Figure 3, top left). Secondly, the yearly selected datasets for each parameter were grouped by stations (station level) to detect the potential stations which fulfilled the premises set above for reference stations. This was achieved by plotting the countries stations datasets along the median value to identify the stations with the lowest concentrations. Those stations were the box-whisker plots fitted within or below the overall median (between Q1 and Q3) were selected as being the country reference stations (Figure 3, top right). Finally, at a local scale (Figure 3, bottom), each selected station was investigated individually for temporal trends. If temporal trends occurred, the selection of the datasets was shortened to fulfil the no-trend premise or to balance the number of data between countries within the aggregated datasets. It should be pointed that continuous data series, as show in Figure 3, are not the most common. Frequently, monitoring datasets were reduced to two or three years of datasets at some stations (or even to the latest available year), to fulfil with the requirements and avoid the introduction of bias. For PAHs, this step was performed solely for Phenanthrene as a model. Therefore, the selected datasets by country had the highest quality to perform the determination and evaluation of BCs and BACs (from reference areas/stations) in the Mediterranean Sea and its eco-regions. This selection process was applied to the large historical mixed datasets submitted through the informal online group on contaminants, as well as to the MEDPOL datasets if required.

3.5. Data aggregation (geo-scales)

For this report, we have undertaken a synoptic approach to determine the Mediterranean BCs and BACs at different spatial scales with the available datasets: the Mediterranean Sea basin as a whole (Med BCs and BACs), the Mediterranean eco-regions (BCs) and the sub-regional seas (BCs). Therefore, we have assigned and grouped the selected stations by eco-regions as shown in the Figure 4 and further detailed in Table 2. It has been recognised that differences between regions and sub-regions within the Mediterranean basins are likely to occur and should be taken into account for the environmental and pollution assessments as will be described later in this document.

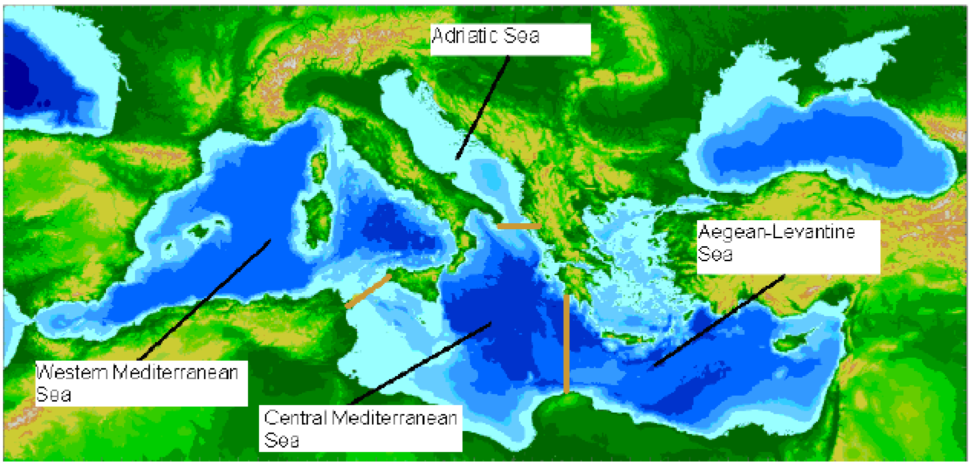

Figure 4. The four Mediterranean MEDPOL eco-regions (WMS, Western Mediterranean Sea; ADR, Adriatic Sea; CEN, Central Mediterranean and AEL, Aegean and Levantine Seas)

The determination of the Med BACs have been performed for the Mediterranean Sea as a whole with the calculated Med BCs (either corresponding to the median (50th percentile) for hazardous chemical substances or the 10th or 90th percentile for selected biomarkers) using the final selected Reference Areas/Stations datasets. The calculations include informative BCs both for the Mediterranean eco-regions and sub-regional seas scales as well. For EACs, those adopted within OSPAR and EU Directives (EU/1881/2006 and EU/629/2008) are further suggested. The datasets and statistical information for each hazardous substance and biomarker considered in this report has been put into tables to understand the distribution of the underlying aggregated datasets, as well as the number of data and their country origin (see Annexes), whilst plots have been included through the present document.

Table 2. The Mediterranean eco-regions and sub-regions aggregation according the database sources and availability within this report.

Eco-regions	Sub-regional seas/basins*
Western Mediterranean Sea (WMS)	Alboran Sea (ALBS) North Western Mediterranean Sea (NWMS) Tyrrhenian Sea (TYRS) Western Mediterranean Islands and Archipelago (WMIA)
Adriatic Sea (ADR)	North Adriatic (NADR) Middle Adriatic (MADR) South Adriatic (SADR)
Central Mediterranean (CEN)	Central Mediterranean(CEN) Ionian Sea (IONS)
Aegean and Levantine Seas (AEL)	Aegean Sea (AEGS) Levantine (LEVS)

3.6. Data filter (QA check)

The datasets distributions observed in the general MEDPOL database could be classified most commonly in three categories: left-skewed, normal-with tail and bimodal distributions. These can be also observed in Figure 5, corresponding to the MEDPOL and online group aggregated datasets used in this report, despite being a selection of reference areas/stations (Figure 5a and 5b). In order to select the statistical approach, the knowledge of the profile distributions within the final aggregated reference areas/stations datasets is of the highest importance (*c.a.* the probability distributions) to undertake the appropriate statistical analysis. In Figure 5, the left-skewed distribution (a) is an example of the aggregated dataset distribution for a hazardous chemical substance which is present naturally in the marine environment and where the tail responds to increased diffuse anthropogenic concentrations for some included country reference areas/stations, (b) corresponds to a natural or non-natural (thus, not shifted towards the zero) occurring hazardous chemical were the tail responds to increased anthropogenic concentrations for some included reference areas/stations, and (c) is a bimodal distribution of a hazardous substance which would be observed with aggregated reference areas/stations datasets when there are differences between natural background concentrations at a given spatial scale, such as Pb in sediments between the Western and Eastern Mediterranean basins. In terms of data profile distributions, the latest is also analogous when country datasets from reference, coastal and hotspot stations are plotted together, and therefore, being the first mode the statistic which would correspond to reference/coastal stations (mixed) and a second mode would represent data from hotspot stations (as observed before the data selection process described in section 3.4).

The complexity in all the cases relies on establishing which data belong to reference stations beyond its definition, thus there is an inherent temporal and spatial datasets variability (at any scale), which makes complex to comprehend what are the natural/background concentrations and to discriminate from potential reference areas/stations with diffuse anthropogenic inputs (either marine, land-based or atmospheric). From a theoretical and general point of view, the selected reference areas/stations datasets should solely exhibit normal probability distributions without any tailing (considering the Central Limit Theorem) and assuming single background concentrations for the whole Mediterranean Sea. In practice, however, the reference areas/stations datasets are “contaminated” with random data for a number of different reasons, such as some data is out of scale for one monitoring year, data for reference stations between countries show considerable absolute differences, etc.; otherwise, eco-regional differences are evident. These facts might cause the 90th percentile selection to be incorrect as representative of the background concentrations (BCs) for reference areas/stations at a Mediterranean basin scale.

In this report the selected and combined datasets from the different countries are not free of these, say “outlier” data (Figure 5a and 5b), and for these reason we have applied a mixtool algorithm (Benaglia et al., 2009) to perform a quality check for the aggregated datasets. The purpose of this statistical tool (in the Mixtool Package for R) is to examine a sample of measurements to discern and describe subgroups of individuals, even when there is no observable variable that readily indexes into which subgroup an individual properly belongs. This is sometimes referred to as “unsupervised clustering" or “model-based clustering” in the literature. In other words, we have reversely applied this method based on the maximum likelihood estimation (MLE) of two normal components (see examples Figure 5a’, 5b’ and 5c’) to track the percentage of data within each normal component (λ value, see Annexes), which gives a measure of the quality of the aggregated datasets to determine BCs. Thus, a parameter described with one of the normal components which includes a high percentage of data (>90%) indicates little influence from “outliers” within reference areas/stations at a Mediterranean Sea basin scale.

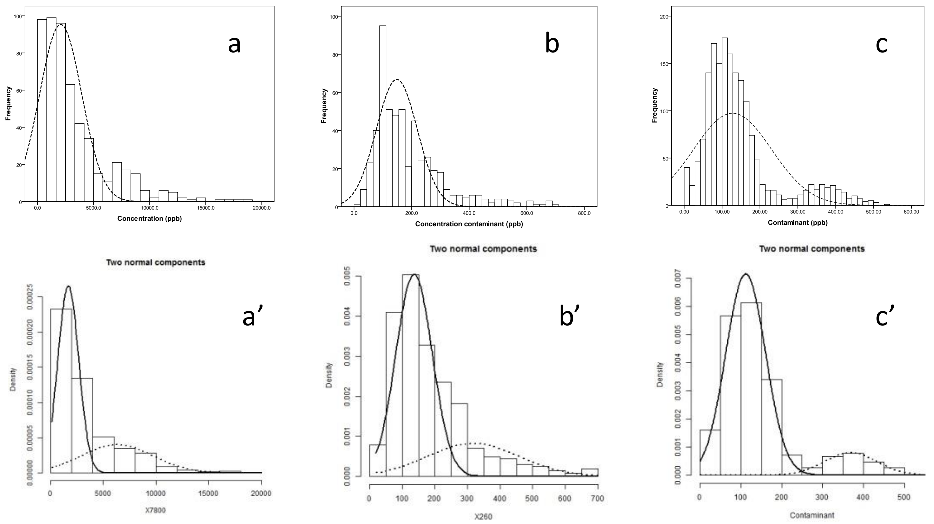

Figure 5. Example of the Mixtool algorithm results applied to perform quality data checks (a, b and c correspond to a’, b’ and c’ with two estimated normal components by MLE method).

On the other hand, the Mixtool algorithm[3] allows obtaining a primary normal probability distribution with a given mean and variance useful to calculate, for example, the upper and lower confidence limits for the population mean. In the Annexes in this report, the statistical results and both BC (medians) and BAC determinations, for each hazardous chemical substance and biomarker, are presented in tables along with the Mixtool algorithm results (mean, variance and % of data within each normal component), including a confidence interval for the mean (90% significance).

Furthermore, our statistical approach calculates the non-parametric statistics for the Mediterranean Sea basin reference areas/stations datasets aggregated by different geographical scales (including eco-regions),rather than for example, the median of the country medians (the5th or 10thpercentile) to evaluate the Mediterranean background concentrations (BCs). The advantages of this approach are to overcome the absolute scale differences (if country medians or percentiles are calculated *a priori*), to avoid a scarce number of medians for calculations which might lead to over or under estimations of Mediterranean BCs, as well as facilitates a full eco-regional and sub-regional approach. As mentioned, the statistical development and results are presented in full for detailed information in the Annexes.

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[3] The Mixtool package is available from the Comprehensive R Archive Network at http://CRAN.R-project.org/package=mixtools. Further statistical analyses were performed using the standard statistical package SSPS 17.0 software (SPSS Inc., Chigago, USA).

4. EVALUATIONOF BACKGROUND CONCENTRATIONS (BCs) AND BACKGROUND ASSESSMENT CRITERIA (BACs)WITHTHE MEDITERRANEAN SEA REFERENCE AREAS/STATIONS

4.1. Threshold values for trace metals (Cd, Hg, Pb) in mussels

Within the national monitoring networks in the Mediterranean Sea, different species of bivalves are sampled to determine trace metals, namely, *Mytilusgalloprovincialis* (MG), *Brachidontesvariabilis* (BV), *Mactracorralina* (MC), *Donaxtrunculus* (DT) and *Ruditapes decussates* (RD). These different species were chosen within the monitoring strategies due to the fact that the *common* Mediterranean mussel (MG) is not distributed in the entire coastline of the Mediterranean basin, archipelagos and islands. The majority of the northern coasts of the Mediterranean Sea, from West to East, have sufficient populations of this species (MG) along the coasts of MEDPOL countries to be used in the majority of the long-term national monitoring coastal networks. In this report, Croatia, France, Greece, Italy, Spain, Slovenia and Turkey contributed with datasets of MG, whilst other countries contributed with other species as follows: Lebanon (BV), Israel (MC and DT) and Tunisia (RD). These different species exhibit different bio-concentration factors of hazardous dissolved chemicals in coastal waters and thus, the determination of BCs and BACs for MG should not be inferred directly if the monitoring is undertaken with other bivalve species. The bivalves sample characteristics and reference stations by country, as well as the statistical results for trace metals are presented in Annex I.

For *Mytilusgalloprovincialis* species (MG), the table below (Table 4.1) shows the Med BCs and BACs calculated for the Mediterranean Sea basin and the BCs for each eco-region, except for the Central Mediterranean basin (no data available). The table also compares the determined Med BACs with the median value (50% of the data) of the MEDPOL Database for each eco-region earlier assessed (UNEP(DEPI)/MED WG.365/inf.4), which included coastal and hotspot stations.

Table 4.1. Mediterranean BCs and BACs (Med BACs) in *Mytilusgalloprovincialis* (µg/kg d.w.)

	Mediterranean Sea Basin		Western Mediterranean (WMS)		Adriatic Sea (ADR)		Central Mediterranean (CEN)		Aegean-Levantine Seas (AEL)	
Trace metal	***MedBCs***	***Med BACs***	*WMS BCs*	*50% MEDPOL Database	*ADR BCs*	*50% MEDPOL Database	*CEN BCs*	*50% MEDPOL Database	*AEL BCs*	*50% MEDPOL Database
Cd	***730.0***	***1095.0***	*660.5*	660 <MedBAC	*782.0*	800 <MedBAC	-	*430* <MedBAC	*942.0*	750 <MedBAC
HgT	***115.5***	***173.2***	*109.4*	130 MedBAC	*126.0*	140 <MedBAC	-	*160* <MedBAC	*110.0*	80 <MedBAC
Pb	***1542***	***2313***	*1585*	2000 <MedBAC	*1381*	1530 <MedBAC	-	*810* <MedBAC	*2300*	2280 <MedBAC

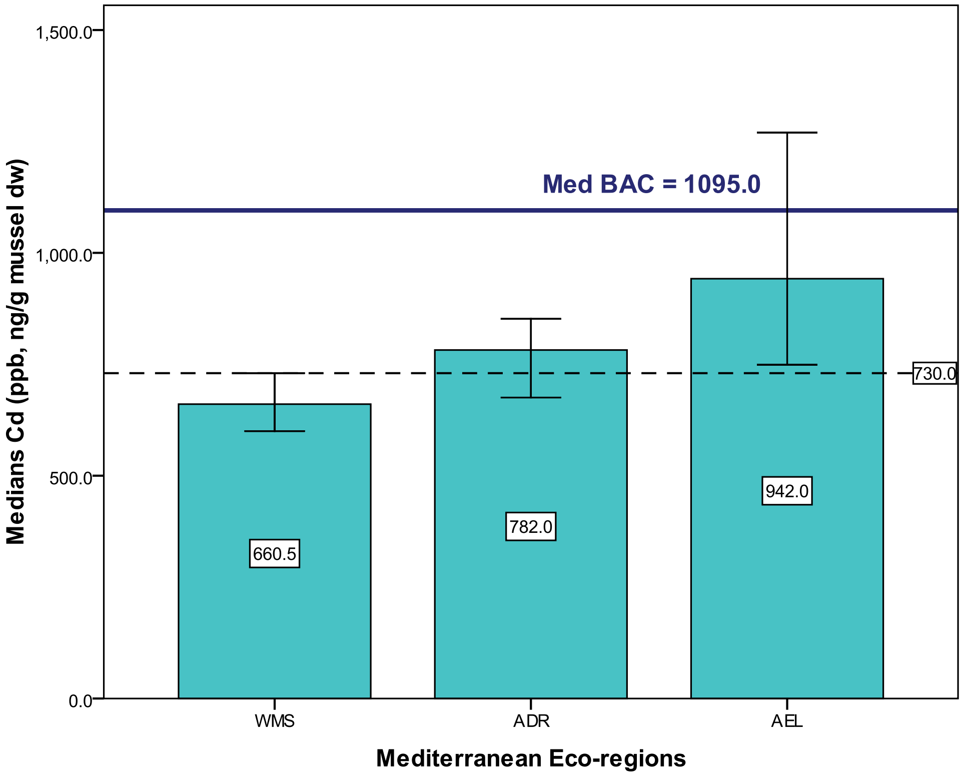
*median value of the MEDPOL Database from UNEP(DEPI)/MED WG.365/inf.4 Report (2011)

The earlier UNEP/MAP work reported data ranges varying over 5 orders of magnitude in the MEDPOL Database, with large asymmetric distributions for hazardous chemical substances (thus, includes both reference/coastal and hotspot stations, see section 3.6). It can be observed in Table 4.1, that at least a 50% or a higher percentage of the data in the MEDPOL Database (grouped by eco-regions) for Cd, HgT and Pb in mussels (MG) are below the Med BACs determined in this document. Consequently, the most impacted coastal sites and known hotspots contribute to the highest ranges observed in coastal environments and therefore, their environmental concentrations would be placed above the Med BACs.The balance between the three types of stations (reference, coastal and hotspots) and the spatial and temporal datasets availability by countries (or geo-scales), which are not homogeneous all over the Mediterranean Sea basin, might influence the calculated MEDPOL Database median. Further, it should be noticed that some calculated BCs for eco-regions (with reference areas/stations datasets) are above the determined Med BCs, although below the Med BACs, for example, both Cd and Pb in the AEL eco-region (Aegean-Levantine Sea) in the Eastern Mediterranean Sea. Here, the explanation is that the major contributor to this eco-region (Turkey) presents slightly higher background values than the average for the reference stations for MG in the Mediterranean Sea (see Annex I).

Nevertheless, the calculated eco-region BCs are well below the determined Med BACs for the trace metals evaluated, as it should be expected, and does not point to any major interference of the selected reference station aggregated datasets. To this regard, the QA check shows that 94%, 88% and 88% for Cd, HgT and Pb, respectively, belong to a major normal component indicating the reliability of the determined Mediterranean BCs and BACs (see Annex I) for trace metals in biota. On the other hand, occasionally higher medians (ca. BCs) at sub-regional scales with higher concentrations than the Med BAC respond to the single contribution of country data exceptionally high despite selected reference stations (e.g. Figure 4.1). Hence, these BCs should be further studied at a sub-regional scale to confirm these reference stations are not influenced by diffuse anthropogenic inputs.

The following figures (Figures 4.1.1-4.1.4) illustrate these findings by eco-regions and sub-regions. It can be observed, for example, the confidence interval for mercury in mussels (MG) in the Northern Adriatic (NADR) is above the Med BAC (Figure 4.2), which is potentially due to the anthropogenic contribution mainly from datasets from Croatia, and Italy to a lesser extent. Similarly, the median level of cadmium in the Western Mediterranean Islands and Archipelagos (WMIA) is above the determined Med BAC and responds to measured cadmium concentrations above the Mediterranean average. For the latter, the inputs of atmospheric cadmium in remote areas (Corsica Island, France) should be considered and thus, these higher data should not be overlooked. In these cases, natural and anthropogenic sources and inputs of trace metals should be further investigated to confirm the causes of exceptionally high concentrations in reference stations. In any case, the median values calculated at a sub-regional seas scale should be carefully examined according the number of stations and datasets employed for each (see Annex I).

In Section 5, tables of the recommended Med BCs, Med BACs and EACs are presented.



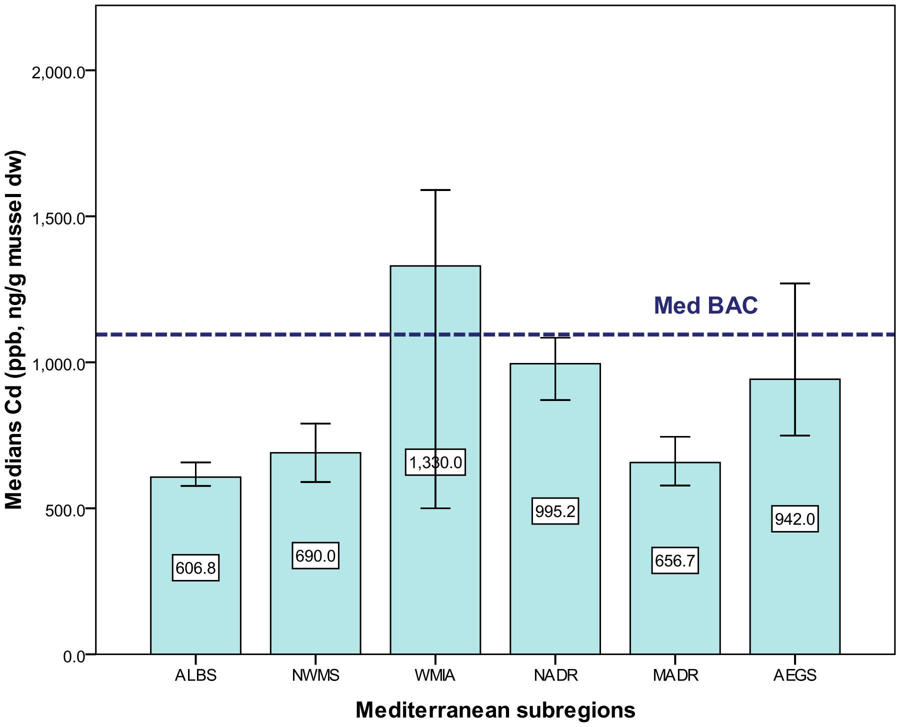
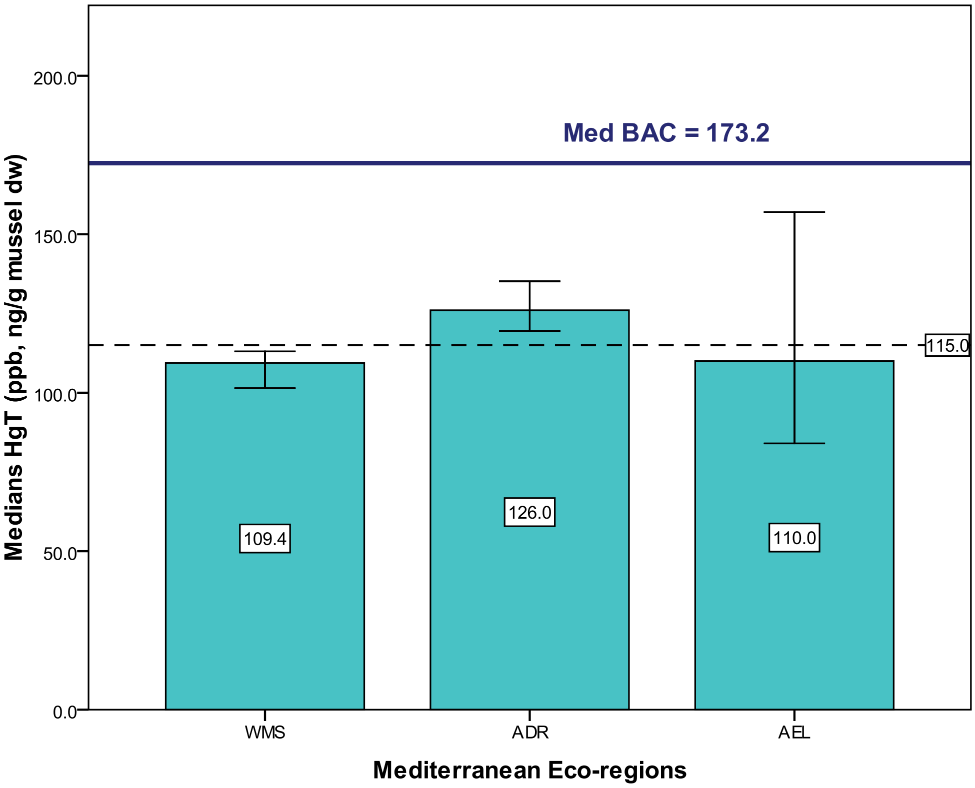


Figure 4.1.1. Plots of cadmium medians (BCs) in mussels by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the dataset median (Med BC).



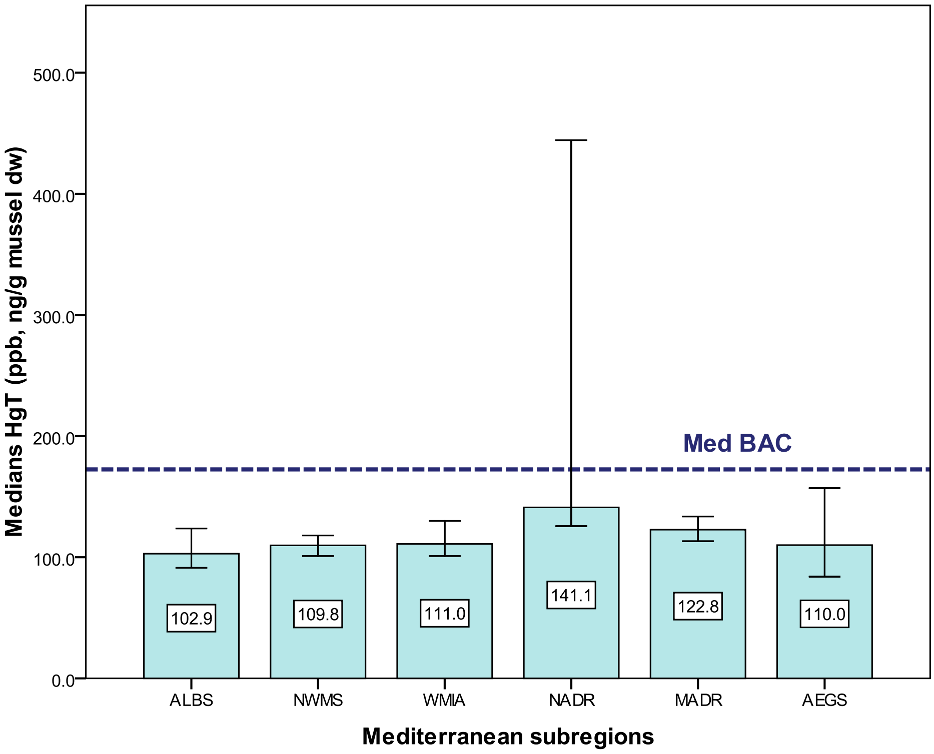
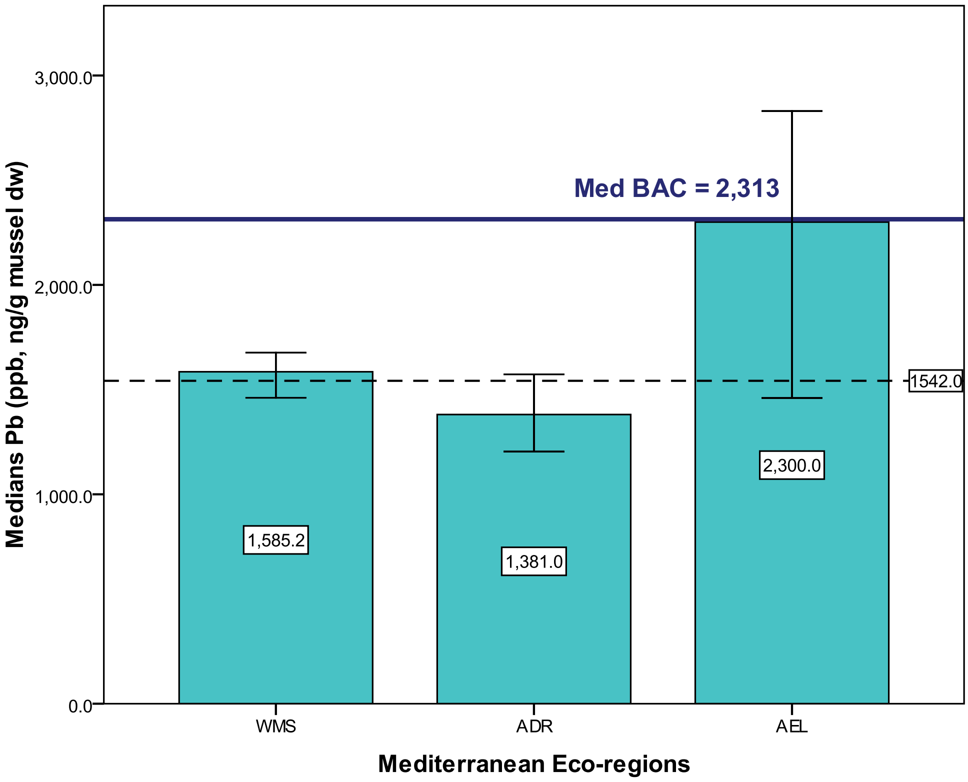


Figure 4.1.2. Plots of total mercury medians (BCs) in mussels by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the dataset median (Med BC).



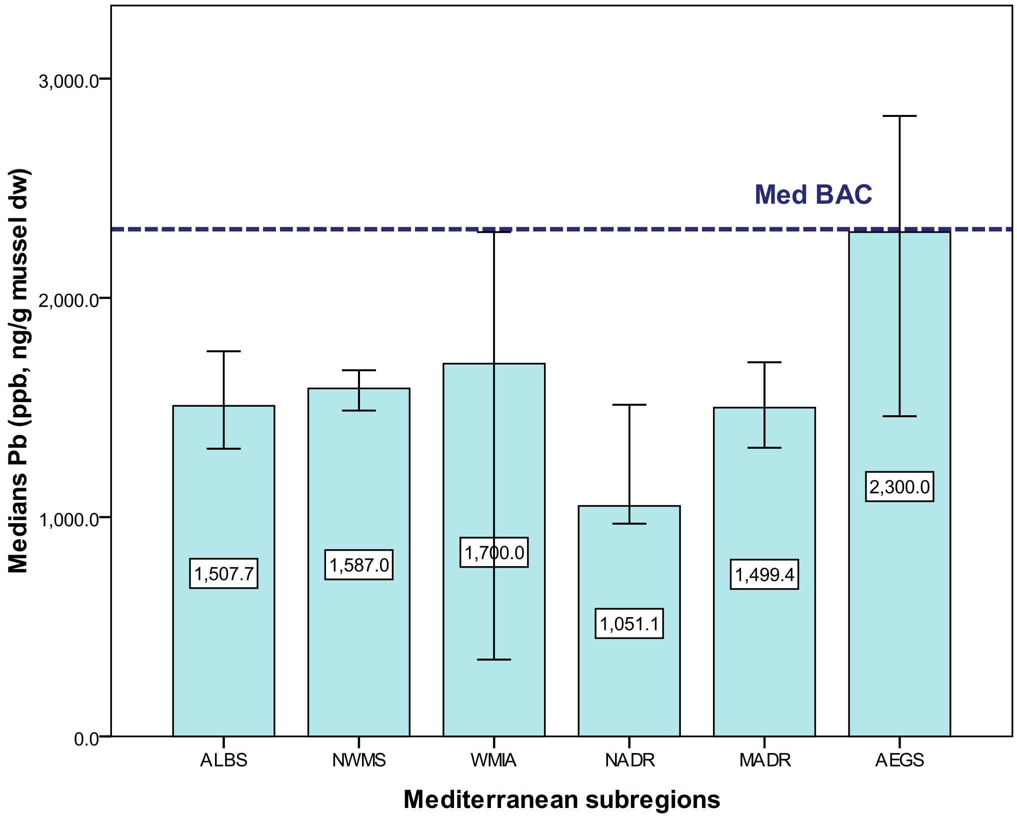
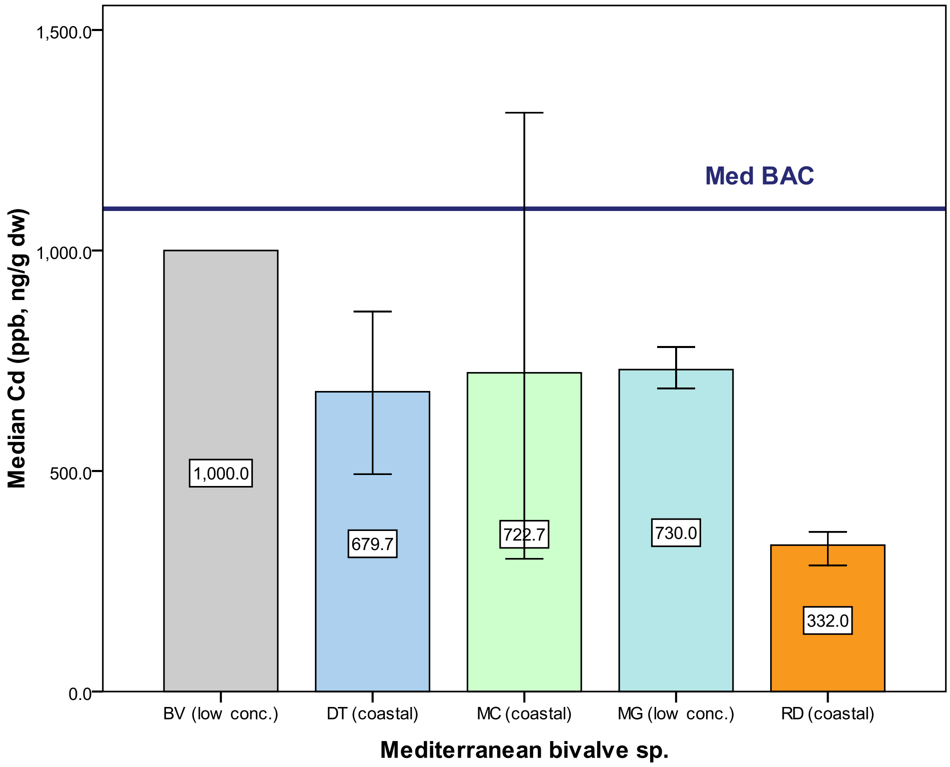
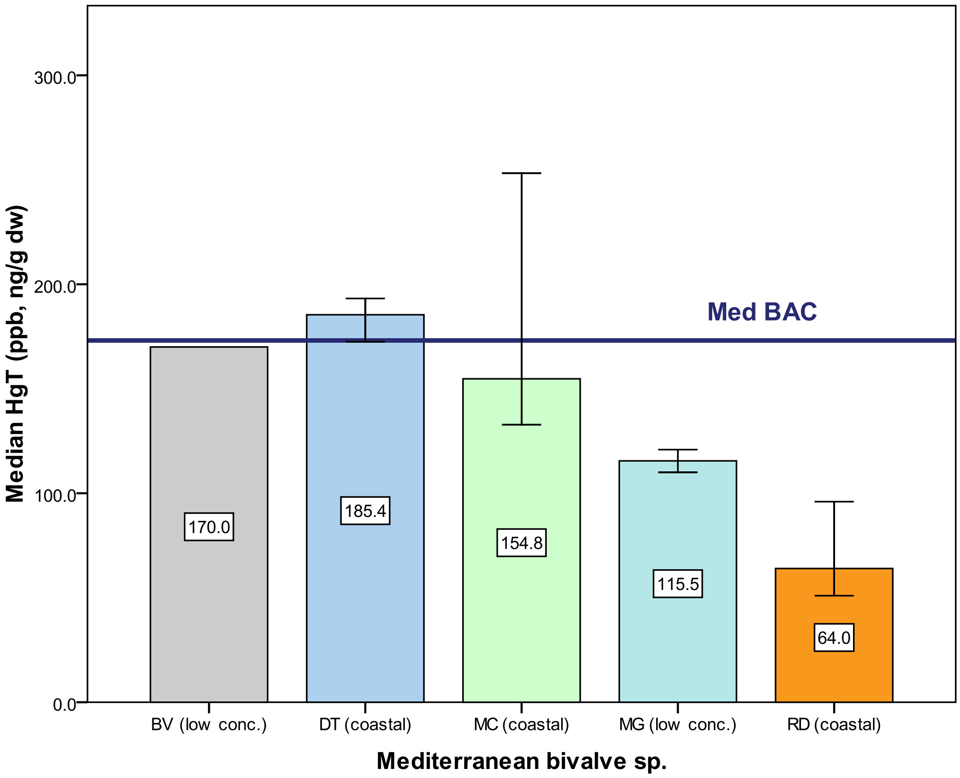


Figure 4.1.3. Plots of lead medians (BCs) in mussels by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the dataset median (Med BC).

As mentioned earlier, different species of bivalves are monitored in the Mediterranean Sea. The figures below compare the levels for Cd, Hg and Pb in the different species from monitoring datasets provided by MEDPOL countries.





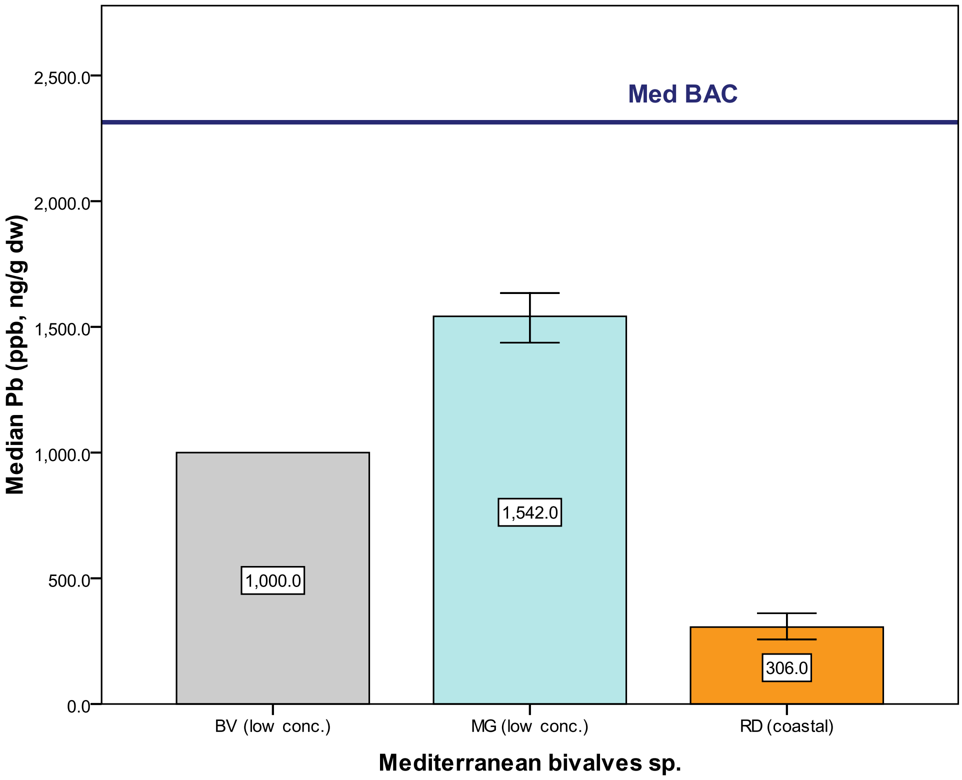


Figure 4.1.4. Plots of cadmium, mercury and lead (BCs) medians for different Mediterranean bivalve species for reference (low concentrations) and coastal stations (coastal) available in the MEDPOL database. The Med BC value for *Mytilusgalloprovincialis* is also shown for comparison. The error bar is a 95% confidence interval for the mean.

Although, the bioaccumulation factors between marine bivalve species might vary (Vázquez-Luis et al., 2016), the plots above shown the order of magnitude of the concentrations found in this species in the Mediterranean coastal environments. It should be observed that *Ruditapes decussates* (RD) in Tunisia, as well as *Donaxtrunculus* (DT) and *Mactracorralina* (MC) in Israel, have been calculated with datasets from coastal locations rather than reference areas/stations. The confidence intervals indicate some data above the determined Med BAC for *Mytilusgalloprovincialis* (MG), especially for Cd and HgT in MC species of Haifa Bay (Israel). However, at present a firm comparison between species could not be done, and the plots should be interpreted with caution against the Med BAC determined for *Mytilusgalloprovincialis* (MG).

4.2. Threshold values for trace metals (Cd, Hg, Pb) in sediment

The countries contributing to develop BCs and BACs assessment criteria for trace metals in sediments were Croatia, Egypt, France Greece, Italy, Spain, Turkey and Israel. In the Annex II, a list of sediment sample characteristics and the statistical results are presented. Within sediment monitoring activities, the sample collection and sample processing methodologies still differ between MEDPOL countries, in particular, for the particle size sieving method which ranged from <63µm to <2 mm fractions. This is an important parameter to allow in-depth comparisons, including regional and sub-regional scales, of the levels of trace metals in surface sediment samples. On the other hand, sediment sample aluminum (Al) normalization has been also investigated to report trace metal concentrations in sediments, despite it could not be performed at present with the available datasets. In this document, the datasets from the reference stations of the countries mentioned above were aggregated according section 3.6 without further considerations for the determination of BCs and Med BACs at a Mediterranean basin scale.

Table 4.2. Mediterranean BCs and BACs (Med BACs) in surface sediments (µg/kg dw)

	Mediterranean Sea Basin		Western Mediterranean (WMS)		Adriatic Sea (ADR)		Central Mediterranean (CEN)		Aegean-Levantine Seas (AEL)	
Trace metal	***MedB Cs***	***Med BACs***	*WMS BCs*	*50% MEDPOL Database	*ADR BCs*	*50% MEDPOL Database	*CEN BCs*	*50% MEDPOL Database	*AEL BCs*	*50% MEDPOL Database
Cd	***85.0***	***127.5***	*91.2*	1600> MedBAC	*92.3*	210 >MedBAC	-	*90* <MedBAC	*56.0*	100 <MedBAC
HgT	***53.0***	***79.5***	*60.0*	160 >MedBAC	*106.8 >Med BAC*	100 >MedBAC	-	*50* <MedBAC	*31.2*	150 >MedBAC
Pb	***16950***	***25425***	*20465*	19400 <MedBAC	*13932*	9830 <MedBAC	-	*4390* <MedBAC	*4920*	16890 <MedBAC >AELBAC**

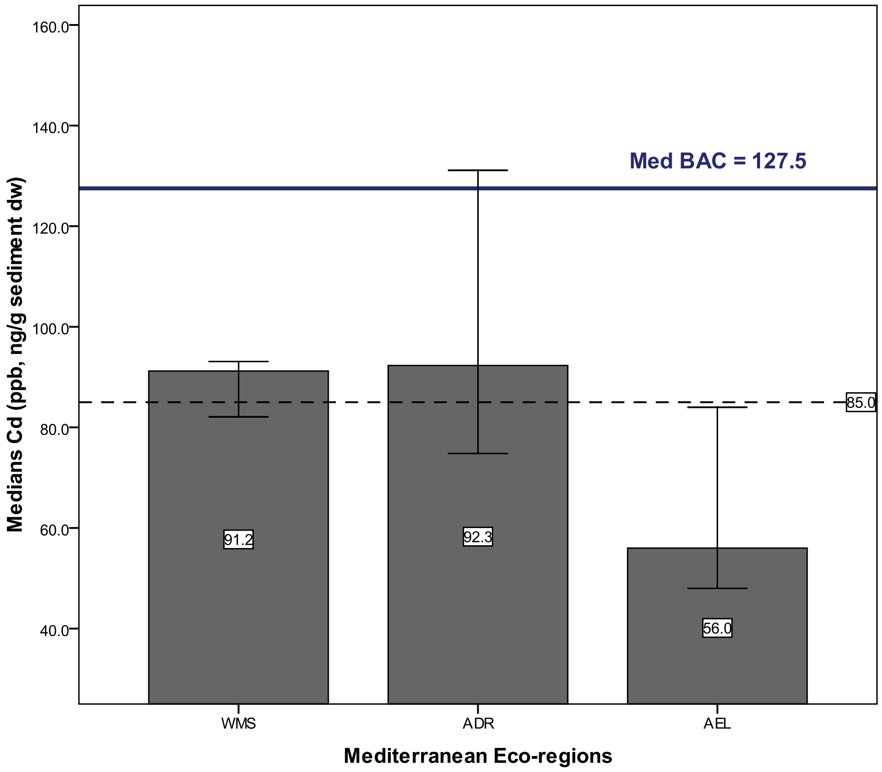
*median value of the MEDPOL Database from UNEP(DEPI)/MED WG.365/inf.4 Report (2011); **see text

The table above (Table 4.2) shows the calculated Med BCs and BACs for the Mediterranean Sea and the BCs for each eco-region, except for the Central Mediterranean basin due to the shorten datasets in this eco-region. The calculated background concentrations (BCs) for each eco-region (aggregated datasets) exhibit values under the calculated Med BACs, except for HgT. Further, the table also compares the determined Med BACs with the median value (50% of the data) from the MEDPOL Database for each eco-region earlier assessed (UNEP(DEPI)/MED WG.365/inf.4), which included reference/coastal and hotspot stations. For surface sediment samples the previous UNEP/MAP assessment report shows data ranges varying over 5 orders of magnitude in the MEDPOL Database, as for the case of mussel samples. As a consequence, it could be concluded from Table 4.2 for both the Western Mediterranean and Adriatic Sea eco-regions that more than the 50% of the MEDPOL Database data for Cd and HgT in sediments would be above the Med BACs and only for HgT in the Aegean-Levantine Sea, whilst the Central Mediterranean shows at least, the 50% of the MEDPOL monitoring data below the determined Med BACs.

Despite unbalanced datasets for each eco-region in the MEDPOL Database, this should be interpreted as preliminary information for the surface sediments contamination originated mostly from the monitoring data of highly impacted coastal sites and known hotspots in the MEDPOL Database, particularly, in the Western Mediterranean Sea. Similarly as for mussels, it should be noticed that some calculated BCs for eco-regions with reference stations datasets are above the calculated Med BCs, although below Med BACs. The Cd, HgT and Pb BCs in the WMS (Western Mediterranean Sea) eco-region are an example (Table 4.2). The exception is the total mercury (HgT) BC in the Adriatic Sea which almost doubles the Med BC and clearly exceeds the calculated Med BAC as well. The explanation here is found in the plots below (Figures 4.2.2-4.2.3), were it can be observed that the higher levels originates from reference stations located in specific areas, such as the Northern Adriatic Sea (NADR). A similar pattern for Pb is also observed. The Northern Adriatic is known as a major area of anthropogenic inputs and therefore the background concentrations, even in reference stations, might be biased in comparison with the Mediterranean Sea average. In the Western Mediterranean, the majority of the datasets from MEDPOL countries contains high concentrations of trace metals in marine sediments and might influence the reference areas, although the geological composition might also contribute to different background concentrations, particularly for Pb.

The aggregated datasets show 90%, 76% and 67% of the data in the primary normal component for Cd, HgT and Pb, respectively, and thus, indicates some discrepancies between the values for each aggregated sediment datasets in the Mediterranean Sea. In the case of mercury (76%), the datasets for reference areas/stations from Croatia show the highest median value (182.5 μg/kg dw sediment, see Annex II), located in the Northern Adriatic Sea coast. For lead, a 67% of the reference stations dataset fit in the primary normal component. In this case, the reason is the differences in the sediment lithological composition between the Western and the Eastern Mediterranean rather than a tailing due to diffuse anthropogenic inputs (see section 3.6). This is shown, here, by the fact that Egypt, Israel and Turkey datasets, which contribute to the AEL eco-region, present a similar median for reference stations (4410, 4063 and 4300 μg/kg dw, respectively, with an AEL BC = 4920 μg/Kg dw sediment), thus, a 33% of the dataset clearly differentiates in a secondary normal component. As a consequence, this fact should be carefully considered to assess the levels of Pb in sediments in the AEL eco-region and would be more appropriate to use a derived AEL BAC (AEL BAC = AEL BC x 1.5), that is 7380 μg/kg dry weight sediment. Therefore, the median value corresponding to the 50% of the MEDPOL data in this eco-region would be then above the AEL BAC rather than below the Med BAC.

The Mediterranean Sea eco-regions in the MEDPOL Database exhibit high concentrations for HgT, except for the Central Mediterranean, pointing to an influence of anthropogenic inputs, thus the Med BAC calculated from reference areas/stations is well below the median (50%) concentration level in the MEDPOL Database (UNEP(DEPI)/MED WG.365/inf.4). On the other hand, the calculated Med BCs and BACs for both Cd and Pb are in the order of magnitude of the background concentrations determined in deep sediment cores in the Western and Eastern Mediterranean basins (Angelidis et al. 2011 and UNEP(DEPI)/MED WG.365/inf.4). Certainly, further determinations of trace metals in sediment core samples and normalization procedures will give more accurate background concentrations to be able to better ascertain the trace metal assessment criteria in the Mediterranean Sea sediments at different regional and sub-regional seas scales. The Figures 4.2.1-4.2.3 illustrates the results for the aggregated sediment datasets from reference stations.



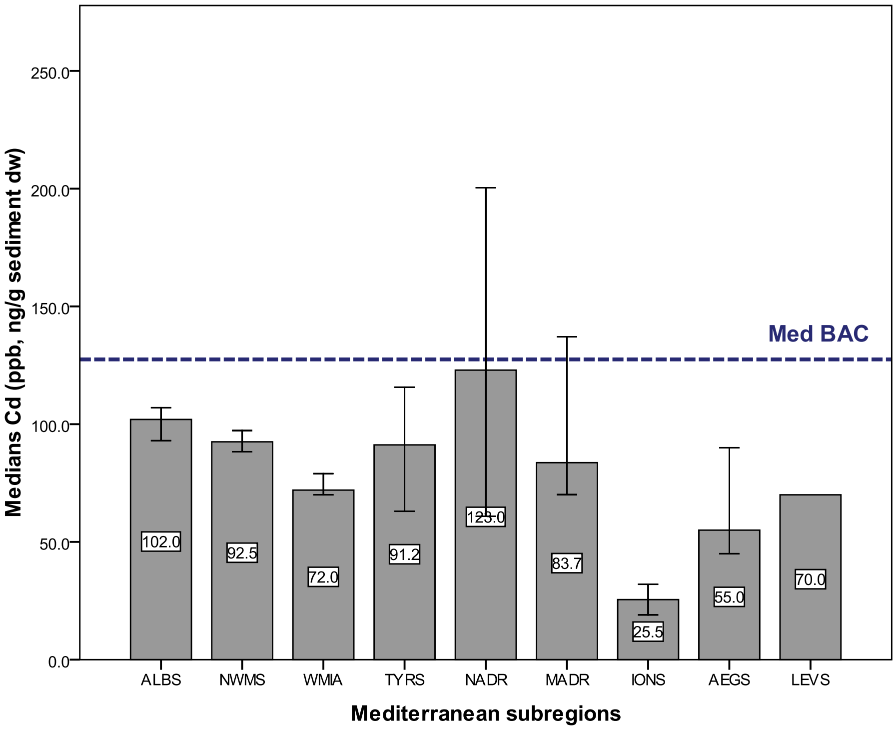


Figure 4.2.1. Plots of cadmium medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the median (Med BC).

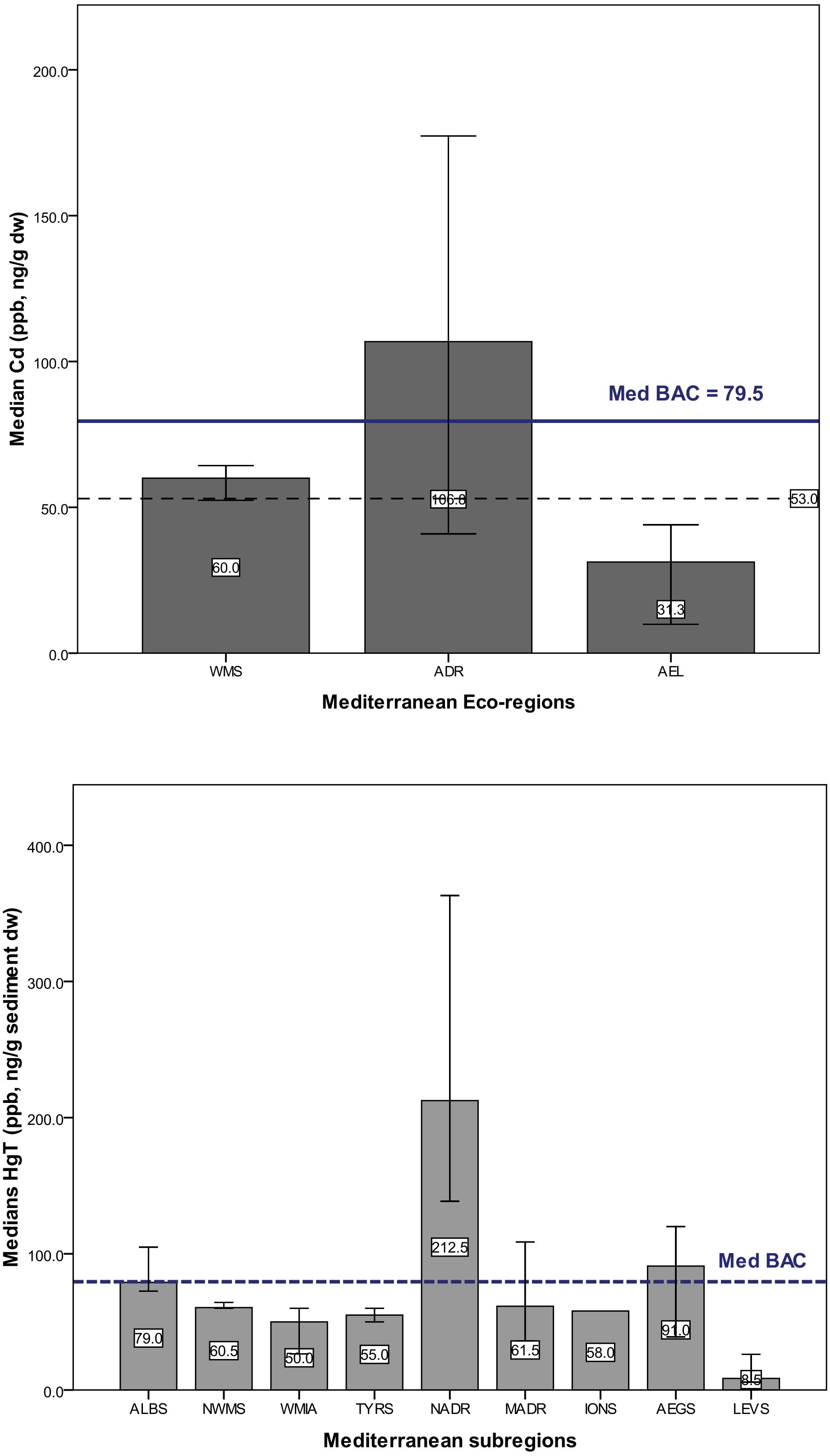
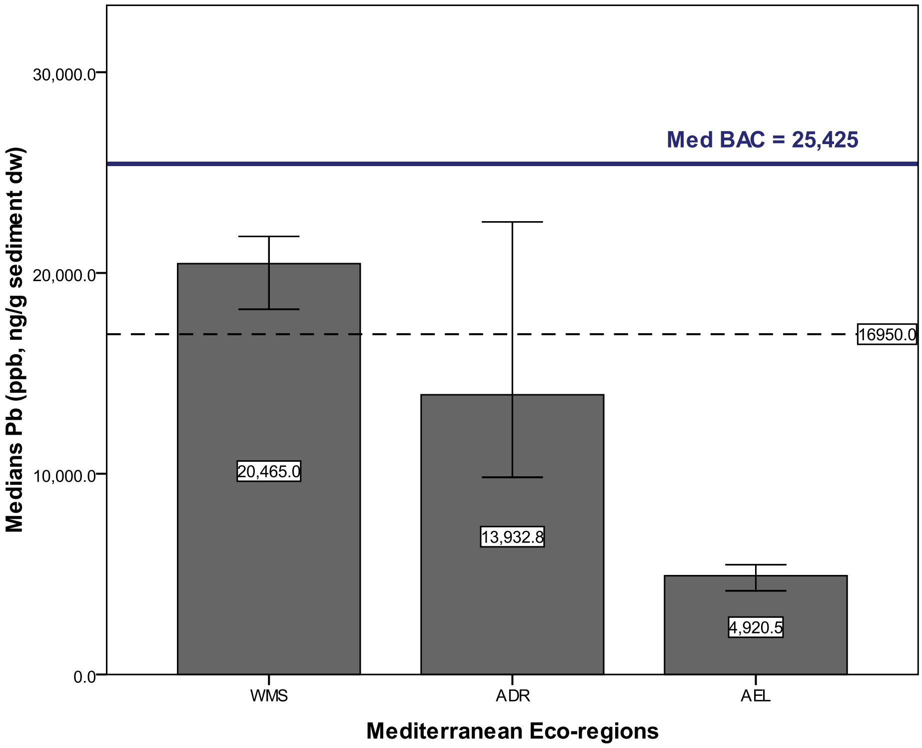


Figure 4.2.2. Plots of mercury medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the median (Med BC).



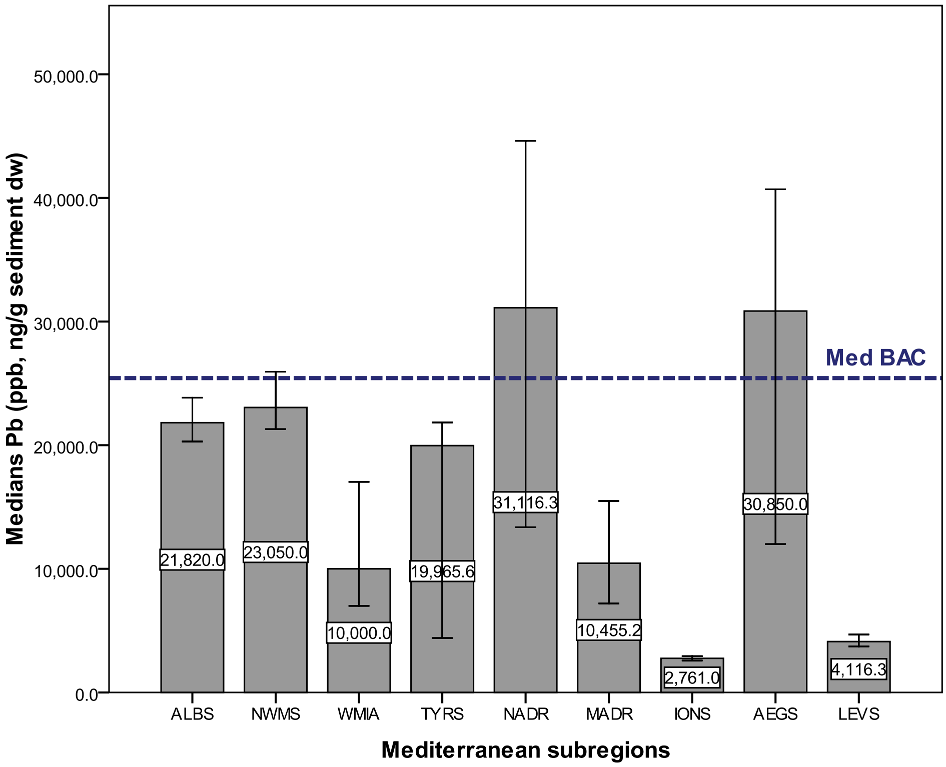


Figure 4.2.3. Plots of lead medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the dataset median (Med BC).

4.3. Threshold values for trace metals (Cd, Hg, Pb) in fish

Trace metals in fish are determined in several species in the Mediterranean Sea, such as *Mullus barbatus* (MB), *Boopsboops* (BB), *Mullussurmuletus* (MS) and *Upneusmollucensis* (UM). The species have been selected in the framework of MEDPOL according their geographical distribution within the national monitoring programs, with some countries monitoring more than one species. The majority of monitoring datasets are available for *Mullus barbatus* (MB), and therefore, this species has been chosen as the reference species to calculate the Mediterranean BCs and BACs for trace metals, although the statistical analysis has been undertaken for all (see Annex III). The countries with available datasets for reference stations for MB were Cyprus, Greece, Italy, Spain, Turkey and Israel. The monitoring of fish species, as in general for biota samples, should take into account the biometrics of the samples to reduce the inherent samples variability. The figure below (Figure 4.3.1.) shows the agreement in terms of length/weight for MB specimens sampled by the different MEDPOL countries. Further, some countries determined trace metals in individual specimens or pooled samples, as well as different fish tissues (see Annex III).

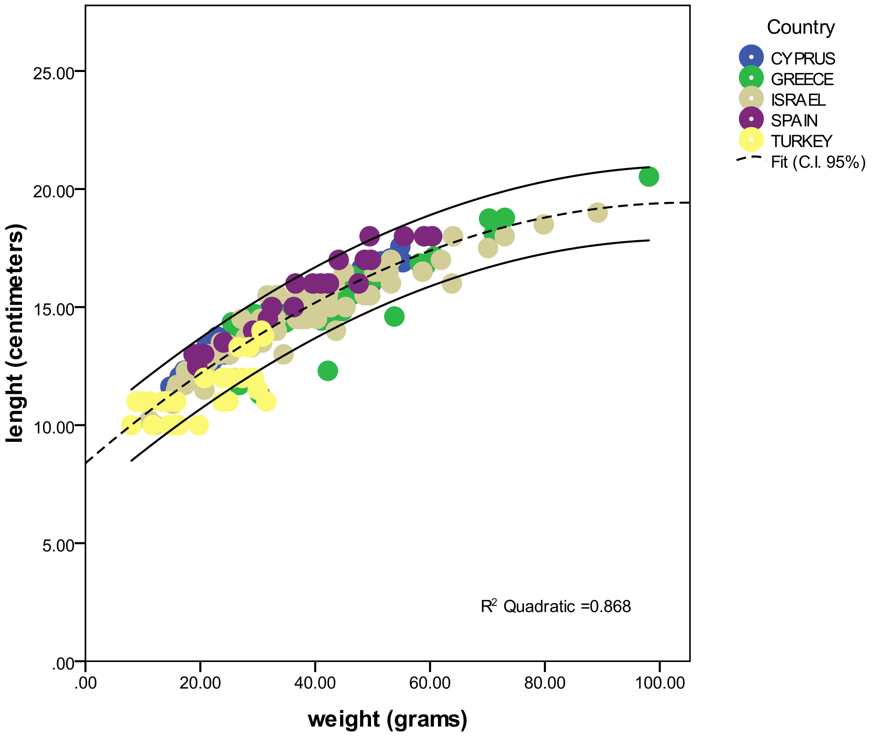
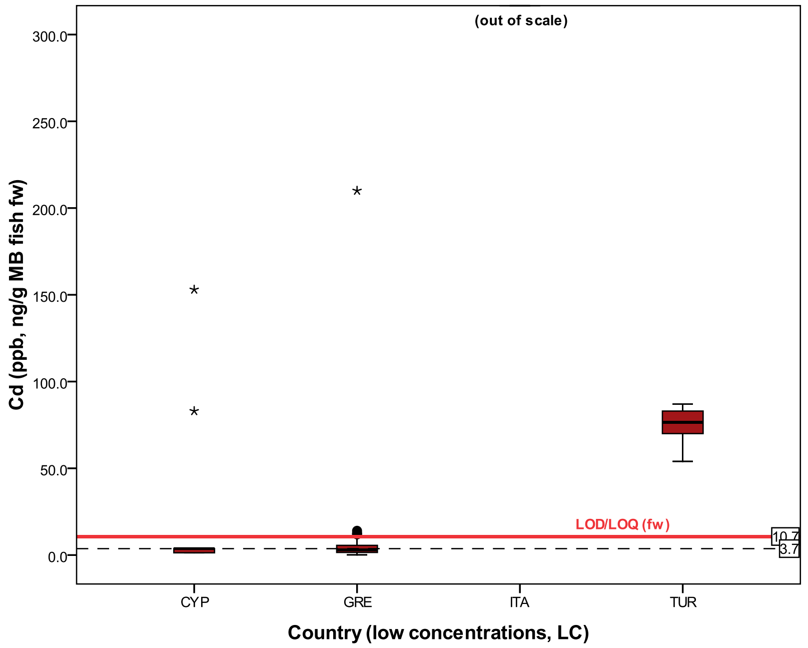
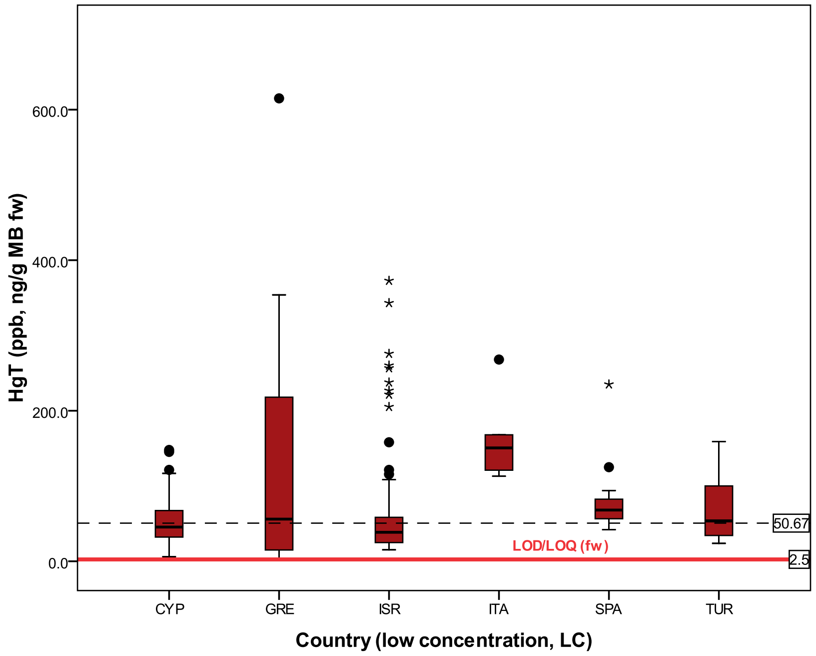


Figure 4.3.1. Length (cm) versus weight (grams) for MB samples collected by different MEDPOL countries in the Mediterranean Sea.

However, in terms of quality assurance, the determinations of Cd and Pb in fillet tissue of MB presents some analytical issues which impede to determine consistent Med BCs and BACs. Particularly, for Cd, the majority of the countries datasets reported over a 90% of the data as BDLs (below detections limit), if not a 100%. Similarly, for Pb, a majority of datasets are reported as either as BDLs or with large values pointing to sample contamination or reporting issues. This is valid for reference stations, as well as for coastal and hotspot stations within the MEDPOL Database. Therefore, it could be concluded that the species MB is not a good proxy for the evaluation of Cd and Pb in Mediterranean fish (fillet tissue). More, the organic contaminant determinations in MB are almost all 100% reported as BDLs (e.g. OCs). Obviously, this impedes to correlate any biological effects with the concentrations of hazardous chemical contaminants in MB sampled from the environment.

The Figure 4.3.2 below shows the reported concentrations of Cd, HgT and Pb in MB fillet tissue by countries in reference stations and includes a reliable estimated limit of detection (LOD) from common analytical methods (and analytical instruments) for these elements in biota matrices reported in the MEDPOL Database.





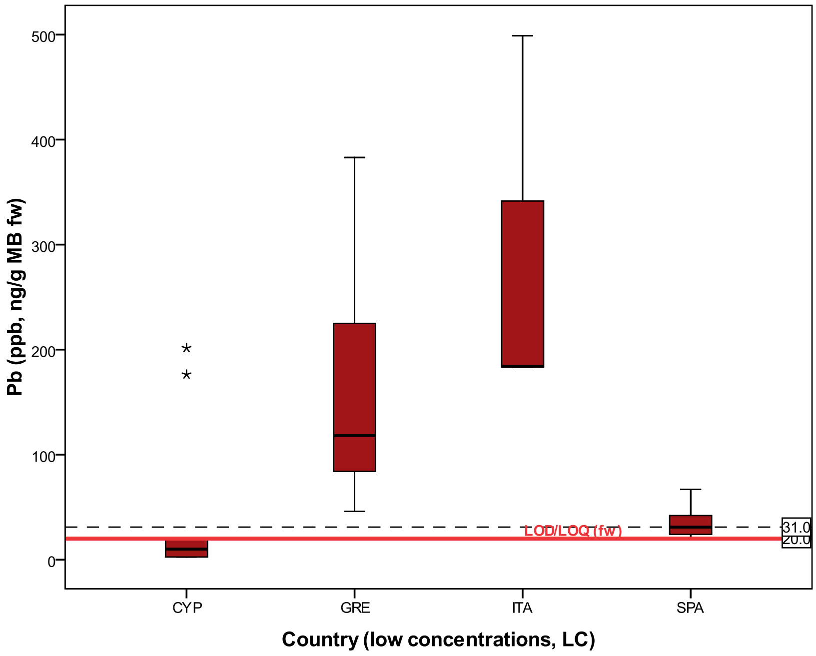


Figure 4.3.2. Datasets of Cd, HgT and Pb reported by countries and reliable limit of detection (LOD) in fresh weight for cadmium, mercury and lead.

It can be observed that the estimated LOD for cadmium (10.7 µg/kg fillet tissue f.w.) is above the median value (Med BC) calculated for this trace metal. For lead, the distance between the Med BC and the LOD (20 and 31 µg/kg fillet tissue f.w., respectively) is very narrow, despite positive. Nevertheless, some countries might be able to lower their analytical detection limits, for example, if ICP-MS for Cd and Pb determinations is chosen (probably Cyprus datasets). In detail, for Cd concentrations are high and do not reflect baseline concentrations for Turkey and Italy. Spain and Israel reported Cd values below detection limits (therefore, not shown). Similarly, Pb concentrations are very low in fillet tissue and the majority of the countries are very close or below analytical detection limits, except for Greece and Italy were Pb concentrations but do not reflect baseline concentrations for reference stations. As a consequence, the datasets for reference stations presents a lack of valid information to conclude Mediterranean BCs and BACs in fish, except for HgT (see also Annex III).To further exemplify this issue, the Table 4.3.1 shows the total error obtained from different QUASIMEME interlaboratory exercises for Pb in different biota species. Despite the type of biota, it can be clearly observed that the total instrumental error increases when the concentrations decrease, which causes a large uncertainty in measurements.

Therefore, the analytical challenges combined with low environmental concentrations (*ca.* lack of bioaccumulation in MB fillet tissue) are the reasons why trace metals in *Mullus barbatus* samples, within MEDPOL Database, are reported as below detection limits (BDLs) for Pb and Cd. On the other hand, this fact has been reflected within OSPAR Convention, and therefore, Cd and Pb determinations are recommended in fish liver, as well as their associated BCs and BACs assessment criteria.

QUASIMEME Code	Material type	Assigned (µg/Kg)	Exercise total error (%)
2011_QTM090BT	**Musseltissue**	**140.1**	**14**
2011_QTM089BT	**Shrimptissue**	**57.7**	**17**
2011_QTM091BT	**Musseltissue**	**427.8**	**13**
2011_QTM092BT	**Hakefishtissue**	**7.8**	**45**
2012_QTM095BT	**Musseltissue**	**432.9**	**13**
2012_QTM096BT	**Plaicefishtissue**	**7.6**	**46**
2014_R1_Sample1	**Turbotlivertissue**	**16.3**	**28**
2014_R1_Sample2	**Musseltissue**	**177.0**	**14**

Table 4.3.1. Colored relationship between concentrations and analytical uncertainty in biota samples for Pb determinations. Source: QUASIMEME Reports, 2011-2014.

A summary of some analytical methods and limits of detection for biota was recently reported by the European Union in the context of the Water Framework Directive (Guidance Document No. 33, ON ANALYTICAL METHODS FOR BIOTA MONITORINGUNDER THE WATER FRAMEWORK DIRECTIVE, Technical Report - 2014 – 084). The boxes relative to Cd, HgT and Pb determinations are shown in a Dialog Box in this section to give an overview of the current analytical capabilities to perform trace metal measurements in biota, as well as to indicate common limits of detection (LOD) and quantification (LOQ).

The table below (Table 4.3.2) presents the calculated Med BCs and Med BACs for fish, the despite the considerations explained above should be observed.

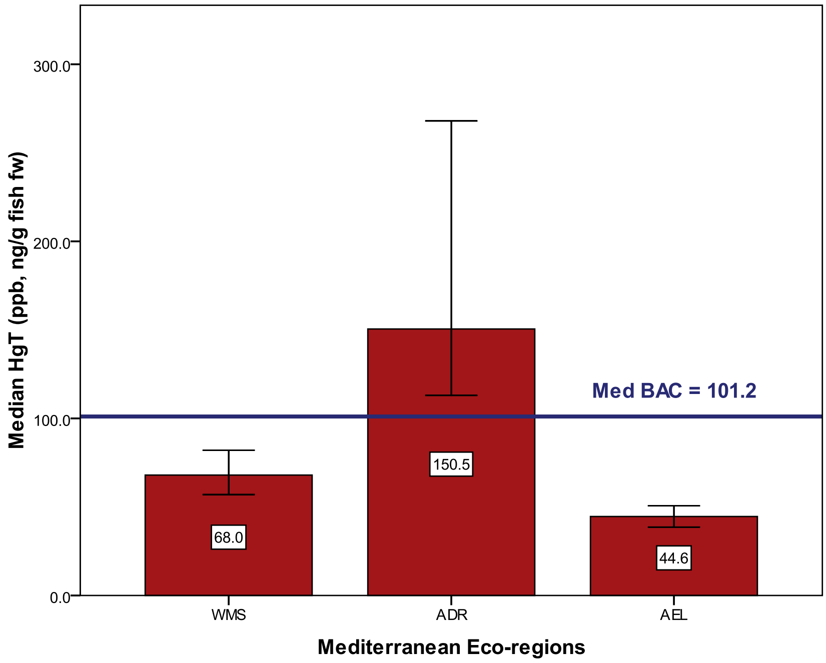
Table 4.3.2. Mediterranean BCs and BACs (Med BACs) in fish (µg/kg fw)

	Mediterranean Sea Basin		Western Mediterranean (WMS)	Adriatic Sea (ADR)	Aegean-Levantine Seas (AEL)
Trace metal	***MedBCs***	***Med BACs***	*WMS BCs*	*ADR BCs*	*AEL BCs*
Cd	***(3.7)***[a]	***(16.0)***[b]	-	-	-
Hg	***50.6***	***101.2***	*68.0*	*150.5* *>MedBAC*	*44.6*
Pb	***(31)***[a]	***(40)***[b]	*38*	-	*20*

[a] Cd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.

[b] estimated BACs from reliable limits of detection (BAC=1.5 x LOD) using both analytical data and certified reference material information (DORM-2). However, liver tissue matrix should be recommended for Cd and Pb as within OSPAR Convention.

The plots below (Figure 4.3.3) present the mercury results for MB for reference areas/stations grouped by eco-regions and sub-regions in the Mediterranean Sea.



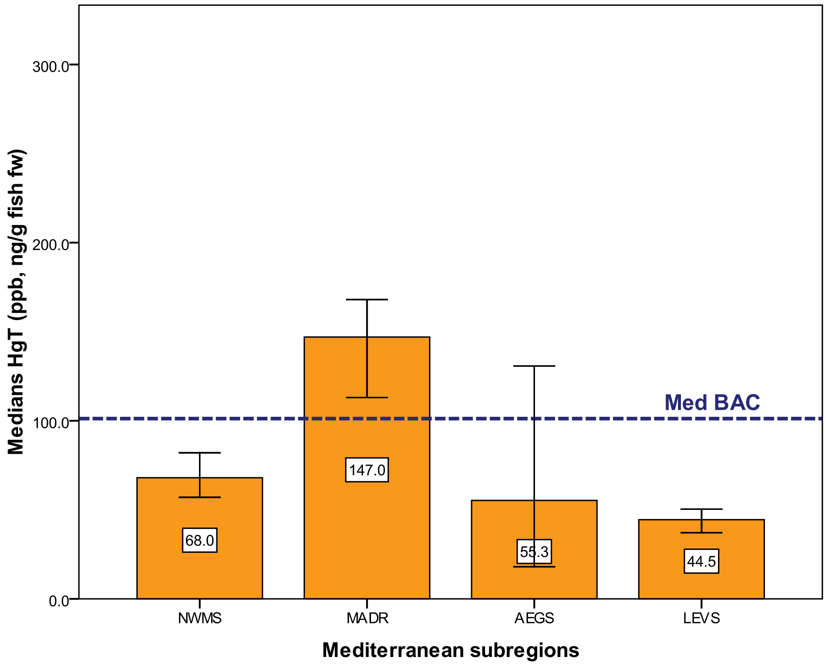


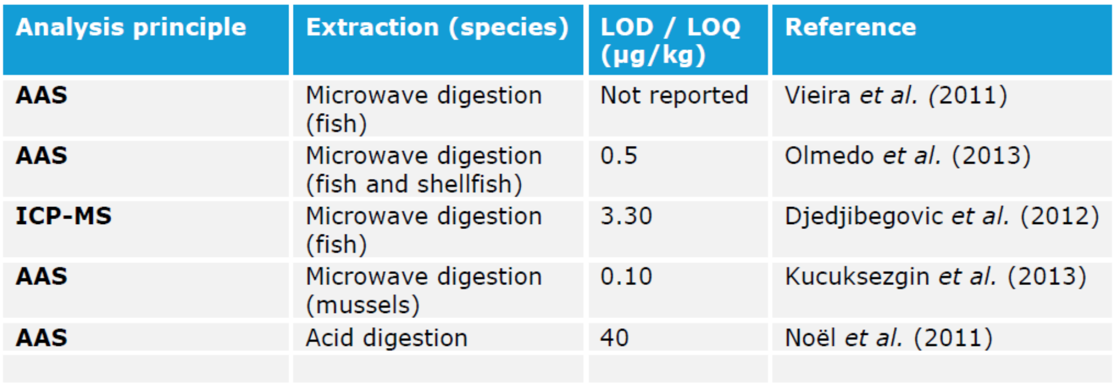
Figure 4.3.3. Plots of mercury medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean; the dashed line (top) is the median (Med BC).

It can be observed that the calculated ADR BC exceeds the Med BAC calculated for mercury due to datasets originated in the Northern Adriatic Sea (see Annex III). As in the case of mussels and sediments, further local studies should confirm theelevated levels in the

Northern Adriatic area. In Section 5, a summary of the proposed assessment criteria for trace metals in fish in the Mediterranean Sea is presented.

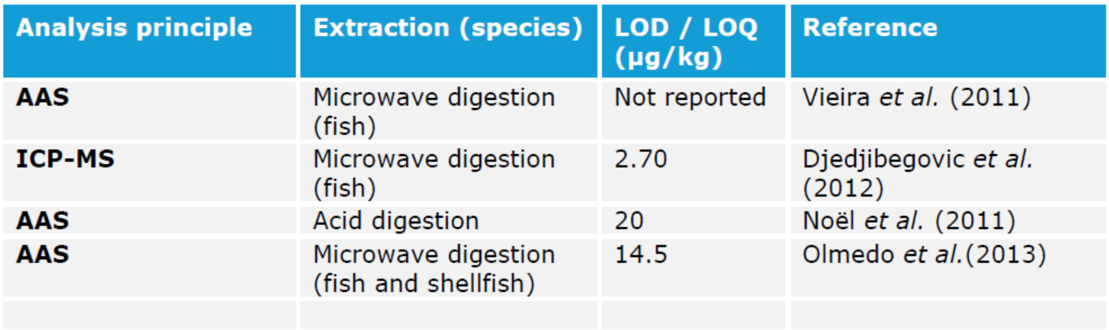
DIALOG BOX: Analytical methods for trace metals in biota.

Cadmium analytical methods (SOURCE: WFD, Guidance Document No. 33, 2014)



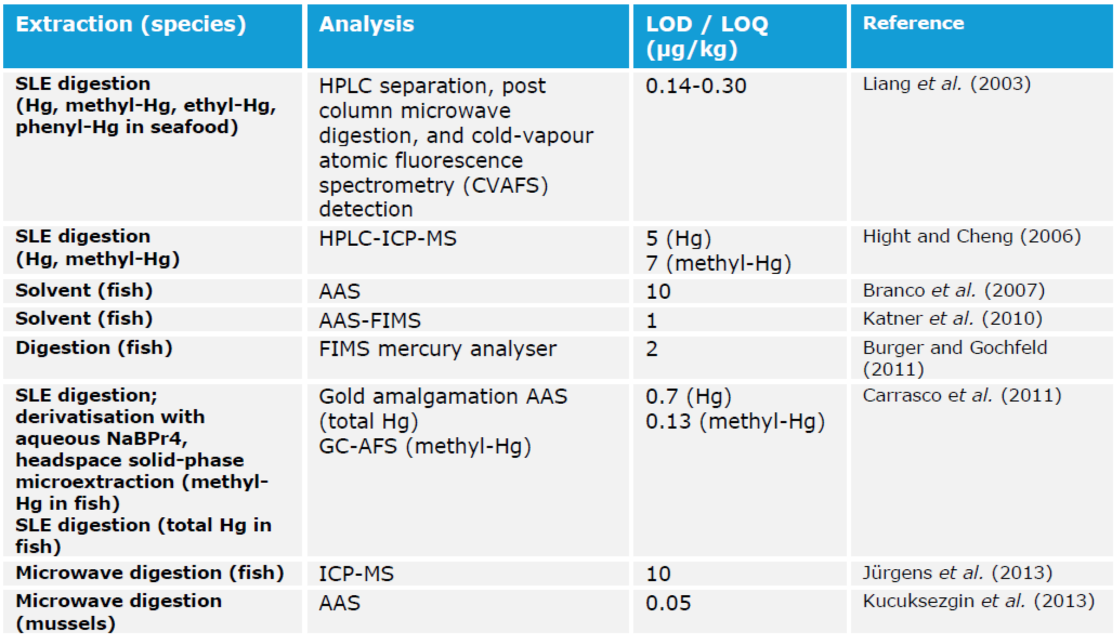
Analysis principle	Extraction (species)	LOD / LOQ (µg/kg)	Reference
AAS	Microwave digestion (fish)	Not reported	Vieira *et al.* (2011)
AAS	Microwave digestion (fish and shellfish)	0.5	Olmedo *et al.* (2013)
ICP-MS	Microwave digestion (fish)	3.30	Djedjibegovic *et al.* (2012)
AAS	Microwave digestion (mussels)	0.10	Kucuksezgin *et al.* (2013)
AAS	Acid digestion	40	Noël *et al.* (2011)

Lead analytical methods (SOURCE: WFD, Guidance Document No. 33, 2014)



Analysis principle	Extraction (species)	LOD / LOQ (µg/kg)	Reference
AAS	Microwave digestion (fish)	Not reported	Vieira *et al.* (2011)
ICP-MS	Microwave digestion (fish)	2.70	Djedjibegovic *et al.* (2012)
AAS	Acid digestion	20	Noël *et al.* (2011)
AAS	Microwave digestion (fish and shellfish)	14.5	Olmedo *et al.*(2013)

Mercury analytical methods (SOURCE: WFD, Guidance Document No. 33, 2014)



Extraction (species)	Analysis	LOD / LOQ (µg/kg)	Reference
SLE digestion (Hg, methyl-Hg, ethyl-Hg, phenyl-Hg in seafood)	HPLC separation, post column microwave digestion, and cold-vapour atomic fluorescence spectrometry (CVAFS) detection	0.14-0.30	Liang *et al.* (2003)
SLE digestion (Hg, methyl-Hg)	HPLC-ICP-MS	5 (Hg) 7 (methyl-Hg)	Hight and Cheng (2006)
Solvent (fish)	AAS	10	Branco *et al.* (2007)
Solvent (fish)	AAS-FIMS	1	Katner *et al.* (2010)
Digestion (fish)	FIMS mercury analyser	2	Burger and Gochfeld (2011)
SLE digestion; derivatisation with aqueous NaBPr4, headspace solid-phase microextraction (methyl-Hg in fish) **SLE digestion (total Hg in fish)**	Gold amalgamation AAS (total Hg) GC-AFS (methyl-Hg)	0.7 (Hg) 0.13 (methyl-Hg)	Carrasco et *al.* (2011)
Microwave digestion (fish)	ICP-MS	10	Jürgens *et al.* (2013)
Microwave digestion (mussels)	AAS	0.05	Kucuksezgin *et al.* (2013)

Source: European Union, 2014.

4.4. Threshold values criteria for Polycyclic Aromatic Hydrocarbons (PAHs) in mussels

In order to develop Mediterranean BC and BACs for PAHs in mussels a limited number of datasets from MEDPOL countries for reference stations were available. The contributions were from France, Greece, Italy, Spain and Turkey. The polycyclic aromatic hydrocarbons were determined in mussel samples of similar lengths; despite each country followed different strategies to pool the samples (see Annex IV). The Mediterranean BCs and BACs have been determined for Naphthalene (N), Acenaphthylene (ACY), Acenaphtene (ACE), Fluorene (F), Phenanthrene (P), Anthracene (A), Fluoranthene (FL), Pyrene (PY), Benz(a)anthracene (BaA), Chrysene (C), Benz(e)pyrene (BeP), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Indeno(1,2,3-cd)pyrene (ID), Dibenz(a,h)anthracene (DA) and Benzo(g,h,i)perylene (GHI). The Table 4.4.1presents the determined assessment criteria for *Mytilus galloprovincialis* (MG) and the selected PAHs.

Table 4.4.1. Mediterranean BCs and BACs (Med BACs) in mussel samples (µg/kg dw).

	Mediterranean Sea Basin		Western Mediterranean (WMS)	Adriatic Sea (ADR)	Aegean-Levantine Seas (AEL)
PAH	***MedBCs***	***Med BACs***	*WMS BCs*	*ADR BCs*	*AEL BCs*
N	(2.4) *	(6.0)	2.24	-	2.80
ACY	(0.6)*	(1.4)	-	-	-
ACE	(0.6) *	(1.4)	-	-	-
F	1.0	2.5	0.96	1.07	0.60
P	7.1	17.8	4.93	9.04	7.55
A	0.5	1.2	0.52	0.38	0.30
FL	3.0	7.4	3.38	2.03	6.60
PY	2.0	5.0	3.02	0.85	5.90 >MedBAC
BaA	0.8	1.9	1.20	0.53	1.60
C	1.0	2.4	1.24	0.27	5.20 >MedBAC
BkF	0.6	1.4	1.27	0.29	1.50 >MedBAC
BaP	0.5	1.2	0.60	0.32	0.70
GHI	0.9	2.3	0.90	-	1.20
DA	0.5	1.3	0.53	-	-
ID	1.2	2.9	1.23	-	0.90

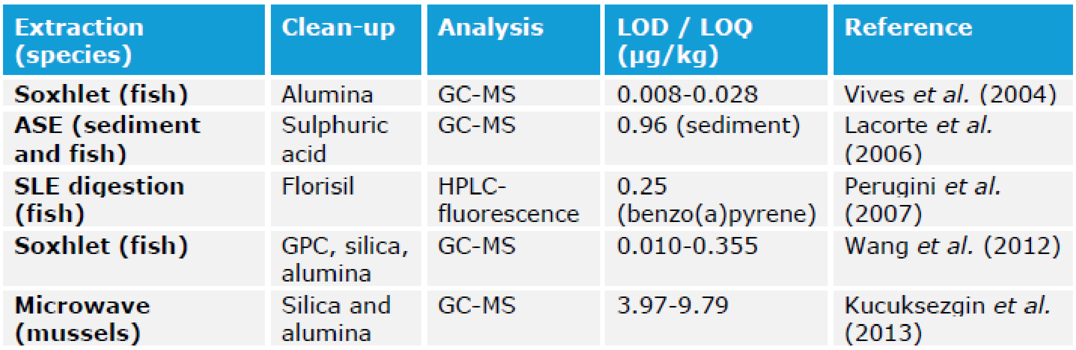
*Naphthalene, Acenaphtylene, Acenaphthene are below detection limits (BDLs) or have limited monitoring datasets, and therefore BACs are preliminary estimations. Benz(e)pyrene and Benzo(b)fluoranthene had not enough datasets (not shown).

Similarly as for trace metals in fish, an estimation of the reliable limits of detection in biota samples (mussel) has been performed and taken into account to evaluate the reference stations datasets, thus a large number of values within the countries datasets were reported as BDLs (see Annex IV). To this regard, the BDLs in the MEDPOL database were not substituted as the halfof the detection limit value of the analytical methodology, thus in the majority of the cases these occurred for more than the 10% of the reported data, and therefore, the datasets distributions become systematically biased. If BDL/2 is used for a large number of data within a dataset, the probability function deviates from normality (i.e. Gaussian), thus becoming "uniform" datasets. Therefore, those with added data were back-corrected (*ca.* BDLs were not used) before the statistical data analysis.

The LODs have been estimated using common reported limits of detection in the MEDPOL Database and based in scientific references for PAHs analytical methodologies and limits of detection (Webster et al., 2009 and Martínez et al., 2004). Further, in the Dialog Box below a revision of LODs and LOQs developed by the European Union in the context of the Water Framework Directive (Guidance Document No. 33, ON ANALYTICAL METHODS FOR BIOTA MONITORINGUNDER THE WATER FRAMEWORK DIRECTIVE, Technical Report - 2014 – 084) is also shown for comparison.

DIALOG BOX: Analytical methods for PAHs in biota.

PAHs (Reference: WFD, Guidance Document No. 33, 2014)

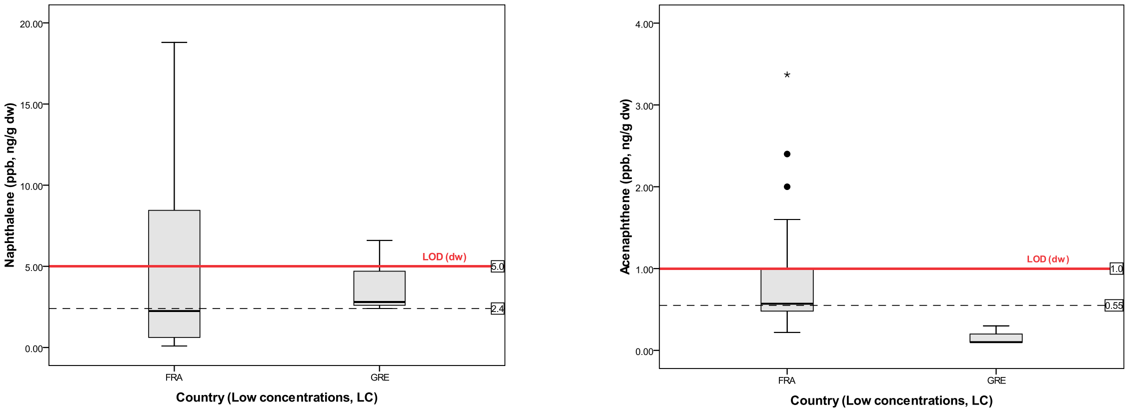


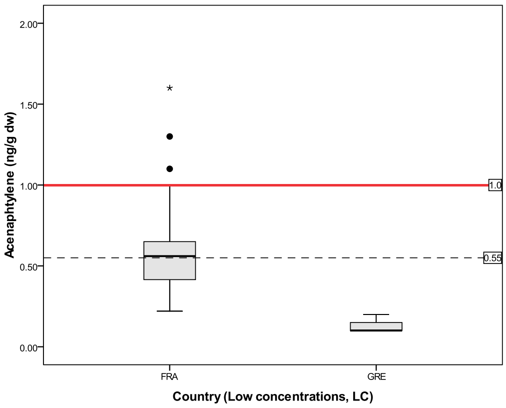
Extraction (species)	Clean-up	Analysis	LOD / LOQ (µg/kg)	Reference
Soxhlet (fish)	Alumina	GC-MS	0.008-0.028	Vives *et al.* (2004)
ASE (sediment and fish)	Sulphuric acid	GC-MS	0.96 (sediment)	Lacorte *et al.* (2006)
SLE digestion (fish)	Florisil	HPLC-fluorescence	0.25 (benzo(a)pyrene)	Perugini *et al.* (2007)
Soxhlet (fish)	GPC, silica, alumina	GC-MS	0.010-0.355	Wang *et al.* (2012)
Microwave (mussels)	Silica and alumina	GC-MS	3.97-9.79	Kucuksezgin *et al.* (2013)

Source: European Union, 2014

Additionally, some data for low molecular weight PAHs presented analytical inconsistencies with high values in reference stations probably due to contamination sources during both the sample treatment and analytical determinations, and thus, were omitted in the statistical assessment. On the other hand, datasets with values reported below the estimated LODs (in this report), as shown in the figure below (Figure 4.4.1), were not used to confirm Mediterranean BCs and BACs for PAH compounds. In general, these semi-volatile PAHs are accurately determined by GC-MS, although depends on the capabilities of analytical laboratories.

In Section 5, a summary of the proposed Mediterranean assessment criteria is presented.



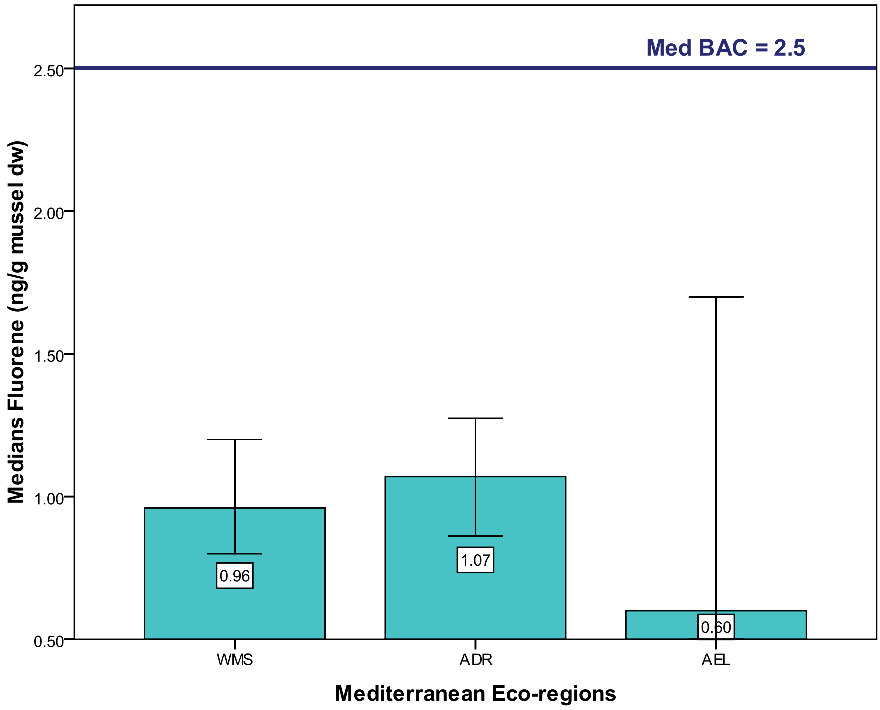


Naphthalene, Acenaphthylene and Acenaphthene were below the estimated LODs/LOQs generally reported in the literature, whilst the largest values have not been taken into account due to potential analytical troubleshooting.

Figure 4.4.1. Napthalene, Acenaphthylene and Acenaphtene box-plots showing country datasets below estimated LODs.The dashed line is the dataset median; the red line the estimated LOD.

In terms of QA data checks, the primary normal component for PAHs ranged between values from 58% to 96% for the aggregated reference stations datasets (see Annex IV). The lowest percentage of data included in a single component was found for Benzo(k)fluoranthene (58%) followed by Pyrene (59%), whilst the largest was found for Phenanthrene (96%) followed by Fluorene (92%) and Benzo(a)pyrene (86%). Here, the causes of discrepancies for the reference areas/stations aggregated datasets distributions point to analytical troubleshooting to determine these low levels of hydrocarbons, although diffuse anthropogenic inputs could not be discarded.

In Figures 4.4.2-4.4.12, the medians (BCs) for individual PAHs are plotted for each Mediterranean eco-regions and sub-regions. It should be noticed, that some eco-regions and sub-regional medians are above the calculated Med BACs. In this case, as mentioned before, this responds to the effect of grouped data by geographical areas with a scarce number of high data. When the medians and confidence intervals are above the Med BACs for a sub-regional sea, the number and magnitude of the data should be further examined (see Annex IV). As an example, Pyrene (Figure 4.4.6), shows a median (BC) below the Med BAC for the WMS eco-region, but when the two sub-regional areas in the WMS are considered (Alboran Sea-ALBS and Northwestern Mediterranean Sea-NWMS), the median and confidence interval for the NWMS is above the Med BAC due to the effect of few high data values which belong solely to this sub-regional area.



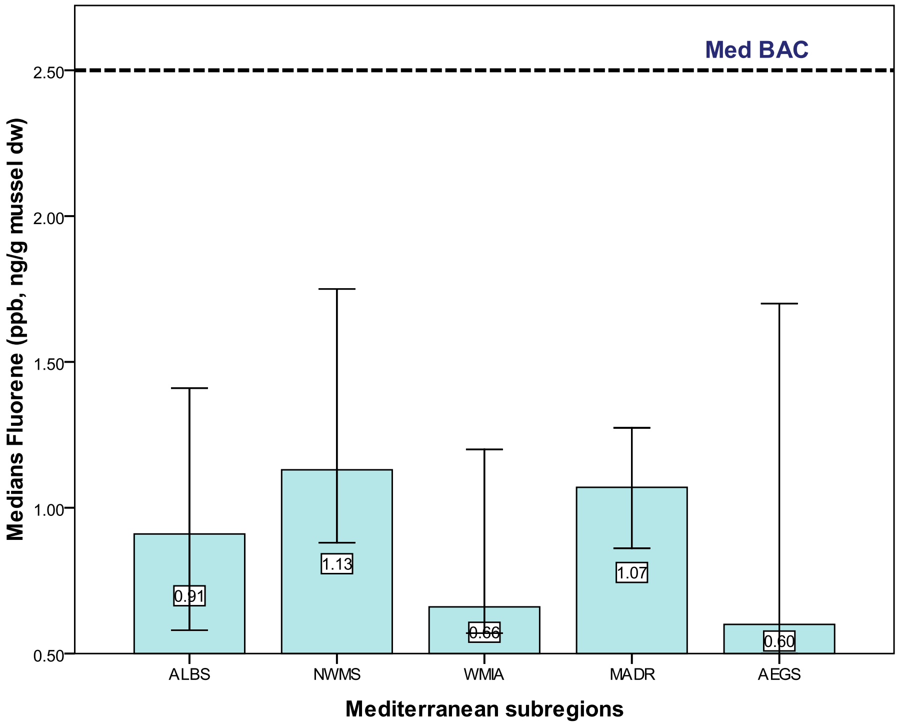
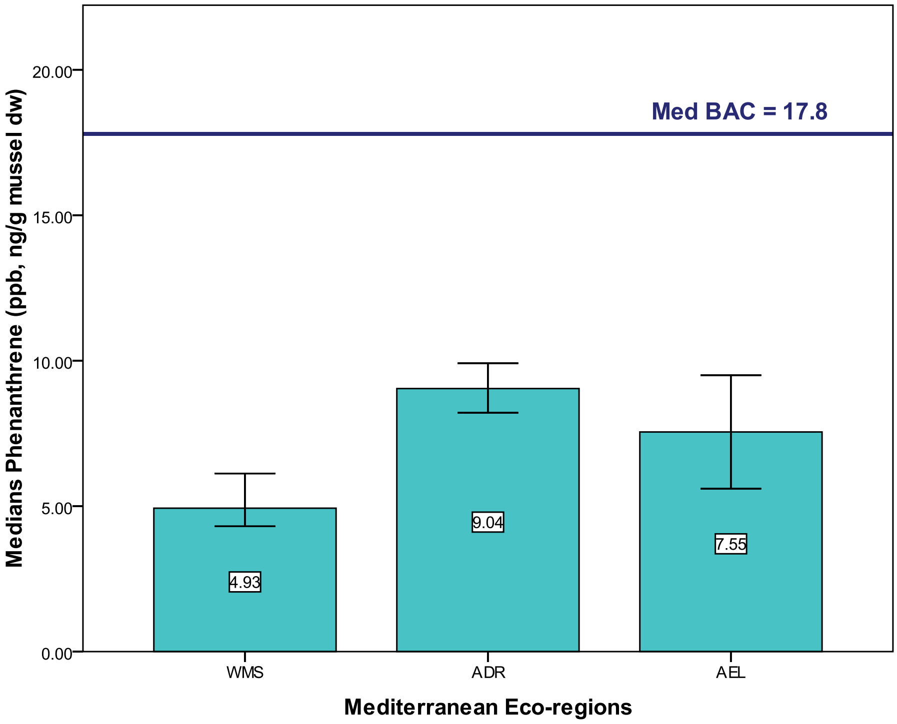


Figure 4.4.2. Fluorene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



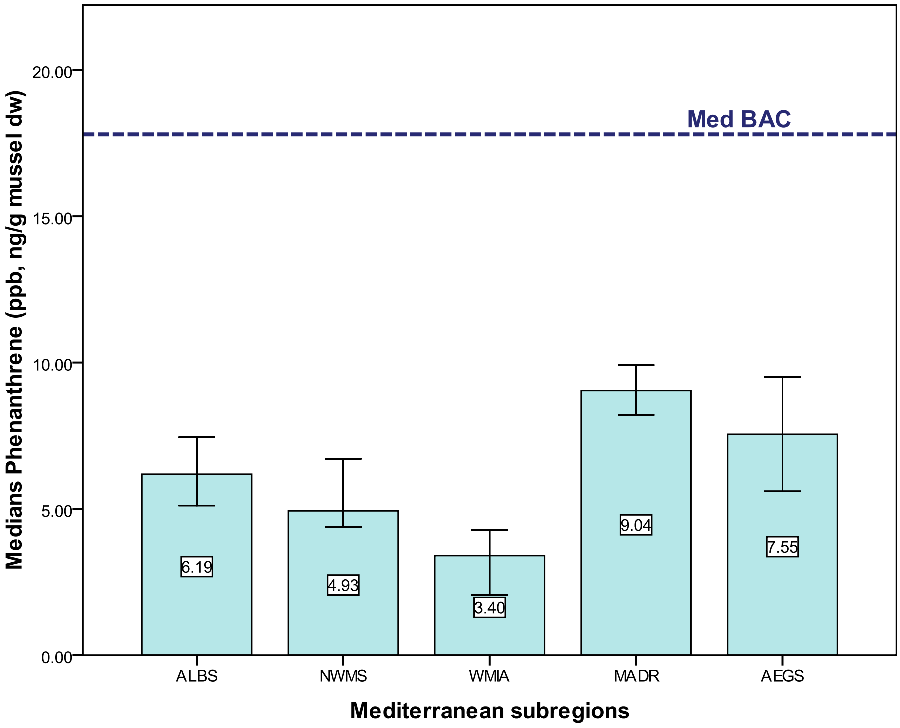
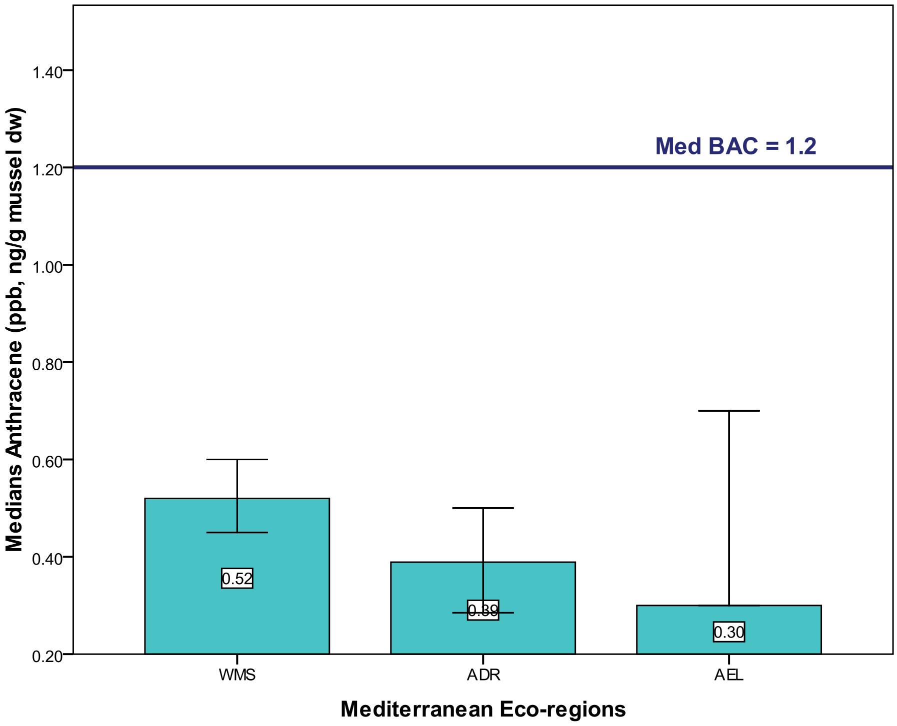


Figure 4.4.3. Phenanthrene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



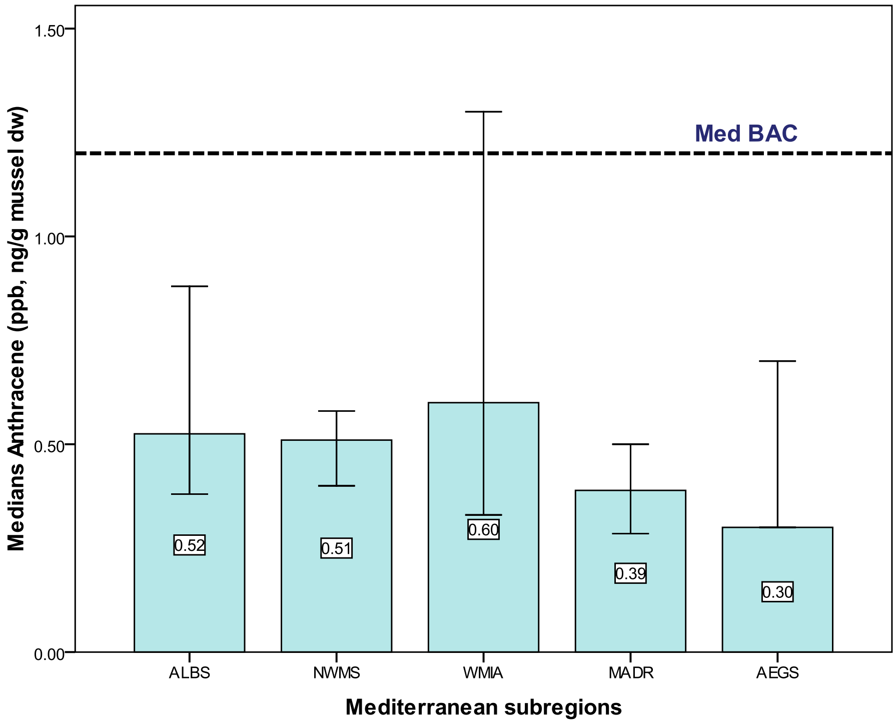
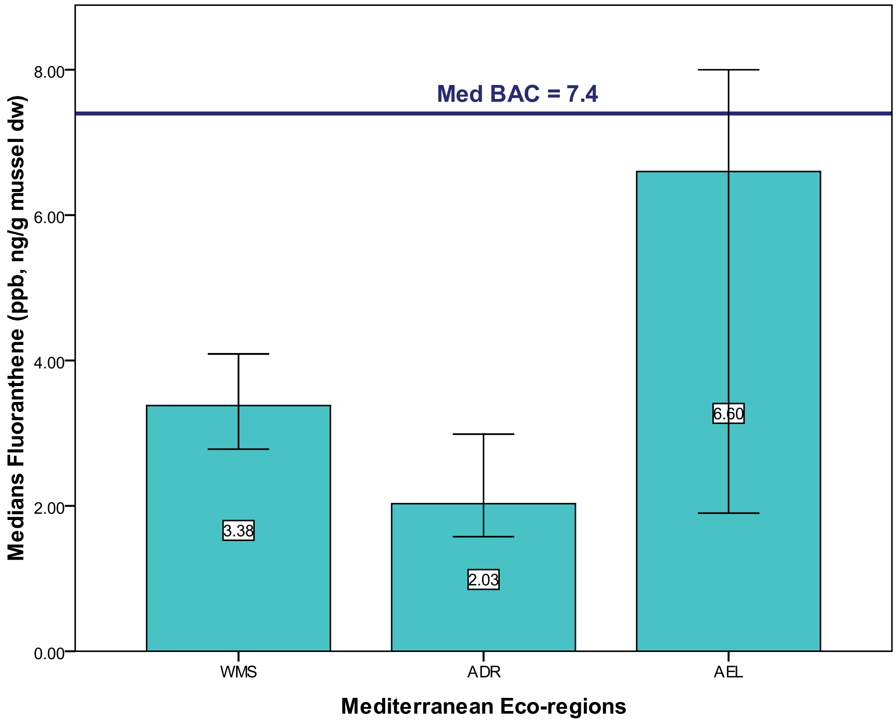


Figure 4.4.4. Anthracene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



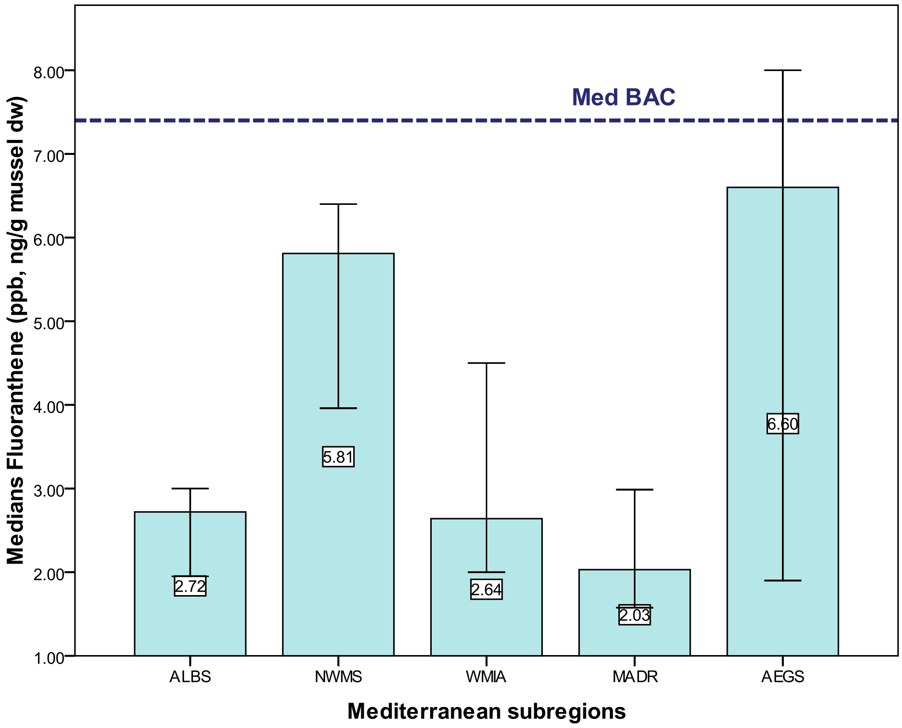
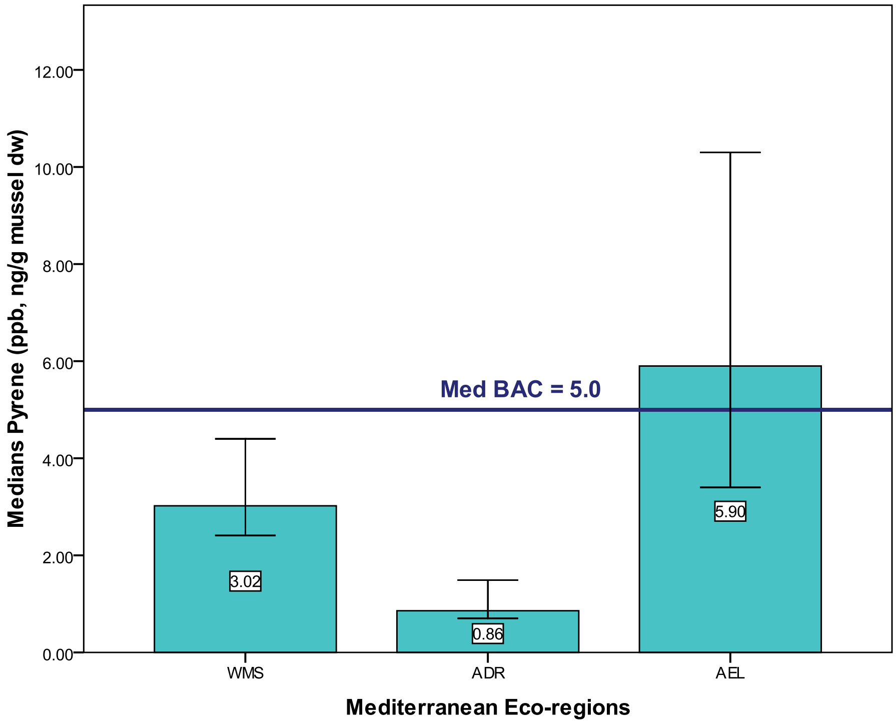


Figure 4.4.5. Fluoranthene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



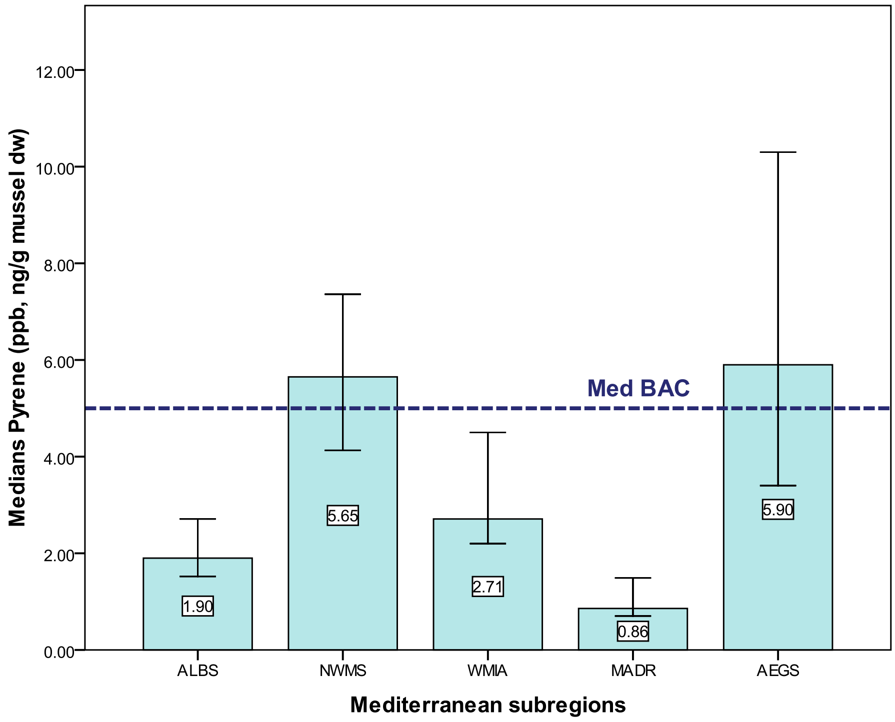
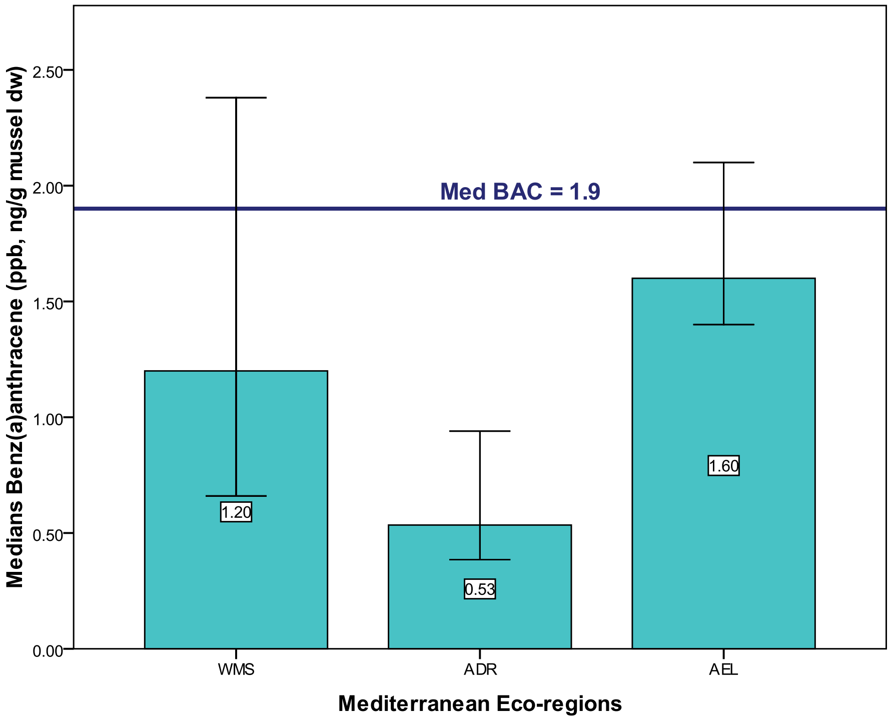


Figure 4.4.6. Pyrene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



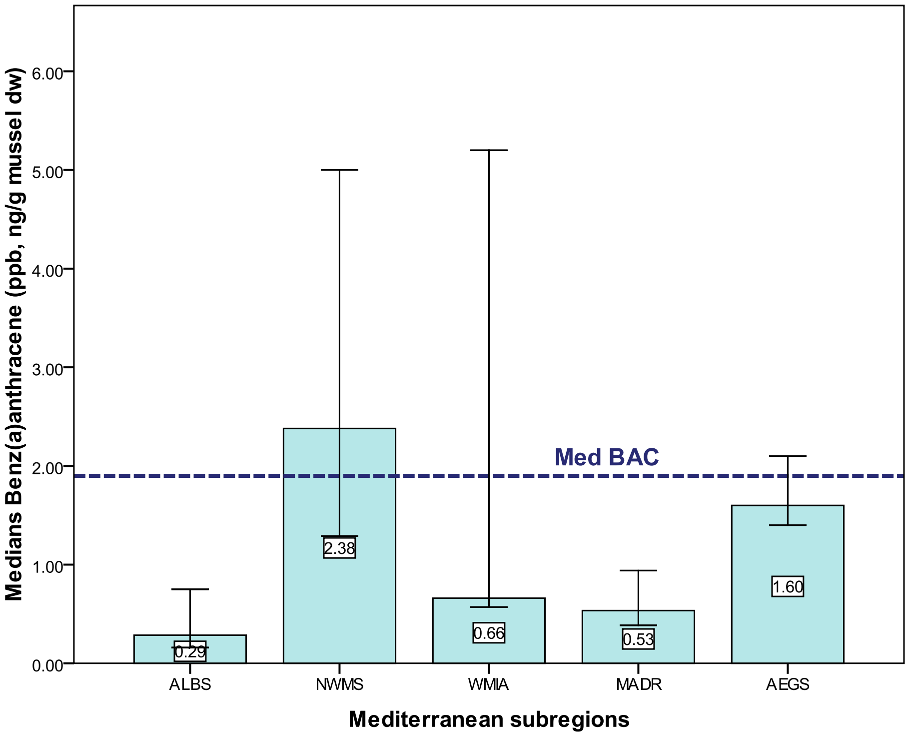
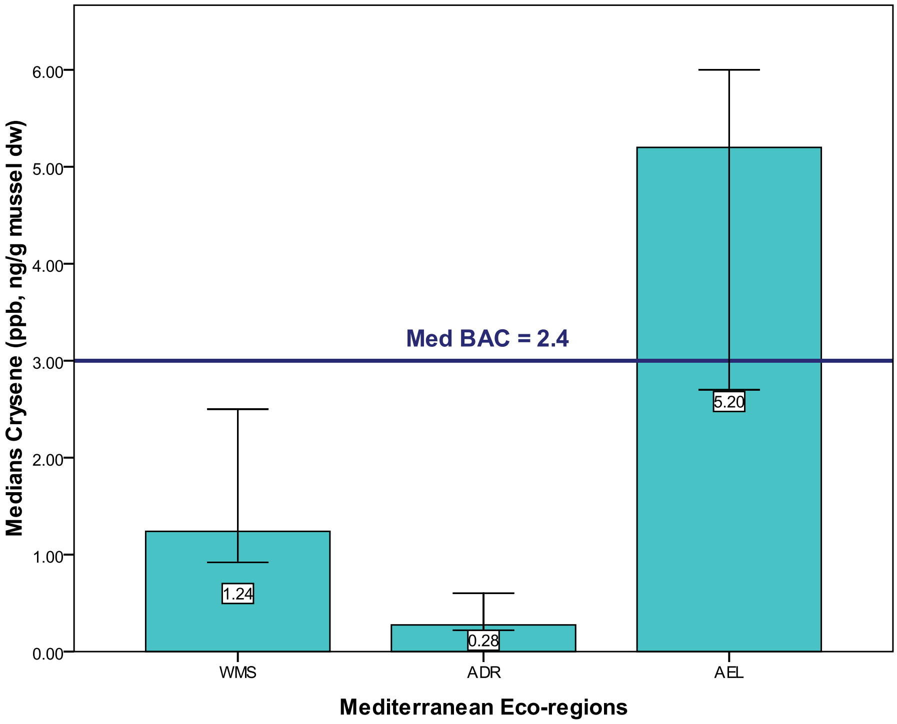


Figure 4.4.7. Benz(a)anthracene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



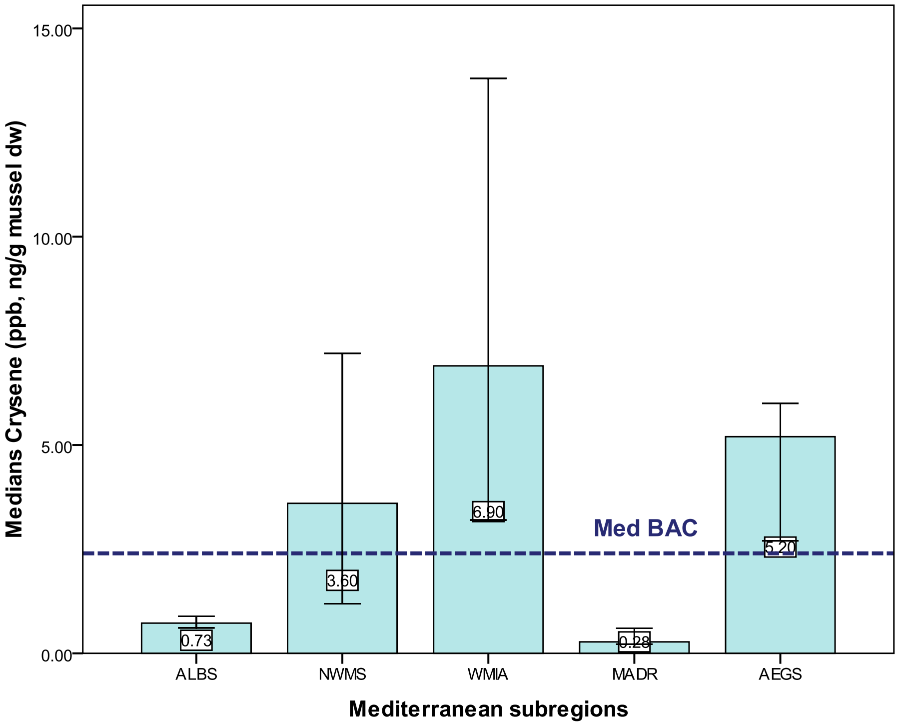
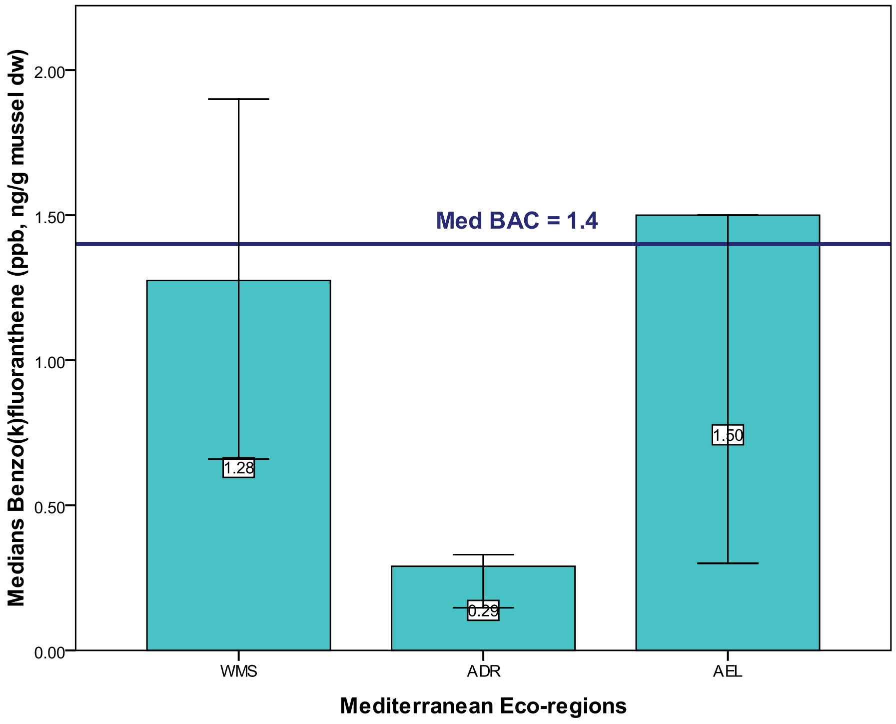


Figure 4.4.8. Chrysene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



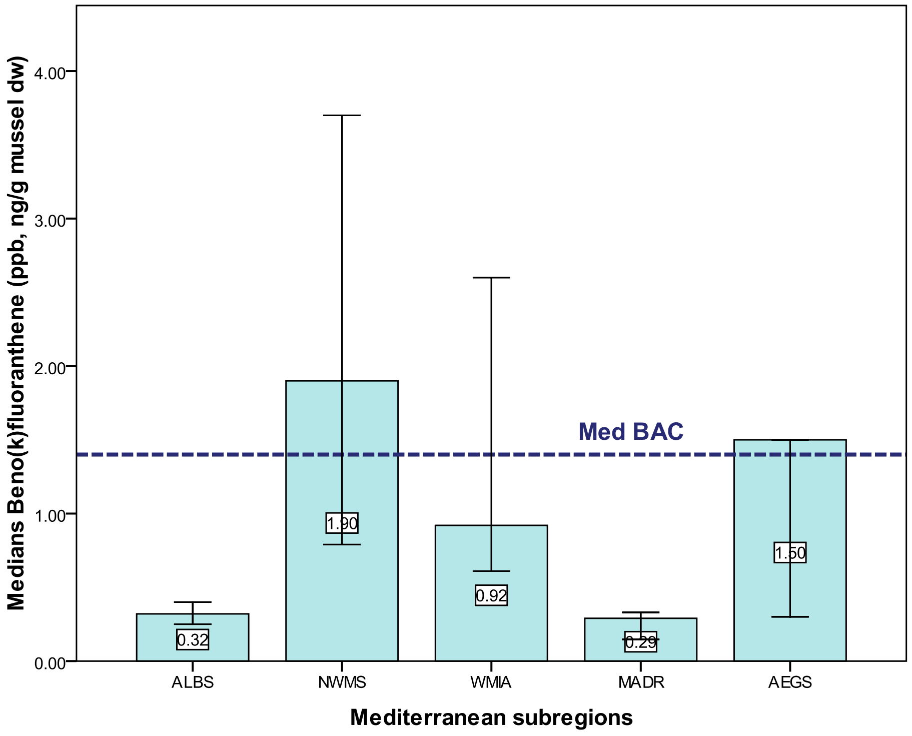
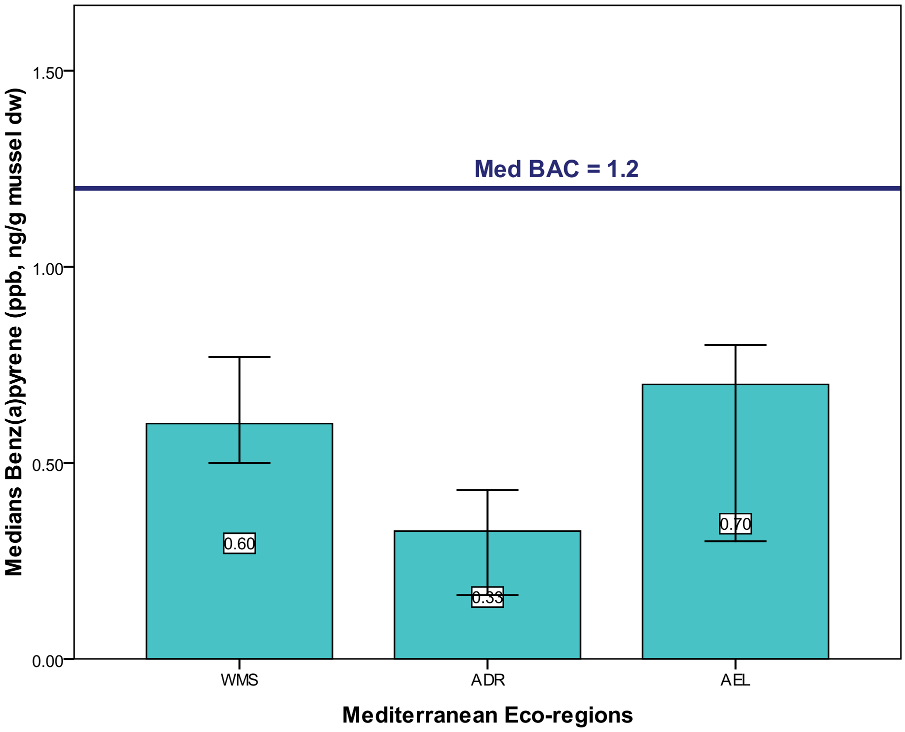


Figure 4.4.9. Benzo(k)fluoranthene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



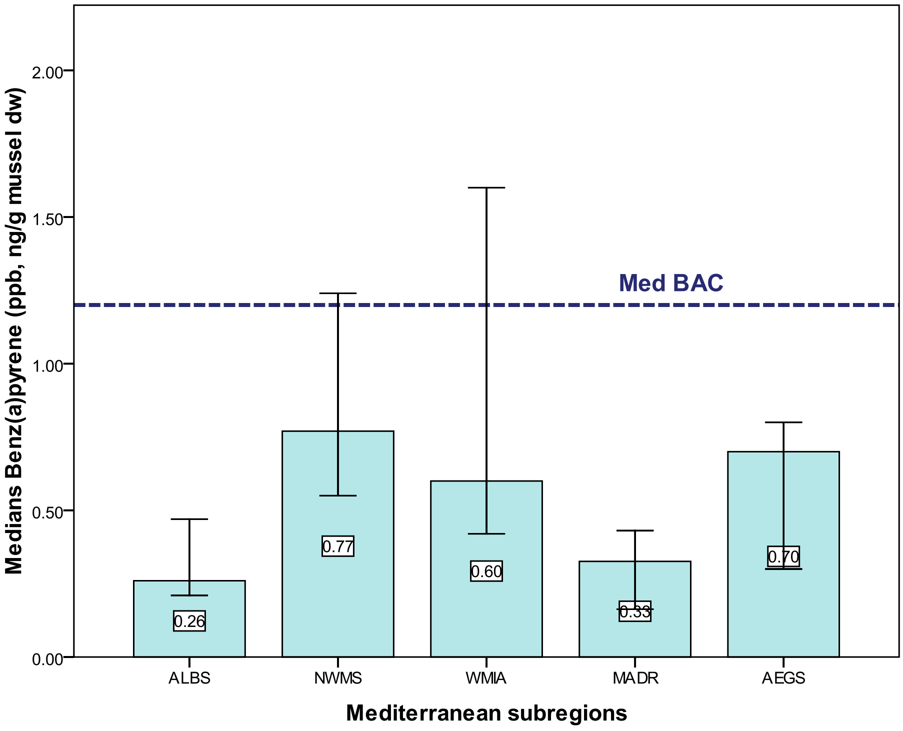
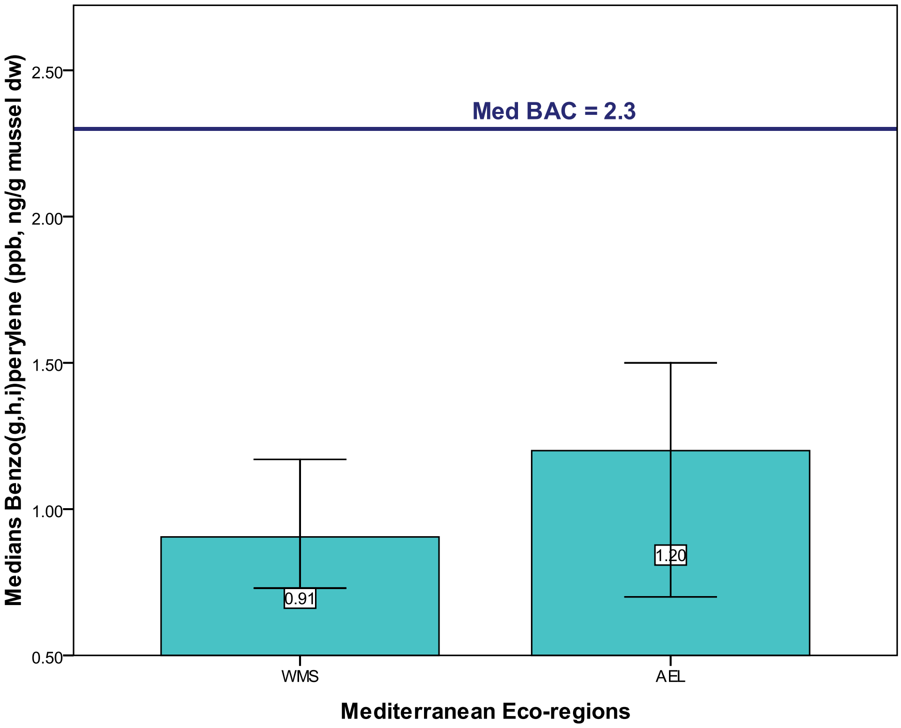


Figure 4.4.10. Benzo(a)pyrene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



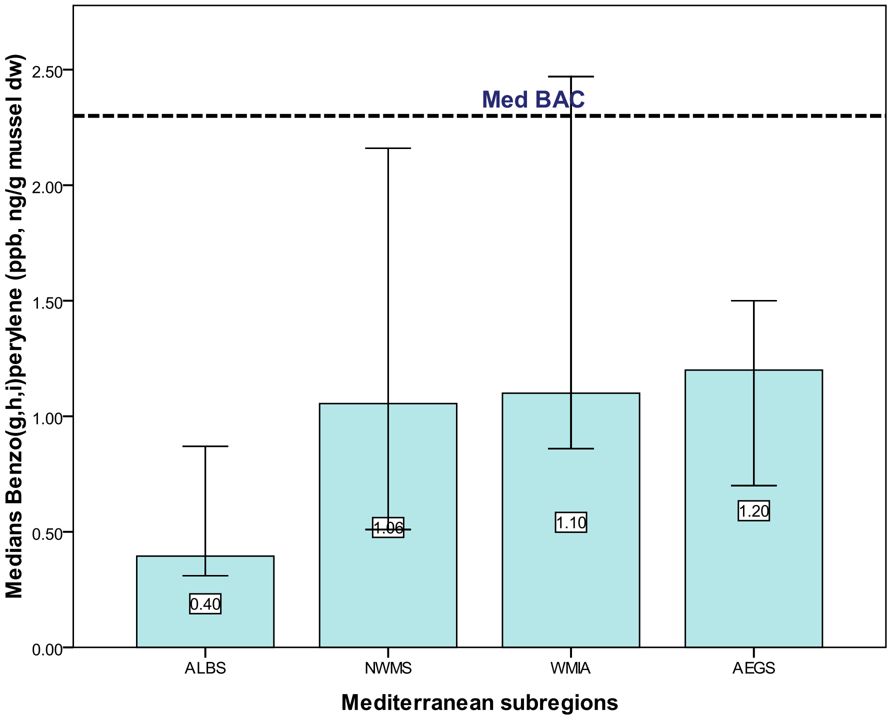
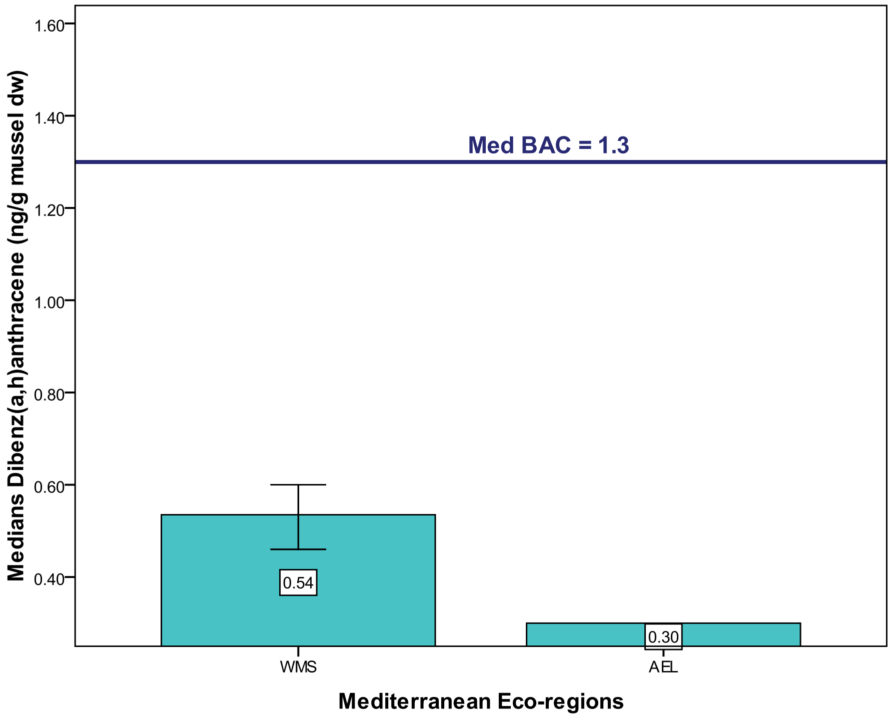


Figure 4.4.11. Benzo(ghi)perylene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



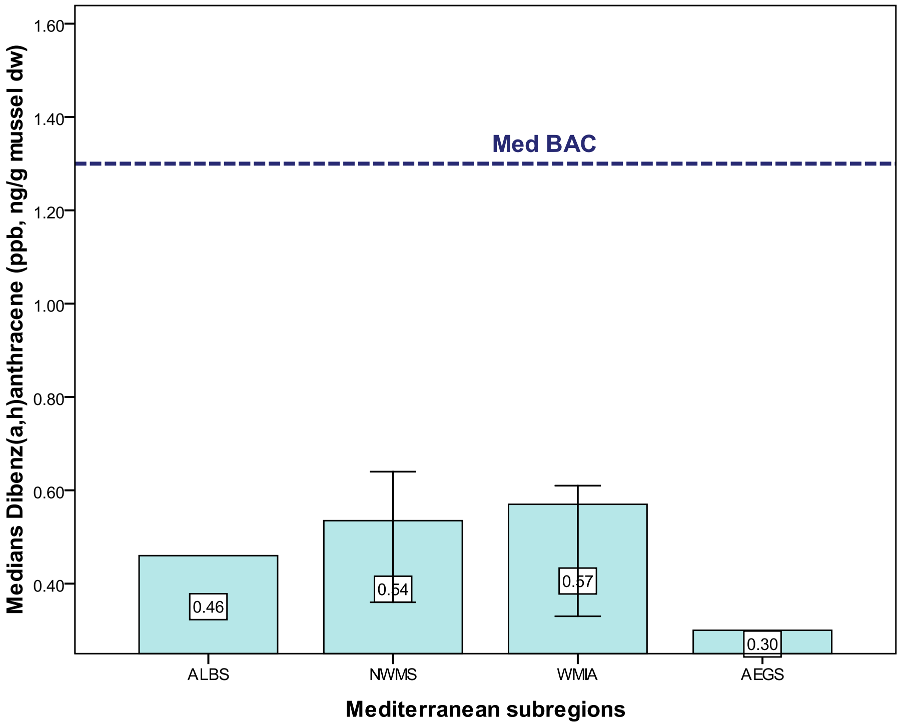
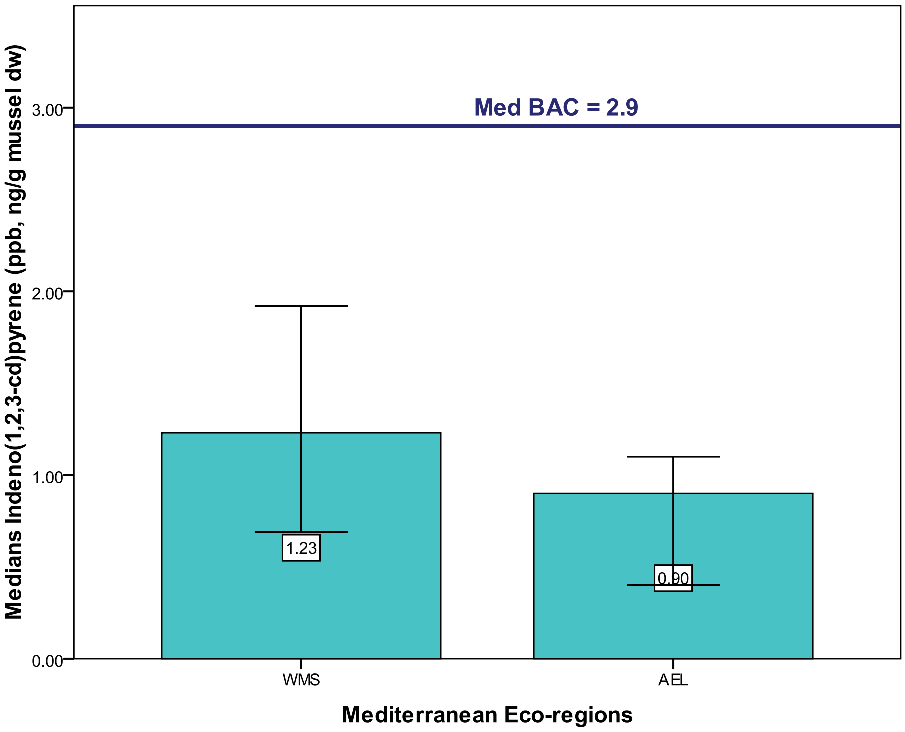


Figure 4.4.12. Dibenz(ah)anthracene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



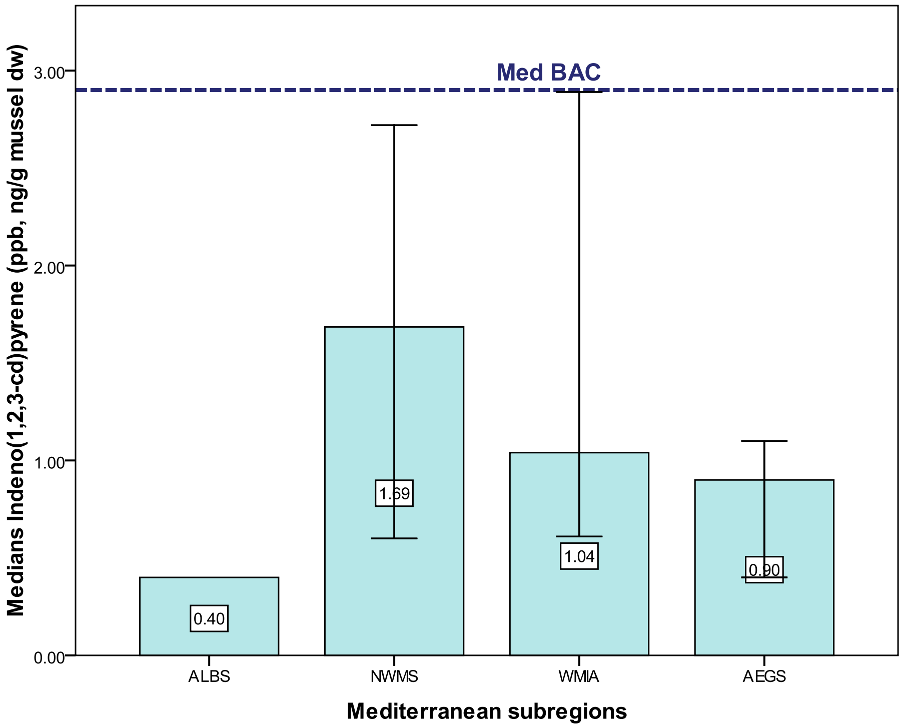


Figure 4.4.12. Indeno(1,2,3-cd)pyrene medians (BCs) in sediment by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.

4.5. Threshold values for biomarkers (AChE, MT, MN, LMS and SoS) in mussels

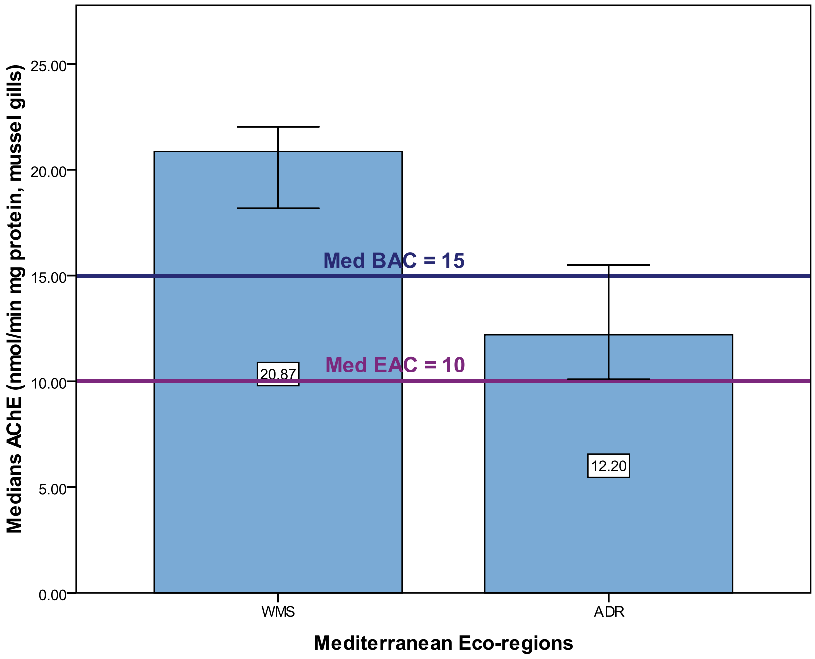
The developments of the assessment criteria using the information from the MEDPOL pilot biomonitoring program were too limited with datasets from Croatia, Italy and Spain. Some datasets from Greece were also available for 2005. Therefore, the WMS and the ADR were evaluated for the majority of biomarkers, whilst the AEL eco-region was evaluated for one biomarker (LMS-LP). The Med BCs and BACs have been developed for Acetylcholinesterase activity (AChE), Metallothioneins (MT), Micronuclei frequency (MN), Lysosomal membrane stability (LMS-NRR and LMS-LP methods) and Stress on Stress (SoS). The biomarker determinations employed different analytical methods and were reported in different units or in different tissues, including different set assessment criteria standards which limits the comparability between the MEDPOL datasets in order to calculate the BCs, such as for the LMS, MT and AChE biomarkers, respectively. The full statistical results and further information can be found in Annex V. The following table (Table 4.5.1) and figures (Figure 4.5.1–4.5.6) explains the determined Mediterranean BC and BACs for biomarkers.

Table 4.5.1. Mediterranean BCs and BACs (Med BACs) for biomarkers in mussel samples.

	Mediterranean Sea Basin		Western Mediterranean (WMS)	Adriatic Sea (ADR)
Biomarker	***MedBCs (median)***	***[a]Med BACs***	*WMS BCs*	*ADR BCs*
AChE activity (nmol/min mg protein in gills)	*21*	*15*	*20.86*	*12.20 <MedBAC*
Metallothioneins (μg/g digestive gland (DG)	*192*	*247*	*191.3*	*200.5*
Lysosomal membrane stability (LMS-Neutral red retention (NRR), minutes)	*(45)*	*120**	*45.0 <Standard*	*47.4 <Standard*
Lysosomal membrane stability (LMS-Liabilisation period (LP), minutes)	*(13)*	*20**	-	*16.8 <Standard*
Micronuclei frequency (per 1000 in haemocytes)	*0.0*	*1.0*	*0.0*	*0.5*
Stress on stress (days)	*11*	*11*	-	-

[a]either the 10th percentile or the 90th percentile are considered for biomarkers to establish the Background Assessment Criteria (BACs), see Information Document; *adopted ICES/OSPAR standard (see Section 5)

The figure below (Figure 4.5.1) shows the AChE inhibition results. It can be observed clearly the differences in datasets for reference stations for both Mediterranean eco-regions and includes the standard recommended values by ICES/OSPAR (see Section 5). It should be noticed that some biomarkers interpretation work in reverse mode than for hazardous chemical contaminants; for example, the AChE measurements correspond to the inhibition of the acetylcholinesterase (the biological effect), and therefore a healthy individual should have higher values (above the BAC).



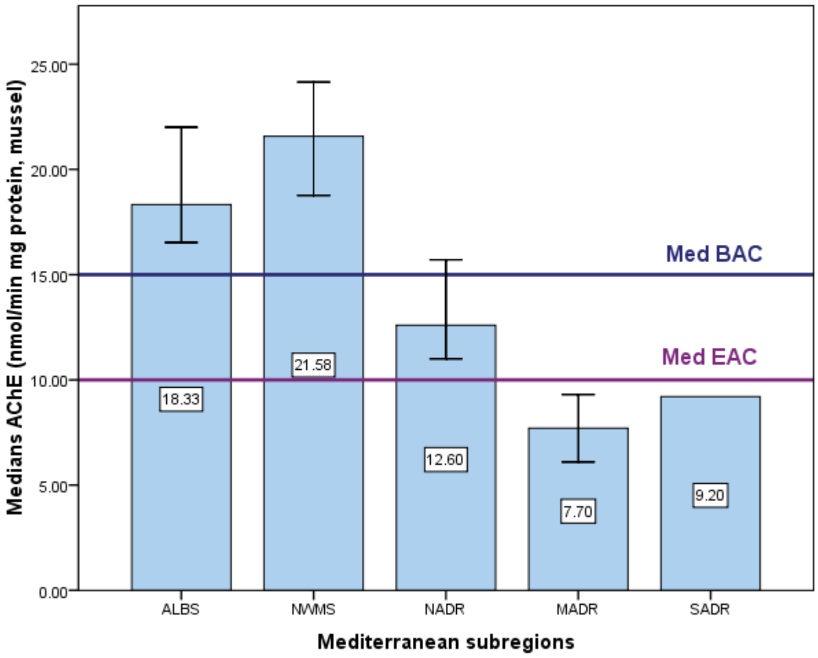
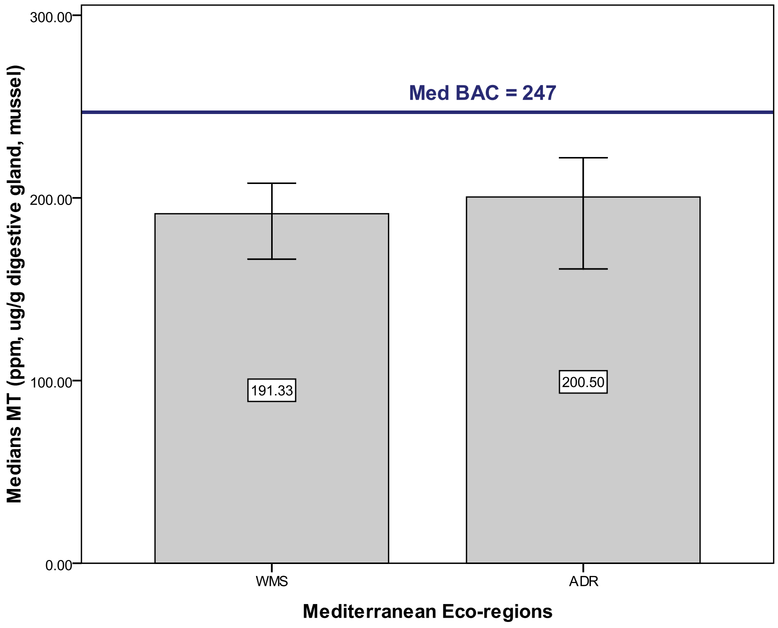


Figure 4.5.1. Acetylcholinesterase activity medians (BCs) in mussels by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.

The Adriatic Sea eco-region show an AChE inhibiton half way to unacceptable levels of biological effects (<BAC and >EAC in the green zone), which should be further discussed, and contrasts with the median level determined in the WMS eco-region, being both reference areas/stations from Croatia and Spain, respectively. For the later, there was a significant decreasing trend (τ-Kendall, coef. = -0.127, α=0.01) at country level (Spain) for reference stations, and therefore only the latest datasets (2010-2012) were selected (see Annex V).



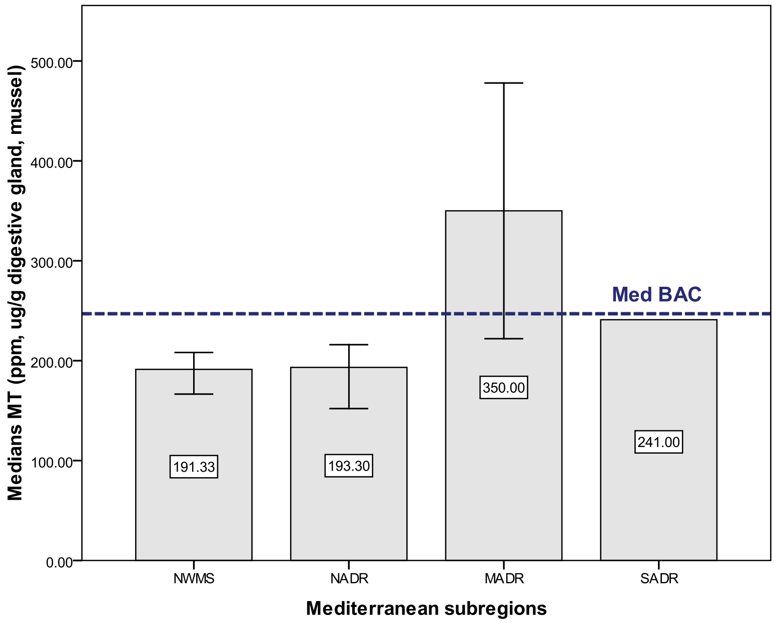
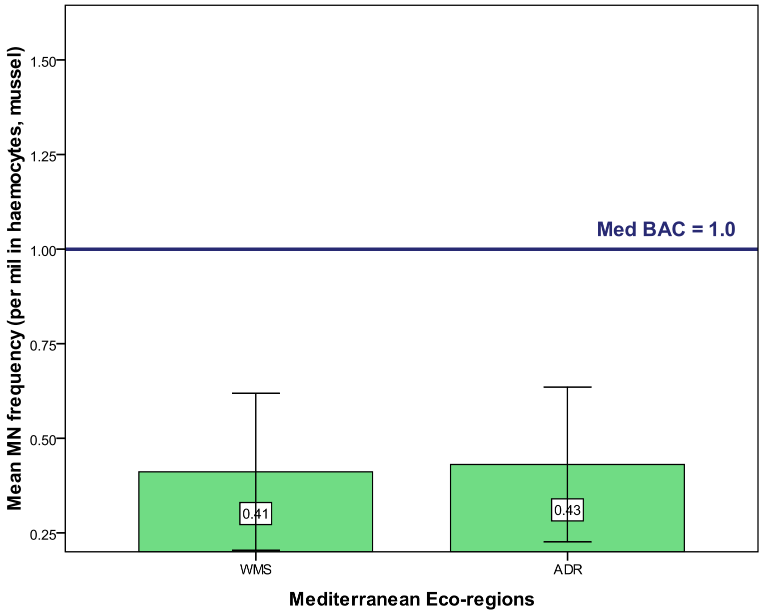


Figure 4.5.2. Metallothioneins medians in mussel digestive gland by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.

From the figure above (Figure 4.5.2) it can be observed that the eco-regions medians for reference stations are safely below the calculated Med BAC for metallothioneins, despite sub-regional BCs above the calculated Med BAC (Northern Adriatic Sea). Further, as for AChE, there was a significant unresolved increasing trend τ-Kendall, coef. = 0.341, α=0.01) at country level (Spain) for reference stations, and therefore only 2010-2012 datasets were selected. The figure below (Figure 4.5.3) plots the measured micronuclei frequencies in mussels with eco-regions and sub-regions medians (BCs) below the Med BACs. However, it should be noticed that these datasets were solely contributed by Spain and Italy for the Mediterranean Sea.



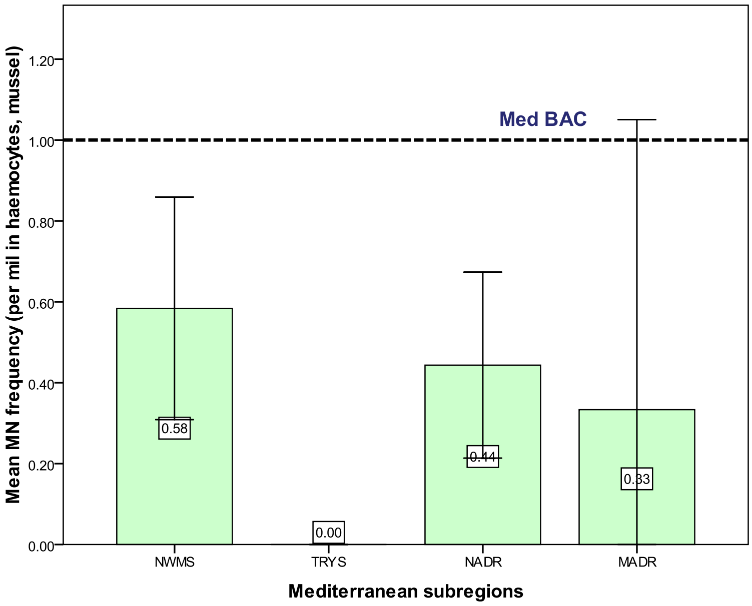
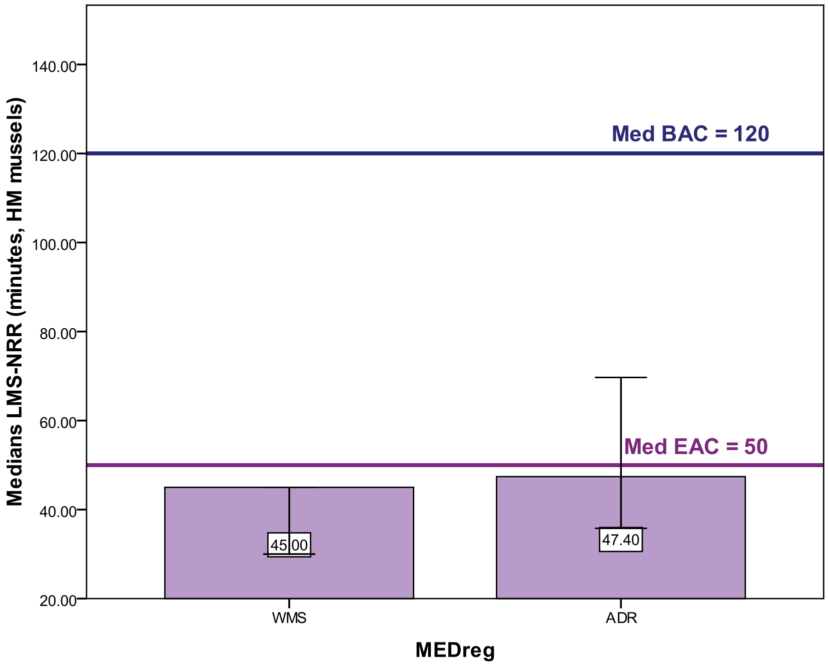


Figure 4.5.3. Micronuclei frequency medians (BCs) in mussel by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.

The figures below (Figure 4.5.4-4.5.5), shows the box-plots for lysosomal membrane stability determinations by neutral red retention (NRR) and labilisation period (LP) cythochemical methods, including the standard assessment criteria for each method. Italy and Spain used the NRR method, whilst Croatia, Greece and Italy as well, used the LP method. Therefore, the geo-scales are assessed accordingly.



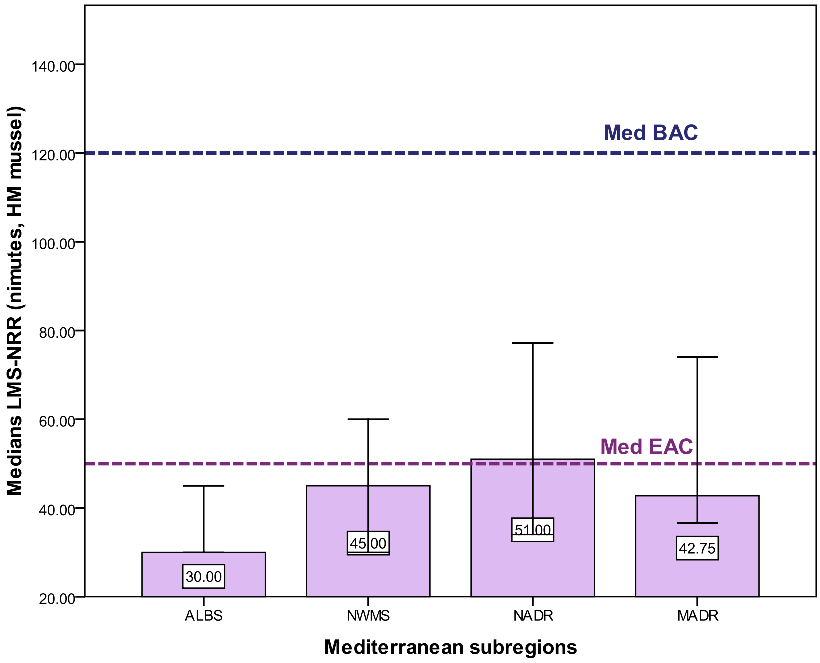
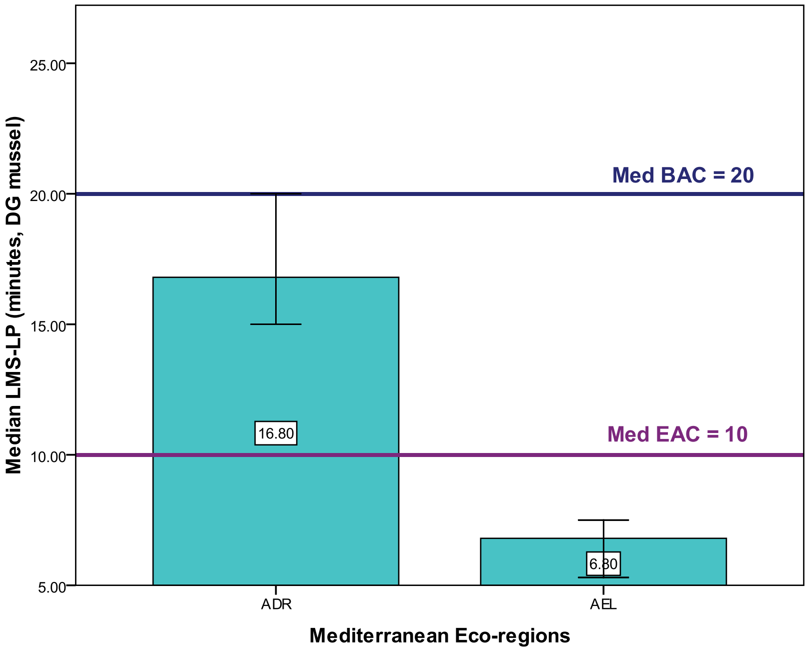


Figure 4.5.4. LMS-NRR (Neutral red retention) medians in mussel by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.



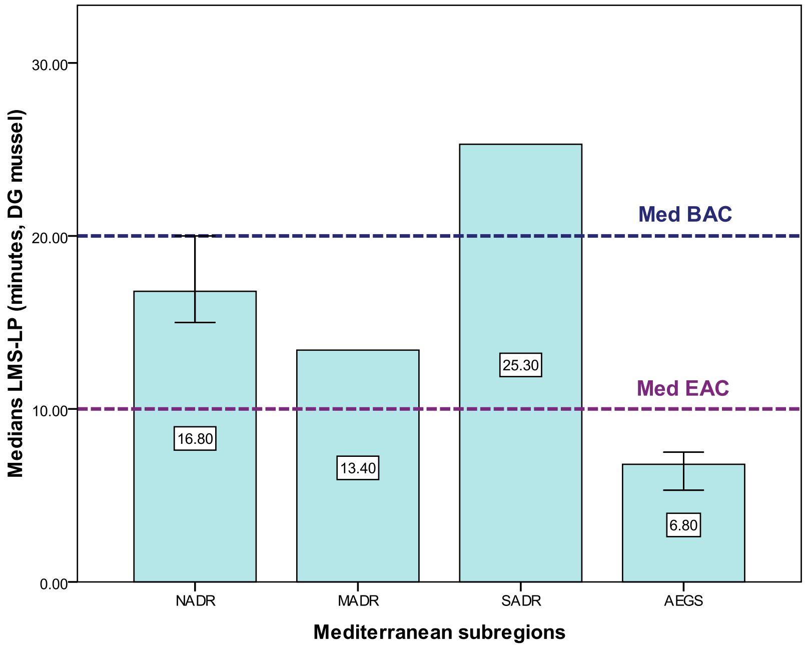


Figure 4.5.5. LMS-LP (Labilisation period) medians in mussel by eco-region and sub-regions for selected reference stations in the Mediterranean Sea. The error bar is a 95% confidence interval for the mean.

It should be noticed in the figures above, that LMS results for the references areas submitted are below the standard times (<BACs and <EACs) for these methodologies to assess healthy biota specimens. Therefore, these discrepancies being datasets for reference stations might reflect the influence of confounding factors in the environment in relation to general stress biomarker responses (e.g. nutritive status, hypoxia, spawning state), and therefore, hinder the correlations with the exposure to hazardous chemical substances, as shown also recently in the literature (Minguez et al. 2012; Cuevas et al., 2015; González-Fernández et al., 2015). In any case, the further development of Med BCs and Med BACs in Mediterranean mussels with

the number of datasets provided is not conclusive within the MEDPOL biomonitoring programme.

On the other hand, the figure below (Figure 4.5.6) summarizes the main datasets for the stress on stress (SoS) parameter in mussels from Spain and Croatia in reference areas/stations. In both cases, a large uncertainty between years is observed. For example, in the reference stations for Spain the variability is almost half of the calculated (90th percentile) in the long-term (see Annex V), which clearly limits the reliability of this measurement to assess unhealthy marine ecosystem conditions.

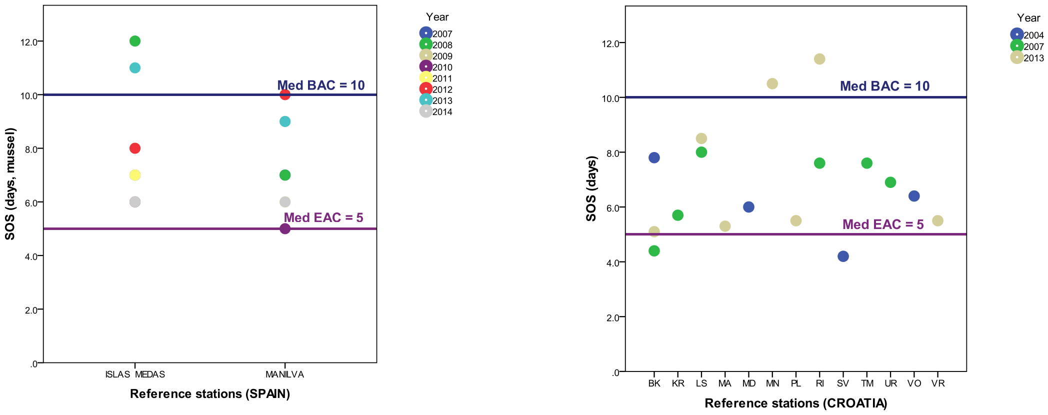


Figure 4.5.6. Stress on stress (SoS) data in mussel by country for selected reference stations in the Mediterranean Sea.

The large variability observed both for biomarkers and biomonitoring parameters; where, biomarker responses are affected by biotic and abiotic factors, or different methods, units, samples and standards are in use, should be reconsidered from a scientific and long-term point of view in order to ensure cost-benefit and cost-effective monitoring activities for the future monitoring of IMAP Ecological Objective 9 (Contaminants cause no significant impact on coastal and marine ecosystems and human health). To this regard, the ongoing work developed in the context of the European Commission Directives could be useful to align the needs for biomonitoring activities in the Mediterranean Sea (EU Technical Report 2014 – 077).

5. REVISION PROPOSAL (TABLES) OF THE BCs/BACs/EACs FOR THE MEDITERRANEAN SEA.

5.1. Table of the proposed assessment criteria for trace metals (TMs)

The tables below (Tables A.1.1 and A.1.2) compare the new proposed/revised BCs, BACs and EACs in this document (using Reference Stations datasets) with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.1.1. Mediterranean Sea: Background Concentrations (Med BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=1.5 x BC (mussel, sediment); BAC=2.0 x BC (fish)

Trace metal	Mussel (MG) µg/kg d.w.			Fish (MB) µg/kg f.w.			Sediment µg/kg d.w.		
	BC	Med BAC	EC*	BC	Med BAC	EC*	BC	Med BAC	ERL**
Cd	730.0	1095.0	5000	(3.7)[a]	(16.0)[b]	50	85.0	127.5	1200
Hg	115.5	173.2	2500	50.6	101.2	1000	53.0	79.5	150
Pb	1542	2313	7500	(31)[a]	(40)[b]	300	16950	25425	46700

[a] Cd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.

[b] estimated BACs from reliable limits of detection (BAC=1.5 x LOD) using analytical data and a certified reference material information (DORM-2). However, liver tissue matrix should be recommended in fish for Cd and Pb as within OSPAR Convention.

*EC/EU 1881/2006 and 629/2008 Directives for maximum levels for certain contaminants in foodstuffs

** Long et al. 1995 (idem OSPAR adopted values) – Effect Range Low values 2.5% TOC normalized (NOAA, USA)

Table A.1.2. Earlier data (2011-2015) from UNEP(DEPI)/MED WG.365/Inf.8, UNEP(DEPI)/MED WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

Trace metal	[a]Mussel (MG) µg/kg d.w.			[b]Mussel µg/kg d.w.	[c]Fish (MB) µg/kg d.w.[f]			Sediment µg/kg d.w.		
	BC	Med BAC	EC	BAC	BC	Med BAC	(EC)	BC	[e]Med BAC	ERL
Cd	725	1088	5000	1000	4	8/16[d]	207	-	150	1200
Hg	125	188	2500	170	296	600	4150	-	45	150
Pb	2500	3800	7500	1000	279	558	1245	-	30000	46700

[a] preliminary data for the NW Mediterranean (Spain);

[b] additional BAC data provided by Lebanon for *Brachidontesvariabilis* species;

[c] preliminary data for the NW Mediterranean (Spain);

[d] earlier estimation wet weight;

[e] estimated from sediment cores (UNEP(DEPI)/MED WG.365/Inf.8, 2011);

[f] a dry/wet ratio of 20 should be used to convert units for MG (f.w. units = d.w. units / 5)

5.2. Table of the proposed assessment criteria for polycyclic aromatic hydrocarbons (PAHs)

The tables below (Tables A.2.1 and A.2.2) compare the new proposed/revised BCs, BACs and EACs with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.2.1. Mediterranean Sea Background Concentrations (BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=2.5 x BC (mussel)

PAH compound	Mussel (MG) µg/kg d.w.			Sediment µg/kg d.w.		
	Med BC	Med BAC	[a]OSPAR EAC	[a]OSPAR BC	[a]OSPAR BAC	[a]ERL
N	(2.4) *	(6.0)	340	5	8	160
ACY	(0.6)*	(1.4)	-	-	-	-
ACE	(0.6) *	(1.4)	-	-	-	-
F	1.0	2.5	-	-	-	-
P	7.1	17.8	1700	4.0	7.3	240
A	0.5	1.2	290	1.0	1.8	85
FL	3.0	7.4	110	7.5	14.4	600
PY	2.0	5.0	100	6.0	11.3	665
BaA	0.8	1.9	80	3.5	7.1	261
C	1.0	2.4	-	4.0	8.0	384
BkF	0.6	1.4	260	-	-	-
BaP	0.5	1.2	600	4.0	8.2	430
GHI	0.9	2.3	110	3.5	6.9	85
DA	0.5	1.3	-	-	-	-
ID	1.2	2.9	-	4.0	8.3	240

[*] Naphthalene, Acenaphtylene, Acenaphthene, Benz(e)pyrene and Benzo(b)fluoranthene are below detection limits (BDLs) or have limited monitoring datasets, and therefore their BACs are preliminary estimations.

[a] OSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota (OSPAR PAHs sediment datasets from Spain, not TOC corrected, except ERL); ERL: Effect Range Low values2.5% TOC normalized (NOAA, USA)

Table A.2.2. Earlier data (2011-2015) from UNEP(DEPI)/MED WG.365/Inf.8, WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

PAH compound	Mussel (MG) µg/kg d.w.			Sediment µg/kg d.w.		
	Med BC	Med BAC	[a]OSPAR EAC	[a]OSPAR BC	[a]OSPAR BAC	[a]ERL
P		24.3	1700		7.3	240
A		4.1	290		1.8	85
FL		6.8	110		14.4	600
PY		6.1	100		11.3	665
BaA		1.3	80		7.1	261
C		2.4	-		8.0	384
BkF		1.8	260		-	-
BaP		1.3	600		8.2	430
GHI		1.3	110		6.9	85
ID		0.8	-		8.3	240

5.3. Table of the proposed assessment criteria for organochlorinated compounds (OCs)

(*Summary of OSPAR values to be used in the Mediterranean Sea*)

Table A.3.1. OSPAR Region (Background Concentrations (BCs), BACsandEACs)[1]

OCs compound	Musselµg/kg d.w.			Fish µg/kg w.w.			[d]Sedimentµg/kg d.w.		
	BC/LC[c]	BAC	EAC	BC/LC[c]	BAC	EAC (lipid w.)	BC/LC[c]	BAC	EAC/ERL
CB28[a]	0.25	0.75	3.2	0.05	0.10	64	0.05	0.22	1.7
CB52 [a]	0.25	0.75	5.4	0.05	0.08	108	0.05	0.12	2.7
CB101 [a]	0.25	0.70	6.0	0.05	0.08	120	0.05	0.14	3.0
CB105 [a]	0.25	0.75	-	0.05	0.08	-	0.05	-	-
CB118 [a]	0.25	0.60	1.2	0.05	0.10	24	0.05	0.17	0.6
CB138 [a]	0.25	0.60	15.8	0.05	0.09	316	0.05	0.15	7.9
CB153 [a]	0.25	0.60	80	0.05	0.10	1600	0.05	0.19	40
CB156 [a]	0.25	0.60	-	0.05	0.08	-	0.05	-	-
CB180 [a]	0.25	0.60	24	0.05	0.11	480	0.05	0.10	12
Σ7CBs ICES[b]	-	-	-	-	-	-	0.20	0.46	11.5*
Lindane [a]	0.25	0.97	1.45		-	11**	0.05	0.13[+]	3.0*
α-HCH [a]	0.25	0.64	-	-	-	-	-	-	-
pp'DDE [a]	0.25	0.63	5-50***	0.05	0.10	-	0.05	0.09[+]	2.2*
HCB [a]	0.25	0.63	-	0.05	0.09	-	0.05	0.16[+]	20.0*
Dieldrin [a]	-	-	5-50***	-	-	-	0.05	0.19[+]	2.0*

[1] OSPAR Commission, 2013.

[a] OSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota, Monitoring and Assessment Series

[b] OSPAR Commission, Background document on CEMP assessment criteria for the QSR 2010, Monitoring and Assessment Series

[c] LC: Low concentrations calculated from QUASIMEME; However, BC values should be considered as zero for OCs

[d] Total organic carbon (TOC) corrected values; [+] LC fromSpain (OSPAR, 2013)

*ERLs values instead EACs: Effect Range Low values2.5% TOC normalized (Long et al. 1995; NOAA, USA); ERL for ICES Σ7CB is total CB concentration/2

**EAC for fish liver derived by applying a conversion factor of 10 on EAC for whole fish (CEMP 2008/2009)

*****Ecotoxicological assessment criteria (earlier data from the QSR2000 Report-Chapter 4)

It should be noted that at present, no quality assured or sufficient datasets exist in the MEDPOL Database to calculate the threshold values for the Mediterranean Sea.

5.4. Table of the proposed assessment criteria for biological markers in mussels

The tables below (Tables A.4.1 and A.4.2) compare the new proposed/revised BCs, BACs and EACs with the earlier proposed threshold values in the Mediterranean Sea. Further details can be found in the information document (UNEP(DEPI)/MED WG.427/Inf.3).

Table A.4.1. Mediterranean Sea and standard reference values; Calculation => BAC = 10th or 90th percentile depending on the parameter.

Biomarkers	Mussel (*Mytilusgalloprovincialis*)	
	Med BAC	EAC
Stress on Stress (SOS, days)	11	5[a]
Metallothioneins (µg/g digestive gland)	247	-
Lysosomal membrane stability (LMS-NNR, neutral red retention method, minutes)	120[a]*	50[a]*
Lysosomal membrane stability (LMS-LP, Cytochemical method, labilisation period minutes)	20[a]*	10[a]*
AChE activity (nmol/min mg protein in gills)[b]	15	10[a]
Micronuclei frequency (per 1000 in haemocytes)	1.0	-

[a]Technical annex: assessment criteria for biological effects measurements. Integrated monitoring of chemicals and their effects. ICES Cooperative Research Report No. 315. Davies, I.M. and Vethaak, A.D.Eds.
[b]subregional differences between assessment criteria are observed by countries
*Moore et al., 2006 (Standard values adopted by ICES)

Table A.4.2. Earlier data (2015) from UNEP(DEPI)/MED WG.417/inf.15 Part3 and Annex to UNEP(DEPI)/MED IG.22/7Decision.

Biomarkers	Mussel (*Mytilusgalloprovincialis*)	
	Med BAC	EAC[a]
Stress on Stress (SoS, days)	10	5
Lysosomal membrane stability (LMS-NNR, neutral red retention method, minutes)	120	50
Lysosomal membrane stability (LMS-LP, Cytochemical method, labilisation period minutes)	20	10
AChE activity (nmol/min mg protein in gills) -France	29	20
AChE activity (nmol/min mg protein in gills) - Spain	15	10
Micronuclei frequency (per 1000 in haemocytes)	3.9	-

6. CONCLUSIONS AND RECOMMENDATIONS

1) A revision of the Background and Environmental Assessment Criteria (BACs and EACs) has been undertaken for the Mediterranean Sea to further establish thresholds for hazardous chemical substances (trace metals and polycyclic aromatic hydrocarbons) and biomarkers in order to perform environmental assessments and to monitor the common indicators 17 and 18 and its Ecological Objective 9 targets in the context of the IMAP/EcAp implementation process.

2) Twenty eight (28) new Mediterranean BACs have been determined based on new Background Concentrations (BCs) calculated with reference areas/stations datasets from both the MEDPOL Database (updated to 2012) and datasets submitted through the informal online expert group on contaminants (2014 - 2015), in line with the methodology followed by the OSPAR Convention. Nine Med BACs are proposed for trace metals, fifteen Med BACs for PAHs and four Med BACs for biomarkers (see Tables A, B and D).

3) The statistical relationship between BCs and BACs for hazardous chemicals established by OSPAR(based on both analytical and monitoring variability) have been used to determine the Mediterranean BACs for metals in sediments, fish and shellfish (for sediments and shellfish BAC = 1.5 x BC, for fish BAC = 2 x BC), as well as PAHs in shellfish (PAHs BAC= 2.5 x BAC).

4) For other groups of hazardous chemical substances (e.g. organ halogenated compounds, pesticides, etc.), there were not enough MEDPOL monitoring datasets for reference areas/stations available to determine Mediterranean BCs/LCs and BACs. Therefore, the assessment criteria by OSPAR are further recommended, despite taking into account the refinements proposed in this document (see Table C.)

5) The Mediterranean Background Assessment Criteria (Med BACs) should be considered as the assessment criteria to define the transition point from non-impacted marine ecosystems (where chemicals are near background concentrations) to those conditions where biological effects could take place (see Revision Proposal Tables, Section 5).

6) Further, the Med BACs have been defined for the Mediterranean Sea basin and additional information on the BCs for the four Mediterranean eco-regions (Western Mediterranean Sea (WMS); Adriatic Sea (ADR); Central Mediterranean (CEN) and Aegean-Levantine Seas (AEL) is also provided in order to refine eco-regional assessments if necessary.

7) Within the four Mediterranean eco-regions, further information values on BCs at a sub-regional seas scale (Alboran Sea, North-western Mediterranean, Northern Adriatic, Middle Adriatic, Ionian Sea, Aegean Sea, Levantine Sea, etc.) is also provided, although the number of data within datasets is limited and should be carefully examined.

8) Regarding EACs it is recommended that the Annex to Decision 22/7 (IMAP) should be considered as the reference for hazardous chemical substances and biomarkers taking into account the refinements proposed in this report (see Tables A, B, C and D).

Future improvements:

i. A number of priority chemical hazardous substances, including organometallic compounds (e.g. methyl mercury), and emerging marine contaminants should be included in the MEDPOL monitoring activities, as suggested in UNEP(DEPI)/ MED WG.417/Inf.4, along with a robust and revised MEDPOL database quality assurance program. Further, Contracting Parties may consider, whether to continue reporting Cd and Pb in fish flesh tissue, or instead report on these metals in liver tissue.

ii. The quality assurance of monitoring activities are still not yet fully implemented within the Contracting Parties of the Barcelona Convention. Datasets showed significant variability (ca. uncertainty) and often below detection limits values, for biological effects and chemical contamination monitoring. This issue should be discussed by Contracting Parties for future biological effects monitoring and quality assurance programmes. It is suggested to ensure more comparable and precise results for biomarkers, as well as standard methodologies to be adopted for all Mediterranean laboratories with regard the IMAP common indicator 18.

iii. For monitoring biological effects in fish, advantage should be taken of fisheries related monitoring programmes (i.e. through GFCM and/or programmes such as MEDITS).

iv. Currently, the MEDPOL Database shows a limited number of datasets related to contaminants in sediment samples. The implementation of long-term sediment monitoring (coastal, off-shore, core samples) should be further developed in order to improve the background and environmental assessment criteria (BACs and EACs) at the different eco-regions and sub-regional seas scales.

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Annex I

Calculations of BCs/BACs for trace metals (Cd, Hg, Pb) in mussels

ANNEX I. Calculations of BCs/BACs for trace metals (Cd, Hg, Pb) in mussels

The following tables summarize the characteristics of the selected dataset from the MEDPOL Database and online datasets and the calculations of the Mediterranean BCs and BACs for cadmium, mercury and lead in *Mytilusgalloprovincialis* (MG).

Table A.1.1. Detailed summary of the selected datasets for BC/BAC calculations in mussel. (WST: Whole soft tissue; SO: Soft organs tissue). Note: it includes other bivalve species; however, these were not included in the Med BAC calculations.

Country	**Eco-regions/Stations**	**Size / Tissue**	**Comments**	**Years**
Croatia	**ADR-NADR**: LS, LU **ADR-MADR**: SI	4.5 ±0.1 cm; 5.4 ± 0.7 cm WST (MG)	5 samples (pooled 15 individuals)	2005-2011
France	**WMS-NWMS**: Banyuls s/mer – LaboratorieAragó, Etang de Leucate, Thau 1, Thau 4, Les StesMaries de la mer, Anse de Carteau 2 **WMS-WMIA**: Etang de Diana, Etangd'Urbino, Sant'Amanza	-	-	2005-2014
Greece	**AEL-AEGS**: GRE1	3.5 cm SO (MG)	Pooled samples 20 individuals	2005-2006
Italy	**ADR-MADR**: Agostino, Amelia, Antares, Antonella, Arianna, Armida, Control Portonovo, Garibaldi	5-6 cm WST (MG)	10 samples-2 depths (duplicate, pooled 3 individuals)	2010
Spain	**WMS-NWMS**: Cadaqués, Medas (Estartit), Cullera **WMS-ALBS**: Torrox, Málaga, Manilva	3.5 ± 0.3 cm (MG)	3 samples (pooled 80 individuals)	2006-2011
Slovenia	**ADR-NADR**: 24, TM			2011
Turkey	**AEL-AEGS**: IZM1B, IZM2B, IZM3B	5.4 ± 0.7 cm		2007-2009
Lebanon	**LEVS** (Halate)	*Brachidontesvariabilis* (BV)	Coastal sample	-
Israel	**LEVS**: ISRTMC18	1-4 cm – *Mactracorralina (MC)*, SO 1-3 cm – *Donaxtrunculus* (DT), SO	MC: 1-3 pooled individuals: DT: pooled ≈10 individuals	1999-2011
Tunisia	**CEN**: M1, G1, S2	(3-4 cm) – *Ruditapes decussates* (RD)	1-3 samples (pooled 20-25 individuals); coastal location	1999-20011

Table A.1.2. Summary of statistics for TM in the reference stations and Mediterranean BCs and BACs calculation in mussel *Mytilusgalloprovincialis*(μg/kg dw). (λ: percentage of data included in the selected normal component; X: mean; σ: standard deviation; μ C.I.: confidence interval for the mean).

Cadmium	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	69	770.4	423.0	502.6	847.0	527.1	1029.7	1125.5		
FRA	81	822.5	434.0	545.0	740.0	435.0	980.0	1394.0		
GRE	5	295.6	141.0	159.0	247.0	297.5	456.5	-		
ITA	82	739.8	366.9	533.9	713.2	401.5	935.4	1099.6		
SPA	93	652.2	348.4	422.0	599.8	398.0	820.0	984.0		
TUR	27	1449.4	730.2	760.0	970.0	1209.0	1969.0	3452.8		
Calculated BC	***357***	***789.1***	***382.5***	***520.0***	***730.0***	***436.5***	***956.4***	***1220.3***	**Med BAC**	**1095.0**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	*94*	*725.4*	*282.9*	*700.0*	*750.8*				*Mixtool BAC*	*1088.1*
LEB (BV)	1	1000.0	-	-	1000.0	-	-	-		
ISR (MC, DT)	23	767.3	281.0	489.5	701.4	432.6	922.0	1395.1		
TUN (RD)	70	368.3	202.2	254.7	322.0	151.8	406.5	517.8		
Mercury	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	69	283.7	85.34	101.86	145.8	253.1	354.9	812.0		
FRA	81	116.4	70.0	94.5	113.0	44.5	139.0	168.0		
GRE	4	154.6	4.3	11.2	128.9	312.8	323.9	-		
ITA	83	125.4	84.9	104.6	120.4	40.2	144.8	169.5		
SLO	2	112.0	105.0	105.0	112.0	-	-	-		
SPA	93	118.2	69.6	90.1	101.3	38.3	128.4	181.8		
TUR	27	118.8	75.0	81.0	110.0	76.0	157.0	171.4		
Calculated BC	***359***	***151.7***	***78.66***	***94.3***	***115.5***	***55.7***	***150***	***210***	**Med BAC**	**173.2**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	*88*	*116.0*	*34.6*	*112.8*	*119.2*				*Mixtool BAC*	*174.0*
LEB (BV)	1	170.0	-	-	170.0	-	-	-		
ISR (MC, DT)	158	188.8	112.3	134.3	179.8	106.0	240.3	285.3		
TUN (RD)	68	95.8	22.8	34.2	64.0	97.8	132.0	246.0		
Lead	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	69	1121.0	513.0	790.5	974.0	530.5	1321.0	1978.0		
FRA	80	1272.0	400.0	592.5	1315.0	1307.5	1900.0	2300.0		
ITA	82	1939.8	991.5	1356.0	1871.7	903.9	2259.9	3239.9		
SPA	77	1820.5	1202.5	1458.7	1634.4	430.6	1889.3	3015.4		
TUR	27	2174.8	944.4	1430.0	2300.0	1500.0	2930.0	3730.0		
Calculated BC	***335***	***1603.2***	***600.0***	***999.1***	***1542.0***	***1008.1***	***2000.0***	***2736.0***	**Med BAC**	**2313**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	88	1399	592	1342	1456				*Mixtool BAC*	2098
LEB (BV)	1	1000.0	-	-	1000.0	-	-	-		
TUN (RD)	66	396.3	151.3	206.7	306.0	266.0	472.7	922.8		

In the table above, few other species of bivalves have been included from the MEDPOL database for Lebanon, Israel and Tunisia as detailed in Table A.1.1., but were not taken into account for the BC and BAC determinations in MG.

Table A.1.3. Summary of statistics for TM BCs in the Mediterranean eco-regions (μg/kg dw).

Trace metal	Eco-region	N	Mean	10th	25th	Median (BCs)	IQR	75th	90th
Pb	ADR	151	1565.7	725.0	952.1	1381.0	1037.0	1989.1	2688.1
	AEL	27	2174.8	944.4	1430.0	2300.0	1500.0	2930.0	3730.0
	WMS	157	1541.0	500.0	1075.4	1585.2	824.6	1900.0	2374.0
HgT	ADR	154	196.2	85.6	104.6	126.0	71.4	176.0	409.0
	AEL	31	123.4	74.2	80.0	110.0	79.0	159.0	177.8
	WMS	174	117.3	70.0	90.8	109.4	43.3	134.0	170.0
Cd	ADR	151	753.8	413.0	510.5	782.0	470.5	981.0	1099.9
	AEL	32	1269.1	297.1	737.7	942.0	1199.0	1936.7	3132.8
	WMS	174	731.5	364.0	520.0	660.5	370.0	890.0	1218.1

Annex II

Calculation of BCs/BACs for trace metals (Cd, Hg, Pb) in sediment

ANNEX II. Calculation of BCs/BACs for trace metals (Cd, Hg, Pb) in sediment

The following tables summarize the characteristics of the selected dataset from the MEDPOL Database and online datasets and the calculations of the Mediterranean BCs and BACs for cadmium, mercury and lead in surface sediments.

Table A.2.1. Detailed summary of the selected datasets for BC/BAC calculations in surface sediments.

Country	Stations	Size fraction	Observations	Years
Croatia	**ADR-NADR**: LS **ADR-MADR**: SI	< 500 μm	Coastal locations (time-series no trend)	2002-2010
Egypt	**AEL-LEVS:** Baghoush	-	1 validdatapoint	-
France	**WMS-NWMS**: Port-Vendres, Banyuls-Cap d'Oune, Tech-tet-Agly 1, Tech-tet-Agly 7, Tech-tet-Agly 8, Aude-Orb-Hérault 5, Orb-Hérault 11, Narbonne Plage, Large Port-La Nouvelle 1, Large Port-La Nouvelle 2, Large Port-La Nouvelle 3, LargeTêt, Large Tech, Large Grau de Leucate, Large Canet Plage, Le Grau du Roi, Pointe de l'Espiguette, Golfe d'Aigues Mortes, Large Frontignan, Large Sète, Large étang de Thau, Large sud Frontignan, Agde Large, Sud Camargue grand large, Petit Rhône 2, 5, 10, 14, 15, 16 and 18,Large Rhône Vif, Emb. Rhône, Fosrade VTC, Fos 46, Argens 8, Argens 19, Raded'Hyères centre, Antibes, Menton **WMS-WMIA**: Aleria, Tavignano, Porto Vecchio, Sant'Amanza, Golfe de Porto Vecchio, Propiano, Porto-Punta Blanca, StfLorendfond, StFlorent	< 2 mm	All stations; single sample; no Cd	2010-2011
Greece	**AEL-AEGS**: A4-A11, THE-6, STR-5, KAV-2, 13, P6, 6, 13, THE-6, STR-5, KAV-2, 6		Aegean Sea; GRE1, 3, 8 and 9 (samples)	2004-2005
Italy	**WMS-NWMS:** Punta Mesco **WMS-WMIA:** Asinara, Arbatax, Villasimius, Oristano, Alghero **WMS-TYRS:** Ucellina, Punta Licosa, Ustica **ADR-MADR:** Tremiti, Capo Rizzuto, Portonovo, Porto San Giorgio, Ancona, Fostespina **CEN-IONS:** Porto Cesareo	< 2 mm	Fontespina unweighted dataset corrected; Uccellina (HgToutlierrs)	2003; 2005-2006: 2009-2011
Spain	**WMS-NWMS**: Castellón **WMS-ALBS**: Castell de Ferro, Málaga **WMS-WMIA:** Mallorca	< 2 mm	Pb excludedfrom Málaga	2006-2011
Turkey	**AEL-AEGS**: IZM1B, IZM2B, IZM3B, Datça, ALBSW1, ALTSW1, ANASWR, DATSWR, GRESW1, MRE, SAMSWR, TASSW1	< 63 μm	2006 and 2010 data excluded; 7 stations 2011 added; Iskenderun and Finike outliers	2007-2009; 2011
Israel	**LEVS**: ISRTMC39, ISRTMC 43, ISRTMC49, ISRTMC 55a	< 250 μm	(time-series, no trends)	2005-2011

Table A.2.2. Summary of statistics for TM in the reference stations and Mediterranean BCs and BACs calculation for sediments (μg/kg dw).(λ: percentage of data included in the selected normal component; X: mean; σ: standard deviation; μ C.I.: confidence interval for the mean).

Cadmium	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	16	177.2	70.9	121.9	143.1	112.0	234.0	348.8		
EGY	1	70.0	-	-	70.0	-	-	-		
GRE	8	204.0	20.0	53.7	139.0	330.5	384.2	-		
ITA	52	87.7	50.0	57.6	74.9	339.7	97.4	125.8		
SPA	51	92.2	71.0	82.1	92.5	20.9	103.0	114.8		
TUR	11	53.5	36.2	44.0	52.0	13.0	57.0	83.8		
Calculated LC/BC	***139***	***103.5***	***50.0***	***67.4***	***85.0***	***40.6***	***108.0***	***149.1***	**Med BAC**	**127.5**
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*90*	*83.3*	*27.2*	*79.3*	*87.3*				*Mixtool BAC*	*124.9*
Mercury	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	14	168.6	48.3	61.8	182.5	172.1	233.9	311.0		
FRA	48	61.6	20.0	30.0	60.0	50.0	80.0	121.0		
GRE	9	89.0	32.5	36.8	44.0	104.2	141.0	-		
ISR	39	8.4	1.1	2.0	5.2	6.2	8.2	15.1		
ITA	37	90.6	32.4	50.0	60.0	60.8	110.8	220.0		
SPA	51	62.5	23.0	26.8	61.1	45.8	72.6	102.7		
TUR	36	69.9	27.1	41.7	56.7	60.2	101.9	123.5		
Calculated LC/BC	***234***	***66.2***	***6.2***	***25.9***	***53.0***	***60.4***	***86.4***	***137.3***	**Med BAC**	***79.5***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*76*	*43.5*	*28.8*	*39.9*	*47.0*				*Mixtool BAC*	*65.2*
Lead	N	Mean	10th	25th	Median	IQR	75th	90th		
CRO	16	23151	11278	13507	21272	17709	31217	39308		
EGY	1	4410	-	-	4410	-	-	-		
FRA	47	18782	6000	14000	18300	10400	24400	29000		
GRE	18	39463	6830	30850	40950	18800	49650	73892		
ISR	33	5894	1900	2655	4063	2739	5394	17782		
ITA	57	14553	3064	5419	16549	16269	21688	27332		
SPA	51	19911	6804	7840	22680	19410	27250	29338		
TUR	33	8242	2244	3549	4300	7636	11185	25310		
Calculated LC/BC	***256***	***16716***	***3290***	***5008***	***16950***	***19697***	***24705***	***30868***	**Med BAC**	***25425***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*67*	*22294*	*11202*	*20906*	*23681*				*Mixtool BAC*	*33441*

Table A.2.3. Summary of statistics for TM BCs in the Mediterranean eco-regions (µg/kg dw).

Trace metal	Eco-region	N	Mean	10th	25th	Median (BCs)	IQR	75th	90th
Pb	ADR	37	16543	3429	7513	13932	19711	27223	32098
	AEL	85	13897	2123	3727	4920	13223	16950	41200
	CEN	2	2761	-	-	2761	-	-	-
	WMS	132	18792	6020	15935	20465	9135	25070	28845
HgT	ADR	27	119.6	28.9	37.8	106.8	149.9	187.7	240.8
	AEL	84	43.4	1.8	5.4	31.2	59.6	65.0	118.5
	CEN	1	58.0	-	-	58.0	-	-	-
	WMS	122	70.3	24.8	40.0	60.0	40.0	80.0	138.4
Cd	ADR	32	125.8	60.0	70.6	92.3	75.4	146.1	268.1
	AEL	20	114.5	35.4	45.7	56.0	42.8	88.5	387.1
	CEN	2	25.5	-	-	25.5	-	-	-
	WMS	85	94.4	50.0	71.5	91.2	32.5	104.0	128.4

Annex III

Calculation of BCs/BACs for trace metals (Cd, Hg, Pb) in sediment

ANNEX III. Calculations of BCs/BACs for trace metals (Cd, Hg, PB) in fish

The following tables summarize the characteristics of the selected datasets from the MEDPOL Database and CORMON datasets and the calculations of Mediterranean BCs and BACs for cadmium, mercury and lead in *Mullus barbatus* (MB). Additionally, other species of fish were also evaluated with datasets obtained from the MEDPOL Database (*Boops boops* (BB); *Mullus surmuletus* (MS) and *Upneus mollucensis* (UM)), but not included in calculations.

Table A.3.1.Detailed summary of the selected datasets for BC/BAC calculations in sediment. (DL: Detection Limits given within datasets; Tissue FI (fish fillet), LI (Liver)).

Country	Stations	DL (ng/g)	Observations	Years
Cyprus	**AEL-LEVS**: Larnaca, Limassol	Cd:0.005-0.02-10; HgT: 0.1-50; Pb:0.02-0.06-20	Coastal; Tissue FI; 6 samples (pooled 8 individuals)	2003-2009
Greece	**AEL-AEGS**: GRE5 (AI9), GR10 (AI14), GRE1 (A/6), GRE9 (A/13), GRE6 (A/3), GRE4 (MB, BB, A/12), GRE8 (A/8), GRE2 (AI10), ALEX/POLI (AIG/1), HANIA (AIG/4), HIOS (AIG/2) **CEN-CEN:** KALAMATA (AIG/7), PARGA (AIG/5)	-	2 species:*Boopsboops* (BB); *Mullus barbatus* (MB); pooled samples (10 individuals)	2004, 2005
Italy	**WMS-TYRS:** PRI-BRUCOLI-03, PRI-PN-04, POR-TORI-SAN-GA, PT-TORRES-05 **ADR-MADR:** PRI-PN-08,	Cd: 1; HgT:?, Pb:?	2 species: Mullussurmuletus (MS); Mullus barbatus (MB); Tissue FI, LI; individual samples	2003-2006, 2008
Spain	**WMS-NWMS**: SANTA POLA	Cd: 9; HgT: 10; Pb: 20	Single reference station; Tissue FI, individual samples	2006, 2010
Turkey	**AEL-AEGS**: CABMB, GEDMB, KMRMB, ILBMB, MESMB **AEL-LEVS:** ANBMB, ALBMB, FETMB, GREMB, TIRMB (TIRTAR only 2009, earlier values too high)	HgT: 20; Pb: 1000 (Pb data too high ≈ 4 ppm, not included)	Tissue FI, 1-3 samples (15-30 pooled individuals)	2009, 2011
Israel	**AEL-LEVS**: TRAWL C and TRAWL S	Cd: 20; HgT: 0.004-0.4	2 species: *Upneusmoluccensis* (UM); *Mullus barbatus*(MB); Coastal; Tissue FI individuals (no pools); time-series	1999-2000;2009-2011

Table A.3.2. Summary of statistics for TM and Mediterranean BCs and BACs calculation for fish (μg/kg fw). (λ: percentage of data included in the selected normal component; X: mean; σ: standard deviation; μ C.I.: confidence interval for the mean). BDL: Below detection limit.

Cadmium	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CYP	14	19	1.3	1.3	3.7	2.4	3.7	118.0		
GRE	47	8.5	0.4	1.5	3.0	4.5	6.0	11.9		
ISR	-	<BDL	-	-	<BDL	-	-	-		
ITA	3	820.0		456.0	572	-	1003.0	-		
SPA	-	<BDL	-	-	<BDL	-	-	-		
TUR	6	74.5	54.0	66.0	76.5	18.0	84.0	-		
Calculated BC	***70***	***51***	***0.8***	***1.4***	***3.7 (BDL<10.7)***	***9.2***	***10.6***	***83.0***	**Med BAC**	**See table**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	*82*	*3.8*	*3.4*	*3.1*	*4.5*				*Mixtool BAC*	*7.6*
	N	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
MB, Italy (LI)	*5*	*417.8*	*102.0*	*114.5*	*141.0*	*745*	*859.5*			
BB, Greece	*55*	*12.0*	*0.8*	*2.1*	*4.4 (BDL)*	*7.0*	*9.3*	*16.7*		
MS, Italy	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*		
UM, Israel	*4*	*32.6*	*20.9*	*23.7*	*32.3*	*18.3*	*41.9*	*-*		
Mercury	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CYP	47	55.0	18.2	31.7	45.4	37.0	68.7	110.4		
GRE	7	162.8	3.2	12.0	56.0	342.0	354.0	-		
ISR	79	68.9	19.9	24.8	38.6	33.6	58.4	221.4		
ITA	6	161.8	113.0	119.0	150.5	74.0	193.0	-		
SPA	24	75.5	50.0	56.2	68.0	26.5	82.7	109.5		
TUR	29	66.9	27.3	33.4	53.7	67.6	101.0	130.0		
Calculated BC	***192***	***72.3***	***20.8***	***30.2***	***50.6 (BDL<2.5***	***52.3***	***82.5***	***146.4***	**Med BAC**	**101.2**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	*73*	*42.5*	*19.6*	*39.8*	*45.2*				*Mixtool BAC*	*85*
	N	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
MB, Italy (LI)	*3*	*181.3*	*123.0*	*123.0*	*179.0*	*-*	*-*	*-*		
BB, Greece	*7*	*83.0*	*9.5*	*10.0*	*52.0*	*137*	*114.0*	*-*		
MS, Italy	*2*	*252*	*-*	*-*	*252*	*-*	*-*	*-*		
UM, Israel	*5*	11.1	5.3	5.7	12.4	10.2	15.9	-		
Lead	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CYP	14	33	3	3	10	18	20	189		
GRE	7	166	46	70	118	220	290	-		
ISR	-	-	-	-	-	-	-	-		
ITA	3	288	183	183	184	-	-	-		
SPA	10	34	20	23	31	20	43	65		
TUR	-	(revise)	-	-	(revise)	-	-	-		
Calculated BC	***34***	***83***	***3***	***10***	***31 (BDL<20)***	***118***	***128***	***245***	**Med BAC**	**See table**
Mixtool	*λ (%)*	*x*	*σ*	*μC.I. (90%) (min./max.)*						
Selected component	*61*	*19*	*15*	*13.8*	*24.4*				*Mixtool BAC*	*38.2*
	N	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
MB, Italy (LI)	*5*	*1002*	*174*	*479*	*874*	*1112*	*1590*	*-*		
BB, Greece	*7*	*145*	*56*	*60*	*63*	*150*	*210*	*-*		
MS, Italy	*5*	*172*	*149*	*156*	*169*	*35*	*191*	*-*		
UM, Israel	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*		

Table A.3.3. Summary of statistics for TM BCs in the Mediterranean eco-regions in fishfillet(μg/kg fw).

Trace metal	**Eco-region**	**N**	**Mean**	**10th**	**25th**	**Median (BCs)**	**IQR**	**75th**	**90th**
Pb	AEL	21	78	3	3	20	136	139	272
	WMS	13	93	20	25	38	100	125	373
	ADR	6	161.8	113.0	119.0	150.5	74.0	193.0	
HgT	AEL	162	68.6	19.9	27.3	44.6	46.0	73.3	130.5
	WMS	24	75.5	50.0	56.2	68.0	26.5	82.7	109.5
	AEL	55	19.5	0.7	1.3	3.7	8.2	9.5	80.6
Cd	CEN	12	3.6	.5	1.6	2.5	1.5	3.1	11.4
	WMS (LI?)	3	820.6		456.0	572.0	-	1003.0	-

Annex IV

Calculations of BCs/BACs for polycyclic aromatic hydrocarbons (PAHs) in mussels

ANNEX IV. Calculations of BCs/BACs for polycyclic aromatic hydrocarbons (PAHs) in mussels

The following tables summarize the characteristics of the selected datasets from the MEDPOL Database and online datasets and the calculations of the Mediterranean BCs and BACs for each individual PAHs in *Mytilusgalloprovincialis* (MG).

Table A.4.1. Detailed summary of the selected datasets for BC/BAC calculations in mussel. WST: Whole soft tissue; SO: Soft organs (tissue)

Country	**Eco-region/Stations**	**Size / Tissue**	**Comments**	**Years**
France	**WMS-NWMS**: Banyuls s/mer – LaboratorieAragó, Etang de BagesEtang de Leucate, Thau 1, Thau 4, Les StesMaries de la mer, **WMS-WMIA**: Ajaccio, Etang de Diana, Etangd'Urbino, Sant'Amanza	-	-	2005-07, 2011-2013
Greece	**AEL-AEGS**: C8B	3.5 cm SO	Pooled samples 20 individuals	2005
Italy	**ADR-MADR**: Alzale, Annabella, Antares, Antonella, Arianna, Armida	(5-6 cm) WST	6 samples-2 depths (duplicate, pooled 3 individuals)	2007
Spain	**WMS-NWMS**: MEDAS **WMS-ALBS**: MANILVA, HERRAD	3.5 ± 0.3 cm	3 samples (pooled 80 individuals)	2007-2011
Turkey	**AEL-AEGS**: IZM1B	5.4 ± 0.7 cm	Winter ; Not included	2006-2009

Table A.4.2. Summary of statistics for PAHs and Mediterranean BCs and BACs calculation for mussel (µg/kg dw). (λ: percentage of data included in the selected normal component; X: mean; σ: standard deviation; μ C.I.: confidence interval for the mean). BDL: Below detection limit; DL has been estimated from datasets and common analytical methods for this report.

Naphthalene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	36	4.70	0.34	0.61	2.24	8.22	8.82	12.66		
GRE	3	3.93	-	0.62	2.80	-	8.45	-		
ITA (too high data)	-	-	-	-	-	-	-	-		
SPA	-	-	-	-	-	-	-	-		
Calculated BC	***39***	***4.64***	***.35***	***.64***	***2.40 (BDL<5*)***	***7.06***	***7.70***	***12.60***	***Med BAC***	***(6.0)***
Mixtool	*λ (%)*	*x*	*σ*	***μC.I. (90%) (min./max.)***						
Selected component	*62*	*7.17*	*4.67*	*5.61*	*8.75*				*Mixtool BAC*	*17.9*
Acenaphtylene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	28	0.62	0.30	0.38	0.56	0.27	0.65	1.12		
GRE	3	0.13	0.10	0.10	0.10	-	-	-		
ITA (too high data)	-	-	-	-	-	-	-	-		
SPA	-	-	-	-	-	-	-	-		
Calculated BC	***31***	***0.58***	***0.20***	***0.33***	***0.55 (BDL<1*)***	***0.31***	***0.64***	***1.08***	***Med BAC***	***(1.4)***
Mixtool	*λ (%)*	*x*	*σ*	***μC.I. (90%) (min./max.)***						
Selected component	*80*	*0.44*	*0.17*	*0.34*	*0.50*				*Mixtool BAC*	*1.1*

Acenaphtene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	29	0.83	0.31	0.41	0.57	0.64	1.05	2.00		
GRE	3	0.16	0.10	0.10	0.10	-	-	-		
ITA (too high data)	-	-	-	-	-	-	-	-		
SPA	-	-	-	-	-	-	-	-		
Calculated LC/BC	***32***	***0.76***	***0.24***	***0.33***	***0.55 (BDL<1*)***	***0.58***	***0.91***	***1.88***	***Med BAC***	***(1.4)***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*71*	*0.44*	*0.16*	*0.38*	*0.49*				*Mixtool BAC*	*1.1*

Fluorene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	43	1.62	0.49	0.60	1.10	1.30	1.90	2.46		
GRE	3	0.93	0.50	0.50	0.60	-	-	-		
ITA	60	1.13	0.66	0.77	1.07	0.59	1.36	1.73		
SPA	33	1.34	0.42	0.58	0.93	0.77	1.36	3.83		
Calculated LC/BC	***139***	***1.33***	***0.50***	***0.69***	***1.00 (BDL<0.27)***	***0.82***	***1.51***	***2.35***	***Med BAC***	*2.5*
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*92*	*1.07*	*0.53*	*0.99*	*1.15*				*Mixtool BAC*	*2.7*

Phenanthrene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	48	7.03	1.79	2.9	4.29	4.28	7.17	10.71		
GRE	2	7.55	5.60	5.60	7.55	-	-	-		
ITA	60	9.25	5.94	7.87	9.04	2.73	10.60	13.92		
SPA	42	7.34	3.39	4.26	6.06	4.25	8.51	12.45		
Calculated LC/BC	***152***	***8.00***	***2.93***	***4.30***	***7.12 (BDL<0.27)***	***5.26***	***9.55***	***12.49***	***Med BAC***	*17.8*
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*96*	*7.00*	*3.34*	*6.55*	*7.46*				*Mixtool BAC*	*17.5*

Anthracene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	25	1.15	0.29	0.37	0.52	0.26	0.62	1.98		
GRE	3	0.43	0.30	0.30	0.30	-	-	-		
ITA	55	0.77	0.21	0.25	0.38	0.33	0.58	2.09		
SPA	28	0.63	0.27	0.38	0.51	0.47	0.85	1.21		
Calculated LC/BC	***111***	***0.81***	***0.24***	***0.30***	***0.46 (BDL<0.31)***	***0.34***	***0.64***	***1.27***	***Med BAC***	***1.2***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*86*	*0.44*	*0.17*	*0.41*	*0.47*				*Mixtool BAC*	*1.1*

Fluoranthene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	48	7.69	1.36	2.68	5.82	7.70	10.38	16.00		
GRE	3	5.50	1.90	1.90	6.60	-	-	-		
ITA	60	2.66	1.23	1.41	2.03	2.32	3.73	4.65		
SPA	42	2.83	1.71	1.86	2.72	1.76	3.62	4.19		
Calculated LC/BC	***153***	***4.34***	***1.32***	***1.73***	***2.96 (BDL<0.25)***	***3.16***	***4.89***	***7.56***	***Med BAC***	***7.4***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*89*	*3.06*	*1.71*	*2.82*	*3.30*				*Mixtool BAC*	*7.7*

Pyrene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	48	7.03	1.98	2.63	4.65	6.67	9.3	15.7		
GRE	3	6.53	3.40	3.40	5.90	-	-	-		
ITA	60	2.82	0.38	0.51	0.85	1.22	1.73	4.43		
SPA	42	3.05	0.78	1.06	1.87	3.26	4.32	7.94		
Calculated LC/BC	***153***	***4.28***	***0.47***	***0.90***	***2.00 (BDL<0.29)***	***3.84***	***4.75***	***12.31***	***Med BAC***	***5.0***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*59*	*1.34*	*0.81*	*1.20*	*1.48*				*Mixtool BAC*	*3.4*
Benz(a)anthracene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	34	3.26	0.52	0.64	1.77	4.36	5.00	7.60		
GRE	3	1.70	1.40	1.40	1.60	-	-	-		
ITA	60	1.02	0.19	0.28	0.53	0.94	1.22	3.17		
SPA	6	0.34	0.16	0.24	0.28	0.17	-	-		
Calculated LC/BC	***103***	***1.74***	***0.21***	***0.37***	***0.76 (BDL<0.29)***	***1.73***	***2.10***	***4.20***	***Med BAC***	***1.9***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*68*	*0.62*	*0.40*	*0.54*	*0.69*				*Mixtool BAC*	*1.5*
Crysene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	26	9.51	3.11	3.97	7.05	8.90	12.87	16.63		
GRE	3	4.63	2.70	2.70	5.20	-	-	-		
ITA	50	0.74	0.12	0.19	0.27	0.93	1.11	2.27		
SPA	42	0.92	0.52	0.61	0.86	0.51	1.12	1.50		
Calculated LC/BC	***121***	***2.78***	***0.19***	***0.33***	***0.95 (BDL<0.31)***	***2.17***	***2.50***	***7.52***	***Med BAC***	***2.4***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*74*	*0.82*	*0.65*	*0.71*	*0.94*				*Mixtool BAC*	*2.1*
Benz(e)pyrene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA										
GRE	3	2.63	1.90	1.90	2.80	-	-	-		
ITA										
SPA	42	1.12	0.36	0.51	0.79	0.79	1.30	2.41		
Calculated LC/BC	***45***	***1.22***	***0.36***	***0.55***	***0.95 (BDL<0.24)***	***0.93***	***1.48***	***2.94***	***Med BAC***	***2.4***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*71*	*0.77*	*0.35*	*0.67*	*0.87*				*Mixtool BAC*	*1.9*
Benzo(b)fluoranthene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	17	7.37	3.4	4.3	5.40	3.95	8.25	14.62		
GRE	3	3.93	1.20	1.20	5.30	-	-	-		
ITA	30	0.59	0.13	0.35	0.43	0.30	0.70	1.09		
SPA	42		0.18	0.24	0.41		0.51	0.70		
Calculated LC/BC	***92***	***1.87***	***0.18***	***0.31***	***0.48 (BDL<0.27)***	***1.11***	***1.42***	***5.37***	***Med BAC***	***1.2***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*68*	*0.38*	*0.16*	*0.35*	*0.42*				*Mixtool BAC*	*1.0*

Benzo(k)fluoranthene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	36	2.54	0.57	0.81	1.69	2.89	3.70	6.73		
GRE	3	1.10	0.30	0.30	1.50	-	-	-		
ITA	24	0.28	0.09	0.14	0.29	0.20	0.33	0.53		
SPA	10	0.34	0.24	0.26	0.33	0.14	0.40	0.53		
Calculated LC/BC	***73***	***1.44***	***0.14***	***0.30***	***0.55 (BDL<0.17)***	***1.39***	***1.69***	***4.26***	***Med BAC***	***1.4***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*58*	*0.37*	*0.19*	*0.33*	*0.42*				*Mixtool BAC*	*0.9*
Benz(a)pyrene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	34	1.45	0.45	0.55	0.70	1.05	1.60	3.60		
GRE	3	0.60	0.30	0.30	0.70	-	-	-		
ITA	27	0.38	0.10	0.11	0.32	0.32	0.43	1.09		
SPA	11	0.28	0.13	0.21	0.23	0.07	0.28	0.67		
Calculated LC/BC	***75***	***0.86***	***0.11***	***0.26***	***0.49 (BDL<0.14)***	***0.64***	***0.90***	***1.72***	***Med BAC***	***1.2***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*86*	*0.48*	*0.31*	*0.42*	*0.55*				*Mixtool BAC*	*1.2*
Indeno(1,2,3-c,d)pyrene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	24	1.80	0.55	0.63	1.24	1.57	2.20	4.59		
GRE	3	0.80	0.40	0.40	0.90	-	-	-		
SPA	1	0.40	-	-	0.40	-	-	-		
Calculated LC/BC	***28***	***1.64***	***0.49***	***0.61***	***1.16 (BDL<0.31)***	***1.52***	***2.13***	***4.11***	***Med BAC***	***2.9***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*62*	*0.85*	*0.34*	*0.75*	*0.95*				*Mixtool BAC*	*2.1*
Dibenz(a,h)anthracene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	25	0.78	0.31	0.36	0.55	0.24	0.60	0.90		
GRE	1	0.30	-	-	0.30	-	-	-		
SPA	1	0.46	-	-	0.46	-	-	-		
Calculated LC/BC	***25***	***0.74***	***0.30***	***0.35***	***0.52 (BDL<0.33)***	***0.25***	***0.60***	***0.87***	***Med BAC***	***1.3***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*66*	*0.49*	*0.18*	*0.41*	*0.57*				*Mixtool BAC*	*1.2*
Benzo(g,h,i)perylene	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
FRA	23	2.09	0.74	0.93	1.45	1.54	2.47	4.83		
GRE	3	1.13	0.70	0.70	1.20	-	-	-		
SPA	27	0.76	0.29	0.35	0.43	0.64	0.99	1.90		
Calculated LC/BC	***53***	***1.36***	***0.31***	***0.41***	***0.93 (BDL<0.38)***	***1.12***	***1.53***	***2.65***	***Med BAC***	***2.3***
Mixtool	***λ (%)***	***x***	***σ***	***μC.I. (90%) (min./max.)***						
Selected component	*73*	*0.78*	*0.41*	*0.53*	*1.02*				*Mixtool BAC*	*1.9*

Table A.4.3. Summary of statistics for PAHs BCs in the Mediterranean eco-regions (µg/kg fw).

PAHs	**Eco-region**	**N**	**Mean**	**10th**	**25th**	**Median (BCs)**	**IQR**	**75th**	**90th**
N	AEL	3	3.93	2.40	2.40	2.80	-	-	-
	WMS	36	4.70	0.34	0.61	2.24	8.22	8.82	12.66
ACY	AEL	3	0.13	0.10	0.10	0.10	-	-	-
	WMS	28	0.62	0.30	0.38	0.56	0.27	0.65	1.12
ACE	AEL	3	0.16	0.10	0.10	0.10	-	-	-
	WMS	29	0.83	0.31	0.41	0.57	0.64	1.05	2.00
F	ADR	60	1.13	0.66	0.77	1.07	0.59	1.36	1.73
	AEL	3	0.93	0.50	0.50	0.60	-	-	-
	WMS	76	1.50	0.49	0.60	0.96	1.19	1.78	3.23
P	ADR	60	9.25	5.94	7.87	9.04	2.73	10.60	13.92
	AEL	2	7.55	5.60	5.60	7.55	-	-	-
	WMS	90	7.17	2.35	3.70	4.93	3.76	7.46	11.59
A	ADR	55	0.77	0.21	0.25	0.38	0.33	0.58	2.09
	AEL	3	0.43	0.30	0.30	0.30	-	-	-
	WMS	53	0.88	0.29	0.38	0.52	0.33	0.71	1.36
FL	ADR	60	2.66	1.23	1.41	2.03	2.32	3.73	4.65
	AEL	3	5.50	1.90	1.90	6.60	-	-	-
	WMS	90	5.42	1.71	2.03	3.38	3.91	5.94	12.51
PY	ADR	60	2.82	0.38	0.51	0.85	1.22	1.73	4.43
	AEL	3	6.53	3.40	3.40	5.90	-	-	-
	WMS	90	5.17	0.97	1.77	3.02	4.75	6.52	13.20
BaA	ADR	60	1.02	0.19	0.28	0.53	0.94	1.22	3.17
	AEL	3	1.70	1.40	1.40	1.60	-	-	-
	WMS	40	2.82	0.29	0.57	1.20	3.70	4.27	7.34
C	ADR	50	0.74	0.12	0.19	0.27	0.93	1.11	2.27
	AEL	3	4.63	2.70	2.70	5.20	-	-	-
	WMS	68	4.20	0.55	0.77	1.24	4.12	4.90	12.83
BeP	ADR	-	-	-	-	-	-	-	-
	AEL	3	2.63	1.90	1.90	2.80	-	-	-
	WMS	42	1.12	0.36	0.51	0.79	0.79	1.30	2.41
BbF	ADR	30	0.59	0.13	0.35	0.43	0.30	0.65	1.46
	AEL	3	3.93	1.20	1.20	5.30	-	-	-
	WMS	59	2.43	0.18	0.26	0.49	3.44	3.70	7.40
BkF	ADR	24	0.28	0.09	0.14	0.29	0.20	0.33	0.53
	AEL	3	1.10	0.30	0.30	1.50	-	-	-
	WMS	46	2.07	0.32	0.50	1.27	2.23	2.73	5.26
BaP	ADR	27	0.38	0.10	0.11	0.32	0.32	0.43	1.09
	AEL	3	0.60	0.30	0.30	0.70	-	-	-
	WMS	45	1.17	0.21	0.38	0.60	0.72	1.10	3.00
GHI	AEL	3	1.13	0.70	0.70	1.20	-	-	-
	WMS	50	1.37	0.31	0.40	0.90	1.23	1.63	2.73
DA	WMS	24	0.76	0.32	0.38	0.53	0.21	0.60	0.88
ID	AEL	3	0.80	0.40	0.40	0.90	-	-	-
	WMS	25	1.74	0.50	0.61	1.23	1.59	2.20	4.47

ANNEX V.

Calculations of BCs/BACs for biomarkers (AChE, MT, MN, LMS and SOS) in mussels

ANNEX V. Calculations of BCs/BACs for biomarkers (AChE, MT, MN, LMS and SOS) in mussels

The following table summarizes the selected datasets submitted through the online group the BC and BAC for each individual biomarkers in *Mytilusgalloprovincialis* (MG).

Table A.5.1. Detailed summary of the selected datasets for BC/BAC calculations in mussel. (AChE: Acetylcholinesterase activity; MT: Metallothioneins content; MN: Micronuclei frequency; LMS-NRR: Lysosonal membrane stability (Neutral red retention method); LMS-LP: Lisosomal membrane stability (Cytochemical method, liabilisation period); SOS: Stress on Stress).

Country	**Eco-regions/Stations**	**Years**
Croatia	**WMS-NWMS**: LS, CO, SV, ML, NA, KV, SI, ST, NK, MR, PU, BR, MD, VO, TM, RI, UP, BK, KR, MA, VR, PL, MN,	AChE: 2002, 2007, 2013; MT: 2007, 2013; LMS-LP: 2004-2008, 2013, 2013 SOS: 2004,2007,2012;
Greece	**AEL-AEGS**: THE2, THE2B, B31, B32, STR4	AchE:2005; MT: 2000; LMS-LP: 2005;
Italy	**ADR-NADR**: Pesaro, Portonovo **ADR-MADR:**Civitanova **WMS-TYRS:** San Gavino	AChE: 2011; MT: 2008, 2011; MN: 2005, 2006, 2011 LMS-NRR: 2008-2010; LMS-LP: 2009, 2011
Spain	**WMS-NWMS**: Islas Columbretes, Cullera, Guardamar, Islas Medas, Islas Medas-Estartit **WMS-ALBS**: Almuñecar, Manilva	AChE: 2010-2012; MT: 2010-2012; MN: 2011,2012 LMS-NRR: 2002-2014; SOS: 2007-2014;

Table A.5.2. Summary of statistics for biomarkers in the reference stations and Mediterranean BCs and BACs calculation for mussel (µg/kg dw). (λ: percentage of data included in the selected normal component; X: mean; σ: standard deviation; µ C.I.: confidence interval for the mean).*adopted ICES standard

AChE (only gills)	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CRO	38	14.05	6.10	8.80	12.40	8.61	17.41	23.31		
GRE (DG)-**not included**	40	153.75	53.14	75.79	104.63	146.73	222.52	296.51		
ITA	12	12.57	6.65	7.31	10.72	10.39	17.70	20.52		
SPA	80	21.32	14.68	17.15	20.86	7.35	24.50	27.38		
Calculated BC	*80*	*21.32*	*14.68*	*17.15*	*20.86*	*7.35*	*24.50*	*27.38*	***Med BAC***	***15***
Mixtool	λ (%)	x	σ	μ*C.I. (90%) (min./max.)*						
Selected component	*98*	*17.92*	*6.43*	*16.7*	*19.1*				*Mixtool BAC*	*16.7*
Metallothioneins	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CRO	14	214.1	148.0	171.5	200.5	47.85	219.4	359.5		
GRE (analytical method in mg/g)-**not included**	40	374.23	102.14	144.42	366.80	359.9	504.4	686.9		
ITA(Eq.(GSH) nmol/mg prot.)-**not included**	8	2.7	1.49	1.86	2.9	1.49	3.34	-		
SPA	60	191.5	136.3	159.2	191.3	68.06	227.3	247.0		
Calculated BC	***74***	***195.8***	***142.8***	***160.9***	***192.38***	***62.34***	***223.0***	***246.7***	***Med BAC***	***247***
Mixtool	λ (%)	x	σ	μ*C.I. (90%) (min./max.)*						
Selected component	*88*	*200.33*	*52.03*	*189.7*	*210.9*				*Mixtool BAC*	*210.9*
Micronuclei frequency	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
ITA	39	0.3	0.0	0.0	0.0	0.5	0.5	1.0		
SPA	31	0.6	0.0	0.0	0.4	1.0	2.1	2.4		
Calculated BC	***70***	***0.41***	***0.0***	***0.0***	***0.0***	***0.7***	***0.7***	***1.0***	***Med BAC***	***1.0***
Mixtool	λ (%)	x	σ	μ*C.I. (90%) (min./max.)*						
Selected component	*92*	*0.29*	*0.39*	*0.2*	*0.4*				*Mixtool BAC*	*0.4*
LMS	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**		
CRO – DG LP	98	16.7	5.9	7.8	15.3	16.6	24.5	30.0		
GRE – DG LP	47	8.2	3.0	3.8	6.8	6.7	10.5	16.0		
ITA – DG LP	15	22.1	15.7	20.0	21.3	2.5	22.5	34.0		
Calculated BC (Labilisation period, LP)	***160***	***14.7***	***4.3***	***6.8***	***12.7***	***14.5***	***21.3***	***26.7***	***Med BAC***	***20****
Mixtool	λ (%)	x	σ	μ*C.I. (90%) (min./max.)*						
Selected	*67*	*19.1*	*7.98*	*17.8*	*20.4*				*Mixtoo*	-

component		*0*						*l BAC*	
ITA – HM NRR	29	54.5	31.3	34.0	47.4	41.60	75.6	88.2	
SPA –HM NRR	428	55.1	15.0	15.0	45.0	75.0	90.0	120.0	
Calculated BC (Neutral Red Retention, NRR)	***457***	***55.0***	***15.0***	***15.0***	***45.0***	***75.0***	***75.0***	***120.0***	***Med BAC***	***120****
Mixtool	λ *(%)*	x	σ	μ*C.I. (90%) (min./max.)*					
Selected component	*59*	*28.03*	*18.54*	*26.2*	*29.9*				*Mixtool BAC*	-
SOS	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**	
CRO	17	7	4	5	6	3	8	11	
SPA	16	8	6	6	7	3	9	11	
Calculated LC/BC	***33***	***7***	***5***	***6***	***7***	***2***	***8***	***11***	***Med BAC***	***11***
Mixtool	λ *(%)*	x	σ	μ*C.I. (90%) (min./max.)*					
Selected component	-	-	-	-	-	-	-	-	*Mixtool BAC*	-

Table A.5.3. Summary of statistics for biomarkers BCs in the Mediterranean eco-regions (µg/kg fw).

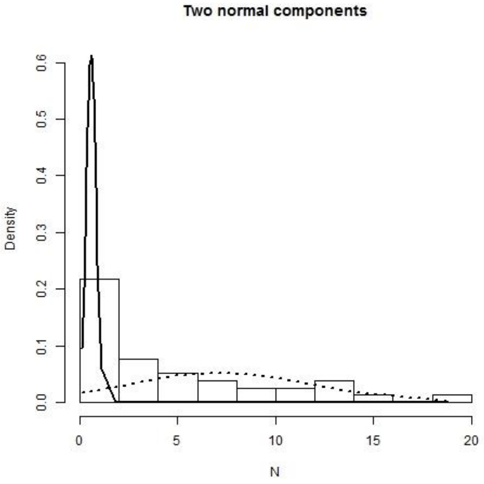
Biomarkers	**Eco-region**	**N**	**Mean**	**10th**	**25th**	**Median**	**IQR**	**75th**	**90th**
AChE	ADR	50	13.69	6.32	8.21	12.20	9.27	17.48	21.95
	WMS	80	21.32	14.68	17.15	20.86	7.35	24.50	27.38
Metallothioneins	ADR	14	214.1	148.0	171.5	200.5	47.8	219.4	359.5
	WMS	60	191.6	136.3	159.2	191.3	68.1	227.3	247.0
Micronuclei frec.	ADR	26	0.4	0.0	0.0	0.5	0.8	0.8	1.0
	WMS	44	0.4	0.0	0.0	0.0	0.7	0.7	1.5
LMS-NRR	ADR	29	54.5	31.3	34.0	47.4	41.6	75.6	88.2
	WMS	428	55.1	15.0	15.0	45.0	75.0	90.0	120.0
LMS-Cytochem.	ADR	113	17.4	6.2	9.4	16.8	13.95	23.3	30.0
	AEL	47	8.2	3.0	3.8	6.8	6.70	10.5	16.0

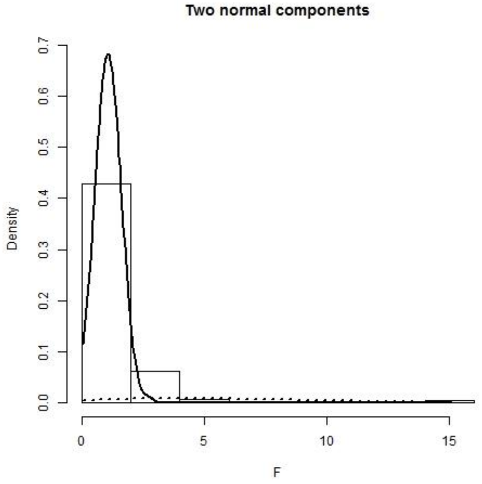
ANNEX VI.

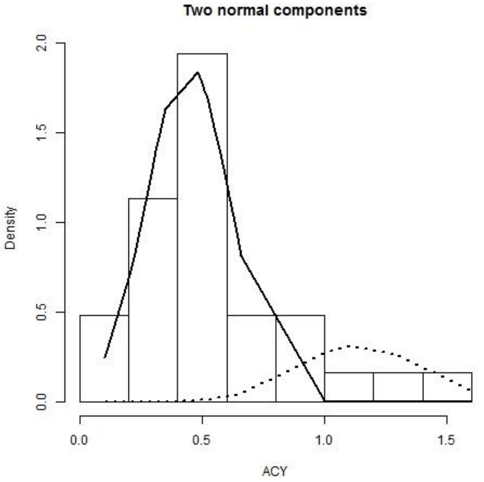
Mixtool algorithm: two normal components plots

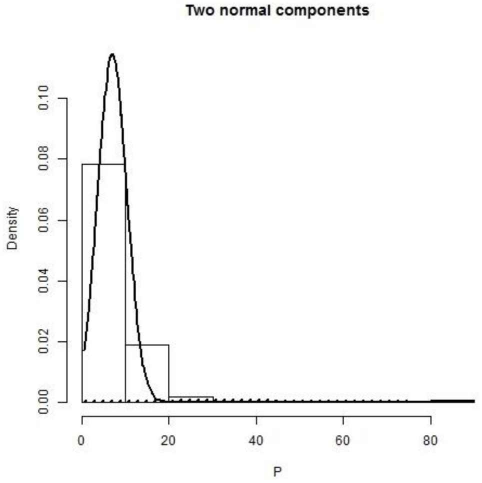
ANNEX VI. Mixtool algorithm: two normal components plots

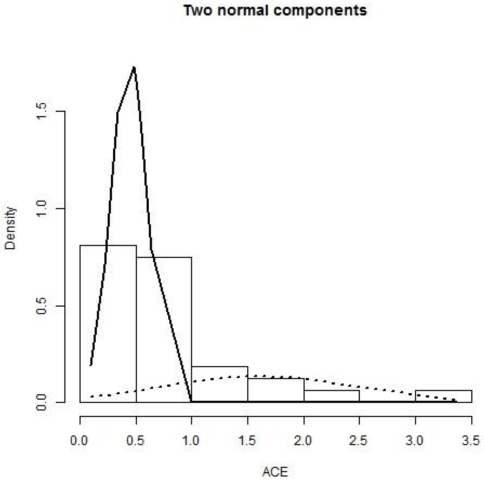
Two normal components (PAHs mussels)

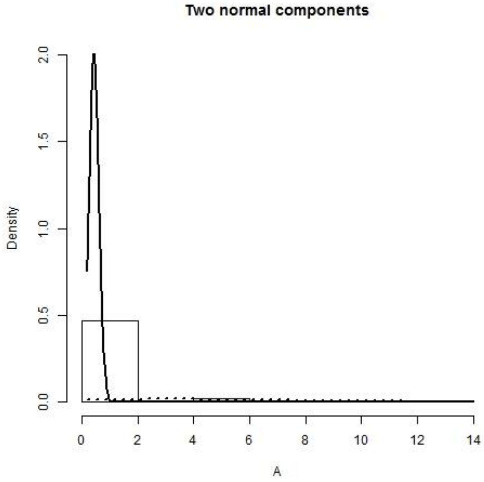


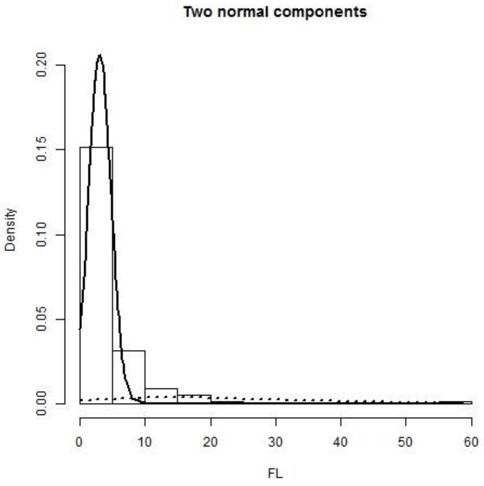








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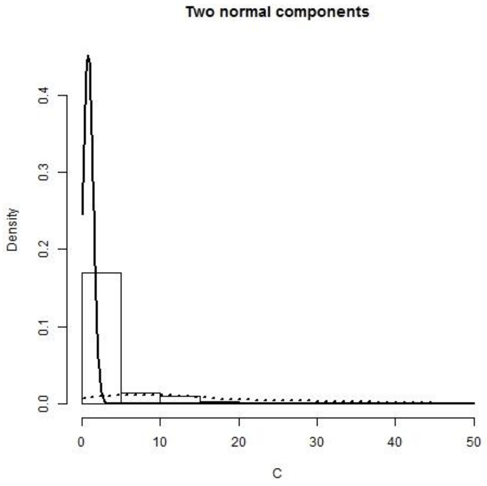
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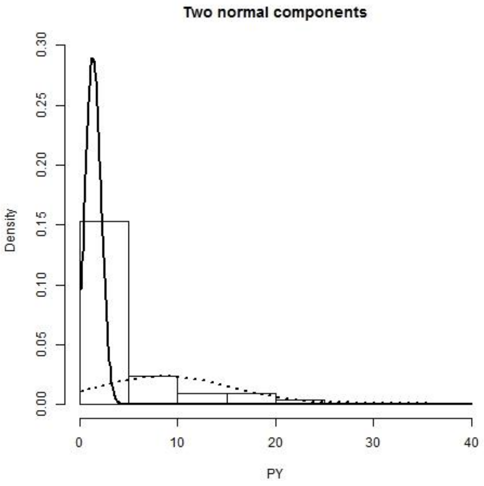
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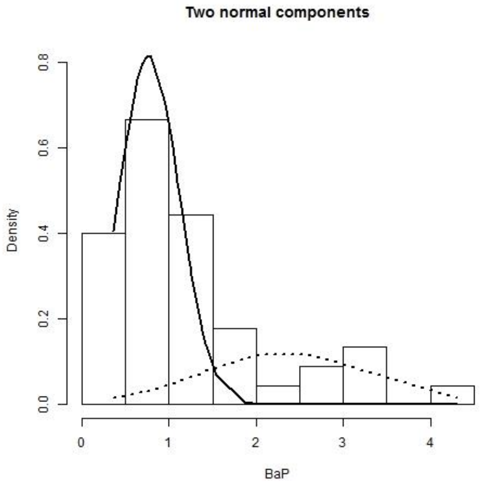
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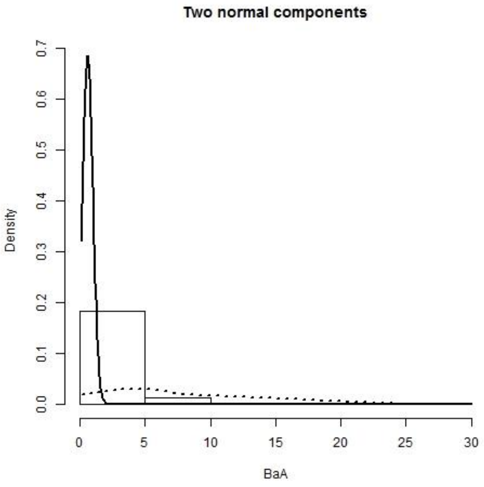
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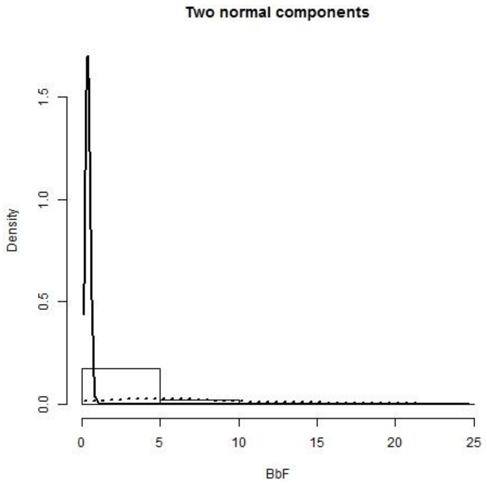
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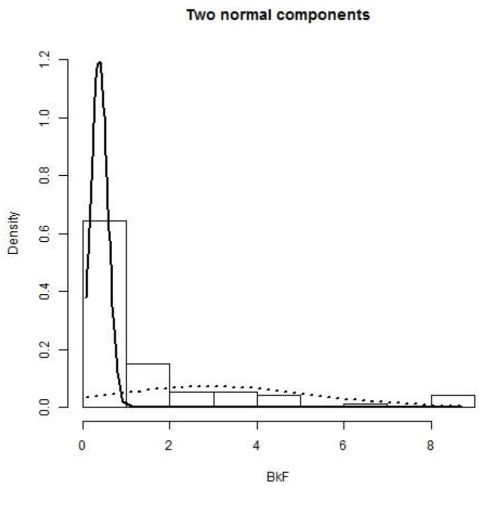
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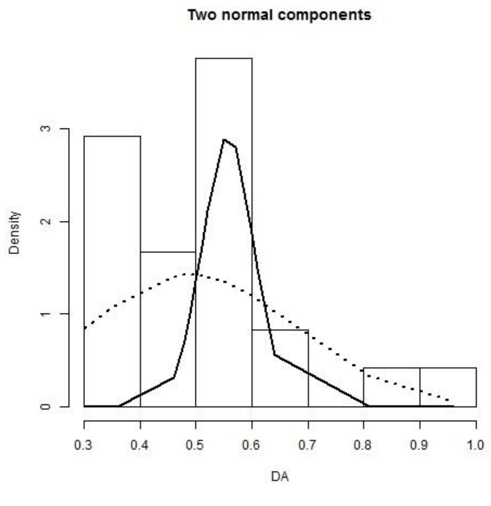
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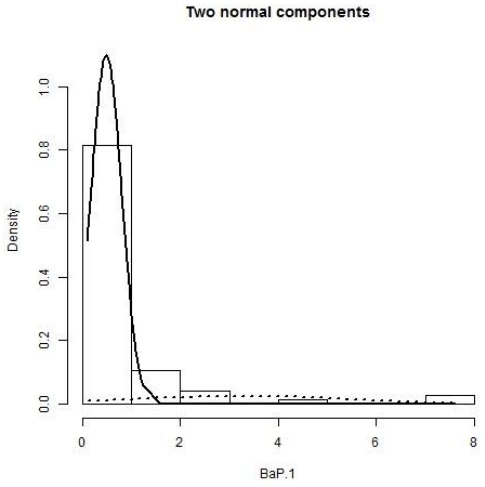
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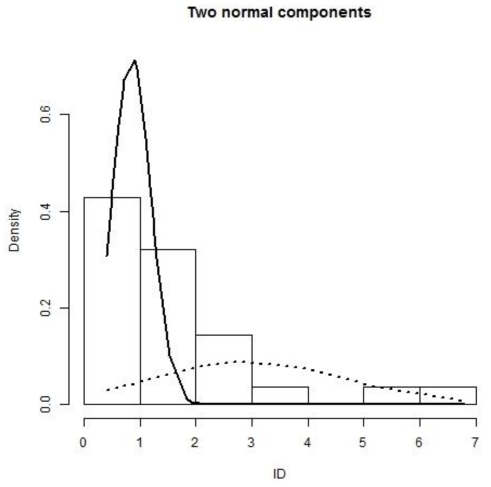
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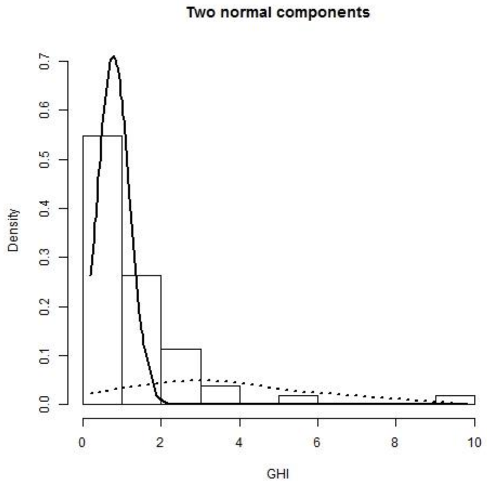
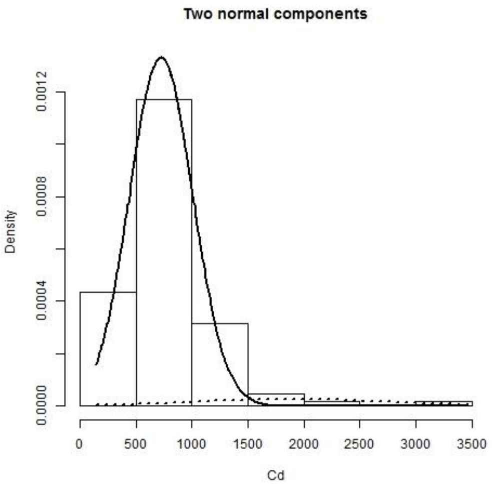
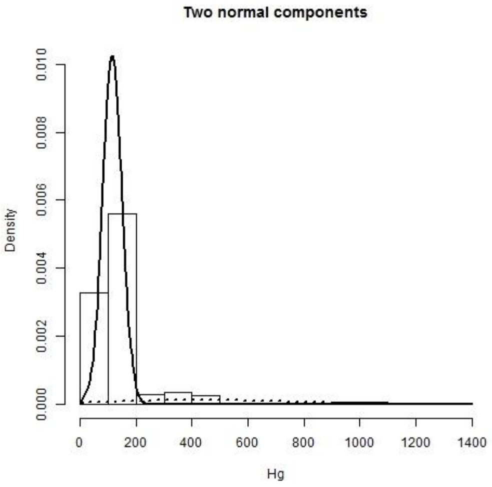
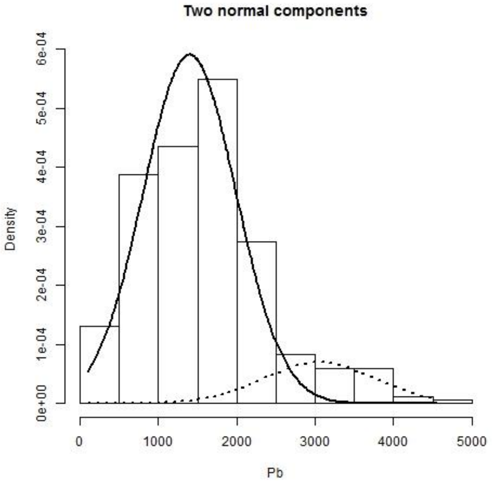

Two normal components

Two normal components (TM mussel)







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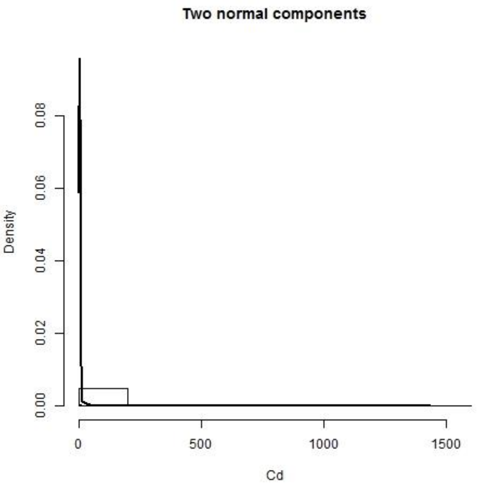

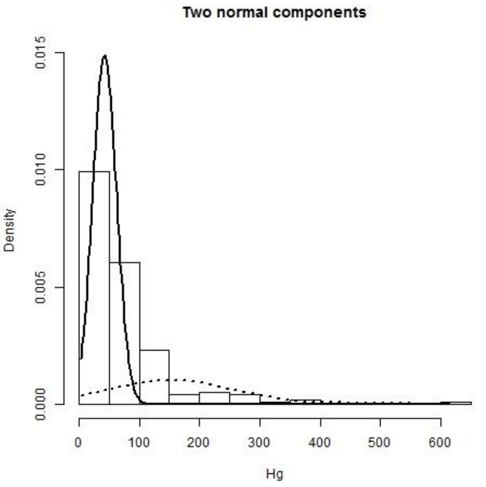

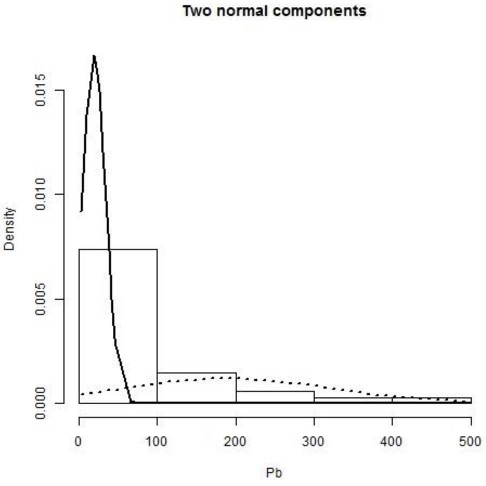

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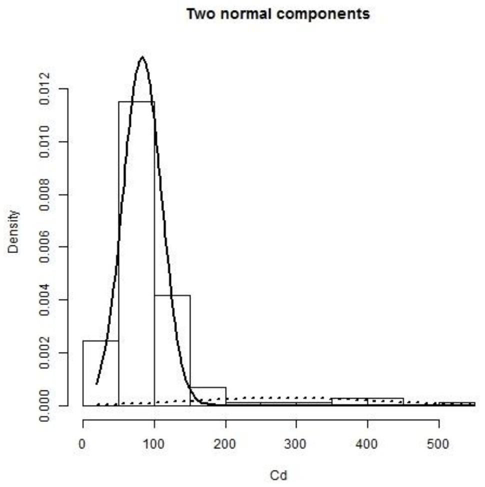
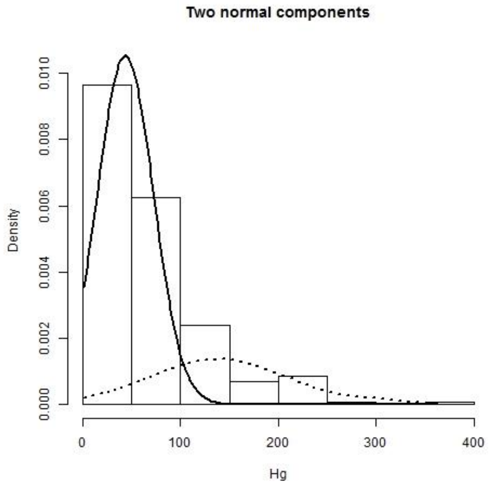
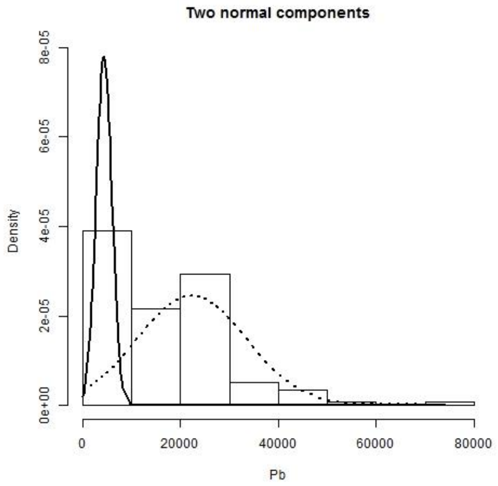
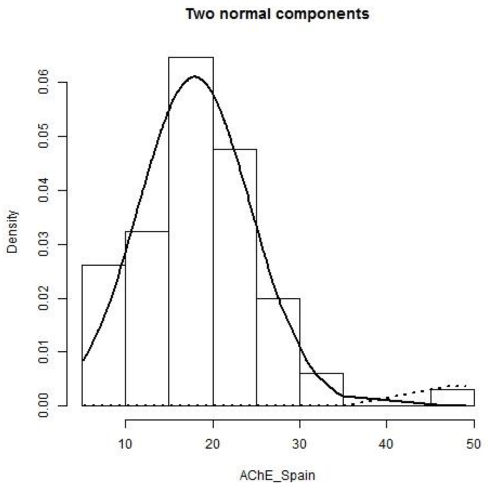
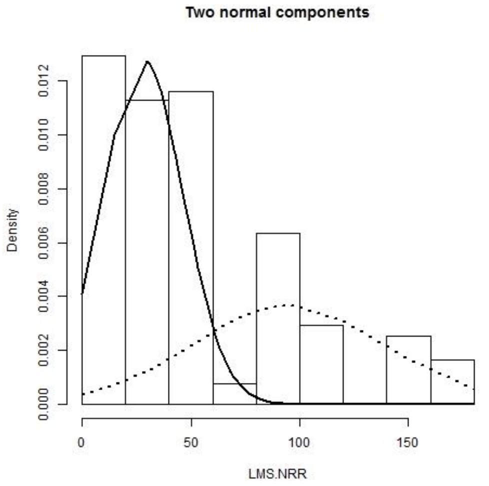


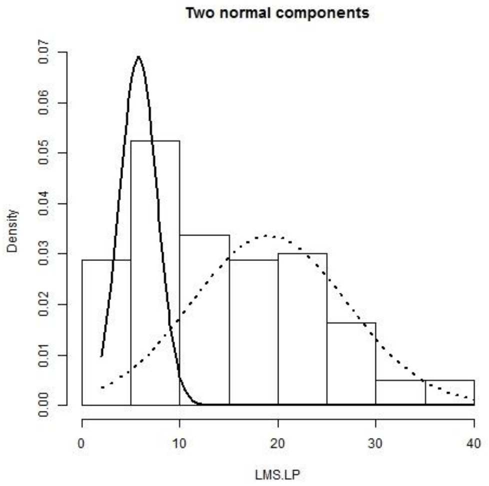


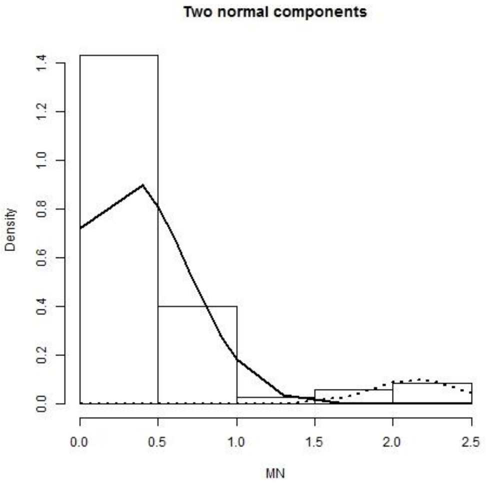


Two normal components (biomarkers)





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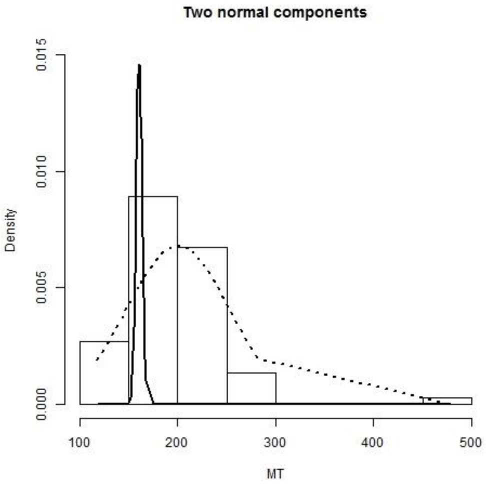
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