BEFORE THE NATIONAL GREEN TRIBUNAL
SOUTHERN ZONE, CHENNAI

Original Application No. 146 of 2024 (SZ)

R. Pratima …. Applicant

-Vs-

The Tamil Nadu Coastal Zone Management Authority,

…Respondents

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DATED AT CHENNAI ON THIS THE 17th DAY OF DECEMBER, 2024

M/s. S SARAVANAN
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BEFORE THE NATIONAL GREEN TRIBUNAL
SOUTHERN ZONE, CHENNAI
Original Application No. 146 of 2024 (SZ)

R. Pratima
W/o (Late) V. Ram Mohan,
No. 339, Kathivakkam High Road,
Ennore, Tamil Nadu – 600 057.
Residing at
1B, Dewside Manor,
58, Mc Nichols Road,
Chetpet, Chennai – 600 031.

.... Applicant

-Vs-

1. **The Tamil Nadu Coastal Zone Management Authority**,
Rep by its Member Secretary,
No. 1, Jeenis Road,
Panagal Building, Ground Floor,
Saidapet, Chennai – 600 015.

2. **The Public Works Department**,
Government of Tamil Nadu
Rep by its Secretary,
Fort St George, Chennai – 600 009.

3. **Tamil Nadu Pollution Control Board**,
Rep. by its Chairperson,
No. 76, Mount Salai,
Guindy, Chennai – 600 032.

4. **The Chennai Petroleum Corporation Limited**,
Rep by its Managing Director,
New No. 536, Anna Salai,
Teynampet, Chennai – 600 018.

.... Respondent(s)

REPLY STATEMENT FILED BY THE 4TH RESPONDENT

बी. कोथंडरामन
B. KOTHANDARAMAN
मुख्य महाप्रबंधक (एचएसइ)
Chief General Manager (HSE)
चेन्नै पेट्रोलियम कॉर्पोरेशन लिमिटेड
Chennai Petroleum Corporation Ltd.
मणली/Manali, चेन्नै/Chennai - 600 068.

I, B. Kothandaraman, aged about 57 years, having registered office at 536, Anna Salai, Teynampet, Chennai 600018, do hereby solemnly affirm and sincerely state as follows:

1. I humbly submit that I am working as the Chief General Manager, in the office of M/s. Chennai Petroleum Corporation Limited, the 4th Respondent herein and hereinafter referred to as CPCL, and as such, well acquainted with the facts and circumstances of the present case, and I am authorised to swear this affidavit on behalf of the 4th Respondent herein.

2. That the present application has been filed before this Hon'ble Tribunal, seeking for the following relief:

   "A. Direct Respondents 1, 3 & 4 to clean up and remediate the lands of the applicant in S. No. 239/1A2A1, 239/1A2A2, 239/1A2A3, 239/1A2A8A and 16/181, Kathlvakkam Village, Ennore, so that the land is completely free of any contamination and in the alternative, if the contamination is not cleaned up/incapable of being remediated, direct the 4th respondent to acquire and provide to the applicant land the same size, value and amenities or acquire the subject lands at market price.
   B. Direct the 4th respondent to pay compensation to the applicant for the contamination of the subject land and exposure to toxins caused on account of leak from their facility and costs of the present proceedings."

3. That nothing contained in this Reply Statement shall be construed as an admission of any statement or averment made in the present Original Application except what has been specifically admitted by the answering Respondent herein.

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4. That the answering Respondent deny all the averments which are contrary to and/or inconsistent with what is stated in this statement, as if the same were specifically traversed.

5. That the instant Reply Statement is being filed in order to bring out the true and correct factual position and to specifically deal with the averments and allegations made by the Applicant in the instant Original Application. The answering Respondent reserves his rights and liberty to file a further detailed Statement/Affidavit and additional documents if the situation so necessitates at a later stage.

6. That before traversing into the para wise reply of this Respondent with respect to the allegations raised in the application, it is necessary to set out the brief facts.

7. The Respondent humbly submits that the flooding of Buckingham Canal and inundation of the Applicant's property, situated in Survey Nos. 239/1A2A1, 239/1A2A2, 239/1A2A3, 239/1A2A8A and 16/18 forming part of Kathivakkam Village, Ennore, is due to the recent Michaung Cyclone.

8. That subsequent to the release of excess water from the reservoir, water level in Manali and neighbouring places raised causing the carryover of surface oil from all industries in Manali and neighbouring areas.

9. The Respondent humbly submits that the Tamil Nadu Government had constituted a High-Level Coordination Committee under Oil Spill Crisis Management Coordination Centre for oil recovery measures, soil and shore clean-up in the affected villages along Buckingham Canal wherein, Tamil Nadu Pollution Control Board, the 3rd Respondent herein and hereinafter referred to as TNPCB; together

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with the Govt of Tamil Nadu Department of Environment and Climate Change; Govt of Tamil Nadu Department of Fisheries and Fisherman Welfare; Govt of Tamil Nadu Revenue and Disaster Management Department; Govt of Tamil Nadu Forest Department; Tamil Nadu Police, Greater Chennai Corporation, hereinafter referred to as GCC; and CPCL, the 4th respondent herein; were part of the above operations.

10. That the cleaning operations were carried out under the careful supervision of the State Government, TNPCB and GCC.

11. The Respondent humbly submits that in order to contain and remove the Oil Pockets, Oil Containment Booms for approximately 1380m were deployed not only near the aforementioned property but also at various places in the river mouth and Buckingham Canal.

12. That the access to the Oil Pocket was not available through the river side; as result of which, as per the directions of the High-Level Coordination Committee, the entry was gained through the Applicant's property with the due presence of the local authorities on an emergency basis.

13. The Respondent humbly submits that CPCL, as a responsible corporate entity, with a view to protect the environment and the public at large had stepped in to the issue and had engaged various experts in the field to contain the oil and other sludge material and took remediation measures.

14. That the photos of the aforesaid property are enclosed herein for the kind perusal of this Hon'ble Tribunal.

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Figure 1 Sludge on the Shores of Private Property Under Ennore Road Bridge South East

Figure 2 Sludge removal Operation

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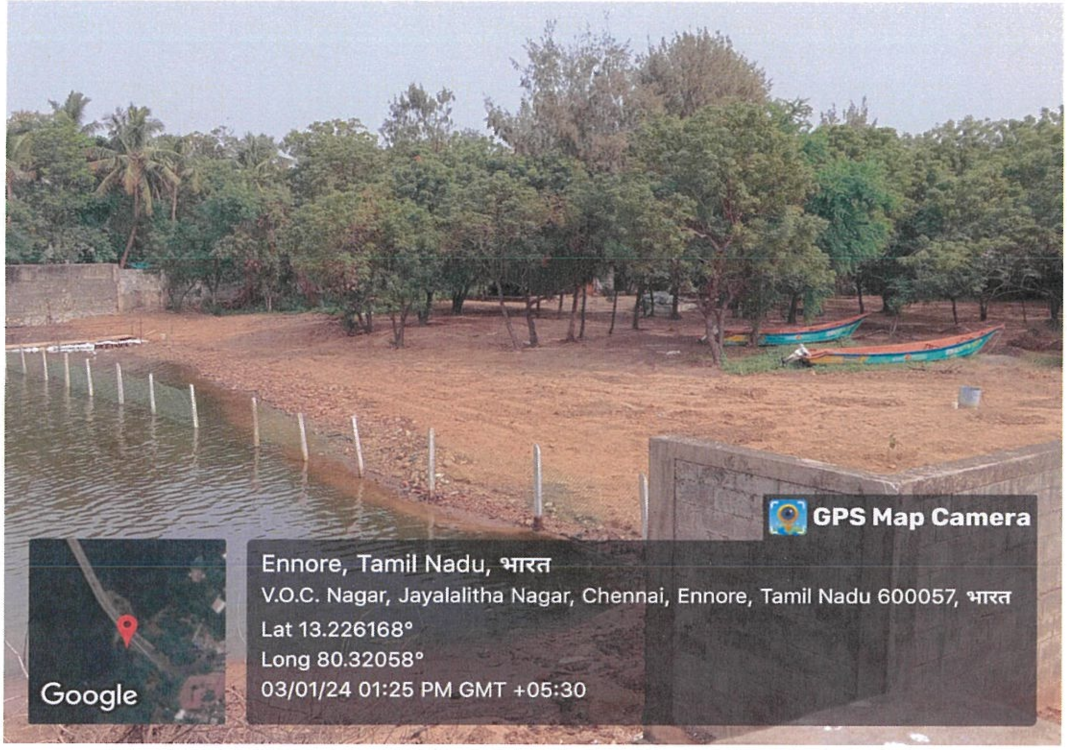

Figure 3(a) Completion of Restoration Works

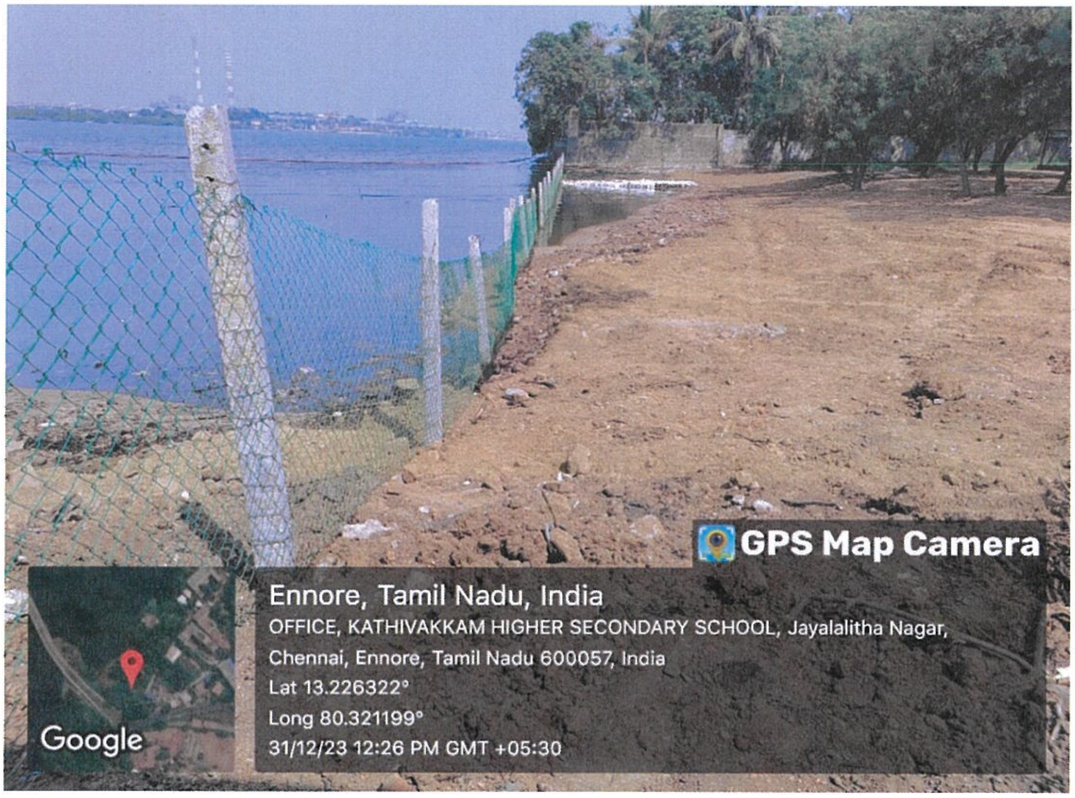

Figure 3(b) Completion of Restoration Works

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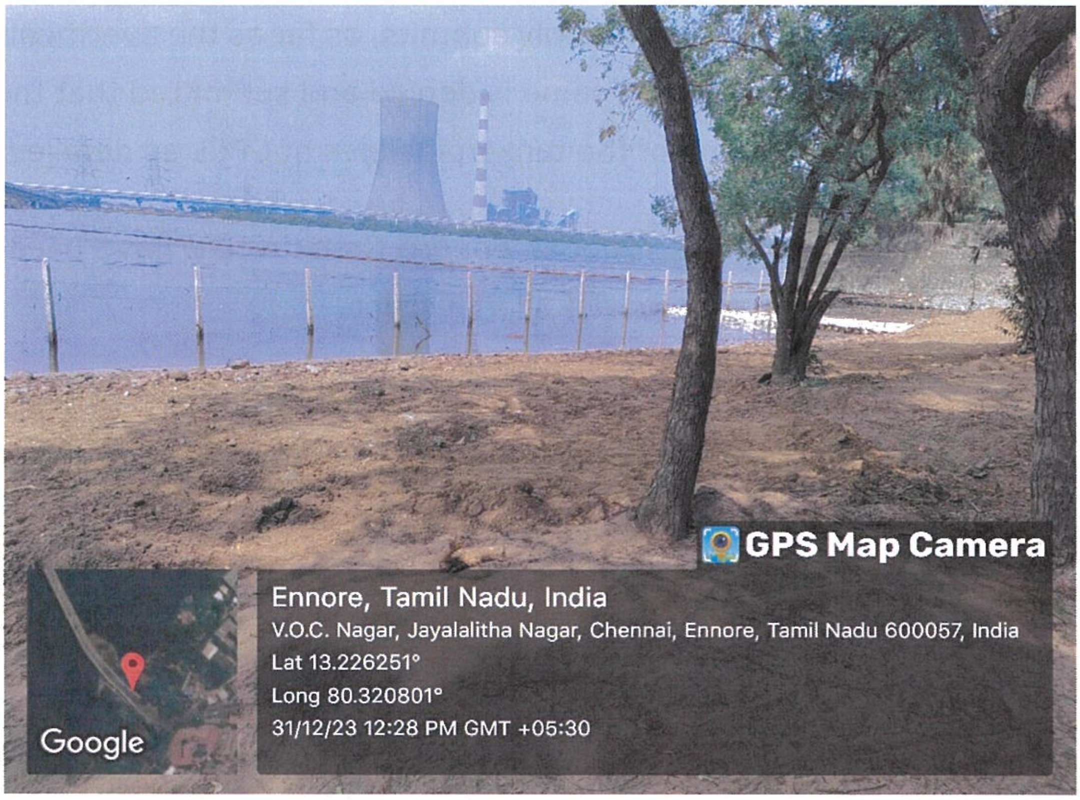

Figure 3(c) Completion of Restoration Works

Figure 4 Plantation at aforesaid property

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15. The Respondent humbly submits, as far as the averment in Para 1 of the application, the same is denied and submitted that there was no oil leak from any of the tanks/pipelines of CPCL as detailed above.

16. That the oil carryout at Ennore Creek would have been due to the surface oil carryover of all industries.

17. That it is necessary to bring to the attention of this Hon'ble Tribunal that CPCL refinery is not the only unit which handles oil and there are various industries in the Manali Industrial area that are handling petroleum products and hence it cannot be concluded that CPCL alone is to be held accountable for the said mishap. CPCL being a responsible corporate entity, had taken up the restoration works.

18. The Respondent humbly submits, as far as the averment in Para 2 of the application, CPCL had completed the restoration work for the aforementioned property utilizing broadly the following resources:

Sl No.	Description of materials	Unit	Quantity
1.	RCC	Cum.	10
2.	Fine aggregate filling	Cum.	439
3.	Brick work	cum	1.173
4.	Reinforcement	MT	0.80
5.	Tipper	Nos.	85
6.	JCB and Poclain	Units	41
7.	Workmen	Nos.	115
8.	Silver Oak planks, cement post and chain link fencing	LS	1
9.	Tree sapling planted	Nos.	30

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19. That subsequent to the completion of the restoration works, as depicted in the Figure 3 of the above enclosed photos herein, CPCL had vacated the aforesaid property.

20. That as previously stated, the authorities from the State Government and officials of TNPCB were overseeing the restoration activities at the Ennore Creek area.

21. The Respondent humbly submits, as far as the averment in Para 4 of the application, that as previously stated, there was no oil leak from the tanks/pipelines of CPCL.

22. That the contention of the Applicant that the oil leaked from CPCL's refinery premises into the Buckingham Canal, reached the Ennore Creek and caused severe damage is not acceptable and the Applicant ought to note that neither any oil was released from CPCL nor there was any leak from the tanks/pipelines.

23. The Respondent humbly submits, as far as the averment in Para 6 of the application, that as previously stated, on the directions of the High-Level Committee, CPCL had entered the property.

24. That since the approach to the property was not suitable for the movement of heavy vehicles due to the absence of kerb walls for ramp, and pavement slabs made of plain cement concrete that were in damaged conditions, the same was temporarily modified by making suitable ramps by earth fills and subsequently the new ramp was provided with kerb wall, along with reinforced cement concrete slabs for approach to the property, thereby making it a strong facility and the same can be seen from the photos enclosed as Figure 5 and Figure 6 herein.

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25. That the removed contaminated soil was not dumped in the said property but was carefully removed, accounted and transported as per the directions of TNPCB.


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Figure 5 Laying of reinforcement for Ramp


OPPO A58
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Figure 6 concreting of Ramp completed

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26. The Respondent humbly submits, as far as the averment in Para 7 of the application, that the length of the said property on the east side of the Ennore Flyover, as depicted in the Figure 7 of the enclosed photo herein, is approximately 60m and CPCL had approached the same for clearing the sludge using heavy machinery and Gully suckers for the removal of the sludge from the bridge.

Figure 7 Said property limit areas on the east of Ennore bridge flyover

27. That on the west side of the said property, CPCL had approached for 133m with earth moving equipment for restorations works, as depicted in Figure 8 of the enclosed photo herein.

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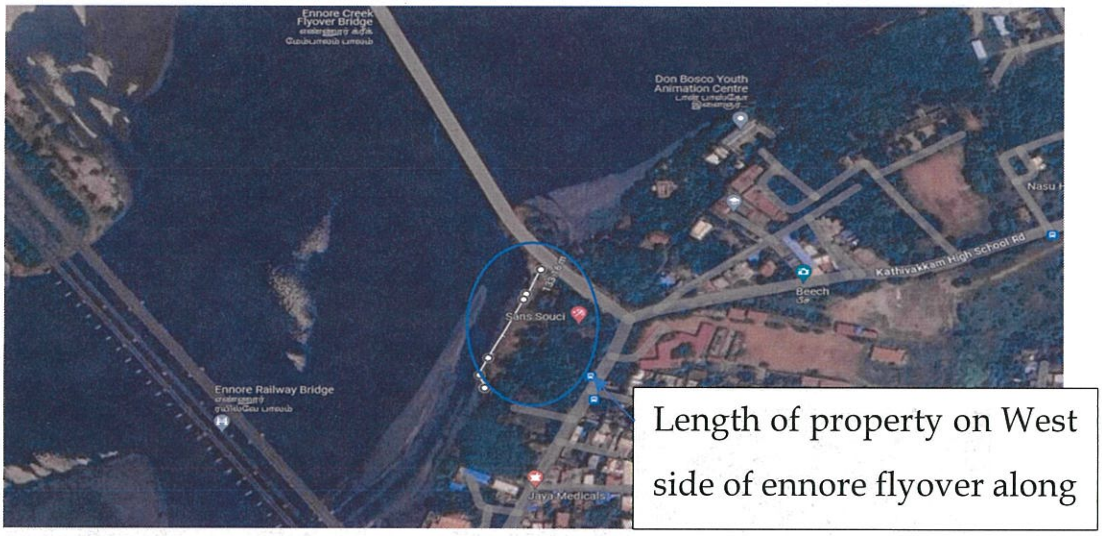

Length of property on West side of ennore flyover along

Figure 8 Said property limit areas on the west side of Ennore bridge Flyover

28. That the black colour of the soil is due to the nature of water carried through the Buckingham Canal and not due to oil.

29. The Respondent humbly submits, as far as the averment in Para 8 of the application, that CPCL had completely removed the sludge on the shoreline adjoining the property limit using earth moving machinery.

30. That since the movement of poclain in the swamp areas of the shorelines was unsafe and frequently causing settlement of poclain track, sludge was shifted to the shore within boom reach of poclain and subsequently the sludge was loaded into the tippers by another poclain/loader and transported out of the said property limit.

31. That as previously stated, the contaminated soil was not dumped in the said property but was carefully removed from the said location, accounted and transported as per the directions of TNPCB.

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32. The Respondent humbly submits, as far as the averment in Para 9 of the application, that as seen from the Figure 3 of the above enclosed photos herein, the restoration works have been completed in the said property limit.

33. That the said property limit lies in a shoreline with a feature of bay/cup like formation, as seen from the Figure 7 of the above enclosed photo herein, wherein the silt/sludge carried by the Buckingham Canal/Kosasthalaiyar river gets deposited as a natural process on southern banks, and the same are not oil spills/oil as claimed.

34. The Respondent humbly submits, as far as the averment in Para 10 of the application, that CPCL had completed the restoration works in aforesaid properties on 01.01.2024 and same was conveyed to the owner vide CPCL letter dated 03.01.2024, subsequent to which no complaint was raised then from the Applicant.

35. The Respondent humbly submits, as far as the averment in Para 11 of the application, that CPCL as a responsible corporate entity had already taken up the restoration works and completed the same in the aforesaid property, the photos of same have been enclosed as Figure 3.

36. That the oil at Ennore creek cannot be attributable to CPCL alone since during the abnormal flood condition, all industries in Manali and Ennore area got affected with most of these industries handling oil.

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37. That the oil carryout at Ennore creek would have been due to surface oil carry over of all industries and it cannot be concluded that CPCL alone is to be held accountable for the said mishap.

38. The Respondent humbly submits, as far as the averment in Para 12 of the application, that the heavy metals mentioned in the application are present in all industrial effluents and also in the city sewage system.

39. That there are various large, medium & small scale industries present in the Manali area that are also handling petroleum products; and the oil carryout at Ennore creek would have been due to surface oil carry over of all industries.

40. That CPCL cannot be held attributable for the presence of heavy metals in the Ennore Creek area.

41. The Respondent humbly submits, as far as the Soil Test Report annexed by the Applicant, that the soil sample has not been taken in the presence of a statutory authority.

42. That there are a total of 54 hydrocarbon components analysed and all the hydrocarbons were observed as Below Limit of Quantification (BLQ) as evidenced from the Test Results.

43. That the Total Petroleum Hydrocarbons (TPH) values range from 190 mg/Kg to 280 mg/Kg and the presence of TPH content could be from the industrial wastewater, automobile service centres, workshops, petrol bunks etc.

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44. That there are more than 20 petrochemical industries in the Manali Industrial area and Ennore area and the TPH content could be from the above said industries.

45. That the reason for TPH may also be from the anthropogenic activities, domestic waste such as detergent, paint, grease, toilet/floor cleaner and industrial waste discharge into the Buckingham Canal.

46. That in India, there is no standard available for the TPH content in soil for comparison purpose and the Guideline value for ecological risk for TPH is 280 mg/Kg as per the sediment quality guideline value from the Australia and New Zealand Environment and Conservation Council (ANZECC). (Copy enclosed as Annexure-1)

47. That the Mineral Oil is in the range from 68.21 mg/Kg to 664.56 mg/Kg and may be from domestic waste such as detergent, paint, grease, toilet/floor cleaner discharge into the Buckingham Canal.

48. That the domestic waste water is let out by various stakeholders into Buckingham canal and that CPCL, being a responsible corporate entity, does not let out any water and is committed to total recycling.

49. That in the soil samples collected from different strata, 10 parameters viz Arsenic, Barium, Chromium, Cobalt, Lead, Nickel, Thallium, Vanadium and Zinc, were present among the 18 tested.

50. The Respondent humbly submits that Mr Karthikeyan et al had conducted a comprehensive heavy metal research work from 2014 to 2016 covering four seasons (post-monsoon, summer, pre-

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monsoon and monsoon) in the Ennore Creek area (Annexure-2) and the team had collected 480 samples and published a research paper.

51. That in addition to the aforementioned research carried out at the Ennore Creek area, a comprehensive research work was carried out by Mr Madurima Bakshi et al published in 2021 in the Sundarban estuarine. (Annexure-3)

51. That the comparison of the heavy metal contents in the soil at Ennore Creek and Sundarban estuarine is as follows:

Heavy Metals	mg/Kg		
	Sediment		
	Mr Karthikeyan et al @ Ennore Creek (2014-2016)	Mr Madurima Bakshi et al @ Sundarban	Applicant @ Ennore Creek
Cd	2.2	0.11	-
Ni	39.1	51.3	8.1
Pb	48.3	22.7	24.7
Cr	90.3	72.4	19
Cu	41.3	73.4	7.8
Zn	-	107.9	40
Co	-	10.74	1.452

52. That it is evident from the above table that heavy metals in the sediments is observed in other estuarine and also in the Ennore Creek before December 2023.

53. That the presence of heavy metals is predominantly due to the domestic waste, inherent nature of soil and due to anthropogenic activities.

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54. The Respondent humbly submits, as far as the Dug Well Water Test Report annexed by the Applicant, that in the Ennore Creek area, the Buckingham Canal and Kosasthalaiyar river join and enter into the sea.

55. That there are many unauthorized domestic and small scale units letting out waste water into the canal and that domestic waste water is let out by various stakeholders in the Buckingham Canal.

56. That in the dug well water sample collected by the Applicant, 54 Hydrocarbon components was found to be Below Limit of Quantification (BLQ) as evidenced from the Test Results.

57. That the slight black colour of the water is due to the sewage water from Buckingham Canal.

58. That in addition to the above, metal content analysis reveal that Barium, Copper, Zinc, Arsenic, Lead, Molybdenum, Nickel and Vanadium were noticed out of the 18 parameters analysed and the balance 10 parameters were observed as Below Limit of Quantification (BLQ).

59. That out the of 8 parameters, Copper, Zinc, Lead, Nickel and Vanadium values are observed to be within the limits prescribed by MoEF&CC as tabulated below:

Sl.No	**Parameter**	**MoEF Limit (in mg/litre)**	**Actual in the dug well water (in mg/litre)**
1	Copper	1.0	0.008
2	Zinc	5.0	0.114

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3	Lead	0.1	0.138
4	Nickel	1.0	0.002
5	Vanadium	0.2	0.004

60. That as per the drinking water standard, as given in the TNPCB & You Guide, the barium concentration should not exceed 1 mg/lit and the limit of Arsenic concentration is 0.05 mg/lit and the actual values found as per the test results for Barium is 0.1 mg/lit and for Arsenic is 0.001 mg/lit, which is well within the limit. (Annexure-4)

61. That as per the World Health Organisation, the maximum permissible limit of Molybdenum in drinking water is 0.7 mg/lit and the actual value observed is 0.003 mg/lit, which is well within the permissible limit. (Annexure-5)

62. That the presence of heavy metals would be due to anthropogenic activities and from the domestic waste.

63. The Respondent humble submits, as far as the observed values of parameters in the Soil and Water samples in the Ennore sea shore area, that CPCL is not attributable to the same.

64. The Respondent humbly submits, as far as the averment in Para 13 of the application, that CPCL has cleared not only the oily sludge but also the sewage sludge accumulated over the years. Please refer Figure 3a, 3b, 3c and 4.

65. The Respondent humbly submits, as far as the averment in Para 14 of the application, that as previously stated, despite there being no leak from the tanks/pipelines, CPCL being a responsible corporate

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entity had executed the restoration works of the said property and the same can be clearly seen from the Figure 3 of the above enclosed photos herein.

66. The Respondent humbly submits, as far as the averment in Para 15 of the application, that the Applicant is repeatedly passing his own verdict that CPCL is to be held accountable instead of appreciating that it was CPCL, a responsible corporate entity, which had immediately taken the restoration works and more reports from various experts are yet to be submitted.

67. That the Oil Booms were deployed at almost 06 locations, for approximately 1350m, which was also at upstream and downstream of the aforesaid property as per the advice of State authorities.

68. The Respondent humbly submits, as far as the averment in Para 16-A, B, C, D, E, F and H of the application, that CPCL had carried out the restoration works in applicant's property and the sludge was removed and, in this regard, necessary communication was made to the applicant, as previously stated, on 03.01.2024.

69. The Respondent would like to bring to the attention of this Hon'ble Tribunal that CPCL had taken immediate steps as directed by the State Government and TNPCB following the December 2023 floods, and that the resultant inundation was an unfortunate incident, being an Act of God.

70. The Respondent humbly pleads that, CPCL had initiated the restoration works immediately after the floods, putting great efforts for immediate mobilization of resources and materials and worked tirelessly as a responsible entity and the allegations raised by the Applicant are all false and denied.

बी. कोथंडरामन
B. KOTHANDARAMAN
मुख्य महाप्रबंधक (एचएसइ)
Chief General Manager (HSE)
चेन्नै पेट्रोलियम कॉर्पोरेशन लिमिटेड
Chennai Petroleum Corporation Ltd.
/Chennai - 600 068.

71. This Respondent is ready and willing to abide by any condition or direction as may be imposed by this Hon'ble Tribunal, in the interest of justice.

Under the above circumstances, it is humbly prayed that this Hon'ble Court may be pleased to dismiss the above application and pass such orders as it deems fit and thus render justice.

DATED AT CHENNAI ON THIS THE 1st DAY OF OCTOBER, 2024

COUNSEL FOR 4TH RESPONDENT

VERIFICATION

I, B. Kothandaraman, son of M. Balu, aged about 57 years, having registered office at No.536, Anna Salai, Teynampet, Chennai 600018, do hereby verify that the contents of paras 1 to 72 are true to the best of my knowledge and are believed to be true on legal advice and that I have not suppressed any material fact.

Verified at Chennai on this the 1st day of October, 2024

4th RESPONDENT

बी. कोथंडरामन
B. KOTHANDARAMAN
मुख्य महाप्रबंधक (एचएसइ)
Chief General Manager (HSE)
चेन्नै पेट्रोलियम कॉर्पोरेशन लिमिटेड
Chennai Petroleum Corporation Ltd.
मणली/Manali, चेन्नै/Chennai - 600 068.

ANNEXURE - 1

WATER FOR A HEALTHY COUNTRY
www.csiro.au

Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines

Stuart L. Simpson, Graeme E. Batley and Anthony A. Chariton

CSIRO Land and Water Science Report 08/07

May 2013

Prepared for the Department of Sustainability, Environment, Water, Population and Communities

Water for a Healthy Country Flagship Report series ISSN: 1835-095X

Australia is founding its future on science and innovation. Its national science agency, CSIRO, is a powerhouse of ideas, technologies and skills.

CSIRO initiated the National Research Flagships to address Australia's major research challenges and opportunities. They apply large scale, long term, multidisciplinary science and aim for widespread adoption of solutions. The Flagship Collaboration Fund supports the best and brightest researchers to address these complex challenges through partnerships between CSIRO, universities, research agencies and industry.

The Water for a Healthy Country Flagship aims to provide Australia with solutions for water resource management, creating economic gains of $3 billion per annum by 2030, while protecting or restoring our major water ecosystems. The work contained in this report is collaboration between CSIRO and [list collaborators]

For more information about Water for a Healthy Country Flagship or the National Research Flagship Initiative visit www.csiro.au/org/HealthyCountry.html

Citation

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Copyright and disclaimer

Important disclaimer

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Executive summary

Sediment quality guidelines (SQGs) were included for the first time in the revised Australian and New Zealand Guidelines for Fresh and Marine Water Quality released in 2000 (ANZECC/ARMCANZ, 2000). At the time, these represented the latest in international thinking, however, in recognition that the science underpinning these guidelines required improvement, the guidelines were termed 'interim' with the intention being that they would be significantly revised in the future. The guidelines were presented as part of two volumes, the first providing a general overview and the second, the more detailed science.

This document, originally prepared for Agriculture Fisheries and Forestry Australia (AFFA), updates the original SQG documents. To facilitate the updating process, it has been prepared in two parts, consistent with the original documents where they appeared as Sections 3.5 and 8.4. The SQG revision builds on the original SQG document with the tiered, decision-tree approach adopted for the interim sediment quality guideline values (SQGVs) maintained, and guidance is provided for use of a weight-of-evidence (WOE) framework to improve the assessment of the potential impacts of contaminated sediments for more complex risk assessments.

The use of SQGVs, originally termed 'trigger values', was initiated in the original SQG document and this approach continues to be used in the revised SQG framework, but with different emphasis. The values are now to be termed sediment quality guideline values (SQGVs). There are numerous uncertainties associated with the SQGVs, and for some assessments too much weight was being given to the chemistry SQGV comparison when following the decision-tree framework of the original SQG document. In cases where there is significant uncertainty in the chemistry SQGV-based initial assessment, the WOE framework expands to consider a greater number of lines of evidence (LOEs). In addition to chemical measures of potential bioavailability (e.g. acid-volatile sulfides, AVS) and the ecotoxicology LOE that were part of the original framework, the revised framework explicitly allows for the considering of bioaccumulation and ecological health as two additional LOEs.

The recommended application of revised SQGs continues to involve a tiered, decision-tree approach, in keeping with the risk-based approach introduced in the water quality guidelines. Following this framework, the total concentrations of contaminants are compared to the SQGVs and if the contaminant concentrations exceed one or a number of the SQGVs, further investigations should be initiated to determine whether there is indeed an environmental risk associated with the exceedance. The SQGVs are not to be used on a pass/fail basis.

The first-level screening compares the SQGV with the measured value for the total contaminant concentration in the sediment. If the SQGV is exceeded, then the next level of screening considers the fraction of the contaminant that is likely to be bioavailable or can be transformed and mobilised in a bioavailable form (based on chemical measurements). The contaminants whose concentrations exceed SQGVs following consideration of contaminant bioavailability are termed contaminants of potential concern (COPCs).

The decision-tree now proceeds to the evaluation of additional lines of evidence (LOEs) to determine whether the COPCs are likely to affect ecosystem health. Chemistry (including bioavailability measures), ecotoxicology, bioaccumulation and benthic ecology are general LOEs, but other LOEs may be added on a case-specific basis. Each LOE can comprise a range of measures, e.g. a number of different toxicity test methods, field- and laboratory-based measures of potential bioaccumulation, etc. These LOEs more accurately identify which of the COPCs are contaminants of concern (COCs). A WOE approach is adopted to evaluate the combination of the individual LOEs. The recommended approach applies numerical scores to each LOE, and brings these together in an overall assessment table that allows ranking of the sediments according to overall risk. High scores for all lines of evidence are indicative of the highest risk of detrimental ecological effects.

Elevated contaminant concentrations (COPCs and COCs), i.e. chemistry, remain the underlying ‘driver’ for the WOE assessment framework for several reasons:

(i) in most sediment quality assessments it is the concentrations of contaminants that is the key concern (i.e. suspected to be the cause of the effects);

(ii) preventing the release of contaminants from, or the dispersion of contaminated sediments, into areas that are pristine or less contaminated is a common value of risk assessments; and

(iii) management options for removal (e.g. dredging), containment (e.g. capping) and other forms of remediation of contaminated sediments will generally be most easily guided by concentrations of COCs.

The scientific background to the proposed revisions is documented in Part 2. This includes some of the information from the original Guidelines, supplemented by advances since 2000.

In addition to the revised SQG framework, suggestions are made for the revision of the recommended guideline SQGVs and upper guidelines (SQG-High values) for a range of metals, metalloids, organometals and organic sediment contaminants. For metals, the SQGVs and SQG-High values are largely unchanged, and remain based on the effects range low (ERL) and effects range median (ERM) values. For organics, threshold effects level (TEL) and probable effects level (PEL) values are now used. For some chemical contaminants for which SQGVs currently exist, published reviews of effects data indicate that SQGVs could be improved (e.g. for PAHs and dieldrin; see Appendix A3 and A4). For other chemical contaminants, there appears to be benefits in re-deriving SQGVs that better consider the influence of sediment type (e.g. for most metals). There are also some common chemical contaminants for which no SQGVs have previously existed, but a SQGV could now be derived (e.g. for total petroleum hydrocarbons, TPHs; see Appendix A5).

Part I The Guidelines

1 Principles, Objectives and Management Framework for Sediment Quality Guidelines

The approach to management of water quality in Australia and New Zealand is based on the protection of environmental values through a consideration of acceptable concentrations of contaminants in receiving waters as well as in effluents and non-point sources. The NWQMS Water Quality Guidelines for Fresh and Marine Waters (ANZECC, 1992) provided a framework for the regulatory control of receiving water quality. The guidelines recognise that the total load and fate of contaminants, particularly to enclosed systems, should also be considered. In 2000, a major revision of these guidelines (ANZECC/ARMCANZ, 2000a) was released that for the first time included sediment quality guidelines (SQGs). The important role of sediments, as both a source and a sink of dissolved contaminants, has been recognised for some time. In addition to their influence on surface water quality, sediments represent a source of bioavailable contaminants to benthic biota, and hence potentially to the aquatic food chain. Defining the extent of the threat to ecosystem health posed by sediment-associated contaminants will assist in prioritising management options.

The sediment quality guideline chapters reviewed the state of knowledge on the environmental impacts of contaminants in sediments, and the approaches used to formulate interim SQGs. On the basis of these, a procedure for the development of appropriate SQGs for Australia and New Zealand was outlined. The sediment guidelines were applicable to slightly to moderately disturbed and highly disturbed aquatic ecosystems (see Section 3.1.4 of the Guidelines, ANZECC/ARMCANZ (2000a)). Consideration of sediment quality followed the hierarchical decision tree approach being adopted in the Guidelines, with a focus on issue identification and the protection necessary to manage these issues.

For aquatic ecosystems considered to be of high conservation/ecological value, a precautionary approach was recommended. In these ecosystems, anthropogenic chemicals should be undetectable, and naturally occurring toxicants (e.g. metals) should not exceed background sediment concentrations (see Section 3.1.4.2 of the Guidelines, ANZECC/ARMCANZ (2000a)). Relaxation of this approach should only be considered when there were considerable biological assessment data showing that such a change in sediment quality would not impact on the biological diversity of the ecosystem.

Since 2000, there have been considerable advances worldwide in the science underpinning sediment quality assessment. These have included the use of weight-of-evidence (WOE) approaches, the development of new toxicity tests, the recognition of limitations in some SQGs and the development of new guidelines, as well as additional information on contaminant bioavailability and uptake pathways (Batley et al., 2005; Simpson et al., 2005; Simpson and Batley, 2007).

Sediment quality assessments take many forms and are used for planning, licencing and approval, and monitoring, assessment and environmental reporting. For many of these, the assessment of potential chemical hazards is undertaken early to assist in formalising the scope of later investigations, e.g. where sediments are to be disturbed by proposed new infrastructure (e.g. a new wharf, maintenance dredging) and contaminant release or disposal options may need to be considered. In some cases, the consideration of the ecology or potential ecotoxicology at the field site may not be necessary (e.g. where operations are confined). Consequently, the decision to use a WOE approach will be dependent on the scale and needs of the specific assessment. A tiered, decision-tree approach, in keeping with the risk-based approach introduced in the water quality guidelines, remains the recommendation of this guideline revision. While guideline values (SQGVs) based on concentrations of a limited number of measured contaminants remain the first tier of the decision tree, where it is suspected that environmentally significant concentrations of contaminants exist with inadequate or no SQGVs, then other lines of evidence (LOEs) should immediately be considered.

This document incorporates these findings in a revision of the interim SQGs for Australia and New Zealand, with Part 1 of the document describing the underlying concepts and philosophy and Part 2 providing greater discussion and literature relating to the approaches taken.

1.1 Underlying Philosophy of Sediment Guidelines

It is important to understand why sediment guidelines have been developed and how and where they might be applied. The establishment of guidelines serves three principal purposes:

(i) to identify sediments where contaminant concentrations are likely to result in adverse impacts on sediment ecological health;

(ii) to make decisions about the potential remobilisation of contaminants into the water column and/or into aquatic food chains; and

(iii) to identify and enable protection of uncontaminated sediments.

Many urban and harbour sediments will fall into the first category, usually being contaminated by heavy metals and hydrophobic organics, resulting from both diffuse and point-source inputs. They are not easily remediated, and *ex situ* treatment or dredging and disposal are currently the most cost-effective options. Knowledge of elevated concentrations of sediment contaminants at a site, and the potential for their biological uptake, may lead to controls on the collection of benthic organisms for human consumption. For the most part, because of the enormous costs involved, there is unlikely to be large scale sediment remediation, unless driven by human health risk assessments. Natural remediation of contaminated sediments may occur via the deposition, above the contaminated zone, of freshly deposited sediments able to support viable biological populations. This will occur through water column inputs and be managed through controls on inputs via water quality guidelines.

Management conflicts can arise when such natural sediment accumulation restricts navigation. It is possible to adopt measures to protect more pristine areas from further contamination, and this is where the application of SQGs will be of greatest value. This will involve the management of inputs.

1.2 The Sediment Quality Guideline Framework

The application of sediment guidelines involves a tiered, decision-tree approach (Figure 1), in keeping with the risk-based approach introduced in the water quality guidelines. Following this framework, the total concentrations of contaminants are compared to SQG values. If the contaminant concentrations exceed the SQGVs, further investigations should be initiated to determine whether there is indeed an environmental risk associated with the exceedance.

It is important to reiterate that the SQGVs should not be used on a pass/fail basis, the major premise of the risk-based approach introduced in ANZECC/ARMCANZ (2000a). The first-level screening compares the SQGV with the measured value for the total contaminant concentration in the sediment. These measurements are made on the <2 mm sediment fraction to exclude low surface area materials with low capacity for binding contaminants (e.g. coarse sand and large debris). If the SQGV is exceeded, the next step in the case of metal contaminants is to look at a dilute acid extractable metal concentration (AEM, by 30 min 1 M HCl extraction) which provides a useful measure of the potentially bioavailable metals. Non-available forms of metals in sediments might include mineralised metals that require strong acid dissolution, as achieved by total particulate metal (TPM) measurements (also referred to as total recoverable metals). For many assessments, AEM measurements may be a useful starting point in the decision tree, rather than TPM determinations. However, for some metal phases that are sparing soluble in 1 M HCl (e.g. sulfide phases of Ag, Cu, Hg) and metals associated with organic polymers that may degrade over time (e.g. antifouling paints, tyre rubber), the measurement of TPM allows the potential future transformation of these metals into more bioavailable forms to be adequately considered (see Part 2). In some jurisdictions, TPM measurements are deemed necessary for comparison with historical data trends.

For organic contaminants, and for metals if the SQGV is still exceeded following the AEM consideration, the next step involves comparison with background concentrations in reference sediments of comparable grain size from appropriate sites. Exceedance of the SQGV is acceptable if it is below the background concentration. Note that for most anthropogenic organic contaminants, the background concentrations should be zero, but for metals it is possible for background concentrations to significantly exceed trigger values. The contaminants whose concentrations exceed SQGVs following consideration of contaminant bioavailability are termed contaminants of potential concern (COPCs).

If the SQGV is still exceeded, the third step involves the more explicit consideration of the bioavailable contaminant fraction (see Part 2, Section 4.1). For metals that form insoluble sulfides, amorphous iron sulfide (FeS) measured as so-called acid-volatile sulfides (AVS), is an important metal-binding phase that reduces metal bioavailability. Measurements of metal concentrations in the pore waters and elutriates also provides valuable information on metal bioavailability. Many organic contaminants are hydrophobic and bind strongly to the organic carbon in sediments. To account for the preferential partitioning of these contaminants to organic matter, organic contaminants and their SQGVs are normalised to the total organic carbon (TOC) concentration of the sediment (i.e. normalised to 1% TOC). This normalisation should only be applied for TOC concentrations between 0.2 and 10%. Advances in the approaches for metals and organics are discussed in Part 2.

In this revised SQG framework, the decision-tree now proceeds to the evaluation of additional LOEs to determine whether the contaminants are likely to affect ecosystem health. In the original Guidelines, the assessment only considered toxicity as the final step. It is important to stress that this approach is still appropriate, and if toxicity testing confirms that adverse effects may be occurring due to contaminants, then that is sufficient and management action can be initiated.

Where the results are ambivalent, in particular where guidelines are exceeded yet no toxic effects are apparent, then the extent of the potential risk needs to be better established through the use of additional lines of evidence in a weight-of-evidence (WOE) approach. Such ambivalence might occur when (i) there are moderate contaminant concentrations and small, but statistically significant effects in chronic tests that could also be due to non-contaminant stressors (e.g. ammonia), or (ii) moderately reduced survival is observed in an acute test, but sub-lethal or chronic tests indicate no effects. A WOE assessment is recommended when contaminants for which no SQGV exists may be present at high concentrations.

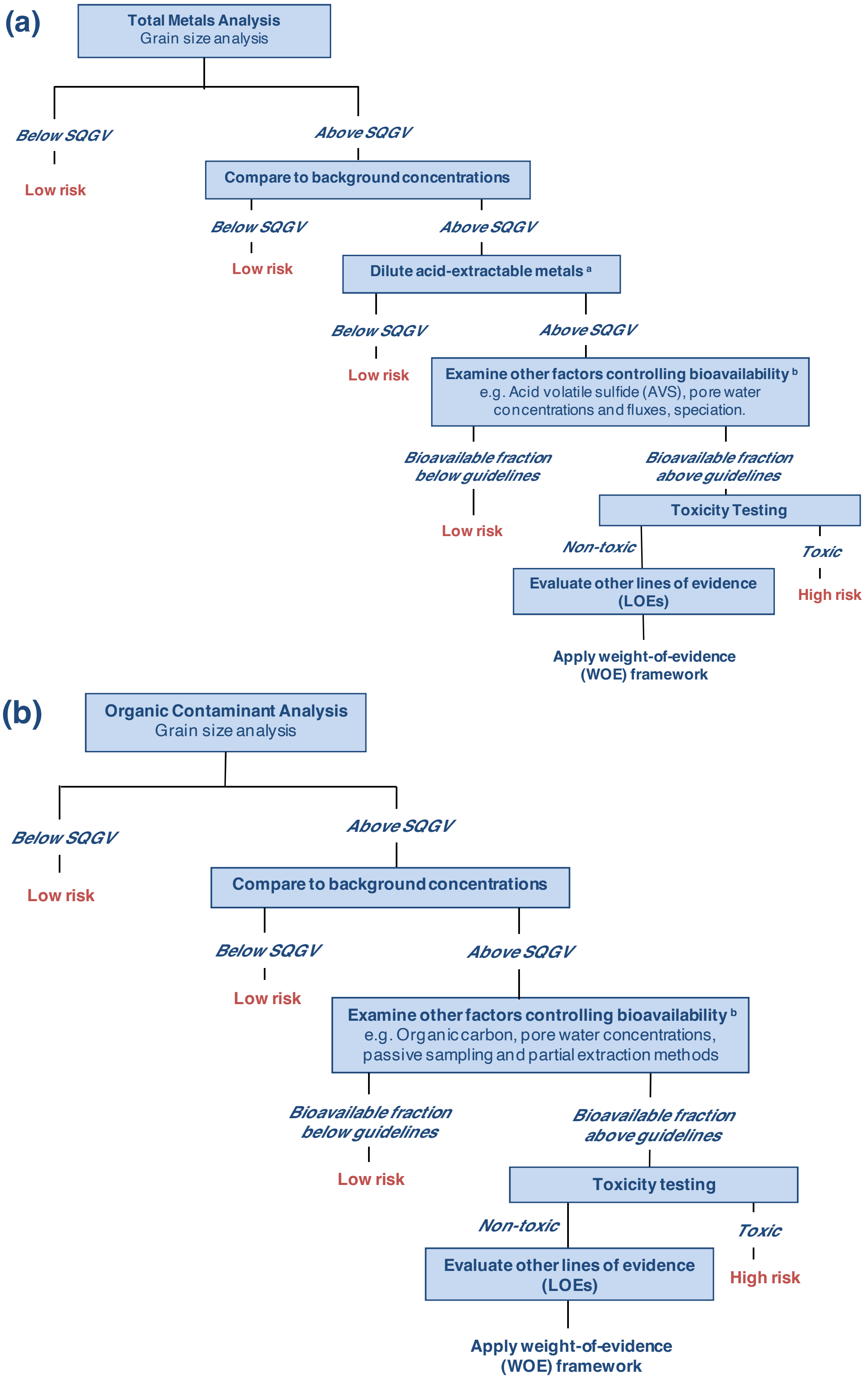

Figure 1. The tiered framework (decision tree) for the assessment of contaminated sediments for (a) metals and (b) organics. SQGV = Sediment quality guideline value. Notes: [a] This step may not be applicable to metalloids (As, Se) and mercury (Hg). [b] See specific methods on how bioavailability test results are used. Other LOE include toxicity, bioaccumulation, ecology, and biomarkers.

The decision trees in Figure 1 imply a purely hierarchical approach to sediment quality assessment, leading to a WOE assessment, however, in other instances, it may be in the interests of those undertaking the sediment study to go directly to a full WOE study, although it is clearly more costly than a consideration only of chemistry, with or without ecotoxicological confirmation. Environmental managers will need to decide whether the costs justify the advantages of a more detailed assessment. For example, defining the area of environmental concern for a dredging activity might involve millions of dollars in additional remediation if the area to be remediated is not clearly defined. The situations that might dictate the undertaking of a full WOE assessment might include:

(i) confounding results are obtained from chemical assessment and toxicity testing (exceeded SQGVs not supported by toxicity tests; or toxicity is seen when no SQGVs exceeded),

(ii) the presence of an unknown mixture of contaminants at a site

(iii) a requirement from a regulatory agency for a full ecological risk assessment of impacts on sediments due either to historical, existing or proposed activities that impact on sediment ecosystem health

(iv) the observation of an apparently degraded ecological environment that requires more detailed evaluation, or

(v) where the site being studied is sufficiently large and the remediation options so expensive that delineation of those sediments posing the greatest risks to ecosystem health is desirable to better target these options.

The WOE approach supplements the chemistry and ecotoxicology LOEs, with measures of bioaccumulation and benthic ecology that are now recognised as important indicators of sediment quality (Batley et al., 2002, 2005; Simpson et al., 2005; Wenning et al., 2005). Assessments of this type require a different framework that considers all of the LOEs together (Chapman et al., 2002; Batley et al., 2002; Chapman and Anderson, 2005; Simpson et al., 2005).

A simplified approach to such an assessment is illustrated in Table 1, with examples showing how different sets of LOEs might be interpreted. The recommended approach applies numerical scores to each LOE, and brings these together in an overall assessment table that allows some ranking of the sediments according to overall risk. High scores for all LOEs are indicative of the highest risk. The application of the WOE assessment framework is discussed in detail in Section 3.

Table 1. Interpretations of likely combinations of LOE responses

LINE OF EVIDENCE				INTERPRETATION
CHEMISTRY	TOXICITY	BIOACCUMULATION	ECOLOGY	
+	-	-	-	Contaminants present at concentrations exceeding guideline values, but not bioavailable
-	+	-	-	Toxic effects due to unmeasured contaminants or an unidentified stressor
+	-	+	-	Contaminants exceeding guideline values and bioaccumulating, but not toxic
+	-	-	+	Toxicity not seen using the test organisms, but effects are still seen on benthic ecology
-	-	-	+	Unmeasured contaminants or other factors contributing to ecological impacts
+	+	-	-	Some resistance to impacts on ecology, or unmeasured contaminants toxic to some species
-	+	-	+	Unmeasured contaminants or stressors are toxic and affecting ecosystem health
+	+	+	-	Measured contaminants are toxic and accumulating, but no significant ecological effects are observed
+	+	+	+	Measured contaminants exceed guideline values, are toxic and affecting ecosystem health

2. Sediment Quality Guideline Trigger Values

2.1 Approach and Methodology used in Guideline Value Derivation

Ideally, sediment quality guideline values (SQGVs) should unequivocally distinguish between sediments that cause biological effects and those that do not. In reality however, the occurrence of biological effects does not show such a clearly delineated relationship (Batley et al., 2005). In a generalised concentration-response model (Figure 2), there are three distinct zones, comprising concentrations (i) below the threshold for effects (TE), (ii) above the probable effects limit (PE), and (iii) in a transition zone between the two (Batley et al., 2005). Currently, there is greater confidence about our ability to define the PE and TE zones, however, the transition zone is poorly defined and may span more than an order of magnitude of metal concentrations. This level of uncertainty needs to be reduced since the transition zone also encompasses the concentration range of many of the contaminated sediments that are of concern to regulators.

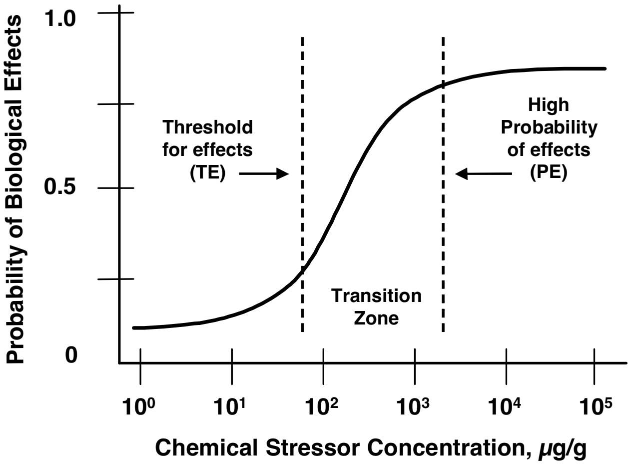

Figure 2. Generalised concentration-response relationship for contaminated sediments

Effects data from toxicity tests, bioaccumulation and ecological assessments, are defined relative to suitable control responses (references sites), and consequently the output is indicative of either ‘effects’ or ‘no effects’. Factors that cause the overlap between effects and no-effects data are numerous, and include unaccounted for contributions from uncharacterised chemicals or stressors, differences in bioavailability, differing responses among organisms, and errors in measurement of chemical and biological response parameters. It is the intent of all SQGVs to minimise the size of the transition zone, however no SQGVs will ever be fully successful in doing this.

The bioavailability of contaminants is greatly affected by sediment properties, and toxic effects due to contaminants are not exhibited in sediments in which the contaminants are not bioavailable, regardless of the total contaminant concentrations. The size of the transition zone shown in Figure 2 is largely a consequence of the uncertainty associated with the effects of sediment properties on contaminant bioavailability.

Because the SQGV derivations were based on total rather than the bioavailable contaminant concentrations, the SQGVs cannot be used alone to predict the onset, or magnitude, of toxic effects. Consequently, use of the SQGVs as strict criteria will likely result in many sediments being classified as toxic when there are no effects evident (a false positive). Conversely, sediments may contain many other chemical contaminants for which SQGVs have not been developed. Consequently, assessing the risk posed by sediments based only on the published SQGVs may result in sediments being classifying as non-toxic when effects may be occurring due to chemical contaminants that have not been considered (a false negative).

The many approaches adopted internationally for the derivation of sediment quality guidelines are more fully described in Part 2. By far the most common approach to guideline derivation has been the use of an effects database for contaminated and uncontaminated sites, based on field data, laboratory toxicity testing and predictions based on equilibrium partitioning of contaminants between sediment and pore water. Unlike the water quality guidelines, which are based on effects data for individual contaminants, the majority of the effects data used to derive SQGs suffer from co-occurrence of contaminants. This prevents the observed effects being confidently assigned to any one contaminant, and is the source of the greatest uncertainty in the guidelines.

With the recent development of appropriate sediment toxicity tests using species that are applicable in Australia and New Zealand, it is increasingly possible to use local species data in the derivation or re-evaluation of SQGVs. However, although such data are slowly being accumulated, without some financial impetus there is little likelihood that sufficient new data will be forthcoming in the immediate future to allow SQGVs to be adjusted for effects on local species or for new SQGVs to be derived where previously none existed. On that basis, and as has been done in many other countries, the option selected for SQGVs is to use the best available effects data from overseas studies and refine these on the basis of our knowledge of existing baseline concentrations, as well as using local effects data as they become available.

The ANZECC/ARMCANZ (2000a) approach recommended two sediment quality guidelines following the empirical approach to guideline derivation (Batley et al., 2005). The lower trigger value, SQGV (equivalent to the effects range low value used by Long and Morgan (1990), represented the threshold for effects and the upper guideline (equivalent to the effect range median) represented the high probability of effects referred to above (Figure 2).

2.2 Recommended Guideline Trigger Values

There is now increased recognition and understanding of the uncertainty associated with the process of deriving SQGVs (Batley et al., 2005; Bay et al., 2012; Di Toro, 2013). As a consequence, revisions are suggested for some of the guideline values from the interim values adopted in 2000 (ANZECC/ARMCANZ, 2000a). However, in general, more recent derivations, using consensus guidelines that are based on a range of differently derived effects guidelines, or guidelines derived from the application of species sensitivity distributions to ecological data, showed SQGVs that were not significantly different from those adopted in Australia and New Zealand (MacDonald et al., 2000; Leung et al., 2005; Lui et al., 2013). In general, there was a good linear (1:1) correlation between freshwater and marine effects-based guidelines (Smith et al., 1996), indicating that, within the uncertainties of the SQGVs, the values are applicable to both ecosystem types. A comparison of national and regional sediment quality guidelines for classifying sediment toxicity in California found relatively small changes in classification accuracy obtained with regional calibration of SQGs (Bay et al., 2012). In future revisions of SQGVs, it should be possible to obtain ecosystem-specific guidelines, however, there are insufficient data to do this currently, although attempted in the Canadian guidelines (CCME, 2002).

The potential to use field-based species sensitivity distributions (f-SSD) to derive sediment quality guidelines for chemical mixtures based on benthic community data and/or ecotoxicology is improving and is discussed in Part 2. However, like the existing empirical and mechanistic approaches, the new approaches also require assumptions that lead to similar levels of uncertainty, and do not currently deal with differences in contaminant bioavailability between sediment types.

The recommended SQGVs and upper guidelines (SQG-High values) for a range of metals, metalloids, organometals and organic sediment contaminants are listed in Table 2. For metals, the SQGVs are largely unchanged, but the discussion of the use of the SQGVs and bioavailability modifying factors has been improved (see Part 2).

For organics, there is greater variability between the various guideline derivations. Here, the effects range low (ERL) values of Long et al. (1995) now appear to be less reliable than the threshold effects level (TEL) values of MacDonald et al. (2000) that have been adopted in Canada (CCME, 2002). The TEL values have therefore been adopted for many of the organics in this revision.

Considerable research has been undertaken into the use of an equilibrium partitioning sediment benchmark (ESB) approach for non-ionic organics (which includes polycyclic aromatic hydrocarbon (PAH) mixtures and a range of other narcotic organic chemicals). The SQGV and SQG-High values for total PAHs have been revised based on improved effects data and guideline derivation approaches, however an ESB approach is recommended for assessments where non-ionic organics represent a dominant class of contaminants or where consideration of individual PAHs is necessary (see Appendix A2). The risks posed by individual PAHs (such as pyrene) should be evaluated using the more advanced ESB approach that is described in Part 2 (US EPA, 2012), and consequently the SQGVs for individual PAHs have been removed from Table 2.

The upper guidelines (SQG-High) are mostly based on the effects-range median (ERM) values (for metals) (Long et al., 1995) and probable effect levels (PELs) of MacDonald et al. (2000) (CCME, 2002) for organics. More advanced approaches are described for considering mixtures of non-ionic organics and the bioavailability of metals. These upper values are in the high probability of effects region of the concentration-response plot in Figure 2.

The adopted effects guidelines were based on the ranking of toxicity based on US effects databases. There was an option to supplement these data with additional data reported since 2000 (and data from Australia and New Zealand). Despite the apparent attraction of this approach, it was seen as counter-productive since many of the data in the original database and some of the new data were compromised by the issues of co-occurrence. A better approach would be to pursue toxicity data for individual contaminants in sediments, since only then can robust guidelines be derived, as is the case for the water quality guidelines.

A more detailed discussion of the origins of the adopted guideline values is presented in Part 2. For some of the contaminants, revised values have been provided based on improved effects data and guideline derivation approaches (Appendix A).

The SQGVs are presented on a dry weight basis. This does not imply that samples should be dried before analysis resulting in potential losses of some analytes, but that results should be corrected for moisture content. For all of the organic contaminants listed and for tributyltin (TBT), values are normalised to 1% organic carbon, rather than expressing as mg/kg organic carbon as is sometimes done. If the sediment organic carbon content is markedly higher than 1%, the SQGV should be reduced accordingly, since additional carbon binding sites reduce contaminant bioavailability. It is recommended that the use of normalisation should, however, be limited to organic carbon concentrations between 0.2 and 10% (Batley et al., 2002). At lower organic carbon concentrations, other physical and chemical factors influence the partitioning process for hydrophobic organics, while at higher values the organic carbon is likely to be dominated by oils or tars.

Table 2. Recommended sediment quality guideline values

CONTAMINANT	GUIDELINE VALUE	SQG-HIGH
METALS (mg/kg dry weight) [a]		
Antimony	2.0	25
Cadmium	1.5	10
Chromium	80	370
Copper	65	270
Lead	50	220
Mercury	0.15	1.0
Nickel	21	52
Silver	1.0	4.0
Zinc	200	410
METALLOIDS (mg/kg dry weight) [a]		
Arsenic	20	70
ORGANOMETALLICS		
Tributyltin (µg Sn/kg dry weight, 1% TOC) [c, d]	9.0	70
ORGANICS (µg/kg dry weight, 1% TOC) [b, c]		
Total PAHs [e]	10,000	50,000
Total DDT	1.2	5.0
p.p'-DDE	1.4	7.0
o,p'- + p,p'-DDD	3.5	9.0
Chlordane	4.5	9.0
Dieldrin [f]	2.8	7.0
Endrin [f]	2.7	60
Lindane	0.9	1.4
Total PCBs	34	280
Total petroleum hydrocarbons (TPHs) (mg/kg dry weight) [g]	280	550

[a] Primarily adapted from the ERL/ERM values of Long et al. (1995).
[b] Primarily adapted from TEL and PEL values of MacDonald et al. (2000) and CCME (2002)
[c] Normalised to 1% organic carbon within the limits of 0.2 to 10%. Thus if a sediment has (i) 2% OC, the '1% normalised' concentration would be the measured concentration divided by 2, (ii) 0.5% OC, then the 1% normalised value is the measured value divided by 0.5, (iii) 0.15% OC, then the 1% normalised value is the measured value divided by the lower limit of 0.2.
[d] Basis of revision is described in Appendix A2.
[e] The SQGV and SQG-High values for total PAHs (sum of PAHs) are described in Appendix A3 and include the 18 parent PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo(a)pyrene, perylene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(ghi)perylene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene. Where non-ionic organic contaminants like PAHs are the dominant chemicals of potential concern (COPCs), the use of ESB approach is desirable, and is applied as outlined in Appendix A3, that includes a further 16 alkylated PAHs (generally listed as C1-/C2-/C3-/C4-alkylated).
[f] Where dieldrin or endrin are the major COPCs, it is recommended that ESB approaches are applied as described in the Appendix A4.
[g] Origin described in the Appendix A5.

2.3 Ammonia, Sulfide, Nutrients and Contaminants without SQGVs

Ammonia, sulfide and nutrients such as phosphate and nitrate are often present naturally at detectable concentrations in sediment pore waters and may become chemical stressors at high concentrations. For ammonia a new SQGV is proposed, but no specific SQGVs apply for sulfide, nitrate or phosphate. For many other chemical contaminants, no SQGVs can be specified for the contaminant due to the lack of research in ecotoxicological effects. However, it is important to identify when the chemicals represent a threat to ecosystem health.

2.3.1 AMMONIA

Ammonia occurs naturally in sediments and pore waters due to the microbial degradation of nitrogenous organic material such as amino acids. Ammonia is highly soluble and total ammonia, generally termed NH_3-N, includes both un-ionised NH_3 and ionised NH_4^+. The extent of ionisation is dependent on the water pH, temperature, and salinity, and 50% NH_3/50% NH_4^+ occurs at pH 9.4 (25ºC, 30 ‰). Unionised NH_3 is the toxic form of ammonia and for seawater with pH 8 and 20ºC, comprises less than 3.8% of the total ammonia (Table 8.3.4, ANZECC/ARMCANZ, 2000a).

The concentration of ammonia will be highest in organic- and nitrogen-rich systems, and in some instances this enrichment occurs where normal nitrogen cycling reactions are disrupted by anthropogenic activities. While most benthic organisms are adapted to deal with elevated porewater ammonia concentrations, and are either insensitive to ammonia or are able to maintain a low exposure to ammonia by regular irrigation of their burrows with overlying water, a guideline value of 4 mg total NH_3-N/L is introduced in these guidelines to better consider potentially degraded systems (Batley and Simpson, 2009). This conservative value is the upper 80th percentile of background data for Sydney Harbour sediments. A value of 4.5 mg total NH_3-N/L was calculated from the 95% species protection trigger value based on 9 acute benthic LC50 values divided by 5 to convert to a NOEC. In whole-sediment toxicity tests, the flux of ammonia from the pore waters can result in significant effects, that in a natural system would be diluted by mixing currents, and requires additional consideration (see Part 2, Section 4.2.3).

2.3.2 SULFIDE

Sulfide is produced in sediments by the biologically-mediated anaerobic decomposition of organic matter and is a major constituent of aquatic sediments. As a consequence, sulfide concentrations are typically higher in more organic carbon-rich sediments and those where the penetration of oxygen is low (e.g. fine silty sediments or low energy environments).

The biological effects of sulfide in sediments are poorly understood. Benthic invertebrates living in close association with sediments are commonly exposed to sulfide in the pore waters. In general, plants are less susceptible to effects from sulfide, although porewater sulfide concentrations greater than 1 mg/L have been shown to be deleterious to seagrasses (Calleja et al., 2007).

The presence of hydrogen sulfide can affect animal behaviour which in turn can alter the toxicity of both sulfide and also other sediment contaminants (Wang and Chapman, 1999). Hydrogen sulfide can be extremely toxic, and can be a naturally occurring chemical stressor. Sediments with high contaminant concentrations may be avoided by larger bioturbating organisms and this will reduce the frequency that oxygen mixes with deeper sediments. Consequently, sediments with high sulfide concentrations can be an indication of poor sediment quality, since normally the bioturbation activities of benthic organisms prevent dissolved sulfide reaching mg/L concentrations. Organisms that do not avoid sulfide may create oxidised niches (e.g. as exist around all burrows), and the oxidative release of metals associated with sulfides may result in the forms of metals becoming more bioavailable near the organism. This leads to the need to consider exposure variability in relation to sulfides. The resuspension of sediments with very high sulfide concentrations has the potential to cause localised deoxygenation of waters and these risks should be considered in relation to the water quality guidelines (ANZECC/ARMCANZ, 2000a).

No guideline value is provided for sulfide as it is considered rare that porewater sulfide concentrations will be elevated due to anthropogenic contamination.

2.3.3 EVALUATING AMMONIA, SULFIDE AND NUTRIENT IMPACTS

Generally ammonia and sulfide should not be considered as contaminants of potential concern (COPCs), however, adequate consideration should be given to the potential contributions of both sulfide and ammonia in sediment quality assessments. Ammonia and sulfide in the pore water that is released from the sediments is rapidly diluted by overlying waters. Organisms that reside at the sediment surface will be exposed to ammonia and sulfide concentrations that are a fraction of the concentrations of those in the pore waters.

Toxicity identification and evaluation (TIE) tests may be used to confirm whether ammonia or sulfide are major toxicants in pore water and whole-sediment toxicity tests (see Part 2 Section 4.3.5).

In sediments where high concentrations of organic matter are a result of anthropogenic contamination, the high dissolved ammonia and sulfide concentrations produced by these sediments may be considered as COPCs. Such concentrations can be toxic and can have effects on benthic ecology.

The ecological effects of high concentration of nutrients, such as nitrate and phosphate, are more an important consideration for water quality. High nutrient fluxes from sediments may stimulate algal or macrophyte blooms. The effects of nutrients will be highly dependent on the ecosystem being assessed. No general sediment quality guidelines for nutrients are proposed.

Note that elevated nutrient concentrations can potentially influence the outcome of toxicity tests that use algae as a test organism.

2.3.4 CONTAMINANTS WITHOUT GUIDELINE VALUES

Thousands of chemicals that enter the environment have no ecotoxicological effects data that can be used to develop SQGVs. The WOE framework is designed to allow decisions to be reached when a single LOE is insufficient for making decisions. Consequently, if SQGVs do not exist for the chemicals, the other LOEs are used to decide whether they represent a likely threat to ecosystem health.

In some situations, site-specific guidelines may be able to be developed for some contaminants. The approach suggested is to derive a value on the basis of median natural background (reference) concentrations multiplied by an appropriate factor. As suggested in the current Guidelines (ANZECC/ARMCANZ, 2000a), a factor of two is recommended, although in some highly disturbed ecosystems a slightly larger factor may be more appropriate, but no larger than three, however, this approach has low reliability. If available, it may be possible to apply the water quality guideline values to the chemical concentrations in the pore waters.

The information available on effects thresholds for contaminants continues to grow, and organisations such as NOAA have provided useful compilations, e.g. the Screening Quick Reference Tables (SQuiRT) for inorganic and organic contaminants in various media (Buchman, 2008).

3. Applying the Sediment Quality Guidelines Framework

3.1 Sediment Sampling and Analysis

The use of appropriate sampling design and sampling techniques is a prerequisite for collection of sediments for chemical analysis, toxicity testing and ecological assessments. Detailed advice is provided in the Australian Guidelines for Water Quality Monitoring and Reporting (ANZECC/ARMCANZ 2000b), as well as in the relevant USEPA document (USEPA, 2001) and CSIRO's Handbook for Sediment Quality Assessment (Simpson et al., 2005), the latter including a discussion of artefacts associated with poor practices). It is important to reinforce the recommended holistic approach of the Monitoring and Reporting Guidelines that begins with determining monitoring objectives, considers sampling design, field sampling, laboratory analyses, data analysis and reporting, with appropriate considerations of quality assurance and quality control built into all stages.

A first concern is that sampling is taken from a depth that is appropriate in terms of effects of contaminants on benthic organisms. Most organisms are found in the upper 10 cm of sediments, while epibenthic organisms might only be exposed to surficial sediment (0-1 cm). Determining contaminants in the top 10 cm or in both the 0-2 and 2-10 cm depth sediments should allow assessment of the major contaminant exposure pathways. The surface layer will represent the most recently deposited sediments, although bioturbation may mix this with deeper sediments. The impact of deeper sediments might be a concern if they are to be disturbed and redeposited, e.g. by dredging.

A second concern is for changes that might occur to the sediment chemistry during field processing, handling and storage of sediments. Particularly for metal analyses, it is important to note that the redox properties of sediments will vary with depth, with oxic sediments confined at best to the upper 2 cm and frequently much less. If anoxic sediments are exposed to air, oxidation of iron (II) will produce hydrogen ions that will release metals into pore waters (with potential toxic effects) in excess of what might be present under ambient conditions. More volatile organic contaminants will be lost through increased handling and storage of sediments.

The concentration of contaminants is typically much greater in the finer sediment fractions. Consequently, the <2 mm sediment fraction that may contain coarse sand and large debris is excluded from the chemical analyses. The <63 µm sediment fraction is considered a suitable representation of the sediment materials that are mostly readily resuspended or potentially ingested by organisms. It is recommended that the binding of contaminants by the fine sediment fraction be considered when more detailed investigations of contaminant bioavailability are required for site-specific assessments.

3.2 Application of the WOE Assessment Framework

If the conclusions from the evaluation of chemical and ecotoxicological evidence are ambivalent and further investigations are required, then this will involve the application of the WOE assessment framework. The WOE investigation should combine assessments of:

(i) sediment chemistry to measure contaminant concentrations, compare to SQGVs (if available) and identify COPCs. These measurements may include chemistry-based bioavailability tests (e.g. porewater measurements, AVS, or biomimetic approaches for hydrophobic organic contaminants).

(ii) toxicity testing (e.g. multiple species, varying exposure pathways, acute and chronic endpoints such as mortality, reproduction, development, growth, avoidance).

(iii) bioaccumulation/biomagnification, and

(iv) benthic community structure (e.g. ecological malfunction)

The WOE framework allows decisions to be reached when a single LOE is insufficient for making decisions, e.g. if SQGVs do not exist for the contaminants, if there are no appropriate toxicity tests that are sensitive to the contaminants of concern.

The WOE approach tabulates and ranks the results of all individual LOEs used, such as sediment chemistry (e.g. exceedance of trigger values), elutriate and bioavailability testing (exceedance of the relevant criteria), toxicity testing (elevated toxicity compared to controls), bioaccumulation (significant differences compared to controls), and benthic community structure (reduced benthic diversity or abundance relative to controls).

The approach outlined builds on previous publications on WOE assessment frameworks (Chapman et al., 2002; Chapman and Anderson, 2005; Simpson et al., 2005; Wenning et al., 2005). The intention of the WOE framework is to provide a process for evaluating LOEs that is sufficiently prescriptive that it can be applied by reasonably informed regulators, consultants and industry personnel, but sufficiently flexible that site-specific assessments can be made in a transparent manner through the application of best professional judgement. Changes to the WOE framework over time will mostly deal with how best to incorporate new LOEs or better utilise existing LOE that are based on more rigorous tools and techniques. Changes to SQGVs will occur as cause-effect relationships are developed for greater varieties of benthic organisms for a wider range of sediment properties.

In applying the WOE approach, it is important to recognise that the science is not yet up to providing a clear prescriptive approach that covers the complexity of environmental contamination. At the end of the day, it is the scientific rigor associated with the various LOEs that is important, not the following of a prescriptive approach.

3.3 Lines-of-evidence (LOEs) and LOE Scoring

The WOE framework is designed to integrate the four major LOEs comprising chemistry, toxicity, bioaccumulation and ecology. Each of these is derived from one or more assessments that together contribute to the scoring for the LOE in that category. The following pages discuss the process for deriving scores for each major LOE.

3.3.1 CHEMISTRY

The LOE for chemistry follows the decision tree outlined in Figure 1. The first step is to compare the measured chemical contaminant concentrations in a sediment with the sediment quality guideline SQGVs provided in Table 2.

If the SQGV for a particular contaminant is not exceeded, it is unlikely that it will result in any biological impact for organisms inhabiting that sediment. COPCs are contaminants that moderately exceed the SQGVs. Contaminants that greatly exceed the SQGVs are termed contaminants of concern (COCs).

The SQGVs apply to total concentrations of individual metal and organic contaminants, and for some contaminant classes (e.g. PAHs that can be grouped to simplify assessments). The predictive ability of SQGVs depends on the procedure used for their derivation, the contaminant class being considered, and whether the main exposure is aqueous or dietary (see Part 2). Future refinement of these SQGVs will result from better defined causality relationships and exposure effects models (Simpson and Batley, 2007).

The total concentration (TC, mg/kg dry weight) of the individual chemical contaminant (or contaminant class) in the sediment is compared to its SQGV and SGQ-high values.

TC<SQGV : effects negligible (LOE score 1)

TC>SQGV<SQG-high : effects possible (LOE score 2: COPC)

TC>SQG-high : effects expected (LOE score 3: COC)

Bioavailability modification of SQGVs

For contaminants that exceed the SQGVs, it is possible to consider only the fraction of the contaminant that is bioavailable, as indicated in Figure 1. There are a wide range of chemistry-based bioavailability tests for contaminants and the value of the information gained from these tests should be considered on a sediment-specific basis. For metal contaminants, total particulate metal (TPM, or total recoverable metals) measurement techniques that utilise concentrated acids are likely to include mineralised forms of metals that are not considered bioavailable. In most cases, it is therefore appropriate to measure the acid-extractable metal (AEM) concentration and compare this value to the SQGV to obtain the LOE score. Caution is noted, however, in applying AEM measurements to sediments which contain metals that are recognised as being sparingly soluble in 1 M HCl. For metals such as copper and mercury, a combination of AEM and TPM measurements may be necessary to accurately describe their forms and potential bioavailability (see Part 2).

Acid-volatile sulfide (AVS) and in some cases organic carbon, are metal binding phases that modify the bioavailability of many metal contaminants (i.e. Ag, Cd, Cu, Hg, Ni, Pb and Zn) and can be used to modify the application of the SQGVs for some metals (see Appendix A6). The presence of AVS also indicates that sediments are sufficiently reducing that chromium should be in the form of Cr(III), rather than the more toxic Cr(VI) form if present in pore waters.

The speciation (forms and accessibility) of sediment organic carbon (TOC) modifies the bioavailability of many organic contaminants, and measurements of TOC can be used to modify the application of the sediment quality SQGV for some organic contaminants. Organic carbon speciation (e.g. black carbon) can be used to modify EqP models, and biomimetic techniques (e.g. using semi-permeable membrane devices (SPMDs) or adsorbants such as Tenax®TA) that examine the lability of sediment-sorbed organic contaminants can be used to assess bioavailability. The application of chemistry-based bioavailability tests to modify the application of the SQGVs is described in more detail in Part 2.

Examining pore waters and elutriates

The examination of sediment-associated pore waters provides another chemistry LOE. If the concentrations of contaminants exceed the water quality guideline values (WQGs), then there is also potential environmental concern, since most benthic organisms ingest pore water. Water quality SQGVs have been derived for many contaminants (ANZECC/ARMCANZ, 2000a), based on data for a range of water-column organisms, however, where sufficient data are available, it is possible to derive SQGVs specific for pore waters, using only toxicity data for benthic organisms.

Because it may be difficult to obtain sufficient pore water for some analyses, an additional option is to undertake a sediment elutriate test and use analyses of the elutriate as an indication of potentially soluble contaminants.

The evaluation of pore water or elutriate results against WQGs is similar to the application of the SQGVs. The concentration of the contaminant in the pore water or elutriate is compared to the WQGs that protect 95 and 90% (HC5 and HC10, respectively) of species respectively where WQGs are available that have been derived on the basis of species sensitivity distributions (SSDs). Results evaluated as follows:

Concentration < WQG HC5: effects negligible (LOE score 1)

WQG HC5 < concentration < WQG HC10: effects possible (LOE score 2: COPC)

Concentration > WQG HC10: effects expected (LOE score 3: COC)

If the WQGs are low reliability and not based on SSDs, the respective scores of 1, 2 or 3 might apply for: Concentration < WQGs, Concentration/ WQGs = 1-5 and Concentration/ WQGs >5. In some cases, a consideration of bioavailability (speciation) of contaminants in the pore waters might be desirable.

Application of the chemistry LOE

(i) The total concentrations of chemical contaminants are measured in the bulk sediment and compared to the SQGVs. For metals, the use of AEM may be suitable.

(ii) If applicable, bioavailability-modifications of SQGVs are applied.

(iii) COPCs and COCs are identified from the exceedances of SQGVs and LOE scores calculated.

(iv) Concentrations of chemical contaminants are measured in the porewaters or elutriates and LOE scores and COPCs and COCs are identified from the WQGs.

(v) A tabular summary is prepared of the LOE scores for the SQGVs (and WQGs for porewaters or elutriates) and a final LOE score for chemistry determined for the WOE assessment matrix.

(vi) Identified COPCs and COCs are listed to aid assignment of causality in the assessment of toxicity and ecology.

For both metals and organics, an overall score for the chemistry LOE, would normally be dictated by the highest score of any of the individual assessments. More than one contaminant with a high score would give more weight to the chemistry LOE score than that for a single contaminant. Examples of the application of the chemistry LOE are given in Part 2.

3.3.2 TOXICITY

The LOE for toxicity includes results from a range of toxicity tests. These tests may comprise a combination of acute, sub-acute and chronic tests, and pore water, elutriate water and whole sediment tests. All toxicity tests may have attributes that make them more or less appropriate for specific situations and the test provider, relevant guidance documents, or regulators should be consulted to determine which tests are most appropriate for the assessment. Whole-sediment toxicity tests are more relevant than porewater tests because:

(i) once isolated from sediments, pore waters are not stable, i.e. losses of contaminants will occur,

(ii) many of the test organisms used do not normally interact with sediment pore waters, although they act as surrogates for species that do.

Marine and freshwater testing with amphipods has been most widely used, although tests using benthic algae, copepods, bivalves, polychaete worms are now in common usage. Chronic toxicity tests are more ecologically relevant than acute or sub-acute toxicity tests. However, there are fewer chronic tests available than acute tests, and the test available may not always be appropriate for the test situation. It is frequently observed that the variability in results is greater for chronic tests than for acute tests, i.e. there will be a greater number of 'false positives' (classifying non-toxic sediment as toxic). With aquatic toxicity, acute to chronic ratios (usually a factor of 10) are applied to acute toxicity data to approximate chronic toxicity. There are insufficient data from whole sediment toxicity tests to yet determine how appropriate such an approach will be.

Guidance for the correct application of some, but not all, toxicity tests is discussed in detail in many documents, e.g. USEPA (1994, 1995, 2001) and Simpson et al. (2005). Toxicity tests other than those shown can also be applied. The intent is to have a balance of tests that respond to the COPC.

There is ongoing debate as to the ecological significance of many sub-lethal and biomarker measures in ecological risk assessment. The use of sub-lethal tests and biomarker responses are only considered appropriate where data exist to demonstrate that a concentration-response exists that corresponds to measurable effects (e.g. toxicity indicators such as growth, reproduction or development). As yet there are few sub-lethal tests and biomarker responses that meet these criteria.

As with chemical testing, it is important that the sample used for toxicity testing has the same concentrations of chemicals and bioavailability that it had in the field situation. The greater the amount of sample manipulation and the longer the storage time before testing, the larger changes to contaminant bioavailability will be. It is recommended that toxicity tests be undertaken as soon as practical after collection of sediments from the field. Storage of sediments for periods longer than eight weeks before testing is not recommended. Staggered sampling and chemical analyses may be required for larger risk assessment project.

The toxicity LOE is used as follows:

(i) Generally, a minimum of three toxicity tests should be undertaken, and these tests should comprise both acute and chronic endpoints, and at least one whole-sediment toxicity test. The test results should be presented as 'effect as a % of control response' (in an uncontaminated sediment with similar properties as the test sediment).

(ii) Determine whether the toxic effects may be attributed to factors other than the COPCs. For example, ammonia and sulfide at naturally occurring concentrations are not COPCs, but may cause significant toxicity. The sediment may have been too compact for burrowing or insufficiently nutritious (e.g. clay), resulting in non-COPC mortality (may be an example of poor test procedure selection). If non-COPC factors are shown to be the cause of the toxicity, these test results should be discarded from the assessment, and alternative tests may be necessary.

(iii) Toxicity data are assessed as non-toxic if the response is <20% toxic effect compared to a control response, toxic with significant effects for 20-50%, and toxic with significant and major effects if the response is ≥50%.

The combined toxicity tests are considered in relation to their robustness and sensitivity (overall value), and scored relative to the likelihood of toxic effects. This may utilise additional knowledge of the test procedures, such as the likelihood of false positives and false negatives caused at random or test artefacts. The scoring system again has three categories: 1, 2, and 3, representing low, moderate and high incidence of toxic effects. Scoring of the various assessment tests uses <20%, 20-50% and ≥50% toxicity on whole sediment or pore water respectively

The overall LOE ranking for a sample will again be dominated by the highest score in the suite of tests, generally giving greater confidence with whole sediment over porewater tests and chronic over acute tests. Causality for COPCs may be assigned for toxicity tests in which effects thresholds for individual contaminants are known.

Normally, toxicity testing will be used to demonstrate the absence of toxicity when the SQGV for a particular contaminant is exceeded. If toxicity is observed, its origins cannot necessarily be attributed to the contaminant of interest because of the possibility of other contaminants either contributing to the observed toxicity or being the primary cause. In some situations, knowledge is required of which contaminant(s) or class of contaminants is responsible for the observed toxicity. Toxicity identification and evaluation procedures (TIE) may be applied for this purpose (Part 2).

Examples of the application of the toxicity LOE are given in Part 2.

3.3.3 BIOACCUMULATION

Bioaccumulation studies involve measurements of contaminant accumulation in:

(i) field-collected native biota,

(ii) field-transplanted biota (specific species placed *in situ* at the field site for a defined period of time),

(iii) laboratory transplanted biota (bioaccumulation assays using specific species and sediments collected from the test site), or

(iv) other indicators of bioaccumulation (e.g. surrogate methods including the use of passive samplers, discussed in Part 2).

The LOE for bioaccumulation also considers biomagnification, which refers to a substance's accumulation through food chains resulting in increased exposure of higher organisms via their diet. Biomagnification is considered for contaminants that are known to undergo trophic transfer up food chains, e.g. dioxins, PCBs, and methylmercury.

The bioaccumulation LOE is used as follows:

(i) A paper study (literature review and calculations) should first be undertaken to consider which contaminants may bioaccumulate to concentrations that cause effects. For example, the presence of dioxins at any concentration would necessitate an investigation of bioaccumulation. The bioaccumulation of many hydrophobic organic contaminants (HOCs) may be first evaluated by simple equilibrium partitioning calculations.

(ii) The results from any bioaccumulation tests undertaken should be presented as bioaccumulated concentrations relative to controls (field or laboratory) and designated as not significantly different from controls ($p<0.05$), significantly different from controls ($p<0.05$) but ≤3× concentrations of control, and significantly different from controls ($p<0.05$) and >3× concentrations of control. Bioaccumulation will be a function of organism age and size and there may be regulation of some metal contaminants by some organisms. The factor of three is chosen simply to provide a graded approach to evaluating the extent of any bioaccumulation, and has no bioaccumulation or toxicological significance. It may be appropriate to modify this factor (e.g. to a factor of 2 or 10) on a contaminant-by-contaminant basis to reflect the propensity of the contaminant to accumulate and any effects-relationships that may exist. However, adequate reasoning would need to be provided if the factor is to be modified. The use of food standards (e.g. FSANZ, 2008) (refer Section 4.4.5, ANZECC/ARMCANZ, 2000a) is relevant where biota are for human consumption, and should be used as an indicator of excessive contamination.

(iii) It should be determined whether any of the bioaccumulating contaminants can biomagnify. For example, if dioxins are present, they will biomagnify. If PCBs are present and bioaccumulating, they will biomagnify.

(iv) The combined results of the bioaccumulation/biomagnification assessment are scored relative to the likelihood of bioaccumulation to high levels. It is unknown whether there will be a physiological response to the bioaccumulated concentration, rather the extent is indicative of the bioavailability of the COPC and that provides an additional LOE to the chemically measured exposure concentrations. The scoring system has three categories: 1, 2, and 3, representing not significant, significant but moderate, and significant and high, respectively. Examples are provided in Part 2.

3.3.4 ECOLOGY

Two main types of ecological data may be considered for the ecology LOE:

(i) benthic community structure data obtained from surveys of test site and reference locations (e.g. Thrush et al., 2008; Chariton et al., 2010a), and

(ii) benthic community response data from manipulative experiments, e.g. sediment transplant recolonisation experiments (e.g. Pettigrove and Hoffman, 2005; Chariton et al., 2011).

The major factors determining whether ecological data are included in an assessment will be cost, and whether appropriate reference locations exist. Manipulative (recolonisation) experiments are costly and would generally only be used if the conclusions from benthic community assessment were confounded and a long-term assessment approach was not possible.

Assessments will generally be restricted to the top 10 cm of the sediments because this is where the vast majority of sediment-dwelling organisms live (Chapman. 2002). Guidance for the correct application of some, but not all, benthic ecology response and structure assessments in relation to COPCs is discussed in Part 2.

Special situations affecting the relationships between benthic ecology and possible effects of COPCs include areas of high activity (anthropogenic or otherwise), unstable bed sediments (subject to regular scouring or resuspension), or areas receiving intermittent freshwater inputs. An additional, but important, external factor that may cause differences between reference and test sites is the spatial and temporal variability in recruitment patterns of relevant biota.

The ecology LOE assessments should:

(i) Determine whether test sites show statistically significant differences compared to reference locations using observational information, univariate analyses and multivariate analyses as indicated in the check list in Part 2.

(ii) Determine whether the ecological effects may be attributed to factors other than the COPCs, e.g. assess effects due to differences in salinity, proximity to unusual structures and activities that may disturb the environment (e.g. stormwater drains, wharves, boat moorings), sediment particle size, etc. If non-COPC factors may be an important factor affecting the ecological assessment, these test results should be discarded from the assessment, and alternative tests may be necessary.

(iii) Based on outcomes of the ecological assessment and consideration of non-COPC factors, reconsider whether reference locations were suitable for reaching the assessment conclusions. Alternative reference locations may be needed, or the significance of the results re-assessed.

The combined results of the ecological assessment are scored relative to the likelihood of effects occurring due to COPCs. The scoring system has three categories: 1, 2, and 3, representing not significant, statistically significant but moderate, and statistically significant and high, respectively. Examples are provided in Part 2.

The ecology LOE will always be difficult to interpret, compared to other LOEs, particularly since there will generally be no proven direct link to the contaminant concentrations in the sediment. Ecological evidence will only be correlative and will be very dependent on the sensitivity of the selected indicator(s) to the contaminants (which are often poorly understood) and the power of the sampling program to actually detect significant changes. Particular attention must be given to other factors driving differences between the test and reference sites that have not been identified or measured, and appropriate caution given to interpretation (Anderson et al., 2006; Thrush et al., 2008; Chariton et al., 2010b, 2011). The primary consideration in the first instance is whether the ecosystem is ‘healthy’, i.e. diverse and abundant biota. It is acknowledged that making the finer distinction as to differences in species between sites and reference locations may be more difficult to link to contaminant impacts.

3.4 Weight-of-evidence Ranking

The WOE framework is designed to integrate the four major LOEs comprising chemistry, toxicity, bioaccumulation and ecology. Each of these major LOEs is derived from single or multiple tests or experiments that represent single LOE. The scores for each LOE are tabulated in a decision matrix as shown in Table 3, with typical examples of its application shown in Table 4. The overall assessment is based on the support of elevated scores in the chemistry LOE by elevated scores in one or more of the other LOEs. The WOE rankings of high, medium and low, equate to findings of significant adverse effects from sediment contamination, possible adverse effects from sediment contamination, and no adverse effects, respectively.

Table 3. Weight-of-evidence decision matrix (not all possible LOE or cases included)

LINE OF EVIDENCE	RANKING		
	3	2	1
Chemistry			
Sediment	Concentration > SQG-high	Concentration > SQGV < SQG-high	Concentration < SQGV
Pore water	Concentration > WQG HC10	WQG HC5 < Concentration < WQG HC10	Concentration <WQG HC5
Toxicity	≥50% effect vs control	20-50% effect vs control	<20% effect vs control
Bioaccumulation	Significantly different (p<0.05) and >3× control	Significantly different (p<0.05) and ≤3× control	Not significantly different from control
Ecology	Significant and high effects on abundance and/or diversity	Significant but moderate effects on abundance and/or diversity	No significant effects on abundance and/or diversity
Weight-of-evidence	**Significant adverse effects**	**Possible adverse effects**	**No adverse effects**

Elevated contaminant concentrations (COPCs and COCs) determined in the chemistry LOE, remain the underlying driver in the WOE assessment framework for several reasons:

(i) In most sediment quality assessments, it is the concentrations of contaminants that are the key concern (i.e. suspected to be the cause of the effects);

(ii) Protecting pristine areas from contaminant impacts is a common focus of risk assessments; and

(iii) Management options for remediation of contaminated sediments will generally be most easily guided by concentrations of COCs.

Table 4. Examples of weight-of-evidence decisions

Case	Line of Evidence [a]				Weight-of-evidence (WOE) Score	Overall Assessment
	Chemistry (metals-organics)	Toxicity	Bioaccumulation / Biomagnification	Ecology		
W1	3	3	2 or 3	3	**3**	Significant adverse effects from sediment contamination
W2	3	3	2 or 3	2	**3**	Significant adverse effects from sediment contamination
W3	2 or 3	3	2	2	**3**	Significant adverse effects from sediment contamination
W4	2 or 3	2	1 or 2	2	**2**	Possible adverse effects from sediment contamination
W5	2	2 or 3	1or 2	2	**2**	Possible adverse effects from sediment contamination
W6	2	2	1 or 2	2 or 3	**2**	Possible adverse effects from sediment contamination
W7	2 or 3	2 or 3	2 or 3	1	**2**	Toxic chemical stressing system but resistance may have developed at community level
W8	1	2 or 3	1	2 or 3	**2**	Unmeasured toxic chemicals causing effects on communities is possible
W9	1	2 or 3	1	1	**2**	Unmeasured physical or chemical causes of toxicity
W10	2 or 3	1	1	2 or 3	**2**	Chemicals are not bioavailable or community change may not be due to chemicals
W11	1	1	1	2 or 3	**1**	Changes probably not due to measured contaminants
W12	1 or 2	1	1 or 2	1	**1**	No adverse effects
W13	1	1	1	1	**1**	No adverse effects
W14	2 or 3	1	1	1	**1**	Contaminants unavailable

[a] Values listed in each line of evidence category are the highest scoring assessment in that category, e.g. under chemistry, metals may score 2, organics 3, so 3 is recorded. The greater the number of 3s recorded in a category, the greater is the weight that line of evidence category assumes.

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Part II Rationale and Background Information

1.Sediments and Contaminants

1.1 Sources of Sediments and Sediment Contaminants

Aquatic sediments are principally derived from weathering processes, with major transportation from terrestrial sources from under high runoff from storms and floods, and deposition of planktonic life forms in oceans. In addition, discharges from urban, industrial and mining activities are potential sources of particulates. Anthropogenic contaminants, including metals, organics and nutrient elements are associated with particulate and dissolved inputs to natural waters. It is important to distinguish between point source and diffuse inputs. The former includes effluent streams, drains or licensed discharges, which can, if required, be the target of management actions. Diffuse inputs generally lead to a gradual build up of contaminants in sediments, especially in coastal lakes, estuaries and marine waters. Diffuse sources include aerial deposition and land runoff of stormwater. The consideration of ongoing inputs from diffuse sources may be necessary for many assessments.

Particulate matter can act as binding sites for contaminants in soluble forms. Biological processes add particulate matter in the form of algal mats, dead cells, degradation and excretion products of animals, and living and dead plant biomass. Suspended particles gradually settle and accumulate as part of the bottom sediments. Rates of sedimentation vary from as low as 1 mm/y in coastal marine waters, to 10–20 mm/y in some riverine and estuarine systems, although higher rates have been reported in New Zealand. Highest values are found in settling basins removed from high currents and close to point sources, whereas more common values are in the range 3–7 mm/y.

Contaminants are also associated with natural colloids, which can precipitate with aging or with changes in water chemistry. The change in salinity from fresh to saline waters will induce the precipitation of iron and manganese oxyhydroxides from both soluble ions and colloids, carrying with them other metals and organics.

1.2 Sediment Properties

1.2.1 PHYSICAL PROPERTIES

Physical properties, such as grain size and density, are important in sedimentation and transport processes. Sediments are a heterogeneous mixture of particles ranging from millimetre to sub-micron in size. Typically, sediments are characterised as coarse material, clay/silt and sand fractions, on the basis of separations using 2 mm and 63 µm sieves. Particles >2 mm may consist of shells, rocks, wood, and other detrital materials, and are usually not a source of bioavailable contaminants (Mudroch et al., 1997). The clay/silt fraction has a high surface area and because of its surface chemistry is more likely to adsorb organic and heavy metal contaminants. Particles <63 µm are more common in the gut of sediment-ingesting biota (Tessier et al., 1984). It is not unusual to normalise contaminant analyses on the basis of the clay/silt fraction.

The sand and coarse silt fractions are generally dominated by quartz, sometimes by carbonates (shell, coral etc), and occasionally by other silicates such as feldspar, or rock fragments. Primary silicates may also be present in the sand fraction, but are less evident in silt particles. Clay particles tend to be dominated by secondary silicates. Other secondary minerals such as oxides of aluminium and iron are prominent in the fine silt and clay fractions. This holds for most terrigenous sediments, and is not dissimilar in coastal marine sediments. Most anthropogenic contaminants (i.e. those associated with human activity) are associated with the clay and silt fractions that generally have high surface area and high concentrations of associated organic matter. In some instances, contaminants may also enter the environment in particulate form, e.g. metal associated with paint flakes and hydrocarbons (including PAHs) associated with deposition of soot, or oil and tar phases.

Sediments are in intimate contact with the water that fills the voids between particles and within the pores of sediment particles. The volume of this interstitial or pore water will be governed by sediment porosity. Sediment particle size is also critical to the ease, and therefore the depths, to which organisms can burrow. This is also dictated by the acceptability to the organism of the chemical environment of the sediment and its associated pore waters. Silty sand is a more acceptable medium for many benthic biota than is more compressible clay.

Physical processes in sediments influence the chemistry of sediments and their associated contaminants. Sediment resuspension can result from wind stirring, tidal currents and boating activities, as well as by biological activities (bioturbation). These processes can lead to particle sorting on the basis of density or size. They also expose particles to a different chemical environment, overlying water versus pore water and usually oxic versus anoxic. In the absence of any physical or biological sediment disturbances, contaminant movement occurs via diffusion processes in sediment pore waters, controlled by factors such as porosity.

1.2.2 SEDIMENT CHEMISTRY

The chemical behaviour and bioavailability of contaminants will be controlled by the sediment geochemistry and the associated pH and redox conditions (dissolved oxygen, sulfide) of the sediment pore waters. The pool of bioavailable metals in sediments is typically much smaller than the total metal concentration and is strongly influenced by metal-binding with sulfides, particulate organic carbon (OC), and iron and manganese oxide solid phases. A significant metal fraction may be present in mineralised forms, and these metals are of little ecological importance as it is unavailable for bioaccumulation. In oxic sediments, the most important metal-binding phases are organic matter and hydrous iron and manganese oxides. In anoxic sediments, many metals (Ag, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Zn) are strongly bound (low solubility) with sulfide minerals, including pyrite and the more reactive acid-volatile sulfide (AVS) phases (e.g. iron monosulfide, FeS). The importance of these metal-binding phases is strongly influenced by particle size, with metal-binding increasing in sediments with greater portions of fine clay or silt sized particles (e.g. <63 µm). The interaction and dynamic equilibrium between the various metal-binding phases creates a great challenge for the development of SQGs for metals in sediments. Future bioavailability models and SQGs should explicitly consider pools of AVS, OC and iron and manganese oxides as phases that reduce toxicity.

Organic contaminants can be divided into hydrophobic (non-polar, water-insoluble) and hydrophilic (polar, soluble) species (hydrophobic organic contaminants), and further subdivided as acidic, basic or neutral compounds. The former distinction based on water solubility can be related to the compound’s octanol:water partition coefficient. Due to their lower solubility and greater propensity to bind to sediments, hydrophobic organics tend to accumulate to higher concentrations. Organic matter, either as discrete particles or as coatings on inorganic substrates, is the primary adsorbing phase for hydrophobic organics, but depending on their charge, inorganic phases may be able to bind some compounds. Black carbon (pyrogenic carbon or soot, including coal) has been shown to be an important phase for binding hydrophobic organic contaminants (e.g. PAHs) in sediments. To assess the bioavailability of HOCs in sediments, the speciation of organic carbon may require measurement as the strength of binding of HOCs is often many orders of magnitude greater for black carbon than for carbon derived from break down of plant or animal matter.

While metals may exist in both complexed and labile forms, they are not subject to the same degradation processes that are common to many organic molecules. In considering the environmental risk posed by organics and metals, the chemical form or speciation will be important, as will be the half-lives of chemical, physical and microbial degradation processes. In practice, hydrophilic organics are typically less persistent than hydrophobic compounds, because they are more amenable to hydrolysis and other solution degradation processes.

The redox state of sediments (i.e. whether they in an oxidising or reducing environment) will be defined by the oxygen content of the pore waters. It is possible for sediments to be oxygen-deficient several millimetres below the surface. Oxygen deficiency will alter the chemistry of metals such as iron and manganese which in turn will affect the behaviour of other heavy metals that were previously bound to oxides of iron and manganese. Iron (III) hydrous oxides will be reduced to more soluble iron (II) species, while hydrous manganese oxides will be reduced to soluble manganese (II) species. Manganese, being more readily reduced than iron, appears in the pore water column at a higher zone in the sediment. The redox boundary is not necessarily stationary, and steady-state conditions may not apply because the boundary may move up and down through the sediments more quickly than the chemistry can respond. Most organic contaminants are not directly susceptible to redox changes, but indirectly the presence of bacteria under specified redox conditions will affect the stability of such contaminants to microbial degradation processes.

The major nutrient elements of environmental concern in sediments are nitrogen and phosphorus. Both are present in organic and inorganic forms. Inorganic forms of nitrogen include nitrate, nitrite and ammonia. Organic nitrogen undergoes bacterial degradation and denitrification via ammonia, nitrite, and nitrate, ultimately to elemental nitrogen, $N_{2.}$ In oxygen-limited systems, these reactions can stop at ammonia. Phosphorus exists as phosphates, both monomeric and polymeric, and in sediments is usually bound with iron. Considerable phosphorus and nitrogen can also be bound by bacteria and it is important to consider living microscopic benthos as part of the sediment structure.

Sediments represent a potential source of contaminants to the overlying water and hence can influence water quality. The natural release of sediment contaminants is controlled by their dissolution into the sediment pore waters. Diffusion of these contaminants to the water column will occur if the pore water concentration exceeds that of the overlying water. The measurement of the fluxes of contaminants can be obtained using dialysis samplers (porewater peepers), benthic chambers or corer reactors. For many metals and some metalloids, the technique of diffusive gradients in thin films (DGT) now provides a useful method for measuring porewater metal concentrations and fluxes at the sediment water interface (Tankere-Muller et al., 2006; Simpson et al., 2012a; Panther et al., 2013).

Burrowing organisms have a significant impact on sediment chemistry and physics. Bioturbation, or burrowing activities, affects the sediment profile, by physically translocating contaminated sediments, mixing and redistributing the contamination.

2. Sediment Sampling

2.1 Collection of Sediments

For the assessment of sediment quality, surface sediments are commonly collected, however, when evaluating the risks of dredging activities to determine their suitability for ocean disposal, deeper sediments may also require collection (Environment Canada, 1994; ASTM, 2000; USEPA, 2001; Simpson et al., 2005). Generally, most epifaunal and infaunal organisms are found in the upper 10 cm of sediments. Some epibenthic species (e.g. shrimps, certain amphipods) might only be exposed to surficial sediments (0-1 cm) while others (e.g. bivalves, polychaetes) that are infaunal irrigators might receive their primary exposure from sediments that are several cm in depth. Determining contaminant concentrations in both the 0-2 and 2-10 cm depth sediments should provide sufficient information to assess major contaminant exposure pathways for most organisms.

A large range of devices is available for the collection of sediments and reviews of their uses and suitability for different collection conditions are available (Mudroch and Azcue, 1995; USEPA, 2001; Simpson et al., 2005). The most important requirement for sediment collection devices is that the integrity of the collected sediment is maintained, because disruption of the sediment's structure will distort its chemical and physical characteristics. Mixing of previously redox-stratified chemical substrates with layers of differing particle size and composition will influence the bioavailability of contaminants and the potential toxicity of the sediment (Simpson and Batley, 2003).

Field measurements and observations are important for any assessment of sediment quality. Measurements should include:

(i) water quality parameters (pH, redox potential, dissolved oxygen, temperature, conductivity/salinity, turbidity and water depth) in the water column 5-20 cm above the sampling site, and

(ii) pH and redox potential of the sediments.

The Handbook for Sediment Quality Assessment (Simpson et al., 2005) provides guidance for making these measurements.

2.2 Sediment Manipulations and Storage

Any form of disturbance to the sediments, whether through the act of sampling, field processing or transportation, will affect the bioavailability of the contaminants (USEPA, 2001; Simpson et al., 2005). Field processing, or manipulation, of sediments may result in changes in the speciation, and bioavailability, of substances by disruption of the equilibrium in the pore water/sediment system. Although disturbances to the sediments cannot be eliminated, it is important that they are minimised (Simpson et al., 2005).

Maximum holding times are governed by sediment type, contaminant characteristics and the tests to be undertaken on the sediments. Following collection, sediment samples should be stored cold (on ice) to reduce loss of volatiles and decrease bacterial activity. The general recommendation is to store sediments and pore water in the dark at 4°C. Sample holding times and storage methods will vary depending on the use of the collected sediments or sediment components (e.g. porewater separation and analysis). Samples for analyses of total metals may be held indefinitely, however changes to metal speciation and partitioning of contaminants between sediments and pore waters will begin occurring within days of collection (Carr and Chapman, 1995; Simpson and Batley, 2003). Changes in bacterial activity will cause changes to the concentrations of ammonia, sulfide, iron(II) and biologically-active sediment components, particularly in pore waters. It is generally recommended that if chemical testing of pore waters is of interest, they should be extracted immediately after collection and appropriate preservation procedures and storage containers used for each analyte (may need separate containers). Most extractable organics (e.g. phthalates, organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), PAHs, hydrocarbons, dioxins) should be extracted from sediments within 14 days of sample collection, while 7 days should be the maximum storage length before extraction of samples for analyses of organic contaminants that are susceptible to losses due to volatility or microbial degradation. Longer storage times may be appropriate depending on properties of the sediments, the concentrations and types of contaminants. Extended storage of sediments may result in losses of labile or volatile contaminants (e.g. ammonia, volatile organics, AVS) or changes to the redox properties of the sediments due to increased or decreased bacterial activity.

Manipulation of sediments in the field or laboratory is often undertaken prior to chemical or toxicity testing. This may involve sieving to remove large particles and debris, or the separation of native biota, or homogenisation so that a large sample can be used for a number of chemical and biological tests. Most manipulations of sediments will alter the properties of the sediments and affect contaminant bioavailability, and the effects of these on the test data need to be evaluated. In particular oxidation of iron (II) will lower the pH or pore waters with enhanced release of heavy metals from sediments. All procedures used to prepare sediment samples for analyses and tests should aim to minimise disturbances and should be fully documented in reports.

It is desirable to undertake some assessment of how sample manipulation may affect the concentrations (e.g. loss of volatiles), bioavailability (e.g. changes to AVS, partitioning in pore waters) and toxicity of contaminants in the collected sediments. For the freshly collected whole-sediments that have been minimally manipulated, measurements of pH, redox potential, TOC, AVS, iron, particle size analysis, and analyses of total and weakly-extractable contaminants, and porewater contaminants will aid interpretation of bioavailability and toxicity test data. For sediments that undergo major manipulation (e.g. sieving) or are stored for long periods of time before testing (e.g. longer than 4 weeks), re-analysis is desirable for those parameters likely to be affected by these manipulations (e.g. pH, AVS, pore waters, volatile organics).

2.3 Sediment Pore Water

Sediment pore water is defined as the water occupying the spaces between sediment particles. Typically pore water will occupy 30-80% of the volume of sediment, the volume being greater for fine-grained (silty) sediments than for sandy sediments. Water currents driven by surface water movements (e.g. currents, tides, wind) or groundwater upwelling will influence porewater composition and stability. In many depositional sediments, pore waters will be relatively static and it is expected that thermodynamic equilibrium will exist between contaminant concentrations in the pore water and in surrounding sediments. The concentrations of contaminants in the pore waters of surface sediments (0-1 mm) will control the flux of contaminants to the overlying waters or into the burrows of organisms. Sediment characteristics (e.g. pH, organic carbon, sulfides, mineralogy, particle size) will greatly affect the partitioning of contaminants between the sediments and pore water. Porewater contaminant concentrations are frequently higher than overlying water concentrations and are easily dispersed and diluted within the system.

Because many benthic organisms are in direct contact with sediment pore waters, this component of sediments is considered to be a major exposure pathway for benthic organisms. The accurate measurement of contaminant concentrations in sediment pore waters is therefore useful for assessing the bioavailability of contaminants. Generally, no evaluation will be required for sediments with coarse particles (sand/gravel) that have little binding capacity for sediment contaminants, or compacted clays that have little accessible pore water with which organisms can interact.

Methods for the isolation of pore waters from sediments are described in Carr and Nipper (2003), Chapman et al. (2002) and Simpson et al. (2005). It is important to recognise that all porewater isolation methods have been shown to alter porewater chemistry and affect metal contaminant bioavailability and toxicity. Because pore waters will generally contain very low dissolved oxygen concentrations, and often have high concentrations of easily oxidisable species (e.g. Fe (II)), maintaining these properties following isolation from sediment is practically impossible.

Passive samplers, such as DGTs for metals and some metalloids (Zhang et al., 1995; Tankere-Muller et al., 2006; Panther et al., 2013) and SPMDs, PEDs, SPME, and POM for non-ionic organics (USEPA, 2012) are increasingly being used to provide information on concentrations of labile contaminants in pore waters and are also finding applications in the assessment of sediments (Maruya et al., 2010; Ding et al., 2012; Dabrin et al., 2012; Mackenbach et al., 2012; Simpson et al., 2013).

3. Derivation of Sediment Quality Guidelines

The many current approaches to the derivation of sediment quality guidelines can be broadly classified as being based on:

(i) empirical approaches that use data from matching sediment chemistry to the effects or WOE database from laboratory or field exposures to contaminated sediments,

(ii) an equilibrium partitioning approach and the application of existing water quality guidelines to sediment pore waters (often termed mechanistic guidelines)

These have been comprehensively reviewed elsewhere (Batley et al., 2005) and have formed the basis, either individually or collectively, of regulatory frameworks in many parts of the world (den Besten et al., 2003; Babut et al., 2005).

3.1 Empirical Guidelines

The use of large effects databases is now the most widely accepted approach to sediment guideline development and formed the basis of the Australian and New Zealand interim SQGs. The first approach of this type was reported by Long and Morgan (1990), based on an assessment of the potential for biological effects of sediment-sorbed contaminants in several hundred sites sampled as part of the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends Program in the U.S. The study examined data obtained from the equilibrium partitioning approach, the spiked sediment bioassay approach and other various approaches to sediment quality criteria. The chemical concentrations observed and predicted by the different methods to be associated with biological effects were sorted, and the lower 10 percentile and median concentrations were identified along with an apparent effects threshold. The lower 10 percentile data were identified as Effects Range-Low (ERL), below which adverse effects on sediment associated species occurred infrequently), and the median as Effects Range-Median (ERM) and above which adverse effects were frequently observed. Data were used to rank sites in relation to the effects range values.

Unlike the water quality guidelines, which are based on effects data for individual contaminants, a large portion of the effects data considered in empirical approaches and used to derive SQGs suffer from the presence of co-occurring contaminants. Although the observed effects are assigned to specific contaminants, it is recognised that these effects are not attributable to that contaminant alone. Empirical guidelines do not explicitly attribute the observed toxicity to either the dissolved or particulate phases.

It should be emphasised that this approach was not originally intended to develop guidelines, but rather to compare and rank sites, to identify priority contaminants, to estimate the relative potential for toxic effects and to itemise and describe the kinds of toxic effects previously observed in association with specific contaminant concentrations. Approaches to matching of biological and chemical data were discussed in a subsequent paper by Long et al. (1995). Recognising the considerable uncertainty arising from co-occurence of contaminants, and that sediments may exist with multiple exceedances of the ERL or ERM values, Long et al. (1998)

suggested that mean sediment quality guideline quotients (SQGQ) should be calculated and used for assessments. This approach involves dividing the measured concentrations of each contaminant by its ERL or ERM value (ERMs were more commonly used), adding up all the ratios and then dividing by the number of contaminants. Usually separate classes of contaminants were identified (e.g. metals, hydrophobic organics, pesticides) and the mean quotient calculated for each class, then the mean taken of the combined contaminant classes (Long and Morgan, 1998; Long et al., 1998; Fairey et al., 2001; Long et al., 2006; Simpson and Spadaro, 2011). Greater mean quotients were taken to indicate greater risk of effects. This approach differs considerably from the toxic unit (TU) approach that is often applied for waters where the TUs are summed, as the quotients are derived solely from effects due to the individual contaminants.

MacDonald and coworkers (including Long) (1992, 1996, 2000) developed an expanded biological effects database for sediments (referred to by the acronym BEDS) for the Florida Department of Environmental Protection (FDEP), which is one of the most comprehensive documentations of sediment quality assessment yet reported. The data were sorted into effects and no effects data. From the no-effects data, the 50th percentile (No Effect Range Median, NERM) and the 85th percentile (No Effect Range High, NER-H) were determined. The threshold effects level (TEL) defines the upper limit of sediment contaminant concentrations of no-effects data and was calculated as the geometric mean of the ERL and NERM. A safety factor of 2 was applied to the TEL values to define a no-observed-effects level (NOEL). The effects range approach has been applied to both fresh (Smith et al., 1996) and marine waters (MacDonald et al., 1996) and was adopted in Canada (CCME, 2002)

$$\text{TEL} = (\text{ERL x NERM})^{1/2}$$

The probable effects concentrations (PEL) defining the lower limit of the range of contaminant concentrations that are usually associated with adverse biological effects was defined as the geometric mean of the ERM and NER-H values:

$$\text{PEL} = (\text{ERM x NER-H})^{1/2}$$

A major issue is that although one or more of these chemicals may have produced the effect, it is ascribed to all chemicals in the mixture. Effects levels entered for some chemicals may therefore be well below actual effects thresholds.

Ingersoll et al. (1996) compared the effectiveness of ERL, ERM, TEL, PEL and no effect concentrations (NEC). They considered the ability of these sediment effects criteria to correctly classify toxicity or no toxicity and the respective abilities to classify non-toxic samples as toxic (Type I error, false positive) or toxic samples as non-toxic (Type II error or false negative). They concluded that ERMs and ERLs were generally as reliable as PELs and TELs in respectively classifying samples as toxic or non-toxic, but stressed the need to use field generated data, noting the problems with other contaminants in contributing to the observed effect.

The fact that the guidelines were primarily developed from estuarine and marine data was not seen as a limitation to their application and in the case of water quality, and the statistical difference between marine and freshwater guidelines is insufficient to preclude their combination.

There is clearly merit in the use of effects databases, provided their limitations are acknowledged and they are applied more as screening tools to delineate areas of concern. It is important that data are continually updated and revised and guideline values that are inconsistent with other findings should be the subject of more detailed investigations.

It is worth remarking that the number of significant figures used in the early empirical guideline values were not justified given the appreciable errors not only in the analyses, but in the general level of confidence in the effects data. Appropriately rounded off numbers will be used in the ANZECC/ARMCANZ guidelines.

Logistic regression modelling was applied in a third form of empirical guideline that has increasingly been applied in sediment assessments (Field et al., 1999; 2002). This approach uses statistical analysis of large matching data sets for chemical (18 contaminants) and effects (survival of marine amphipods) to relate chemical concentrations to the probability of sediment toxicity. The data used for each regression analysis were first screened to reduce the influence of samples that did not contribute to the toxic effects associated with that contaminant. The individual models were then combined in a single mixture effects model. While equally as effective as the other empirical approaches (Bay et al., 2012), this approach does not provide specific guideline values, and is therefore a little more complicated to apply.

A number of other effects based approaches have been used, including apparent effects thresholds, sediment effects concentrations, and screening level criteria. These are reviewed in detail elsewhere (Batley et al., 2005). Where several different methods for deriving SQGs result in a quantitatively similar value, consensus guidelines have been developed that are typically calculated as the geometric means of the SQGs from the different methods (MacDonald et al., 2000).

3.2 Mechanistic Guidelines

A mechanistic approach to SQGs uses equilibrium partitioning based on the assumption that the critical factor controlling sediment toxicity is the concentration of contaminant in the sediment pore water (Di Toro, et al., 1991; Di Toro, 2013). Water quality guidelines can be applied to porewater contaminants, and the sediment quality guideline value can be defined by the concentration of contaminant in the sediment that is in equilibrium with the water quality guideline concentration in the pore water. The ratio of the contaminant concentration in the sediment (C_S) and its concentration in the surrounding water (C_W) is defined as the partition coefficient, $K_{D.}$

The approach is attractive to many regulators because toxicity can be predicted using LC50 values obtained from water-only toxicity tests. The major research effort has been devoted to attempts to predict the factors controlling the partitioning of contaminants within the sediment solid phases, into pore waters. The approach is most readily applicable to hydrophobic organic chemicals and has been incorporated for these chemicals in the derivation of equilibrium sediment benchmarks (ESBs) and the NOAA and FDEP guidelines.

An implicit assumption of applying the equilibrium partitioning approach to sediments is that pore waters represent the major uptake route for sediment contaminants. Mechanistic guidelines (the EqP approach) attribute the observed toxicity to the dissolved phase, where organisms receive equivalent exposure from water or from any equilibrated phase, either from pore water via respiration, from sediment via ingestion or other sediment-integument exchange, or from a mixture of exposure routes. This is quite likely to be the case for many hydrophobic organic contaminants (HOCs), although dietary uptake cannot be discounted.

Dietary uptake of metals is increasingly being demonstrated as a major route of metal exposure and accumulation (Luoma and Rainbow, 2005; Simpson, 2005). For deposit or detritus feeders, which comprise a major portion of benthic invertebrates, particulate phases may represent a major route of metal exposure (Rainbow, 2007; Luoma and Rainbow, 2008; Camusso et al., 2012). Only a few studies have attempted to quantify the toxic effects of dietary metal exposure to benthic organisms (Croteau and Luoma, 2009; Casado-Martinez et al., 2010; Campana et al., 2012). Despite the increasing evidence that dietary uptake of contaminants is a significant exposure route, dietary exposure has not been explicitly accounted for in the development of concentration−response models for the prediction of sediment metal toxicity. Although not demonstrated, it may well be that the most sensitive organisms are those that respond to the porewater concentrations of contaminants only, despite the greater 'available' pool of particle-associated contaminants in some sediments.

3.2.1 ORGANICS

For non-ionic organic chemicals, it has been well-established that the partitioning is dominated by sediment organic carbon (Di Toro et al., 1991). For sediments having >0.2 % organic carbon (dry weight), sediment quality guidelines normalised to mg/kg organic carbon, have been shown to be valid for a range of sediment types. This cut-off in organic carbon content is judged to be necessary because at lower organic carbon contents, second-order effects such as particle size and adsorption to non-organic mineral fractions become more important.

The sediment/pore water partition coefficient K_D, is related to the organic carbon partition coefficient, K_{OC}, and f_{OC}, the fraction by weight of organic carbon:

$$K_D = f_{OC} K_{OC}$$

Note that K_{OC} is empirically related to the readily-determined octanol/water partition coefficient K_{OW}.

The equilibrium partitioning model predicts that sediments will be toxic when the pore water concentration exceeds the water-only toxic concentration. Thus if WQG (µg/L) is the no-effect concentration in water, then the sediment quality guideline value SQG (µg/kg) is given by the partition coefficient K_D(L/kg) between pore water and sediment according to the equation:

$$SQG = K_D \, WQG$$

in terms of measurable parameters:

$$SQG = f_{OC} K_{OC} \, WQG$$

Using published data for the toxicity to marine and freshwater amphipods, Di Toro et al. (1991) demonstrated that the incidence of mortality increased dramatically when the ratio of the pore water concentration to water-only LC50, exceeded a value of 1. This agreement implied that benthic organisms were as sensitive as water-column organisms. They rationalised that, if the pore water and sediment are in equilibrium, then the effective exposure concentration is the same regardless of the exposure route.

Using a predicted sediment toxic unit (STU) as given by:

$$STU = (C_S/f_{OC})/(K_{OC}.\ LC50_{\text{water-only}})$$

where C_S/f_{OC} is the organic-carbon-normalised concentration of contaminant in the sediment, a similar plot to that for pore water toxicity can be obtained for a range of organics, with 100% mortality occurring where STU = 1.

The USEPA has accepted the equilibrium partitioning approach for hydrophobic organics and have commissioned a number of studies addressing the derivation of criteria for specific organic chemicals. The criteria have been derived on an organic carbon basis by determining K_{OC} and the WQG according to:

$SQG_{OC} = K_{OC}\ WQG$

The current water quality criteria are used for WQG.

It is important to recognise that the normalisation to organic carbon is only valid when f_{OC} >0.2%, as discussed earlier. For polar organics, the K_{OC} model can overestimate the bioavailable concentration, because adsorption can be enhanced by factors other than hydrophobicity. The general behaviour of such organics will also differ because they are susceptible to a range of degradative and removal processes.

The consideration of mixtures of organics was first applied to PAHs (Swartz et al. 1995) with the development of the ΣPAH sediment toxicity model that used a combination of equilibrium partitioning and quantitative structure activity relationships (QSARs) to derive a mixture toxicity threshold. This was improved by Di Toro et al. (2000) in an approach applicable to all non-ionic organic chemical mixtures.

Equilibrium partitioning sediment benchmarks (ESBs) represent site-specific concentrations for non-ionic organic chemicals in sediments which are protective of the presence of freshwater and marine benthic organisms (USEPA, 2003a,b,c,d; 2008; 2012). This approach utilises EqP relationships to estimate the bioavailability of non-ionic organic contaminants based on their measured sediment concentrations and sediment organic carbon content. A toxic unit approach (based on final chronic values) is then applied to the calculated porewater concentrations of the individual organics to predict the toxicity of the mixture. The EqP relationships are intended to account for the influence of different sediments on the observed biological effects. As a consequence, the ESBs are causally linked to the specific chemical, applicable across sediments, and are considered protective of benthic organisms.

A full spectrum of forms of carbon may exist in sediments, and the differences in these forms will influence the partitioning of the organics. In recognition of this, a two-carbon model incorporating black carbon along with organic carbon is now available for making EqP-based predictions (US EPA, 2012). The direct measurements of non-ionic organic contaminant concentrations in pore water using passive samplers is increasingly being used to validate the EqP model predictions, calculation of their toxic units, and derivation of site-specific ESBs (Maruya et al., 2009; 2010).

The ESB approach has been used for almost 10 years, with increased used for deriving site-specific ESBs (US EPA, 2012). The ESBs do not consider the antagonistic, additive or synergistic effects of other sediment contaminants in combination with the chemical(s) of potential concern or the potential for bioaccumulation and trophic transfer of chemical(s) of potential concern by organisms.

Currently, the ESB models represent an excellent starting point for considering the potential effects of mixtures of non-ionic organic contaminants, and passive sampling techniques provide a basis for validating predictions. Appendix A2 and A3 provide greater details on the application of ESBs for PAHs, endrin and dieldrin.

3.2.2 METALS

The application of the EqP approach to metals is less advanced, and is confounded by the dependence of metal bioavailability on more than one phase in the sediments, and the fact that bioavailability can be ameliorated in the pore waters by complexation with dissolved organic matter.

In anoxic sediments, reactive forms of sulfide described as AVS will regulate the solubility of metals such as cadmium, copper, mercury, nickel, lead, silver and zinc, which form relatively insoluble sulfides (Ankley et al., 1996; USEPA, 2003b; Rickard, and Morse, 2005). The significance of AVS partitioning in controlling metal bioavailability in marine sediments spiked with cadmium was demonstrated by Di Toro et al. (1990, 1992). The key parameter is the difference between AVS and the concentration of the simultaneously extracted metals (SEM=∑Cd,Cu,Ni,Pb,Zn), where both AVS and SEM are operationally-defined respectively as the sulfide and metals liberated from wet sediment by treatment for 30 min with cold 1 M hydrochloric acid. If there is a molar excess of AVS over SEM, then the excess of sulfide will imply no dissolved metals and, therefore, no metal toxicity. If there is no excess of AVS over SEM, then the metals may be present in the pore waters or associated with phases that bind them less strongly and the sediments may be toxic. Based on AVS-SEM theory, ESBs have also been developed for metal mixtures (cadmium, copper, lead, nickel, silver and zinc) (USEPA, 2003a, 2005). Appendix A6 provides greater details of the application of AVS-SEM and ESB approaches for metal mixtures.

Several studies have pointed out limitations to the application of AVS-SEM approach to metals (Simpson et al., 1998, Rickard, and Morse, 2005). The sulfides of Ag, Co, Cu, Hg, and Ni are sparingly soluble in dilute HCl (Simpson et al., 1998; Cooper and Morse, 1998) and so will not appear as AVS, although oxidative release of these metals can occur (Simpson et al., 2000). The tendency may be to overestimate SEM:AVS. They also found examples of metal oxides particles in sediments armoured with sulfide coatings, in which the bioavailability of the metals was minimised by the sulfide coating, yet both oxides and sulfides appeared in the SEM fraction. The reverse may also occur, whereby significant concentrations of AVS are measured, but the AVS is present within particles that are coated with oxidised phases which ultimately control the metal solubility. The latter is more likely to occur near the sediment-water interface (or the wall of organism burrows), where sulfidised particles from deeper in the sediment have been brought into contact with oxygenated overlying water.

Many organisms reside close to the sediment-water interface or create burrows, so the surface sediments with which they are in contact are more oxidized and often contain low AVS concentrations (Naylor et al., 2004; Gallon et al., 2008; De Jonge et al., 2010; 2012; Teuchies et al. 2012). The oxidation of AVS in surface sediments has the potential to increase the bioavailability of both dissolved and particulate metals (Peterson et al., 1996; Eriksson Wiklund and Sundelin, 2002; De Lange et al., 2008; Simpson et al., 2012b). For oxic/sub-oxic sediments, the bioavailability of these metals is strongly influenced by the concentrations of OC, iron and manganese oxyhydroxides and sediment particle size (Besser et al., 2003; Costello et al., 2011; Strom et al., 2011; Campana et al., 2012).

While EqP models have not been developed for oxic sediments, it has recently been demonstrated that toxicity thresholds based on the OC-normalised copper concentration of the <63 µm sediment fraction were effective in predicting sub-lethal and lethal effects to a range of benthic organisms (Simpson et al., 2011; Strom et al., 2011; Campana et al., 2012; Campana et al., 2013). This approach is suggested for copper in Appendix A7.

A significant outstanding issue in the development of SQGs for metals in oxidized sediments is the applicability of the SQG approach to a wide suite of metals, as is achieved for Ag, Cd, Cu, Cr, Ni, Pb and Zn with the AVS-SEM and ESB approaches. Costello et al. (2011) showed that the bioavailability of nickel in surface sediments was more strongly influenced by the concentrations of iron and manganese oxides than by OC. The study reinforced the need for SQGs that vary with the metal-binding properties of the sediments, but indicated that there was not yet a universally applicable model. If future models of metal bioavailability are to be applicable to metal mixtures, then it is likely that the models should explicitly consider pools of AVS, OC and iron and manganese oxides as phases that reduce toxicity.

To validate EqP approaches for metals, the direct measurements of metal concentrations in pore waters is necessary, but this can be challenging due to the influence that small disturbances can have on these concentrations. The DGT technique can potentially provide time-integrated concentrations in pore waters and fluxes of bioavailable metals at the sediment water interface (Tankere-Muller et al., 2006; Simpson et al., 2012). Like passive sampling techniques for organics, the application of DGT is expected to increase, and provide useful information for validating EqP approaches.

3.3 Field-based SSD-derived Guidelines

Most forms of SQGs have been derived from effects data generated from laboratory ecotoxicity bioassays, supplemented with ecology data (Long et al., 1995; MacDonald et al., 2000). As discussed above, there are many limitations to these approaches. There have been attempts to derive SQGs from field-based species sensitivity distributions (f-SSD), utilizing field data on benthic communities and contaminant loadings concurrently measured in sediment samples (Leung et al., 2005; Kwok et al., 2008; Hewitt et al., 2009). The f-SSD method can use a quantile regression method to derive SQGs for single contaminant with consideration of the presence of other contaminants and biological interactions. More recently, a field-based community sensitivity distribution (f-CSD) approach has been proposed that utilises an empirical Bayesian method to model the toxicity effect of contaminants on the species density of benthic infauna (Leung et al., 2005). The CSD distribution allows the percentage of species being affected (a drop in species density) to be calculated for a given contaminant concentration and provides a lower confidence limit (Lui et al., 2013).

These new approaches appear particularly useful for the validation of existing guidelines and for the derivation of multiple-stressor site-specific guidelines that better account for the interactions of chemical stressors and naturals stressors, e.g. variations in temperature, salinity, dissolved oxygen, and food availability.

The f-SSD approach of Kwok et al. (2008) was applied to sediments from Hong Kong and resulted in threshold values for a range of metals, PAHs and PCBs that fell within the range of current SQGs. This is not the case with measurements of copper, lead and zinc in a New Zealand study by Hewitt et al. (2009), where values were significantly below both the ANZECC/ARMCANZ (2000) and the ERL values. The endpoint in these studies is effects on communities rather than toxicity per se and unless there is a distinct concentration gradient, relating the observed effects to specific contaminants remains confounded by co-occurring contaminants, factors that influence contaminant bioavailability, as well as other physical and chemical parameters. Such studies are better included as part of an ecology LOE.

3.4 Porewater Guidelines

In some instances pore waters may represent the dominant phase in which a contaminant or naturally forming chemical stressor is found. This may be a consequence of its formation in this phase as a result of chemical and microbiological processes, and/or because of its high aqueous solubility. With respect to naturally forming chemical stressors, ammonia is a case in point, as are nutrients such as nitrate and nitrite. As discussed in Part 1, in such cases it is generally appropriate to apply the WQGs, or equivalent values derived using pore water toxicity testing with benthic organisms. In the case of porewater ammonia, a WQG of 4 mg total NH_3-N/L is introduced in these guidelines, based on the data discussed below.

Ammonia is a potentially highly toxic naturally-occurring constituent of sediment pore waters, and is generally not considered a contaminant of concern in the regulation and management of sediments (e.g. dredged material). The toxicity of ammonia is influenced by the temperature and pH of the water and at elevated levels has the potential to confound interpretation of sediment toxicity tests using sensitive species, and influence the distribution of infaunal species measured in ecological impact studies.

Canada has no recommended guideline for ammonia in marine waters (CCREM, 2000). The European Union directive for fresh water has guidance and imperative values of 200 and 1000 µg total NH_3-N/L respectively (EU, 2006). The USEPA have a chronic saltwater criterion of 35 µg NH_3/L (equivalent to 760 µg total NH_3-N/L at 20^{o}C and pH 8.0), developed from two saltwater and four freshwater species (USEPA, 1989). Boardman (2004) has suggested that this value be increased by a factor of 2.31 based on the inclusion of his toxicity data for four additional species. All are, however, acute data, converted using acute to chronic ratios.

A review of acute and chronic effects data for marine waters and sediment pore waters by Batley and Simpson (2009) found that it was appropriate to revise the guideline for ammonia and proposed a threshold value for ammonia in sediments. Using species sensitivity distributions, a new trigger value of 360 µg total NH_3-N/L was derived for slightly to moderately disturbed systems (95% protection concentration, PC95), with a value of 100 µg total NH_3-N/L applying to waters of high conservation value (PC99).

Several large data sets are available for ammonia in estuarine and marine sediments that represent background porewater concentrations. A review of porewater ammonia concentrations in 322 estuarine/marine sediments in the United States showed a log-normal distribution of data with a mean concentration of ammonia concentrations (mean ± standard deviation) of 9.0 ± 15 mg total NH_3-N/L (10.9 mg total NH_3/L) for marine sediments excluding dredged material, and 40.7 ± 38.9 mg total NH_3-N/L (49.4 mg total NH_3/L) for dredged materials only (Sims and Moore, 1995; Moore et al., 1997). Studies from Australian sites show similar results. In 68 sediment samples from 17 locations (4 replicates/location) in Sydney Harbour, measured porewater ammonia concentrations were 3.7 ± 2.4 mg total NH_3/L, with a maximum value of 13.6 mg total NH_3/L (Chariton et al., 2010a). Despite these observations, in most situations the sediment ecological health appeared to be unaffected by the elevated porewater ammonia concentrations (Chariton et al., 2010a).

In sediments from ship navigation channels, ammonia concentrations can exceed 200 mg NH_3-N/L (Ankley et al., 1990; Schubauer-Berigan and Ankley, 1991; Stronkhorst, 2003). In general, the pore waters of silty sediments that have high concentrations of organic matter are expected to contain higher concentrations of total ammonia (e.g. 2-20 mg NH_3-N/L), while for sandy sediments with low organic content the concentrations of total ammonia are expected to be lower (e.g. <0.5 mg NH_3-N /L).

The large amount of variability in porewater ammonia data and lack of suitable of cause-effect relationships prevents the derivation a risk-based guideline for ammonia. However, to better consider potential degradation of nitrogen cycling reactions that may be occurring due to anthropogenic activities, a WQG of 4 mg total NH_3-N/L is recommended. This value was derived from the 80th percentile of background data from Sydney Harbour.

In whole-sediment toxicity tests, the flux of ammonia from the pore waters can result in significant effects, that in a natural system would be diluted by mixing currents, and requires additional consideration (see Part 2, Section 4.2.3). A comparison of the toxicity of ammonia in spiked-sediment versus water-only exposures has shown good correspondence between the LC50 values for the infaunal *L. variegatus* and the chironomid *C. tentans*, indicating that ammonia bioavailability and toxicity may be accurately predicted from porewater concentrations for some species (Whiteman et al., 1996). However, the epibenthic *Hyalella azteca* exhibited a behavioural response and apparently avoided the spiked sediments being frequently observed in the overlying waters. Ammonia toxicity tests on New Zealand invertebrate species suggest that they may be among the more sensitive species (Hickey and Vickers, 1994).

For Australian marine organisms used in whole-sediment toxicity tests, EC50 values range from ~20 mg NH_3-N/L for 10-day lethality to juvenile *M. plumulosa* to >450 mg NH_3-N/L for 24-h esterase inhibition in the benthic algae, *Entomoneis* cf *punctulata,* (CSIRO unpublished results). For the Australian marine benthic bivalve, *Tellina deltoidalis*, the 10-day whole-sediment LC50 is approximately 67 mg NH_3-N/L (King et al., 2010). These effects thresholds can be compared to marine amphipods used for toxicity tests in North America (*Ampelisca abdita, Rhephoxius abronius, Eohaustorius estuarus, Grandidierella japonica, Leptocheirus plumulosus)* that have 96-h LC50 values for total ammonia ranging from 50 mg NH_3-N/L (*A. abdita*) to 130 mg NH_3-N/L (*E. estuaries*) (Kohn et al., 1994; Moore et al. 1997). For porewater toxicity tests using Australia biota, EC50 values are ~10 mg NH_3-N/L for the chronic 72-h growth rate for the algae *Entomoneis* cf *punctulata* and *Nitzschia closterium* (CSIRO, unpublished results), ~3 mg NH_3-N/L for 48-h larval development for the oyster *Saccostrea* sp., and 1-h fertilisation of the sea urchin *Heliocidaris tuberculata* (Ecotox Services Pty Ltd, unpublished results).

3.5 Guidelines Based on Toxicity Testing

As is done for water quality guidelines, toxicity testing of spiked sediments can be used to generate SQG trigger values. The success of this approach depends on the number of test species used. In the European Union’s Water Framework Directive recommendations for sediment quality standard derivation (EU WFD, 2010), one option is the use of long-term whole sediment laboratory toxicity tests with sediment organisms and spiked field sediments. Assessment factors are applied to the tests as follows: for 1 long-term test (EC10 or NOEC), divide by a factor of 100; for two long-term tests with species representing different living and feeding conditions, divide by 50, and for three such tests, divide by 10. A factor of 1000 is used for short-term tests. Again, as noted for water quality guidelines, the use of assessment factors is not the preferred approach, with the application of species sensitivity distributions (SSDs) to datasets containing At least seven species from four taxonomic groups recommended. In the case of sediments, however, it was recognised that these minimum data requirements will rarely be met.

One successful application of SSDs to SQG derivation is the case of copper, as discussed in Appendix A7. This is a more generic approach taken into account sediment particle size and chemical composition. Site-specific guideline derivation based on the spiking of field sediments, could be quite an expensive exercise.

3.6 Approaches to SQGs in the EU and Norway

3.6.1 EU GUIDELINES

After a prolonged consideration, the European Union (EU) as part of its Water Framework Directive has recently provided technical guidance for the derivation of sediment quality guidelines (standards in the EU) (EU WFD, 2010). This encompasses all of the methods that have been discussed above. The recommendations suggest that the following are suitable for deriving the sediment quality standards ($QS_{sediment}$):

(i) ecotoxicity data from experiments with benthic organisms

(ii) water column ecotoxicity data used with equilibrium partitioning

(iii) empirical field or mesocosm data.

Unless a large number of tests undertaken, guidelines based on spiked sediment toxicity testing (Section 3.5) are likely to be highly conservative. Equilibrium partitioning requires some knowledge of the sediment:water partitioning coefficient, which, as already noted, will be problematic for metals, and marginally more reliable organics. The EU document acknowledges the uncertainty in uptake pathways when $\log K_{ow}>5$ by dividing any such derived $QS_{sediment}$ value by 10.

The empirical field data refer to ERLs and TELs, and other empirical derivations, as well is the use of field-based SSDs. Their acceptability is qualified by the following:

1. If the TEL or ERL or mesocosm NOEC/EC10 is higher than or equal to the quality standard derived from available ecotoxicity data, either the latter is used as the $QS_{sediment}$, or there may be a case for reducing the size of the application factor applied to the laboratory data, but only if the field or mesocosm data are reliable.
2. If the TEL or ERL is lower than the $QS_{sediment}$, derived based on ecotoxicity tests, there might be case are increasing the size of the application factor if the field or mesocosm data are reliable.
3. If the TEL or ERL is higher than or equal to the value calculated by applying equilibrium partitioning, the latter is used to the derivation of the $QS_{sediment}$.
4. If the TEL or ERL is lower than the value calculated by applying equilibrium partitioning, the former value is used with an assessment factor to derive the $QS_{sediment}$. This factor would be set at five.

More specific details are provided on the bioavailability issues, background concentrations and dealing with substances that bioaccumulate or biomagnify.

3.6.2 NORWEGIAN GUIDELINES

One of the more different approaches to SQG derivation was that adopted by Norwegian authorities (SFT, 2007; Bakke et al., 2010). Their 1997 guidelines had been based not on biological effects, but on intervals of excesses of concentrations over background concentrations. Those guidelines considered 5 levels of contamination determined from frequency distributions of selected contaminants in the field, including for mercury and dioxins, a consideration of health as well as environmental effects. In updating this in 2007, they largely followed the approaches being developed by the EU Water Framework Directive

(EU WFD, 2010), but retaining the five classes of contamination, background, good, moderate, bad and very bad. The boundaries between these classes now represented respectively, the upper limit of background concentrations, the predicted no effects concentration for chronic exposure PNEC$_{chronic}$, the PNEC for acute exposure, and 2-10 times the acute PNEC. Where a sediment quality standard has been proposed in the European Union Water Framework Directive, it was used instead of the PNEC$_{chronic}$.

The Norwegian approach goes further than ANZECC/ARMCANZ (2000) in identifying more than two levels of protection for sediments in the way that ANZECC/ARMCANZ does for waters. Multiple levels of protection can also be derived from any SSD-based guideline derivation, such as that of Kwok et al. (2008).

3.7 Reliability of the Australian and New Zealand Effects-based Guidelines

The effects-based guidelines that were adopted in 2000 for Australia and New Zealand were primarily derived from the effects range-low (ERL) and effects-range median (ERM) values from Long et al. (1995) and were based on a single large biological effects dataset of North American sediment data with appropriate rounding off in keeping with their precision. They also included some metal guidelines adopted for Hong Kong (Chapman et al., 1999). In this revision, the guideline values for metals and metalloids are unchanged, however the values for organics now mostly correspond to the threshold effects levels (TEL) and probable effect levels (PEL) derived by MacDonald et al. (1996). For some of the contaminants (e.g. PAHs, TPHs, TBT), revised or new values have been provided based on improved effects data and guideline derivation approaches (see Appendix).

Concerns continue to be expressed for the reliability of particular guideline values, however, it is important to recognise that as multiple stressors will be present in most sediments, and organisms will have the varying responses to different stressors, that the guidelines will never provide an ability to predict when ecotoxicological effects will occur. The purpose of the SQGVs is to act as a guide to when effects will not occur in any sediments, and the upper guideline values as a guide to when effects may become more likely to occur.

In this context, it is important to consider the methods of derivation of the guidelines (see Section 3.1). It has been commented that agreement within a factor of three between freshwater and marine guidelines is considered good (Smith et al., 1996) and that might reflect one view of the uncertainty. We know that because the empirical guidelines were derived from a ranking of toxicity and other effects data, and because contaminants typically co-occur (e.g. metals and organics), then any toxicity was equally attributed to all components of the mixture. For example, toxicity may be due to high concentrations of PAHs in a sediment, but the toxicity is equally ascribed to say cadmium, which may be present but at concentrations that were not causing effects. The cadmium guidelinethen becomes overly conservative. For sediments where the toxicity is truly a result of the cumulative effects of multiple contaminants, e.g. from PAHs, cadmium and other contaminants in combination, the approach is more appropriate, but the individual guidelines derived still remain quite conservative.

Further, it is recognised that the database is largely derived from acute toxicity effects, albeit to sensitive species. As newer tests evolve with chronic endpoints, it is clear that effects will be seen at lower concentrations. Until a wide range of single species chronic guidelines are developed, we will be unable to reliably define ‘safe’ concentrations for individual contaminants in sediments with respect to longer-term chronic effects.

For the most part, the concern will be for defining potential impacts when the defined trigger values are exceeded, and this will cover the uncertainties with conservative guidelines, co-occurrence and mixture toxicity. Where there is a concern that the trigger value is not sufficiently conservative, it is here that a manager might request more detailed investigations using other LOEs. Note that it has always been recognised that chemical assessment is but one LOE, although it is the common starting point. When other LOEs might suggest that effects may be occurring, where the chemistry does not, and this is the opportunity for the WOE approach to provide the solution.

In other cases, there have been concerns that the guideline SQGVs are not sufficiently protective. This has largely been observed in attempts to link contaminant concentrations to ecological effects in field studies in New Zealand (Hewitt et al., 2009). WOE studies would also be useful in such cases to try and deconvolute the effects of multiple stressors. More research is required, however, before f-SSDs from such studies can be reliably used to derive site specific guidelines.

3.7.1 LEVELS OF PROTECTION

As already noted, the SQGVs effectively only consider one level of protection, based on the 10th percentile of effects data. These SQGVs, if exceeded, are triggers for further investigation. There will, however, be instances in which the sediments might require a lesser level of protection. For example, sediments in a dockyard or marine environment would clearly be classified as highly disturbed, and while ever this usage remained, the likelihood of a pristine or even a slightly-to-moderately disturbed environment being achieved in these locations would be remote. In such instances, the upper guideline, SQG-High, might be a more reasonable concentration limit. In instances where large datasets for either toxicity or ecology have been accumulated, the delineation of 95th, 90th, and 80th percentiles from SSDs, would provide values for additional levels of protection. However, while large error bars and areas of uncertainty remain with the derived guidelines, attempting to delineate these cannot be justified.

3.8 Guidelines for the Ocean Disposal of Dredged Sediments

The Australian National Assessment Guidelines for Dredging (NAGD, 2009) have recently been released, replacing the National Ocean Guidelines for Dredged Material released in 2002. The document outlines methods for sediment sampling and analysis, sediment quality assessment and biological testing. The guideline values are identical to those proposed in this document, with the lower and upper guideline values being referred to as Screening and High Levels respectively.

The sediment quality assessment procedure used in the ocean disposal guidelines is as follows:

(i) Where data were available to establish the regional concentrations in the sediments of the receiving area, the mean value of such concentrations was used as the background level for naturally occurring substances. A lower (reference) screening level is developed by multiplying the background level by two to account for sampling and analytical variability and the range of natural values in the area. Where background data were not available, the screening values were used.

Dumping is permitted where the mean of all contaminants to be dredged is below the Screening Level. It is likely that where clay/silt sediment is being dumped on sand that even uncontaminated sediment would fail the twice background criteria, and then the Screening Guidelines are applied.

(ii) Where the mean concentration of one or more contaminants is between the Screening and High Levels, further assessment is required including the determination of acute sediment toxicity on suitable test organisms. Where one or more contaminants is above the High Level, the sediment is unsuitable for disposal at sea, although this may be justified if the results of further sediment bioassays, including an evaluation of sub-lethal toxicity and bioaccumulation, show that the material is non-toxic.

The Screening Level values are considered to be tentative and are to be revised as international criteria are updated, and/or Australian criteria are developed. The value for radionuclides is the maximum specified by Australian ocean dumping legislation. For organochlorine pesticides where reliable detection levels are close to screening levels, it was recommended that a case-by-case assessment be made.

Sediment toxicity testing using protocols such as those developed in Australia (Simpson et al., 2005) or used in the US (USEPA, 1994) were considered to be the most appropriate for predicting the bioavailability, toxicity and bioaccumulation potential of contaminants in sediments.

Elutriate testing (USEPA, 1994) was used to determine the water quality impacts of disposal. Using a 1:4 dilution and a four-hour mixing, the results are appraised against ANZECC/ARMCANZ (2000) marine water quality guidelines, taking into account appropriate dilution factors.

The NAGD (2009) have slightly different objectives to the ANZECC/ARMCANZ SQGs since they are considering the impact on biota both during ocean disposal and transport through the water column and on their deposition on the sea floor. In the latter case, the same guidelines will apply, whereas the release during mixing with seawater will be estimated by elutriate test data judged against water quality guidelines. The NAGD guidelines follow a similar WOE approach.

4. Sediment Quality Assessment

4.1 Sediment Chemistry and Bioavailability Testing

The assessment of sediment chemistry, involves the measurement of total contaminant concentrations, together with supporting information on modifying factors such as TOC, AVS, grain size, as already discussed in Part 1. The initial screening for COPCs is made on the <2 mm sediment fraction to exclude low surface area materials such as coarse minerals, shell and wood debris, that bind limited amounts of contaminants. As noted earlier, the <63 µm sediment fraction is mostly readily resuspended or potentially ingested by organisms, and assessment of the concentrations and bioavailability of contaminants associated with this fine sediment fraction can be considered when more detailed investigations of contaminant bioavailability are required.

It is possible for the total concentrations of particular contaminants to exceed their respective SQGVs and even the SQG-high values, but no toxic effects can be observed, because not all of the contaminants are in bioavailable forms (Simpson and Batley, 2007; SERDP/ESTCP, 2008; Ahlf et al., 2009; Maruya et al., 2010; Burgess et al., 2013).

The bioavailability of metals in sediments is complex and influenced strongly by:

(i) speciation (e.g. metal binding with particulate sulfide, organic carbon, and iron hydroxide phases, pH and redox potential),

(ii) sediment-water partitioning relationships,

(iii) organism physiology (uptake rates from waters, assimilation efficiencies from particulates), and

(iv) organism feeding and other behaviour (feeding selectivity, burrow irrigation) (Luoma and Rainbow, 2005; Simpson and Batley, 2007).

The bioavailability of hydrophobic organic contaminants (HOCs) (e.g. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)) is also affected by organism physiology and sediment characteristics (Moore et al., 2005; Cornelissen et al., 2005; Qui and Davis, 2004). The bioavailability of HOCs is complicated by the large range of different types of particulate organic carbon (OC) phases to which HOCs may bind (e.g. black carbon), the concentrations of these OC phases, and the properties of the HOCs that determine their binding strength and desorption rates.

In the case of PAHs, if the source is petroleum, the potency will be strongly influenced by the alkylated compounds and an assessment that only analyses the parent PAHs may grossly underestimate the overall potency of the mixture (USEPA, 2003d; Hawthorne et al., 2006; Driscoll and Burgess, 2007). Consequently, it is recommended that 34 PAHs are measured, including 18 parent PAHs and 16 C1 to C4 alkyl PAH derivatives (see Appendix A2.1, USEPA, 2003a).

The use of bioavailability tests in sediment quality assessment can be used to modify SQGVs for specific COPCs or to provide additional information on cause-effect relationships.

4.1.1 BIOAVAILABILITY TESTS TO MODIFY SQGVS

Mineralised forms of metals

Mineralised forms of metals are not considered bioavailable as they do not easily dissolve in the water or during the passage through organism guts. The comparison of concentrations of total metal concentrations and dilute acid-extractable metals provides information on the extent to which the metals are mineralised.

Total particulate metals (TPM) analyses are usually analysed using microwave-assisted digestion using a mixture of nitric, hydrofluoric and hydrochloric acids (or combinations). For most metals, the most reactive and bioavailable fraction (i.e. the metal fraction of interest in sediment quality assessments) are those that can be easily extracted with cold dilute acid. Weakly extractable, dilute-acid extractable metals (AEM) are determined by reacting the sediment (1-10 g/L) with cold 1 M hydrochloric acid for 30 min (although variations on the HCl concentration 0.5-2 M and time 0.5-1 h have also been used). The use of higher acid molarities (e.g. 6 M HCl) will result in metal extractabilities that are more similar to TPM and this will reduce the benefits of the AEM measurement for assessing metal bioavailability (Simpson and Batley, 2007). The 1 M HCl extraction is analogous to the extraction of metals used in the SEM (simultaneously extractable metals) analysis and aids comparison of results between sites USEPA, 2003b).

For metals such as Ag, Cu and Hg, that are recognised as being sparingly soluble in dilute HCl (Simpson et al., 1998; Cooper and Morse, 1998), a combination of AEM and TPM measurements may be necessary to accurately describe the forms and potential bioavailability. For example, metal sulfide phases or metals associated with organic polymers (e.g. antifouling paints, tyre rubber) may change over time, and the measurement of TPM allows the potential future transformations of these metals into more bioavailable forms to be adequately considered (Turner and Hallett, 2012; Simpson et al., 2013). Furthermore, the potential contribution of metals associated with fine sediments to dietary effects in benthic organisms that ingest sediments is not yet fully understood (Simpson and Batley, 2007).

Acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM)

As already discussed, the AVS content of a sediment is a key modifier of the bioavailability of several metals (USEPA, 2003b; Simpson and Batley, 2007). Trace metals in sediments are generally believed to react with FeS (the major component of AVS) to form metal sulfides according to:

$$\mathrm{Me}^{2+} + \mathrm{FeS(s)} \rightarrow \mathrm{MeS(s)} + \mathrm{Fe}^{2+}$$

In general, appreciable concentrations of Ag, Cd, Co, Cu, Hg, Ni, Pb and Zn are not expected to be observed in pore waters until the reservoir of FeS is exhausted. Measurement of AVS concentrations (mmol/kg) and comparison against the molar sum of acid-soluble metals (often termed simultaneously extracted metals or SEM, mmol/kg) is a useful indicator of the bioavailability of metals in sediments. If AVS is greater than SEM, the metals are likely to be bound in sulfide complexes with greatly limited bioavailability. However, if AVS < SEM, metals may or may not be toxic due to other controlling factors (e.g., TOC, iron hydroxides).

AVS is operationally defined as the fraction of sulfides extracted from sediments by cold dilute HCl (usually 0.5-1 M HCl for 0.5 to 1 h) (Simpson et al., 2005). Iron and manganese monosulfides constitute the majority of the sulfide extracted by AVS methods. SEM is calculated as $\sum$(Ag,Cd,Cu,Ni,Pb,Zn) in mmol/kg. The differential approach (SEM-AVS) is the preferred representation of results, rather than the more traditional ratio approach (SEM/AVS), which tends to misrepresent available concentrations of SEM at low AVS concentrations.

In evaluating AVS, it is obviously important to specifically consider surficial sediments where AVS concentrations will be lower, as well as analysing deeper sediments, rather than a pooled sample over a range of depths. Seasonal changes can also influence the AVS-SEM relationship.

The application of AVS-SEM measurements to modify SQGVs is described in the Appendix A6.

Other modifying factors

The pH and redox potential of the sediments may also significant influence metal speciation. For example, in sediment having a low redox potential, iron and manganese oxyhydroxide (e.g. FeOOH, MnO_2) phases may reductively dissolve to form Fe(II) and Mn(II) resulting in the release of metals which were adsorbed to these phases. At lower redox potentials, AVS will begin to form and chromium if present in pore waters should be in the form of Cr(III), rather than the more toxic Cr(VI) form. Despite this knowledge, pH and redox potential measurements cannot be used to accurately predict metal partitioning or bioavailability and their use alone is not recommended for sediment quality assessments. It is recommended that pH and redox potential be recorded to assist in the evaluation of factors influencing the toxicity of the sediments.

4.1.2 ADDITIONAL BIOAVAILABILITY TESTS

Porewater contaminants

Contaminants in dissolved forms are considered to be more bioavailable than those associated with solids. Consequently, direct measurement of COPCs in sediment-associated waters, including pore water, burrow water and overlying water, can provide valuable information for assessments. Because many benthic organisms are in direct contact with sediment-associated waters, they can be considered to be a major exposure pathway for benthic organisms. The concentrations of contaminants in the pore waters of surface sediments (0-1 mm) will control the flux of contaminants to the overlying waters or into the burrows of organisms. For sediments where pore waters cannot be easily extracted, sediment elutriates prepared by shaking sediments with water will often provide a useful surrogate for pore waters.

Concentrations of COPCs in sediment-associated waters should be compared to WQG concentrations unless effects data are available for sediment-associated waters (e.g. copper).

Hydrophobic organic contaminants (HOCs)

Traditionally the bioavailability of sediment-bound HOCs has been determined using field-collected organisms or bioaccumulation assays of 2-6 week test duration (Moore et al., 2005). Measurement of the bioavailable fraction of HOC is sediments may also be undertaken using passive samplers and biomimetic methods designed to mimic the uptake and accumulation of organic contaminants from both water and sediment phases. Passive sampling techniques have developed considerably recently and the use of semi-permeable membrane devices (SPMDs), polyethylene devices (PEDs), solid phase microextraction (SPME), polyoxymethylene (POM) samplers, and Tenax desorption techniques and gut fluid mimics (MacRae and Hall, 1998; Leppänen and Kukkonen, 2000; Cornelissen et al., 2001; Vinturella et al., 2004; Qiu and Davis, 2004; Maruya et al., 2010; Ding et al., 2012; Mackenbach et al., 2012; USEPA, 2012). Biomimetic approaches include gut fluid mimics (MacRae and Hall, 1998; Voparil and Mayer, 2004), and while these are not substitutes for organisms, they provide complementary or preliminary information for toxicity testing, e.g. allowing a more efficient use of biota and a more refined understanding of toxicant exposure. The advantages and disadvantages of biomimetic devices have been discussed by Moore et al. (2005).

Hydrophobic organic contaminants are generally much more strongly associated with (partitioned to) black carbon (pyrogenic carbon, soot, coal particles) than other forms of natural organic matter (Koelmans et al., 2006). Relationships between toxic effects of HOCs and sediment organic carbon concentrations, organic carbon forms (e.g. black carbon) and sediment particle size may improve concentration-response relationships (Simpson et al., 2007).

Combined organic carbon and black-carbon-normalised EqP models that use Freundlich isotherms for the combined adsorption behaviour (Accardi-Dey and Gschwend, 2002, 2003; Qui and Davis, 2004; Koelmans et al., 2006) may be used where sufficient information on the speciation of carbon is available (USEPA, 2012).

4.1.3 EXAMPLES OF THE CHEMISTRY LOE

Examples of the chemistry LOE scoring are provided in Table 5, using both sediment and porewater data. The sediment measures include chemistry-based measures of bioavailability modifiers. Similar bioavailability considerations could also be applied to porewater analyses. The examples shown are representative of real cases. In case C1, a single (class of) contaminants has a score of 3 and the WQGs are considered quite robust. In case C5, a single contaminant is driving the score of 3 and given the uncertainties of the GVs, this score is not as strong as one in which there are other contaminants exceeding GVs. In case C7, the fact that acid-extractable metals (AEM) are below GVs makes that score at best 2, which is considered conservative. In case 8, the fact that there is a molar excess of AVS compared to SEM (equivalent to AEM) offers protection against effects, but does not completely eliminate possible effects from metal sulfide oxidation or dietary exposure so the score is 2.

4.2 Sediment Toxicity Testing

The evaluation of sediment toxicity through laboratory or field bioassays is an important LOE for assessments. The toxicity tests are designed to determine whether the whole sediment, or sediment-associated water in the case of pore water tests, may cause toxic effects to individual species of biota (ASTM, 2003; Carr, 1998; Environment Canada, 1998; Simpson et al., 2005; USEPA, 1994).

The exposure route to sediment contaminants for other benthic organisms has been assumed to principally involve pore waters, but it is now recognised that the ingestion of sediment particles and dermal exposure can also be important exposure mechanisms. Benthic biota can include surface-dwelling filter feeders (mussels, oysters) and grazers (amphipods, harpacticoid copepods, snails, shrimps), burrowing organisms that may filter feed and/or deposit feed (amphipods, bivalves, crabs, polychaete worms, and shrimps). In addition, there are organisms living in intimate contact with the sediment, such as benthic algae or rooted plants that are incapable of ingesting particulate materials. The assessment of toxicity should include organisms with a range of behaviours.

4.2.1 TOXICITY TEST SPECIES

The toxicity test procedure used can have a major influence on the outcome of LOE score for ecotoxicology. No one organism is best suited for all sediments and generally, a range of organisms, having differing exposure pathways and test endpoints (e.g. survival, growth, reproduction) should be used for ecotoxicological assessment of contaminated sediments. Different benthic species have different sensitivities to different toxicants. It is therefore important in sediment quality assessments to use a suite of tests with organisms having different feeding strategies and behaviours, to cover all potential routes of exposure.

Whole-sediment toxicity tests use sediment-dwelling organisms or biota that live in close association with the sediments should be used in preference to toxicity tests on sediment-associated waters that have been isolated from sediments (e.g. pore water, elutriates). In general, elutriate tests are not preferred, except for assessing the release of contaminants from dredged sediments as part of the assessment process of sediments for ocean disposal. Solvent extracts of sediments are also not recommended due to their lack of environmental relevance. Porewater chemistry can change rapidly following extraction from the sediment, and particularly important is the loss of contaminants through adsorption to containers or volatilisation. In addition, many benthic species ingest sediments and this dietary route of exposure to contaminants is not measured in porewater tests.

Simpson et al. (2005) discussed a variety of pore water and whole-sediment toxicity tests, however, a range of more robust sub-lethal tests have since been developed using Australian species (Mann et al., 2009; 2010; Ward et al., 2011; Perez-Landa and Simpson, 2011) In summary, it is now possible to routinely undertake sub-lethal whole-sediment toxicity tests using benthic alga, benthic copepods, and epibenthic amphipods, and acute lethality tests with benthic copepod, amphipod, bivalve and polychaete worm species. Apart from algae, that live at the sediment-water interface and are exposed to dissolved contaminants, most benthic organisms have dissolved and sediment ingestion contaminant exposure routes, although the relative importance of the exposure pathways will vary between tests species. Juvenile life stages are more sensitive than adult life stages. Sub-lethal, partial life-cycle toxicity tests (10 days) are now available for whole-sediment that utilise the amphipod, *Melita plumulosa* (Mann et al., 2009) and the benthic copepod, *Nitocra spinipes* (Perez-Landa and Simpson, 2010). For *M. plumulosa*, full lifecycle chronic tests were initially developed for *M. plumulosa* (Gale et al., 2006), but due to the duration and labour required to perform, they are not useful for most assessments. The partial life-cycle toxicity tests consider ovarian to embryo development in *M. plumulosa*, and reproductive output of the copepod *N. spinipes* following exposure to undiluted test sediments and can provide useful results for 10-day exposures. Both species are amenable to testing for the effects of contaminants in a full spectrum of sediment types (sand to silt, high to low organic carbon) and are proving to be robust organisms for assessing sediment toxicity. For both tests, sub-lethal effects were consistently observed when sediment contaminant concentrations exceeded SQG concentrations (Simpson and Spadaro, 2011; Campana et al., 2012). Variations in sediment particle size and organic carbon content did not affect endpoint variability, which was typically less than 10% (standard error).

A 10-day whole-sediment acute toxicity test using juveniles of the epibenthic amphipod species, *Melita plumulosa,* is currently the most robust acute test available in Australia (King et al., 2006a,b; Simpson et al., 2005, Spadaro et al., 2008). Of six amphipods (endemic to Australia) evaluated as possible species for use in whole-sediment toxicity tests, *M. plumulosa* was the most sensitive to contaminants and has the advantage of being easily cultured in the laboratory. The tube-dwelling amphipod, C*orophium colo*, also used for whole-sediment toxicity tests, was significantly less sensitive to contaminants. Research has shown that *M. plumulosa* ingest significant amounts of sediment while feeding and therefore are exposed to contaminants from both dissolved and sediment pathways (King et al., 2005, 2006a). For *M. plumulosa,* 7-day old juveniles are approximately three times more sensitive than adults (CSIRO, unpublished results).

Of the other species currently used in Australia for whole-sediment toxicity tests, the benthic marine microalga, *Entomoneis* cf *punctulata*, is the most thoroughly developed and ultilised (Adams and Stauber, 2004; Simpson et al., 2007). The test endpoint is based on 24-h inhibition of esterase activity within the algae, and while it can be criticised for being a 'biomarker' response rather than a toxicity endpoint, good concentration-response relationships are achieved for the assessment of hydrocarbon-contaminated sediments (Simpson et al., 2007). Whole-sediment acute toxicity test methods have been developed that use Australian bivalves (*Tellina deltoidalis)* and polychaete worm (*Australonereis ehlersi*) species (King et al., 2004), and other amphipods (*Corophium minor*), but they are not considered as robust as the tests using *M. plumulosa.*

Increased numbers of false-positives (toxic effect due to non-contaminant stressors or test artefacts) are often encountered (and expected) when moving from acute lethality endpoints to chronic test endpoints. For this reason, in many assessments more useful information may be obtained by undertaking a greater number of acute lethality tests using robust methods, than undertaking fewer chronic tests.

The number of available tests continues to grow, and these can be followed through the literature or through local experts.

Table 5. Line-of-evidence decision matrix examples for chemical assessment [a]

Case	Assessment based on SQGVs				Assessment based on WQGs			LOE Score	Explanation
	1	2 (COPC)	Modifier	3 (COC)	1	2 (COPC)	3 (COC)		
C1	1						OCs	3	SQGV not exceeded; WQG exceeded (>HC10) for OCs. (sandy sediments not binding high OCs)
C2		Cr, OCs/OPs					TPHs	3	SQGV exceeded (<SQG-high) for Cr, OCs; WQG exceeded (>HC10) for TPHs (oils).
C3		Cr, Pb, Zn PAHs		Cu	1			3	SQGV exceeded (<SQG-high) for Cd, Cr, Pb, Zn, PAHs; SQGV>SQG-high for Cu.
C4		As, TPHs					As	3	SQGV exceeded (<SQG-high) for As, TBT; WQG exceeded (>HC10) for As.
C5				Dieldrin	1			3	SQGV exceeded (>SQG-high) for dieldrin.
C6		Ag, Cd, Ni, Hg, Pb, Zn	SEM-AVS <0					2	SQGV exceeded (<SQG-high) for Ag, Cd, Hg, Ni, Pb and Zn, **but** bioavailability tests using SEM-AVS method indicate that these metals are of low bioavailablity.
C7		2 (3)	AEM low, mineralised	Ag, Cr, Hg, Ni, Pb, Zn				2	SQGV exceeded (>SQG-high) for Ag, Cr, Hg, Ni, Pb and Zn, **but** bioavailability tests measuring AEM show that the metals are mineralised and not bioavailable. SQGV score changed from 3 to 2.
C8		PCBs, PAHs	Biomimetic tests					2	SQGV exceeded (<SQG-high) for PCBs and PAHs, **but** bioavailability tests using SPMDs show that the PCBs and PAHs are not bioavailable.
C9		Pb, Zn, PAHs				Zn		2	SQGV exceeded (<SQG-high) for Zn, PAH;, WQG exceeded (>HC5) for Zn.
C10		Cr, Cu, V, TBT				Cu		2	SQGV exceeded (<SQG-high) for Cr, Cu, V, TBT; WQG exceeded (>HC5) for Cu.
C11		PAHs, Zn			1			2	SQGV exceeded (<SQG-high) for Zn, PAHs.
C12		TBT, OCs			1			2	TBT sediment TC>SQGV<SQG-high
C13	1				1			1	No SQGVs exceeded. No COPCs or COCs identified.

[a] LOE-Scores for SQGVs and COPCs and COCs listed; PAHs = polycyclic aromatic hydrocarbons; OCs/OPs = organochlorine/organophosphate pesticides; PCBs = polychlorinated biphenyls; TPHs = total petroleum hydrocarbons; TBT = tributyltin; AVS = acid-volatile sulfide; SEM = simultaneously extractable metals sum: Σ(Ag,Cd,Cu,Ni,Pb,Zn); AEM = acid-extractable metals by cold 1 M HCl; not all possible LOEs or cases included.

4.2.2 TEST SEDIMENTS

The type of sediment collected for toxicity testing depends on the study aims. For most monitoring and toxicity assessment studies where historical contamination is not an issue, the upper 0-10 cm of sediment is generally the sediment fraction of interest. For studies where the oxic/sub-oxic layer is well defined a smaller depth, e.g. 0-3 cm might be more appropriate. Oxic sediments are appropriate for toxicity testing as most sediment infauna will reside in the top oxic layer or irrigate their burrows with oxygenated overlying water.

The integrity of the test sample may have a significant influence on the test outcomes and the suitability of methods for collection, manipulation and storage (ASTM, 2000; Environment Canada, 1994; USEPA, 2001; Simpson et al., 2005). It is recommended that toxicity tests be undertaken as soon as practical after collection of sediments from the field. Storage of sediments for periods greater than eight weeks before testing is not recommended (Casado-Martinez et al., 2006; USEPA/USACE, 1998; Geffard et al., 2004).

Any form of disturbance to the sediments, whether through the act of sampling, field processing or transportation, will affect the bioavailability of the contaminants (Simpson et al., 2005; USEPA, 2001). Although disturbances to the sediments cannot be eliminated, it is important that they are minimised. Following collection, sediment samples for toxicity tests should be stored cold (on ice) to reduce loss of volatiles and decrease bacterial activity, and stored refrigerated until the time of testing. It is desirable to undertake some assessment of how sample manipulation may affect the concentrations (e.g. loss of volatiles), bioavailability (e.g. changes to AVS, partitioning in pore waters) and toxicity of contaminants in the collected sediments.

Measurements of non-contaminant stressors such as ammonia and sulfide should be made as soon as possible after sample collection and also monitored during toxicity tests so the their effects on test outcomes can be quantified and concentration compared to what was present in the sediments *in situ.*

Many toxicity tests will require several weeks to prepare the test organisms before test can commence, then 1-2 weeks to perform and potentially another week to analyse and report the data. Consequently, the costs associated with toxicity tests will often be high and the collection of test samples for larger risk assessment projects may need to be staggered so that sufficient time is available for undertaking these tests.

4.2.3 DATA ANALYSIS AND INTERPRETATION

Quality assurance, including criteria for test validity, consideration of control or reference sediments, replicates and reference toxicants, is important when considering the significance of the test results (Environment Canada, 1994; ASTM, 2003; Simpson et al., 2005).

The data analysis and quality assurance procedures should be sufficient to determine if these effects are significantly different from those occurring in the controls or reference sediments (USEPA, 1994). It is important to ensure that the tolerable range of the test organism to physicochemical characteristics of the test sediments, pore waters and overlying waters, such as DO, pH, salinity, particle size, ammonia and sulfide, are not exceeded.

Different criteria can be used to determine whether or not the test sediment is toxic. For most acute lethality tests, the use of pass/fail criteria based on mortality being significantly different and exceeding that of the reference sediment by more than 20% (Environment Canada, 1998; Simpson et al., 2005)

Sediments may have 'natural' toxicity due to the presence of ammonia or hydrogen sulfide, decomposition products of organics in aerobic and anoxic sediments respectively. Ammonia was found to have a probability of up to 18% of being the only cause of toxicity in a series of 322 marine sediments in the eastern USA (Moore et al., 1997). The presence of ammonia may therefore confound interpretation of results and determining the risk associated with contaminants. In situations where ammonia concentrations are high and suspected of cause the observed toxicity, additional tests may be require to quantify the influence of ammonia. When high ammonia concentrations are measured in overlying waters during whole-sediment toxicity tests, it is recommended that the water is renewed regularly to reflect dilution of ammonia with overlying water that would occur naturally in the field.

There are a number of other factors that affect test organism response aside from those related to sediment-associated contaminants. They include effects of sediment heterogeneity, sediment manipulations, temperature, light, food availability (sediment nutrition) and organism mobility. These factors may result in significant toxicity (negative effects relative to control responses) when contamination is low, greater toxicity than expected, or no (or less) observed toxicity when contaminants are present at concentrations expected to cause great toxicity.

Photo-induced toxicity has been observed to be an important factor in controlling the toxicity of some contaminants associated with sediment (e.g. certain PAHs). The light conditions used during tests may need to reflect lighting conditions at the field site (e.g. at a certain water depth or sun exposure on a mud flat) (Ankley et al., 1994; Swartz et al., 1997).

4.2.4 WEIGHTING OF TOXICITY TESTS FOR LOE SCORING

It is recommended that three or more toxicity tests, using different species, are evaluated to determine the final LOE Score for toxicity. It is recommended that at least one, and preferably 2 or 3, of these tests is a sub-lethal whole-sediment toxicity test, and at least one test should have a chronic test endpoint. The relevance, sensitivity to the suspected COPCs, reliability (lower numbers of false positives of false negatives), and applicability to the sediment types or assessment environment will be different for every test. Consequently, some best-professional judgment may be necessary to determine the final LOE score when the toxicity results are believed to be influenced by stressors that are not sediment-associated contaminants.

4.2.5 IDENTIFICATION OF TOXICANTS IN SEDIMENTS

Procedures exist for the identification of the cause of toxicity observed in pore water and whole-sediment toxicity tests. Toxicity identification and evaluation (TIE) procedures, as they are commonly referred, involve the manipulation of sediments, or sediment components (e.g. pore waters) to remove or mask the effects of particular classes of contaminants (e.g. PAHs, metals, ammonia), thus allowing identification of the chemical class(es) responsible for the observed toxicity (Ankley and Schubauer-Berigan, 1995; Ho et al., 2002; Simpson et al., 2007; USEPA, 2007). The TIE methods for whole sediments generally target the three main toxicant classes in sediments: metals, organics and ammonia (USEPA, 2007). Manipulations include the addition of the green macroalga *Ulva lactuca* and zeolite resins to remove ammonia, cation exchange resins to sequester metals, and powdered coconut charcoal to sorb organics. The use of knowledge of contaminant exposure pathways (pore waters, whole sediments) (Simpson and King, 2005), species-specific sensitivity to selected contaminants is also useful for identifying which contaminants or stressors may be responsible for the observed toxicity (Simpson and Batley, 2007).

4.2.6 *IN SITU* TESTING

Laboratory-based effects assessments may sometimes be poor measures of field-based exposures (Chappie and Burton, 2000; Burton et al., 2005). This occurs because exposure dynamics and interactions occurring at the field site, including the heterogeneity of natural sediments, cannot be correctly mimicked in the laboratory.

In situ tests may be defined as environmental measurements that are taken in the field, without removal of a sample to the laboratory to minimise manipulation (Baird et al., 2007; Crane et al., 2007). *In situ* effects measures may more accurately represent the effects occurring to single organisms or populations in the field. Currently few routine *in situ* test procedures exist in Australia (Pettigrove and Hoffman, 2005). This is due both to the lack of research into these methods and some difficulties associated with logistics associated with placement and retrieval of *in situ* chambers) and integrity during placement (e.g. vandalism, high energy sites) (Liber et al., 2007). The incorporation of results from *in situ* tests in the WOE decision-making process is desirable, especially as standardised techniques may become available in the future (ASTM, 2001; Chappie and Burton, 2000).

4.2.7 EXAMPLES OF THE TOXICITY LOE

Examples of hypothetical cases of toxicity testing LOE results are provided in Table 6. If the only toxicity result was from the Microtox assay (cases T8 and T9), the ecological relevance of this bacterial test might make this questionable, whereas if this was supported by other toxicity tests, the LOE score would be higher. The consideration of ammonia and sulfide effects is important. If separate toxicity is seen from these at the concentrations present in the test sites (e.g. case T10), then that is a concern in itself, in addition to any effects that might be due to other toxicants. The management of ammonia as a COPC might however be different to the management of other contaminants.

4.3 Bioaccumulation and Biomagnification

Bioaccumulation refers to the accumulation of contaminants in the tissues of organisms through any route, including respiration, ingestion, or direct contact with contaminated sediment or water (USEPA, 2000a, b; Moore et al., 2005; Simpson et al., 2005). Bioavailability and organism physiology are the two most important variables affecting chemical contaminant body burdens. Moore et al. (2005) review and discuss the many factors that affect the assessment of bioaccumulation.

Many non-ionic (hydrophobic) organic chemicals (HOCs), such as PCBs and PAHs, are lipophilic and readily taken up by many organisms and accumulate in fattyl tissues. In general, substances with an organic carbon adsorption coefficient (Koc) of <500–1000 L/kg are not likely to be sorbed to sediment. Generally, only HOCs with a high octanol-water partition coefficient, log Kow >4.5, need to be considered for potential to bioaccumulate. While PAHs are readily taken up by many organisms, many are rapidly metabolised. Synthetic chemicals such as PCBs that are persistent (highly resistant to metabolic degradation) and exhibit high lipophilicity may accumulate to high levels. Many HOCs may also biomagnify (increase in concentration through three or more trophic levels) through food chains. The biomagnification of metals does not occur, although secondary poisoning (trophic transfer of two trophic levels) may be observed.

The bioaccumulation of metals by invertebrates is complex and strongly affected by organism feeding behaviour (ingestion rates, selectivity of feeding) and the different sources of metals (overlying waters, pore waters, sediments and speciation) (Luoma and Rainbow, 2005; Moore et al., 2005; Simpson and Batley, 2007). Measurements of body concentrations (body residues) of metals may provide useful information on possible effects of non-essential and non-regulated metals, but only if strong and clear relationships exist between bioaccumulation and toxic effects (Borgmann, 2000; Simpson and Batley, 2007). For metals that are sequestered into non-toxic forms or are regulated over the concentration range of interest, the use of body concentrations to predict affects is not appropriate (Borgmann, 2000; Luoma and Rainbow, 2005).

Field measurements (body residues in field–collected organisms), laboratory measurements (bioaccumulation tests with laboratory animals), surrogate measures of bioaccumulation (biometric methods, gut fluid extraction) and modelling (biota-to-sediment accumulation factors (BSAF) and theoretical bioaccumulation potential (TBP)) can all be used for assessing bioaccumulation of organic chemicals. Each approach has a number advantages/disadvantages and assumptions/uncertainties associated with it (USEPA, 2000a; Moore et al., 2005; Simpson et al., 2005). Care should be taken to use a suitable depuration period (e.g. 24 h) for gut clearance before body concentrations of organisms are determined.

Examples of typical applications of the bioaccumulation LOE are given in Table 7.

Table 6. Line-of-evidence decision matrix for toxicity test examples (using estuarine marine tests)[a]

Case	Assessment using Toxicity Tests (% toxic effect)				Ammonia (A), Sulfide (S)	LOE Score	Explanation
T1	Algae growth inhibition ≥50% (WS) (3)	Sea urchin, 72-h larval development 20-50% (SC, PW) (2)	Amphipod, 10-d reproduction ≥50% (C, WS) (3)	Copepod 10-d reproduction ≥50% (C, WS) (3)	Ammonia = low Sulfide = ND	**3**	Significant chronic toxicity in all tests. Chronic toxicity to algae in pore water. Toxicity not caused by ammonia or sulfide. Chronic toxicity to amphipod and copepod in whole-sediments.
T2	Oyster, 48-h larval development 20-50% (SC, PW) (2)	Bivalve 10-d survival <20% (A, WS) (1)	Amphipod, 10-d reproduction ≥50% (C, WS) (3)	Copepod, 10-d reproduction ≥50% (C, WS) (3)	Ammonia = low Sulfide = ND	**3**	Mixed results for acute (survival) tests. Chronic toxicity to amphipod and copepod in whole-sediment. Toxicity not caused by ammonia or sulfide.
T3	Bacteria (Microtox®) <20% (A, PW) (1)	Amphipod 10-d survival <20% (A, WS) (1)	Sea urchin, 72-h larval development <20% (SC, PW) (1)	Amphipod, 10-d reproduction ≥50% (C, WS) (3)	Ammonia = low Sulfide = ND	**3**	No toxicity for acute tests. Chronic toxicity to amphipod in WS. Toxicity not ammonia or sulfide. Chronic toxicity certain when ≥50%.
T4	Bacteria (Microtox®) <20% (A, PW) (1)	Sea urchin, 72-h larval development <20% (SC, PW) (1)		Algae 72-h growth ≥50% (PW, C) (3)	Ammonia = low Sulfide = ND	**3**	No toxicity for acute tests. Chronic toxicity to algae in pore water. Toxicity not ammonia or sulfide.
T5	Algae growth inhibition <20% (WS) (1)	Amphipod 10-d survival ≥50% (A, WS) (3)	Copepod 10-d reproduction 20-50% (C, WS) (2)		Ammonia = low Sulfide = mid	**3**	High toxicity for one acute tests. No toxicity in other acute tests. No chronic toxicity to algae in pore water.
T6	Algae Growth inhibition 20-50% (WS) (2)	Amphipod 10-d survival 20-50% (A, WS) (2)	Sea urchin, 72-h larval development 20-50% (SC, PW) (2)	Algae 72-h growth <20% (PW, C) (1)	Ammonia = mid Sulfide = ND	**2**	Moderate toxicity for acute tests. No chronic toxicity to algae in pore water.
T7	Polychaete 10-d survival <20% (A, WS) (1)	Sea urchin, 72-h larval development 20-50% (SC, PW) (2)	Amphipod, 10-d reproduction 20-50% (C, WS) (2)		Ammonia = mid Sulfide = ND	**2**	Mixed results for acute test. Moderate chronic toxicity that may, in part, be non-contaminant effects.
T8	Bacteria (Microtox®) ≥50% (A, PW) (3)	Polychaete 10-d survival <20% (A, WS) (1)	Sea urchin 1-h fertilisation <20% (SC, PW) (1)		Ammonia = mid Sulfide = ND	**2**	High toxicity only in bacteria test, but is probably not important. Other tests are not necessarily very sensitive or are inconclusive.
T9	Bacteria (Microtox®) 20-50% (A, PW) (2)	Amphipod 10-d survival <20% (A, WS) (1)	Oyster, 48-h larval development <20% (SC, PW) (1)	Copepod 10-d reproduction <20% (C, WS) (1)	Ammonia = low Sulfide = ND	**1**	Toxicity only in bacteria test. No toxicity in the other acute tests. No chronic toxicity to copepod in whole sediment.
Special cases (toxicity from naturally occurring ammonia or sulfide)							
T10	Algae growth inhibition <20% (WS) (1)	Amphipod 10-d survival ≥50% (A, WS) (3)	Sea urchin, 72-h larval development 20-50% (SC, PW) (2)	Algae 72-h growth ≥50% (PW, C) (3)	Ammonia = high Sulfide = ND	**2**	Mixed results for acute tests. Chronic toxicity of PW to algae. High PW-ammonia. No other toxicants! Definitive algae test indicates NH_3 toxicity. NH_3 ≥LC50 for amphipod.

[a] WS = whole sediment; PW = pore water; A = acute test; C = chronic test; SC = sub-chronic; ND = not detected; not all possible LOE or cases included.

Table 7. Line-of-evidence decision matrix for bioaccumulation/biomagnification examples[a]

Case	Bioaccumulation Assessments		Biomagnification	LOE Score	Explanation
B1	Field-collected biota bioaccumulation (2) Significant, <3× controls (HOCs)	Transplanted biota – laboratory bioaccumulation assay (3) Significant, ≥3× controls (HOCs)	Not present (1)	**3** (Significant concern)	Bioaccumulated HOC concentrations are five or more times greater than that measured in the controls (treatments or sites)
B2	Transplanted biota – *in situ* bioaccumulation assay (3) Significant, ≥3× control (HOCs)		Not present (1)	**3** (Significant concern)	Bioaccumulated HOC concentrations are five or more times greater than that measured in the controls (treatments or sites)
B3	Field-collected biota bioaccumulation (2) Significant, <3× controls (HOCs)		Dioxins present and bioaccumulated (1-2)	**3** (Significant concern)	Bioaccumulated HOC concentrations are statistically greater than that measured in the controls (treatments or sites). Biomagnification likely.
B4	Transplanted biota – *in situ* bioaccumulation assay (1) Not significant	Surrogate method (SPMD) (3) Significant (HOCs), ≥3× control		**3** (Significant concern)	No bioaccumulation measured, but surrogate method finds (statistically) that contaminants are very bioavailable.
B5	Field-collected biota bioaccumulation (2) Significant, <3× controls (HOCs)	Surrogate method (GFM) (2) Significant, <3× controls (HOCs)		**2** (Possible concern)	Bioaccumulated HOC concentrations are statistically greater than that measured in the controls (treatments or sites)
B6	Transplanted biota – laboratory bioaccumulation assay Not significant (HOCs)	Surrogate method (XAD-2) (2) Significant, <3× controls (HOCs)	Dioxins present (1-2)	**2** (Possible concern)	No bioaccumulation measured, but surrogate method finds (statistically) that HOCs are bioavailable. Dioxins present.
B7	Field-collected biota bioaccumulation (2) Significant, <3× controls (HOCs)			**2** (Possible concern)	Bioaccumulated concentrations are statistically greater than that measured in the controls (treatments or sites)
B8	Transplanted biota – laboratory bioaccumulation assay (3) Significant, ≥3× controls (Metals)	Surrogate method (Tenax) (1) Not significant	Mercury present – little bioaccumulation (1-2)	**2** (Possible concern)	Bioaccumulated metal concentrations ≥3× measured in the controls, but effects due to metal bioaccumulation are unlikely.
B9	Field-collected biota bioaccumulation (1) Not significant	Transplanted biota – *in situ* bioaccumulation assay (1) Not significant	Not present (1)	**1** (No concern)	No significant difference in the bioaccumulation data relative to controls (treatments or sites)
Special cases (metal bioaccumulation)					
B10	Field-collected biota bioaccumulation (2) Significant, <3× controls (metals)		Not present (1)	**1** (No concern)	Field-bioaccumulation of metals, but effects due to metal bioaccumulation is unlikely.

[a] HOCs = hydrophobic organic contaminants (e.g. PAHs, PCBs, PAHs); surrogate method = laboratory procedure used to measure contaminant release of contaminants (generally HOCs) from sediment particles to simulate route of uptake by biota, e.g. laboratory-based use of resins (Tenax and XAD-2); semi-permeable membrane devices (SPMD) or gut fluid mimics (GFMs); not all possible LOE or cases included.

4.4 Ecological Assessment

The objective of an ecological LOE in a WOE assessment is to obtain information that can help ascertain whether a location's ecology has been negatively or extensively impacted. For this purpose, benthic macrobenthic invertebrate communities are commonly used as they are ecologically important and often respond to significant changes in sediment chemistry (Pearson and Rosenberg, 1978; Dauer 1993). Although the primary objective is to protect biota, paradoxically, ecological lines of evidence are often negated due to their prohibitive labour cost. Ecological indicators are also poorly utilised because of a general lack of understanding of the direct cause-effect pathway and hence lack of specificity to a particular contaminant or set of contaminants.

Because ecological LOEs are to used in concert with other measures in a WOE, the rigour with which this assessment needs to be undertaken is less than might be required for a comprehensive stand-alone benthic survey. The latter would need to take into account the spatio-temporal distributions of benthic assemblages; and would entail multiple reference and impacted sites to ensure adequate statistical power and to minimise Type II errors, i.e. failing to observe a difference when a difference actually occurs. As a result, the likelihood of a Type II error will be substantially greater in a LOE study than that of a rigorously designed benthic community monitoring program. Even so, the fundamental principles of experimental design and the employment of suitable statistical techniques are still pertinent issues which require adherence to, as these dictate the quality and relevance of the data collected and the amount of weight which can be assigned to any findings.

4.4.1 LINES OF EVIDENCE USING BENTHIC COMMUNITIES

Observational indicators of sediment health

Benthic communities have a profound influence on the productivity of surficial sediments by altering the physical and chemical conditions of the sediment-water interface. In environments were the total abundance of benthos has been severely impoverished, marked changes to the morphology of the sediment-water interface should be clearly evident. Such changes include: the absence of irrigation burrows; the presence of single-type of burrow, indicating the overriding dominance of a single taxon; a very thin oxic layer which is predominately a furry-like, surficial biofilm; and algal mats. Even though some resilient taxa are ubiquitous in even the most grossly-contaminated locations, they are generally small, shallow burrowers whose morphological imprint on the sediment is significantly less pronounced than found in diverse environments with a high level of biotic integrity. The use of such observational indicators can form a useful component of the ecology LOE.

Univariate indicators of sediment health

Univariate measurements are frequently used to summarise the structural attributes of a community, and generally include the total number of individuals, indices for richness, diversity and evenness, and the abundances of *a priori* selected taxa (Antrill and Depledge, 1997; Clements, 1997). An advantage of this approach is that the variables are amenable to several commonly used univariate statistical techniques (e.g. ANOVA, correlation and regression analyses). However, in order to provide robust, ecologically useful information, univariate community attributes need to show a high level of response to contaminants and possess a low level of natural variability.

Community indices provide a simple approach for gaining information regarding the ecological integrity of a site. These include techniques which individually measure or encompass the concepts of richness, sample size and evenness, e.g. Shannon-Weiner Index, Simpson's Index, and Pielou's Evenness (Shannon and Weaver, 1963; Pielou, 1966; Krebs, 1994). Numerous studies have shown a strong correlation between many of these variables and an increase in the concentration of contaminants, and consequently these measurements continue to form the basis of many benthic community studies (e.g. Gray et al., 1990; Lindegarth and Hoskins, 2001; Johnston and Roberts, 2009).

The differential loss, replacement, proliferation and relative abundance of taxa are represented in indices of diversity, richness and evenness, with a decline in these indices often symptomatic of ecological stress (Newman, 1998; Rogers and Hsu, 2001). The underpinning assumption is that contaminants can induce stress which affects the structure and stability of communities, which is subsequently observed as a change in the number of species, and their relative abundances (Krassulya, 2001). It is emphasised that these do not necessarily manifest as a change in total abundance (the sum of all individuals from all taxa). Some taxa are relatively resilient to perturbation and can thrive under conditions of attenuated inter-specific competition and increased resources. The high prevalence of such taxa commonly results in an increase in total abundance. Consequently, total abundance should not be used in isolation when attributing contaminant exposure to changes in community metrics.

A decline in abundance of sensitive taxa can be expected as contaminant exposure can amplify the incidence of mortality; reduce the physiological condition of animals, attenuate fecundity; and increase disease susceptibility (Newman, 1998). Declines have been observed across all coarse taxonomic groups (e.g. polychaetes, decapods, bivalves, gastropods), although some taxa appear to be more intolerant than others. For example, amphipods are frequently scarce or absent in contaminated environments (e.g. Rand and Petrocelli, 1985; Warwick, 2001). Identification of potential study or regionally specific sensitive taxa must be made prior to analysis, enabling the testing of taxa specific hypotheses. The numeric dominance of deposit-feeding taxa such as Capitellidae often reflects a shift from larger, species in stable, unpolluted environments, to small-bodied, short-lived and highly fecund species in contaminated and disturbed environments (Pianka, 1970; Weston, 1990). In cases of nutrient enrichment, this shift in species can result in an overall increase in the total abundance of individuals within a population, possibly due to a concurrent increase in the availability of food used by deposit-feeders (Pearson and Rosenberg, 1978; Tsutsumi, 1990). However, there is no evidence to suggest that this response is elicited when exposure is due to of metals and organic contaminants.

Measurements of diversity and richness are the mostly commonly examined metrics of community structure. The simplest measurement of diversity is species (or taxa) richness. However, this measurement has three serious limitations (Krebs, 1999): (i) it is improbable that all species will be counted; (ii) the boundary for the community being quantified is arbitrarily defined in space and time; and (iii) the measurement negates the concept of heterogeneity, and the relative abundance of each species.

Graphical techniques complement rather than replace the more formal statistical analyses (univariate and multivariate). *K*-dominance curves plot the cumulative ranked abundance against taxa rank (or log taxa rank), providing useful information about dominance, and the number of species (or taxa) that are dominating a community (Lambshead et al. 1983). *K*-dominance curves are often modified to create Lorenz curves, in which the taxa rank (x-axis) is rescaled and is measured against the cumulative abundance, enabling dominance to be partitioned from the number of taxa. The underpinning assumption for these curves is that 'stressed' communities will contain few taxa that will contribute to a significant proportion of the overall abundance, i.e. a reduction in evenness due to the dominance of a few taxa. Consequently, the curves for the less even communities will sit above those that are more even.

Although commonly used, there is much criticism of univariate measurements due to the loss of information which occurs when assemblages are reduced to a series of variables (Warwick and Clarke*,* 1991). For example, two locations may have similar levels of diversity, even though the locations contain very different taxa. There are also some concerns about the suitability of applying these measurements to standard univariate statistical techniques (e.g. ANOVA), as their distributions often deviate from the assumptions of normality and the homogeneity of variances which underlie these techniques (McArdle and Anderson, 2004). Nevertheless, many univariate measurements are founded on frequently observed ecological observations and hypotheses, and commonly provide relevant information which may aid in discriminating between putatively impacted and unimpacted locations

Multivariate measurement

There is a range of multivariate tools that can provide a powerful means of isolating the relationships between ecological communities and co-varying contaminants (Hewitt et al., 2005; Anderson et al., 2006; Thrush et al., 2008; Hewitt et al., 2009; Chariton et al., 2010a, 2011). In contrast to univariate approaches, multivariate techniques do not require the data to be reduced to a single variable, but rather comparisons are made between two or more sites by quantifying the similarities (and differences) in their taxa and relative abundances. As a result, multivariate approaches can capture and reflect differences in whole assemblages.

Non-metric multidimensional scaling (nMDS) is one of the most commonly used ordination techniques as it is conceptually simple to understand. In nMDS, the output is presented as either a 2- or 3-dimensional ordination 'map' in which samples that are more similar to each other are positioned closer than those that are less similar. Although the graphical representation provided by nMDS is intuitive, the complex number of multivariate relationships that occurs in a large dataset, and the reduction of this information down to a 2- or 3-dimensions can distort the spatial relationships between samples (Clarke and Warwick, 1994). In most statistical packages, the level of distortion is calculated as a measure of 'stress', with a lower stress value indicating a more accurate representation of the spatial relationships on the ordination map, thereby increasing the power of the interpretation. The variance among or within the samples as traditionally measured by ANOVAs is not quantified by nMDS and other analogous ordination techniques. Additional analysis is required to establish if pre-defined groups or treatments (i.e. location, site or time) contain significantly different assemblages. The most commonly-used approaches for benthic studies are Analysis of Similarities (ANOSIM) and Permutational Multivariate Analysis of Variance (PERMANOVA formally known as NPMANOVA), both routines are available in the Primer 6+ statistical package (PRIMER-E Ltd, Plymouth, United Kingdom).

The greater sensitivity of multivariate techniques can also be one the pitfalls, as significant differences between reference assemblages are frequently observed. To accommodate this, differences in the variability within treatments should also be evaluated. In cases of obvious perturbation, variability will be greater among the reference than the impacted assemblages, however, these two treatments should still be spatially discrete. This may be done qualitatively by examining ordination plots; via the use of distance similarity matrices (e.g. Primer's SIMPER); or by using a priori defined groups (e.g. Canonical Analysis of Principal coordinates) (Hewitt et al., 2005; Anderson et al., 2006; Hewitt et al., 2009; Chariton et al., 2010a). Once differences between treatments have been identified, follow-up analysis with procedures such as Primer's SIMPER or CANACO's triplots enables users to identify those taxa which are characteristic of each treatment.

A more explicit investigation of the links between observed differences in biological communities and other environmental data (e.g. sediment chemistry) requires other statistical approaches. The simplest approach is to overlay nMDS ordination maps with measurements of suspected correlating environmental variables. A more formal approach is Primer's BIO-ENV (Primer 5, 2001), which examines correlative relationships between abiotic and biotic matrices. Alternatively, constrained (or direct) techniques, e.g. Canonical Correspondence Analysis (CCA) and distance-based redundancy analysis (dbRDA) can be employed (Clarke and Ainsworth, 1993; Jongman et al., 1995; Legendre and Legendre, 2012). These techniques may be advantageous as they enable the environmental data to be integrated directly into the analysis. In addition, the influence of covariables such as grain size can be partitioned from the analysis, potentially providing clearer patterns between benthic communities and sediment chemistry (ter Braak and Verdonschot, 1995). Although the intent of the WOE assessment is to use the extent of co-occurrence of effects in each LOE to support a finding that the system is impacted, additional information on covariables is valuable for this assessment.

A number of new approaches are showing great promise for extracting additional detail on how biological communities (and the taxa they encompass) may be being modified by environmental variables. Two notable examples are Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010) and Gradient forest analysis (Ellis et al., 2012), both freely available for the R software environment (http://www.r-project.org/). TITAN is designed to identify where the greatest change in the species abundance and occurrence occurs along an environmental gradient. For example, in a hypothetical scenario examining changes in benthic macroinvertebrate communities along pronounced salinity gradient, TITAN can identify which taxa declined or increased as a response to incremental changes in salinity. In addition, evidence for a community level threshold can be obtained from the program running independent comparisons using the normalized changes in both increasing and decreasing taxa. An additional advantage of TITAN is that the program uses boot-strapping to produce estimates of uncertainty, with narrow confidence limits adding credence to the evidence of a community threshold, while wider confidence limits are indicative of other responses (e.g. random and modal) (Baker and King, 2010). Presently, TITAN is limited to detecting species and community threshold responses to a single environmental variable (predicator), with a multivariable version currently in development (pers comm. Matthew Baker, University of Maryland, Baltimore County).

Random forest models have been shown to be to a powerful tool for exploring the importance of environmental predictors on individual taxa, and for extrapolating the position along gradients where pronounced changes in abundance may occur (Cutler et al., 2007; Peters et al., 2007; Knudby et al,. 2010). Gradient forest analysis extends this approach from taxa specific responses to biological assemblages, providing information on where along a range of environmental gradients marked compositional changes may be occurring, permitting the identification of key environmental thresholds (Ellis et al., 2012). The authors have since demonstrated the capacity of the technique to detect key environmental variables that correlated with composition changes in marine benthos, and the threshold values at which composition turnover was greatest (Ellis et al., 2012). Gradient forest analysis has several favourable attributes which distinguish it from approaches such as CCA and dbRDA. Firstly, due to its machine-learning heritage, it has greater capacity than other approaches to capture the complex relationships between multiple environmental predictors and species. Secondly, environmental variables do not require transformation, nor do correlative variables require removal prior to computation. Thirdly, the approach is less subject to distortion than dissimilarity measurements which are constrained, e.g. Bray-Curtis dissimilarity is bounded between 0 and 1. It is emphasised that Gradient forest analysis is not an alternative approach for more formalised techniques (e.g. CCA and dbRDA), but rather, an exploratory tool which can be used to provide additional evidence for teasing out the often complex relationships which occur between biological communities and environmental variables.

4.4.2 RECOLONISATION EXPERIMENTS

In many instances, it may not be possible to find reference sites suitable for comparisons, or additional ecological evidence may be warranted which extends past the correlative findings of benthic field studies. In such cases recolonisation experiments may prove beneficial. The underlying objective of these experiments is to examine differences in recolonised benthic assemblages between defaunated sediments translocated from impacted and reference locations (e.g. Roach et al., 2000; Pettigrove and Hoffman, 2005; Chariton et al., 2011). Communities can be analysed using similar procedures as field studies, however, direct comparisons of measurements are not possible due to differences in spatial scales (sites verses containers) and recruitment patterns (established verses recruited fauna). As with all experiments, the methods employed will influence outcomes, with major considerations including: the handling and preparation of the sediment; container effects; larval dispersal and mobility; and the length, timing and positioning of the experiments. Due to a lack of a true control, recolonisation experiments using translocated sediments cannot identify causality. However, they enable sediments that were formerly spatially separated to be compared under similar environmental conditions, removing the confounding influence of space that occurs in traditional field studies.

4.4.3 ECOGENOMICS

Ecological studies are an important line of evidence in the assessment of sediment quality. In marine systems ecological data are commonly derived from the collection and enumeration of macrobenthic organisms (e.g. polychaetes and bivalves) (Thrush et al., 2008; Chariton et al., 2010b). However, macrobenthic data have many significant limitations:

(i) they are costly to collect; labour intensive;

(ii) they require regionally-specific taxonomic expertise;

(iii) they require a large number of replicate samples; and

(iv) it is impractical to include juvenile and cryptic taxa.

From a risk assessment perspective, a critical concern of macrobenthic studies is that it represents only a small fraction of the total diversity. Often less than 40 taxa are being used to make assumptions about total ecosystem health. This is despite that fact that size, trophic position, diet, behaviour and life-stage influence the resilience and resistance of organisms to environmental disturbances.

Whilst the inclusion of meio- and microfauna (including algae and diatoms) has been demonstrated to be of great benefit, with many of these taxa shown to be sensitive indicators of environmental condition, their size and taxonomic issues have made it impractical to include these organisms in routine monitoring programs. New DNA-based ‘ecogenomic’ approaches to monitoring sedimentary environments are currently under development that enable a more rapid and comprehensive examination of the biotic composition of sediments, regardless of size or taxonomy, providing a more realistic view of the ecological status of a system (Chariton et al., 2010b).

Ecogenomics can broadly be defined as the examination of genetic materials from the environment (van Staalen and Roelofs, 2011). The use of molecular tools in community ecology are by no means new, however, the need to isolate and sequence individual genes in order to obtain taxonomic information has previously constrained their viability and subsequent adoption into routine monitoring programs. Since the advent of pyrosequencing in 2005 (Margulies et al., 2005), the molecular sciences have undergone a paradigm-shift, with high throughput sequencers eliminating the need for cloning, and the time, costs and biases associated with this practice. High throughput sequencers continue to evolve rapidly, improving in quality, output and read length (Shokralla et al., 2012). As it is currently technically impractical to study the complete genomic constituents of complex environment samples (i.e. the genomes of all sampled taxa) with the spatio-temporal replication required for environmental monitoring programs, a ‘gene-centric’ approach, commonly referred to as ‘metabarcoding’ or ‘environmental-DNA surveys’ is used to produce biodiversity information by examining single or multiple genes of interest. Chariton et al., (2010b) demonstrated that high throughput sequencing could be applied to examine the ecological composition of estuarine sediments. In this study, the macro-, meio- and micro-biota of sediments were sampled from a reference and contaminated estuary. Using multivariate techniques commonly applied to marine community data, the ecogenomic data were able to discriminate between the assemblages sampled from the reference and impacted locations, regardless of the biological fraction (macro-, meio- or micro-), or taxonomic level (sequence to Phyla). Conversely, comparisons between these two systems using traditional macrobenthic data (approx. 50 taxa) produced ambiguous results (Chariton et al., 2010b). To date, environmental DNA (e-DNA) surveys have been performed on a wide range environmental of matrices (e.g. Deagle et al., 2009; Bik et al., 2012; Baldwin et al., 2013), with the approach continually demonstrating its capacity to provide far broader coverage of biodiversity than is available using traditional means. As the field matures, it is becoming increasingly evident that comprehensive biological coverage requires the targeting of a number of genes, and that downstream bioinformatic pipelines can have a pronounced effect on the quality and ecological interpretation of the data (Coissac et al., 2012; Taberlet et al., 2012; Tang et al. , 2012). In its current capacity, one notable limitation of the approach is its inability to provide accurate quantifiable data, however; it is foreseeable that this issue will be resolved as ‘PCR-free’ techniques become more refined and suitable for e-DNA applications.

4.4.4 ECOLOGY LOE SUMMARY

In order to make valid judgement regarding the ecological integrity of a specific environment, comparisons must be made with suitable reference locations. Furthermore, several statistical lines of ecological information (e.g. univariate and multivariate interpretations) must be incorporated to compensate for the accuracy of specific indicators in over and under-estimating the ecological condition of perturbed and health systems, respectively. Multivariate analysis, and graphical assessments (K-dominance curves), supplemented by information from CCA analyses, are the most valuable approaches to assess field data. Multivariate analyses should also be used to analyse data from recolonisation experiments.

The following checklist can be used to assess the ecological evidence:

(i) Observational: Is there evidence of burrowing? Is there oxygen penetration into surface sediments? Is there a prevalence of some taxa?

(ii) Univariate analyses: What is the diversity of taxa in impacted vs reference sites (possible use of Lorenz curve)? Is there a loss of sensitive taxa? Is there an increase in robust taxa?

(iii) Multivariate analyses: What is the difference in the dispersion between impact and reference sites? Is there different clustering of impact vs reference sites? Are there statistical differences in community composition between impacted sites vs reference sites? Which taxa are contributed to the differences between impact and reference sites? Are there strong correlative relationships between the biotic and contaminant datasets? Is there evidence that biological communities change along certain points of an environmental gradient?

The above also be applied to transplant/recolonisation experiments for additional evidence, however usually sufficient data will be obtained without this more costly exercise. On the basis of the above analyses the extent of difference between control and reference sites can be ranked. Examples of the applications of the ecology LOE are given in Table 8.

Table 8. Line-of-evidence decision matrix for ecological assessment examples [a]

Case	Ecological Assessments		Factor	LOE Score	Explanation
E1	Benthic community structure analysis: benthic survey (3) Significantly different to reference	Manipulative transplant assessment (3) Significantly different to reference	Normal	3	Effects significant and high
E2	Benthic community structure analysis: benthic survey (2) Significantly different to reference, but moderate	Manipulative transplant assessment (3) Significantly different to reference	Possible salinity effects	3	Effects significant and high
E3	Benthic community structure analysis: benthic survey (3) Significantly different to reference		Possible grain size effects	3	Effects significant and high
E4	Benthic community structure analysis: benthic survey (3) Significantly different to reference		Water column effects detected due to intermittent boating activity	2	Effects significant but possibly confounded by anthropogenic activity. Note such confounding factors should be considered before site selection
E5		Manipulative transplant assessment (3) Significantly different to reference	Major differences between native and colonising species	2	Effects significant but species recolonising significantly different from test site species
E6		Manipulative transplant assessment (2) Significantly different to reference, but moderate	Normal	2	Effects significant but moderate
E7	Benthic community structure analysis: benthic survey (1) Not significantly different to reference			1	Effects not significant
E8		Manipulative transplant assessment (1) Not significantly different to reference		1	Effects not significant

[a] not all possible LOE or cases included.

5. Uncertainties in WOE Assessments

It is important to understand the uncertainties and limitations in the various LOEs that comprise a WOE assessment, as well as those associated with the SQGs (Batley et al., 2002). Many of these have been discussed in the preceding pages. The potential for errors begins with sediment sampling, handling and storage. Sediments are very heterogeneous, both physically and chemically and it is typical to spatially average sampling. How appropriate this is must be assessed against the objectives of any study. Similarly study objectives will determine to what depth a sediment should be sampled.

Perturbation of sediment and porewater chemistry due to oxidation can significantly alter pore water metal concentrations, and indicate toxicity when none would be seen at the concentrations that would exist in the field.

There are many uncertainties associated with toxicity testing. A full range of tests are seldom applied and these are not always the most sensitive. The relationship between laboratory tests and field responses is poorly understood as is the extrapolation to population level effects. Consideration of different exposure routes (water vs sediment vs diet) is often poorly covered.

Benthic community studies are a major challenge and subject to confounding stressors that are both physical and chemical.

The uncertainties in data from both chemical and biological studies need to be understood not only for measurement variability but accounting for sample variability. The same is true for trigger values, where values may be confounded in their derivation by co-occurrence issues, making them unreliable. The saving grace is that multiple LOEs give greater confidence in the delineation of cause and effect relationships.

Table 9 provides a checklist to alert practitioners to the uncertainty issues in WOE assessments.

Table 9. Checklist for evaluating data uncertainties/limitations (adapted from Batley et al., 2002)

PARAMETER	SOURCES OF UNCERTAINTY	RECOMMENDATIONS
Sampling, Transport and Storage	Choice of reference sites	Ensure physico-chemical and biological characteristics similar between reference and exposed sites; use multiple reference sites
	Choice of sample sites	For ecological studies, ensure sample sites are selected so as to avoid confounding factors, e.g. disturbance due to boating activities
	Sediment heterogeneity	Spatial averaging appropriate to study purposes and chemical and biological measurements. Undertake a pilot study to determine the number of samples required to ensure sampling is representative of the sediments to be assessed.
	Pore water and sediment sampling depth	Depth-integrated samples; depth based on study objectives, physico-chemical and particularly biological realities; same depths for chemistry, toxicity and biology. Note that AVS is depth dependent, so sample homogenising over large depths will give misleading results

PARAMETER	SOURCES OF UNCERTAINTY	RECOMMENDATIONS
	Pore water sampling methods	No “best” method; minimise oxidation, sample under nitrogen gas atmosphere
	Obtaining sufficient pore water	*In situ* peepers best for minimally changed chemistry; centrifugation best for rapidly generating large volumes
	Sample storage	Store cool in the dark, excluding oxygen; test as soon as possible, reporting storage time and conditions
	Changes prior to testing/analysis	Take all reasonable precautions; recognize possibility of such changes, some of which can be predicted from knowledge of sediment physico-chemical characteristics
Sediment Chemistry	Appropriate measurements	Measure all contaminants of potential concern, and key modifiers (e.g. water: pH, DOC; sediment : AVS/SEM, grain size, TOC)
	Metals bioavailability	Measure easily extractable metals (e.g. cold 1M HCl), not total metals, plus parameters that affect bioavailability (e.g. AVS)
	AVS/SEM comparisons	A useful qualifier of metal bioavailability, but possibility of Type I errors for some metals. Note possible depth dependence
	Organic carbon normalisation	Recommended that organic carbon normalisation be applied to non-polar organics only over the range 0.2-10% organic carbon
	Sediment quality values	Use only for screening and not to infer cause of toxicity
Ecotoxicology	Grain size effects	Similar grain sizes for reference and test sediments; grain sizes must not adversely affect test organisms
	Sieving – removal of coarse particles	Not encouraged; if necessary, do under nitrogen and allow redox equilibria to re-establish
	Test species: exposure routes, sensitivity, residency	Appropriately sensitive range of ecologically important taxa including species from reference areas; exposure routes complete
	Sediment spiking	Adequate equilibration times; recognize laboratory artefacts; environmentally realistic concentrations; complete exposure routes; full physico-chemical documentation
	Laboratory vs field	Separate lines of evidence; one does not validate the other. Do not use alone for decision-making
	Cause-and-effect	Correlative (e.g. gradient) analyses coupled with TIE or CBR determinations
Benthic Community Structure	Spatial and temporal scales	Tailor to purpose; ensure common sampling protocols in comparing temporal data
	Sieve sizes	Based on study objectives and key species present / species relationships
	Species identifications	Species designations may not be reliable; conduct concurrent higher level taxonomic community structure assessments and/or consider functional groupings
	Exposure routes	Must be known for at least key (dominant) and keystone species, along with relative sensitivities via these routes

PARAMETER	SOURCES OF UNCERTAINTY	RECOMMENDATIONS
	Stressor identification	Physico-chemical and biotic stressors must be characterised, including interactions (spatial and temporal)
Data Uncertainties and QA/QC	Data - significant figures	Generally no more than two significant figures are warranted or defensible
	Data analysis	Estimate Type II errors for chosen design
	QA/QC	Ensure QA/QC are adequately addressed

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Appendix. Revision and Derivation of Guideline and SQG-High Values for Contaminants

A1. Introduction

In general, the interim SGQ values adopted as the SQGVs and the interim upper values (ISQG-High) have been useful, given their intended purpose. These values were based primarily on the effect range low (ERL) and effect range mean (ERM) values from Long et al. (1995), and to a lesser extent empirical effects data from MacDonald et al. (1996) and (Chapman et al., 1999). In the context of Australia and New Zealand, the study commissioned by the Auckland Regional Council to develop regional models of benthic ecosystem health (Anderson et al., 2006) indicated biological effects were occurring when these thresholds were exceeded. Likewise, studies of toxicity along a concentration gradient of trace metals, including Pb, Zn, Cd, and Cu, in the sediments, have demonstrated toxicity at metal concentrations approaching the SQG trigger values (Mann et al., 2009; Simpson and Spadaro, 2011).

While the interim SQGs have been generally useful, the application of these and other guidelines to assessments in Australia, New Zealand and other nations has indicated that it would be better to use the threshold effect level (TEL) and probably effects level (PEL) values than the ERL/ERM values for organic contaminants (MacDonald et al. 2000; CCME, 2002). Therefore, in the SQG revision, the TEL and PEL values are used as the SQGV and upper guideline values for most of the organic contaminants. The guideline for tributyltin (TBT) has been revised based on published reviews of effects data and new approaches. For non-ionic organic chemicals in sediment, the ESB approach developed by the USEPA (2003-2012) represents the best current approach for considering the potential effects of individual and mixtures of non-ionic organic contaminants. Consequently, for these organic contaminants, including PAHs, dieldrin, and endrin, the guideline values have been revised and the ESB approach is recommended. The basis for the default values in Table 2 is described below. Total petroleum hydrocarbons (TPHs) are common sediment contaminants and there are now sufficient effects data to derive a SQGV. For metals in anoxic sediments, the approach for metal mixtures (Ag, Cd, Cu, Pb, Ni, and Zn) based on AVS-SEM theory and the ESB approaches are reviewed and the scope and limitations discussed. For metals in oxic/suboxic sediments, possible procedures for the modification of SQGVs for metals that consider the influence of sediment type are described, using copper as an example.

A2. Revision of Guideline Values for Tributyltin

The proposed revisions of the SQGV and SQG-high values for TBT are based on reviews of effects data and proposed ESB approaches (USEPA, 2003b,c). Tributyltin is a biocide used in antifouling coatings applied to the hulls of coastal and ocean-going boats (USEPA, 2003d). It is now banned on small vessels, but contamination continues in many ports and harbours in Australia as a result of leaching from large commercial ships. Tributyltin is highly toxic to a wide range of aquatic species and is linked to imposex in snails and immuno-supression in bivalves. TBT is hydrophobic and adsorbs strongly to the organic carbon in sediments, and is relatively persistent, with a half-life of years.

The USEPA criteria for acute (FAV) and chronic (FCV) protection from effects of TBT are 460 ng TBT/L (acute) and 72 ng TBT/L (chronic) in freshwater, and 420 ng TBT/L (acute) and 7.4 ng TBT/L (chronic) in saltwater (USEPA, 2003d). These criteria are based on an extensive review of effects to aquatic organisms (USEPA, 2003d). The toxic effects of TBT are considered to occur due to exposure of organisms to TBT in the dissolved phases.

Stronkhorst et al. (2002) investigated the toxicity of TBT to the burrowing amphipod *Corophium volutator in* 10-d acute toxicity tests and to the heart urchin *Echinocardium cordatum* in 14-d and 28-d tests. For *E. cordatum* and *C. volutator,* the LC50s for pore water (222 and 329 ng Sn/L) equated to toxicity at a TBT concentration in the sediment (2% organic carbon) with LC50s for *E. cordatum* and *C. volutator* of 1,600 and 2,200 µg Sn/kg dry weight, respectively. Meador (2000a) recommendations a criterion of 60 µg Sn/kg (1% OC) based on effects to salmonoids.

The partitioning of TBT between the dissolved and particulate phases will depend on the sediment properties, particularly the organic carbon concentration (Langston et al., 1995; Meador et al., 1997; Meador, 2000b; Hoch et al., 2000; Burton et al., 2005). The partitioning of TBT between the dissolved phase and particulate organic carbon (OC) can be described by a partition coefficient, Koc (L/kg) = [TBT-sediment, mg/kg]/foc/[TBT-dissolved, mg/L], where foc is the fraction of OC in the sediments. Note that the fraction of sediment OC should be determined by TOC analysis, rather than loss-on-ignition. Koc values vary greatly in sediments, but are typically of the order of 3000 L/kg (1% OC) (Langston and Pope, 1995; Berg et al., 2001; Burton et al., 2005). Based on a FCV of 7.4 ng TBT/L, a sediment criterion of 22 µg TBT/kg (1% OC) or 9 µg Sn/kg is calculated.

There is no scientific basis for modifying the ISQG-High value and it is proposed that this value should remain unchanged.

SQGV and SQG-high value for TBT:

For all sediments:

SQGV = 9 µg Sn/kg (normalised to 1% organic carbon, dry weight)
SQG-High = 70 µg Sn/kg (normalised to 1% organic carbon, dry weight)

A3. Revision of Guideline Values for Total PAHs and the Equilibrium Sediment Benchmark (ESB) Approach for PAH Mixtures

This suggested revision is based on: (i) reviews and modelling of effects data for PAHs in sediments (Di Toro et al., 2000a,b; Di Toro and McGrath, 2000), and (ii) the equilibrium sediment benchmark (ESB) approach developed and proposed by the USEPA for mixtures of PAHs (USEPA, 2003a; Driscoll and Burgess, 2007; USEPA, 2012).

Based on extensive review and modelling of effects data, Di Toro and McGrath (2000) proposed a final acute value (FAV) and final chronic value (FCV) for total PAHs of 29 and 5.7 µmol/g OC respectively. The scientific basis for these threshold values is considered to be an improvement on the interim SQGV and ISQG-high values (ANZECC/ARMCANZ, 2000) and it is recommended that the SQGV and SQG-high value be set respectively as 10 and 50 mg/kg total PAHs, normalised to 1% organic carbon (the conversion from mole to mass being based on the PAHs having an average molecular weight of 173, the conversion factor used by Di Toro and McGrath (2000)).

When comparing the total PAH concentration with the SQGV and SQG-high, the 18 parent PAHs should be included: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo(a)pyrene, perylene, benzo(b)fluoranthene, benzo(k)-fluoranthene, benzo(e)pyrene, benzo(ghi)perylene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene.

The total PAH concentration is normalised to 1% organic carbon (OC) is undertaken within the limits of 0.2 to 10%. Thus if a sediment has (i) 2% OC, the '1% normalised' concentration would be the measured concentration divided by 2, (ii) 0.5% OC, then the 1% normalised value is the measured value divided by 0.5, (iii) 0.15% OC, then the 1% normalised value is the measured value divided by the lower limit of 0.2.

The guideline values for individual PAHs were removed because, for the majority of assessments, the concentration of total PAHs represents contributions from a large number of individual PAHs, with each being a small percentage of the total. While it is recognised that the toxicities of the individual PAHs differ significantly, it is considered unlikely that an individual PAH will, by itself, either dominate the total PAHs concentration or the Chemistry LOE within the WOE framework. Consequently the use of total PAH concentrations is considered suitable for most assessments. Where PAHs are likely to be the dominant COPCs in the sediments, the use of ESB approach is desirable, and includes 16 alkylated PAHs (Table A2.1).

The ESB for PAH mixtures is based on narcosis theory (USEPA, 2003a; Driscoll and Burgess, 2007; USEPA, 2011). The toxicities of mixtures of narcotic chemicals in water are considered to be approximately additive, and the combined toxic contribution of all PAHs in the mixture is the sum the effects predicted for each PAH. The EqP model is based on sediment organic carbon (OC) being the major PAH-binding phase in the sediments. The EqP model holds that non-ionic chemicals, such as PAHs, in sediment, partition between sediment OC, pore water and benthic organisms. At equilibrium, if the concentration in any one phase is known, then the concentrations in the others can be predicted. It was demonstrated that biological responses of benthic organisms to non-ionic organic chemicals in sediments are different across sediments when the sediment concentrations are expressed on a dry weight basis, but similar when expressed on an organic carbon basis (mg/kg OC). Similar responses were also observed across sediments when porewater concentrations were used to normalise bioavailability.

The ESB approach calculates an FCV concentration for each specific PAH in sediment ($C_{OC,PAHi,FCVi}$, mg/kg OC). The ESB for total PAHs is then calculated as the sum of the quotients of the 34 individual PAHs in a specific sediment divided by the $C_{OC,PAHi,FCVi}$, of each individual PAH. This sum is termed the ESB Toxic Unit ($\sum ESBTU_{FCV}$). For freshwater or saltwater sediments, if $\sum ESBTU_{FCV}$ <1.0 then no effects from PAHs are expected, and if the $\sum ESBTU_{FCV}$ >1.0, then sensitive benthic organisms may be unacceptably affected.

Advances have been made in the ESB approach to better consider the forms of carbon that exist in sediments and influence the partitioning of the organics. Black carbon (which includes pyrogenic carbon, soot, and coal particles) has been shown to be an important phase for binding hydrophobic organic contaminants (e.g. PAHs) in sediments. Provided suitable site-specific data are available on the form and concentration of black carbon (BC) and the influence of the BC concentration on the PAH-BC partition coefficients, then site-specific ESBs may be utilised that account for adsorption of PAHs onto BC and other forms of sediment organic carbon (Driscoll and Burgess, 2007). The ESB model now includes a two-carbon model, incorporating black carbon along with organic carbon, and is now available for making EqP-based predictions (US EPA, 2012). However, the partition coefficients for black carbon are often difficult to parameterise. Thus where significant amounts of black carbon are suspected to be present in sediments, measurements of interstitial water (porewater) PAH concentrations directly, or through passive sampling, is recommended.

For PAHs, the hydrocarbon narcosis risk model requires the measurement on sediment of 18 parent PAHs and 16 groups of prominent C1 to C4 alkyl PAH derivatives (so-called 34 PAHs) (Table A2.1). This creates a challenge for implementing the ESB approach is the analytical measurement of the 34 PAHs specified in the ESB document (USEPA, 2003a). There are quantification challenges for the alkyl compounds, as there are not standards for all compounds. The default approach is to assume that the response factors for the alkylated compounds are the same as the parents. Consequently, not many laboratories would analyse these routinely.

The interim Guidelines (ANZECC/ARMCANZ, 2000) considered the contribution of only 16 non-substituted PAHs. However, the ESB approach does allow for assessments that measure fewer than 34 PAHs through the use of an uncertainly factor. Although this greatly increases the uncertainty, it is based on a 95% confidence level and can be considered conservative.

The USEPA have developed an Excel-based spreadsheet as an ’autocalculator’ for the PAH ESBs (Mount, 2009, personal communication). Based on measured concentrations, this calculates the acute and chronic ‘potency ratio’ for measurement in waters or sediments. The calculations include an ‘alkylation multiplier’ to account for alkylated PAHs when parent PAHs were all that was measured. The reason for this is that if the source of PAHs is petroleum, the vast majority of the PAH potency is in the alkylated compounds, so analyses that measure only parent PAHs grossly underestimate the overall potency (USEPA, 2003a; Hawthorne et al., 2006).

While an ESB-based approach may be suitable for assessing possible toxicological effects due to PAH mixtures, it increases the complexity of the assessment and a single effects threshold based on the total PAH concentration is currently recommended for general guideline use. The proposed guideline should be applied to the sum of the concentrations of the 16 individual PAHs specified in the interim Guidelines (ANZECC/ARMCANZ, 2000).

Validation of model predictions is always desirable. The direct measurements of non-ionic organic contaminant concentrations in pore waters using passive samplers is increasingly being used to validate the EqP model predictions, calculation of their toxic units, and derivation of site-specific ESBs (Maruya et al., 2009; 2010).

SQGV and SQG-high value for total PAHs:

For all sediment types:

SQGV = 10 mg for total PAHs/kg (normalised to 1% organic carbon, dry weight)

SQG-High = 50 mg for total PAHs/kg (normalised to 1% organic carbon, dry weight)

where total PAHs = the sum of the 16 individual non-alkylated PAHs
(also known as unsubstituted or parent PAHs - listed in ANZECC/ARMCANZ, 2000)

In cases where the assessment allows for the measurement of both parent and alkylated (substituted) PAHs, the ESB benchmark approach may be applied (USEPA, 2003a; Driscoll and Burgess, 2007).

ESB_{PAHs} (applied as described in USEPA, 2003a):

For freshwater or marine sediments for 34 individual PAHs:

$\sum \text{ESBTU}_{\text{FCV}} < 1.0$ then no effects from PAHs are expected

$\sum \text{ESBTU}_{\text{FCV}} > 1.0$, then sensitive benthic organisms may be unacceptably affected.

The uncertainly factor should be applied when fewer then 34 PAHs are measured.

Table A2.1. Thirty-four individual PAHs to be analysed when applying the ESB

1	Naphthalene
2	2-Methylnaphthalene
3	1-Methylnaphthalene
4	C2 Naphthalenes
5	C3 Naphthalenes
6	C4 Naphthalenes
7	Acenaphthylene
8	Acenaphthene
9	Fluorene
10	C1 Fluorenes
11	C2 Fluorenes
12	C3 Fluorenes
13	Phenanthrene
14	Anthracene
15	C1 Phenanthrenes/anthracenes
16	C2 Phenanthrenes/anthracenes
17	C3 Phenanthrenes/anthracenes
18	C4 Phenanthrenes/anthracenes
19	Fluoranthene
20	Pyrene
21	C1 Fluoranthenes/pyrenes
22	Benz[a]anthracene
23	Chrysene
24	C1 Benz[a]anthracenes/chrysenes
25	C2 Benz[a]anthracenes/chrysenes
26	C3 Benz[a]anthracenes/chrysenes
27	C4 Benz[a]anthracenes/chrysenes
28	Benzo[b1k]fluoranthene
29	Benzo[e]pyrene
30	Benzo[a]pyrene
31	Perylene
32	Indeno[1,2,3-cd]pyrene
33	Dibenz[a,h]anthracene
34	Benzo[ghi]perylene

A4. ESBs for Dieldrin and Endrin

The revised SQGV and SQG-High values for dieldrin and endrin are the TEL and PEL values rather than the ERL and ERM values for organic contaminants (MacDonald et al., 2000; CCME, 2002). In addition, reviews of effects data for dieldrin and endrin have been made and ESB approaches proposed that may be useful for assessments where these compounds are major COPCs (USEPA, 2003b,c, 2012). ESBs for dieldrin and endrin are based on EqP theory that considers sediment OC (with options for BC) as the major binding phase for these chemicals.

For dieldrin, the ESB-based FCV is 12 (5.4-27) µg/g OC for freshwater sediments and 28 (12 to 62) µg/g OC for saltwater sediments.

For endrin, the ESB-based final chronic value (FCV) is 5.4 (2.4-12) µg/g OC for freshwater sediments and 0.99 (0.44-2.2) µg/g OC for saltwater sediments.

The confidence limits (in parentheses) were calculated using the uncertainty associated with the degree to which toxicity could be predicted by multiplying the partition coefficient for binding of endrin or dieldrin to organic carbon (K_{OC}) and the water-only effects concentration. It is suggested that the upper confidence limit is used as the SQG-High value.

It is suggested that these values are adopted as SQGVs.

ESB-based SQGs for dieldrin:

For freshwater sediment:

SQGV = 0.12 mg dieldrin/kg (normalised to 1% organic carbon, dry weight)
SQG-High = 0.27 mg dieldrin/kg (normalised to 1% organic carbon, dry weight)

For saltwater (marine) sediment:

SQGV = 0.28 mg dieldrin/kg (normalised to 1% organic carbon, dry weight)
SQG-High = 0.62 mg dieldrin/kg (normalised to 1% organic carbon, dry weight)

ESB-based SQGs for endrin:

For freshwater sediment:

SQGV = 0.054 mg endrin/kg (normalised to 1% organic carbon, dry weight)
SQG-High = 0.12 mg endrin/kg (normalised to 1% organic carbon, dry weight)

For saltwater (marine) sediment:

SQGV = 0.01 mg endrin/kg (normalised to 1% organic carbon, dry weight)
SQG-High = 0.22 mg endrin/kg (normalised to 1% organic carbon, dry weight)

A5. Derivation of a Guideline Value for Total Petroleum Hydrocarbons

Total petroleum hydrocarbons are common sediment contaminants and there are now sufficient effects data to derive a SQGV. They comprise a broad group of hydrocarbons, including crude and refined oils that are usually classified according to the number of carbon atoms contained in their alkane chains. Generally TPH concentrations are reported as C6-C9, C10-C14, C15-C28, C29-C36 TPHs, where the numbers refer to the number of carbon atoms. Once in the sediment, TPHs will generally adsorb to sediment particles, particularly those with high concentrations of organic matter. Some TPHs may redissolve, disperse and evaporate, while other TPHs may undergo biological or photo-degradation. The solubility, reactivity, transport, and degradability of TPHs generally decreases as their size increases (i.e. as the number of carbon atoms increases). As a consequence of the poorly defined nature of TPHs contaminants and of these processes, the composition of TPHs in contaminated sediments is very complex and will vary considerably from site to site. Therefore, the toxic effects of TPH-contaminated sediments will also vary greatly.

The concentration of TPHs in a sediment is typically determined using an operationally defined procedure (e.g. hexane/dichloromethane or supercritical fluid extractions). The extraction procedures can be non-specific to petroleum compounds, and a number of other non-polar organic substances may contribute to the result (e.g. peat, humic acids, or organic waste associated with pulp and paper mills). The potential for interferences and false measurements of TPHs may therefore need to be considered. Equally important to consider is that not all types of petroleum hydrocarbons will be equally harmful, and their bioavailability will be influenced by their carbon chain length, how long they have been in the sediments, and the concentration of organic carbon. Typical limits of reporting (LORs) for TPHs by most analytical laboratories are 25 mg/kg for C6-C9, 50 mg/kg for C10-C14, 100 mg/kg for C15-C28, and 100 mg/kg for C29-C36. This equates to an overall LOR of 275 mg TPHs/kg.

In studies by Brils et al. (2002), the effect of a concentration series of TPHs spiked into sediments on three toxicological endpoints was investigated: *Vibrio fischeri* (10-min test, endpoint bioluminescence inhibition), *Corophium volutator* (10-d test, endpoint mortality), and *Echinocardium cordatum* (14-d test, endpoint mortality). The study determined EC50s for gas oil and hydraulic oil, respectively, of 44 and 2,680 mg/kg dry weightfor *V. fischeri*, 100 and 9,100 mg/kg dry weight for *C. volutator*, 190, and 1060 mg/kg dry weight for *E. cordatum*. The most toxic fraction was the C10-C19 fraction. For TPHs in general, a no-observable-effects-concentration (NOEC) of 200 mg C10-40 TPHs/kg was calculated.

Pettigrove and Hoffmann (2005) observed significant impairment of freshwater macroinvertebrate assemblages for freshwater sediments that had been spiked with a synthetic motor oil. They estimated a Threshold Effect Concentration (TEC) of 860 mg TPH/kg.

Studies by Simpson et al. (2006) investigated the effect of hydrocarbon-contaminated sediments on the sub-lethal response of the benthic marine alga, *Entomoneis* cf *punctulata.* The sediments contained high concentrations of both PAHs and TPHs, and there was a strong relationship ($p<0.01$) between the concentrations of total PAHs and total TPHs in the sediment: TPHs = 2.2×total PAHs + 1470 mg/kg ($r^2 = 0.77$), indicating a possible common source for these contaminants. The NOEC determined based on the total PAH concentration was approximately 490 mg total PAHs/kg (dry weight), which equates to a concentration of 1080 mg TPHs/kg.

Brils et al. (2002) observed that the toxicity of diesel marine oil in sediments decreased significantly over time. Following 27 months of weathering of soil-spiked sediments, Jonker et al. (2006) observed significant losses, attributed to microbial degradation, of C10-C16 TPH fractions. The weathered sediments were significant less toxicity than the freshly-spiked sediments, and the toxicity was attributed to oil constituents rather than the co-occurring PAHs.

The USEPA has a convincing case that “oil” in sediment has an effect on some invertebrates beyond that from PAHs alone (Mount, presentation at SETAC North America meeting, New Orleans, 2009). Normalization to volume appeared important for interpreting concentration-response relationships. In whole sediment TIE studies where charcoal is added to remove organics (USEPA, 2007), the response of oil and PAHs differed. For estuarine/marine benthic organisms, the effects threshold determined for mineral oil were: 10-d LC50 of 20,000 mg/kg *Ampelisca abdita* (Burgess, unpublished results); 10-d LC50 210 mg/kg for *Leptocheirus plumulosus*; and 28-d NOEC > 18750 mg/kg for *Neanthes arenaceodentata* (Mount, presentation at SETAC North America meeting, New Orleans, 2009).

Verbruggen et al. (2008) have undertaken the most comprehensive study of the toxicity of TPHs in sediments and proposed a method for deriving thresholds. For freshwater and marine sediments spiked with either a light gas oil or a heavier lubricant, they conducted a suite of acute and chronic toxicity tests and a series of equilibrium partitioning calculations to estimate narcosis or baseline toxicity. Excellent concentration-response relationships (based on baseline toxicity) were obtained for the marine amphipod *Corophium volutator*, the sea urchin *Echinocardium cordatum,* and freshwater midge larvae *Chironomus riparius* and the amphipod *Hyalella azteca* (all 10-day tests). Useable responses were obtained for the bacterium *Vibrio. fischeri* and the mayfly *Ephoron virgo* (freshwater, 10-d), but poor responses were obtained for the nematode *Plectus acuminatus* (freshwater, 10-d), although these were still used by Verbruggen et al. (2008) in the threshold derivations. Both EC50 and EC10 values were calculated for each endpoint, although very wide 90% confidence intervals existed for the EC10s. Toxic effects were attributed to TPHs for the lighter gas oil, but for the heavier lubricant, toxicity was attributed to physical effects of the soil on the sediment (e.g. effecting burrowing and oxygen levels).

Verbruggen et al. (2008) derived TPH fraction-based environmental risk limits for a range of TPH carbon-numbers. For sediment with 5-10% TOC these ranged from 0.7 mg/kg (dry weight) for lighter 5-6 carbon TPHs to 15 mg/kg for heavier 21-35 carbon TPHs. These derivations would indicate that chronic effects to some species are quite possible for sediments with TPH concentration <50 mg/kg. For comparision, the LC50s were 160 (*C. volutator)*, 200 (*E. cordatum)*, 500 (*H. azteca*) and 3200 (*C. riparius*) mg TPH/kg and the EC10s were 100, 110, 170 and 2200 mg/kg respectively (the four species with the best concentration response relationships). Higher effects thresholds were determined for the heavier lubricant.

There are some interesting analytical and methodological challenges in the Verbruggen et al. (2008) study. For the TPH-spiked sediments, the background TPH concentrations ranged from 30 to 110 mg/kg and were believed to be of biogenic. The average recoveries of the spiked and background TPHs were used to calculate the carbon numbers for aromatic and aliphatic parts. Between 15 and 65% of the spiked TPHs were not recovered in the analyses (this is not considered unusual), but believed to remain present in the sediments in a non-bioavailable form. A number of other assumptions were necessary to estimate the exposure concentration used to derive the effects thresholds.

The limited effects threshold data available for total TPHs make setting a SQGV difficult. As the chronic effects threshold for some species may be below the typical LOR for TPHs of 275 mg/kg (sum of 25/50/100/100 mg/kg TPH C6-C9//C10-C14/C15-C28/C29-C36), then, if TPHs can be detected, they should be considered above the trigger level. Based on the available effects data and the routinely achieved LOR for TPHs, it is proposed that the LOR of 280 mg/kg be used as a SQGV. An arbitrary ISQG-High value of 550 mg TPH/kg is also proposed. While this initial approach is considered simplistic, and does not consider the differences in toxicity of the various TPH fractions, without improved LORs and stronger cause-effects relationships more complex guidelines are not appropriate.

Note that while TPHs will also partition strongly to sediment organic carbon, insufficient information is available to derive a SQGV that is modified using the TOC concentration of the sediments (as is done for other hydrophobic organic substances such as PAHs).

SQGV for TPHs:

For all sediments: SQGV = 280 mg TPH/kg (dry weight)

SQG-High value = 550 mg TPH/kg (dry weight)

A6. AVS-SEM and ESBs Applications for Metal Mixtures

This suggested revision, or modification of the application of the AVS-SEM approach, is based on the much greater understanding of the interaction of metals with sulfide phases in sediments that has been achieved since the release of the original Guideline document (ANZECC/ARMCANZ, 2000).

An ESB approach for the metal mixtures (ESB_{MM}) of Ag, Cd, Cu, Pb, Ni, and Zn in sediments has been proposed by the USEPA (2005). The EqP theory is used to calculate ESBs based on the metal partitioning in the sediment between acid-volatile sulfides, pore water, benthic organisms, and other sediment phases such as organic carbon (USEPA, 2005). The basis of these ESBs is that AVS is the key partitioning phase controlling cationic metal activity and metal-induced toxicity in the sediment–porewater system (Di Toro et al., 1992; Berry et al., 1996). AVS binds Ag, Cd, Cu, Co, Hg, Pb, Ni, and Zn, forming insoluble sulfide complexes with minimal biological availability. When there is a molar excess of AVS over SEM (SEM=ΣAg, Cd, Cu, Co, Hg, Pb, Ni, Zn), these metals are predicted to be bound as sulfide phases and not present in the pore waters at concentrations that can cause biological effects. When SEM - AVS <0, the sediments should not exhibit toxicity that can be attributed to these metals. The SEM - AVS approach was used as a bioavailability qualifier in the interim guidelines (ANZECC/ARMCANZ, 2000).

The AVS and SEM fractions are usually defined as the molar amounts (mmol/kg) of sulfide and metal respectively, that are released from the sediments by extraction in dilute HCl (0.5 -1 M) in 0.5 to 1 h (Di Toro et al., 1992; Allen et al., 1993; Simpson et al., 1998; Simpson et al., 2000). AVS concentrations are operationally defined, as there is no true measure of the accuracy of the results. It has been observed that there can be great variability among laboratories analysing the same samples (Hammerschmidt and Burton, 2010).

ESBs can also be calculated on a porewater basis, i.e. if porewater metal concentrations are below WQGs. The ESB metal-mixture procedure of the USEPA (2005) proposes further refinement of the EqP approach to consider partitioning of these metals to both AVS and particulate organic carbon: (SEM - AVS)/f_{OC}, where f_{OC} is the fraction of sediment that is organic carbon.

While the ESBs based on the SEM - AVS theory adequately predict which sediments are not toxic, when SEM-AVS >0 there is not a sound basis for predicting thresholds for when toxicity should occur. Seasonal changes can also influence the AVS-SEM relationship, and surficial sediments typically have much lower AVS concentrations than the deeper sediments (Naylor et al., 2004; Gallon et al., 2008; De Jonge et al., 2010; Teuchies et al. 2012). As many organisms reside within the more oxidised surface sediments or create oxidised microniches within more anoxic sediments, the protection from metal provided by AVS is not boundless (Simpson et al., 2012). There is still much uncertainty regarding the contribution of dietary-metal exposure to toxicity in benthic organisms (Rainbow, 2007; Simpson and Batley, 2007; Luoma and Rainbow, 2008; Campana et al., 2012; Camusso et al., 2012). Consequently, upper limits to the (SEM - AVS) threshold and ESB_{MM} are considered necessary, e.g. if the concentration of AVS is 50 µmol/g, the models predict a capacity to bind 3000 mg Cu/kg, but we doubt this would provide adequate protection to all benthic organisms.

For the revised guidelines, it is recommended that an upper threshold is applied for each metal ESB (Ag, Cd, Cu, Ni, Pb, Zn). As insufficient biological effects data exist to set risk-based limits, it is suggested that these thresholds should be set as a multiple of the existing SQGVs. The metals Ag, Cd, and Hg have low SQGVs and generally occur in sediments at low concentrations unless a major point-source of these metals in present. The metals Cu, Ni, Pb and Zn have higher SQGVs and generally occur at moderate concentrations in sediments that receive diffuse anthropogenic inputs (e.g. urban stormwater). Upper thresholds for the application of AVS-SEM and ESB-based SQGV modification for these metals are suggested as: Hg = 3 mg/kg (20× the SQGV), Ag = 10 mg/kg (10× the SQGV), Cd = 15 mg/kg (10× the SQGV), Cu = 300 mg/kg (~5× the SQGV), Ni = 200 mg/kg (~5× the SQGV), Pb = 500 mg/kg (10 × the SQGV), and Zn = 1000 mg/kg (5× the

SQGV). The use of these arbitrary upper thresholds applies greater conservatism to the application of AVS-SEM theory than existed in the original guidelines (ANZECC/ARMCANZ, 2000).

AVS concentrations are frequently highly variable in surface sediments and an excess of AVS over SEM in sediments sampled over a 0-10 cm depth, will not mean a similar excess of AVS exists for the 0-3 mm surface layer in which many meiofuana reside. For sediments with metal concentrations exceeding the SQG-High values, it is not uncommon to observe toxicity to epibenthic invertebrates despite an excess of AVS over SEM in the bulk sediment sample being tested. This may occur due to the oxidation of surface AVS during the toxicity tests, and but similar changes in metal bioavailability may occur when deeper sediments are brought to the surface in field locations.

It is important to note that benthic ecosystem health may be directly influenced by sulfide and high AVS concentrations in surface sediments (e.g. greater than 30-50 µmol/g), may cause a reduction in species diversity, or abundance some species (Chariton et al., 2010).

In summary, the ESB_{MM} should be applied with caution and the SEM – AVS and (SEM - AVS)/f_{OC} should be applied as modifying factors, rather than benchmarks, with careful attention to upper thresholds values for application to each metal.

To validate EqP approaches for metals, the direct measurements of metal concentrations in pore waters are still considered useful. However, as noted earlier, accurate porewater metal measurements are difficult due to the influence that small disturbances can rapidly have on metal concentrations. For many metals, the DGT technique now provides a useful method for measuring porewater metal concentrations and fluxes at the sediment water interface (Tankere-Muller et al., 2006; Simpson et al., 2012). It is anticipated that increased use of this technique will be valuable for validating EqP approaches for metals.

AVS-based modification of SQGVs for Ag, Cd, Cu, Hg, Pb, Ni, and Zn:

Any sediment in which the molar difference SEM > AVS should pose a low risk of adverse biological effects due to Ag, Cd, Cu, Hg, Pb, Ni and Zn; where SEM = $\sum$(Ag,Cd,Cu,Hg,Ni,Pb,Zn (µmol/g))

When this condition is met, the SQGV for each metal may be modified to a maximum set by the upper threshold. For assessments where these upper thresholds are exceeded, SEM-AVS or ESB should not used as 'bioavailability modifiers'.

Upper thresholds for metals when applying AVS-SEM or ESB approaches

Hg = 3 mg/kg (dry weight) (20× the SQGV)
Ag = 10 mg/kg (dry weight) (10× the SQGV)
Cd = 15 mg/kg (dry weight) (10× the SQGV)
Cu = 325 mg/kg (dry weight) (5× the SQGV)
Ni = 210 mg/kg (dry weight) (5× the SQGV)
Pb = 500 mg/kg (dry weight) (10 × the SQGV)
Zn = 1000 mg/kg (dry weight) (5× the SQGV)

For AVS, an arbitrary upper threshold of 50 µmol/g is suggested, whereby different ecological effects due to anoxia may become more apparent.

A7. Derivation of Sediment Property-Specific Guideline Values for Metals

This is a discussion topic rather than a suggested SQG revision, that considered how factors that affect the bioavailability of metals in more oxidised sediments may be used to modify trigger values for metals. The discussion is predominantly based on an extensive data set for copper, but it is expect that similar approaches may be applicable for other metals.

While equilibrium partitioning models have not been developed for oxic sediments, it has recently been demonstrated that toxicity thresholds based on the organic carbon (OC)-normalised copper concentration of the <63 µm sediment fraction were effective in predicting sub-lethal and lethal effects to a range of benthic organisms (Simpson et al., 2011; Strom et al., 2011; Campana et al., 2012; Campana et al., 2013). However a universal applicable model for metal bioavailability in oxidised sediments is not yet available. Costello et al. (2011) observed that the bioavailability of nickel in surface sediments was more strongly influenced by the concentrations of iron and manganese oxides than by OC. If future models of metal bioavailability are to be applicable to metal mixtures, then it is likely that the models should explicitly consider pools of AVS, OC and iron and manganese oxides as phases that reduce toxicity.

A7.1 Background

The Australian and New Zealand interim SQGV for copper in sediments is 65 mg/kg (ANZECC/ARMCANZ, 2000) and the ISQG-High value is 270 mg/kg. The SQGV was based on empirical effect data from Hong Kong (Chapman et al., 1999). The values may be compared to the effects range low (ERL) and effects range mean (ERM) values of 34 and 270 mg/kg derived by Long et al. (1995) and the threshold effects level (TEL) and probably effects level (PEL) values of 18.7 and 108 mg/kg derived by MacDonald et al. (1996). Each of these guideline values was generally derived from a ranking of toxicity and other effects data, and because contaminants typically co-occur (e.g. metals and organics), any toxicity was equally attributed to all contaminants within the mixture. This type of guideline derivation approach is very different to that used for the water quality guidelines (WQG), in which the WQG is derived from species sensitivity distributions of effect thresholds, for a range of species, where the effects are entirely from the contaminant of concern. The implication for the interim SQGs is that their value is greatest when applied to sediments with broad mixtures of contaminants (Long et al., 2006), but they should not be considered thresholds for any of the individual contaminants in isolation.

As discussed in the review by Simpson and Batley (2007), the toxicity of metal contaminants in sediments to benthic organisms is dependent on the bioavailability of metals in both the water (via exposure to pore water, burrow water, or overlying water) and sediment phases (via ingestion of particles), and on the sensitivity of the organism to these metal exposures. The bioavailability of metals in sediments is controlled by:

(i) speciation (e.g. metal binding with particulate sulfide, organic carbon, and iron hydroxide phases);
(ii) sediment-water partitioning relationships;
(iii) organism physiology (uptake rates from waters, assimilation efficiencies from particulates); and
(iv) organism feeding and other behaviour (feeding selectivity, burrow irrigation).

Where toxic effects have been attributed directly to copper, whole-sediment toxicity tests of both naturally contaminated (field-collected) sediments and artificially contaminated (copper-spiked) sediments using a range of benthic organisms indicate that toxic effects from copper are observed over a very broad range of total particulate copper concentrations (Roper et al., 1995; Costa et al., 1996; Bat and Raffaelli, 1998; Marsden and Wong, 2001; Marsden 2002; King et al., 2004; Simpson, 2005). However, for the majority of these studies, insufficient information was provided on the sediment properties and partitioning of copper between the dissolved and particulate phases to allow the interpretation of whether the copper-spiked sediments used would provide environmentally realistic exposures (Simpson and Batley, 2007). It has been demonstrated that both aqueous and dietary exposure to copper can cause toxic effects to some invertebrates (King et al. 2005, Simpson and King 2005). For many of the past studies, very little time was allowed for the spiked copper to equilibrate with the sediments before tests were commenced. This has the effect of resulting in very high porewater and overlying water copper concentrations compared to what is typically measured for field-contaminated sediments with similar total copper concentrations (Bat and Raffaelli, 1998). Such abnormally high dissolved copper concentrations results in a misinterpretion of the sensitivity of organisms to sediment copper (Simpson, 2005).

A7.1.1 EFFECTS DATA FOR EXPOSURES TO COPPER-SPIKED SEDIMENTS

Using copper-spiked fine sands, equilibrated for 24-48 h before toxicity tests, Roper et al. (1995) observed that the burial behaviour of the juvenile bivalve, *Macomona liliana* (endemic to New Zealand) was affected at 25 mg Cu/kg, and avoidance occurred at 10 mg Cu/kg. As no measurements of dissolved copper was made for pore waters or overlying waters, it is not possible to determine the significance of these results in terms of sediment copper and effects that might occur for more environmentally realistic exposures.

Using copper-spiked sediments with varying sediment particles size and organic carbon ranges, Costa et al (1996) determined that 10-day LC50 for the amphipod *Gammarus locusta* (endemic to Europe) of 6.8, 57 and >200 (no effects) mg/kg for sediments with the properties fine-fraction (%FF)/organic matter (by loss-on-ignition) 0.5%FF/0.9%LOI, 25%FF/1.9%LOI, 75%FF/7.1%LOI respectively. The sediments had been spiked with copper and equilibrated for just 24 h before toxicity tests were undertaken and it is likely that most of the effects were due to dissolved copper that had not bound to the sediments. Despite the likely artefact associated with inadequate equilibration of the spiked copper, the influence of particle size and organic matter (note that a LOI typically provides a high estimate of TOC) on the effects threshold was consistent with the revision of the Cu-SQG being proposed.

Studies by Marsden and Wong (2001) and Marsden (2000) determined a 10-day LC50 for the amphipod *Paracorphium excavatum* (endemic to New Zealand) of 55 mg/kg, and effects to juvenile recruitment at less than 20 mg/kg. The sediment used contained 57% fine material and 1.6% organic matter (by LOI), but like many of the past studies, the spiked copper was not given adequate time to equilibrate, with dissolved copper concentrations not representing environmentally realistic exposures (no measurements were made to check).

Bat and Raffaelli (1998) spiked sediment of particle size <300 µm with a mixture of Cd, Cu and Zn, and equilibrated for a few days (actual time not reported), before assessing survival of the amphipod *Corophium volutator* (endemic to the UK). An LC50 of 37 mg Cu/kg was determined, however as dissolved Cd, Cu and Zn concentrations exceeded 300, 400 and 100 µg/L during the tests, the effects were likely to be due to a combination of these dissolved metals. These dissolved concentrations greatly exceeded those observed for similarly contaminated field sediments, making the effects data of little use for guideline development.

For metal-metal spiked muddy sediments, equilibrated for 9 days before testing, Hagopian-Schlekat et al. (2001) determined a 96-h LC50 of 282 mg Cu/kg for survival of the copepod *Amphiascus tenuiremis*. Dissolved metals measured in the pore water indicated the effects were due to the dissolved exposure of the combined metals, with the LC50 for dissolved copper being 124 µg/L. Due to presence of the high dissolved mixed-metal concentrations, these data are also of little use for development of an GV for copper.

In the studies discussed by Simpson (2005) in which copper-spiked silty sediments were prepared to achieve environmentally-realistic partitioning between the dissolved and sediment phase, survival was unaffected at copper concentrations at the ISQG-High value of 270 mg/kg for any of the nine benthic organism considered. These studies indicated that ‘single value’ SQGVs are ineffective for predicting the toxicity of copper in sediments and that a better approach would be to have SQGVs that vary according to changes in sediment properties.

A7.2 Guideline Value Based on Porewater Copper Toxicity

One approach to assessing sediment quality is to derive a WQG for pore waters based on the database of dissolved copper toxicity for benthic organisms only. There is sufficient information on the sensitivity of benthic biota to dissolved copper in water-only toxicity tests to develop a copper guideline for exposure to benthic biota via sediment-associated waters (e.g. pore water, burrow water, and closely-associated overlying water). The derivation of a trigger value for copper in sediment-associated waters should only consider data from benthic organism life-stages that are in intimate contact with the sediment pore water and not organisms that don't reside in the sediments (benthos), or life stages of benthic organisms that occur solely in the water column, such as planktonic/larval life stages that exist in the water column before settling on the sediment substrate. A review of effects data for dissolved copper and the derivation of acute and chronic effects thresholds for copper in sediment-associated waters was undertaken by Simpson (2005).

The compiled copper effects data for benthic organism data are provided in Simpson et al. (2011). The most commonly reported effects data were LC50 values for water-only tests, of which there were values for 65 different species, comprising 1 benthic algae, 13 amphipods, 15 bivalves, 3 crabs, 3 gastropods, 10 harpacticoid copepods, 12 polychaetes, and 8 prawns/shrimps. Looking at the data in more detail, there were 44 different species with 96-h LC50 values for adults and 20 for juveniles. For 10-d tests there were 20 species with LC50 values for adults and 5 for juveniles. Chronic LC50 values were reported for 13 species and it was possible to calculate acute to chronic ratios (ACRs) (acute LC50/chronic LC50). Ratios of adult LC50 to juvenile LC50 and 96-h LC50 to 10-d LC50 could also be calculated for a range of species. Cumulative frequency plots of the acute LC50 values (Figure A7.1) indicate the position of the organism types (algae, amphipods, bivalve, etc.). These findings are from a range of geographic locations (North America, Europe, Australasia, etc.) with no specific differences based on location. These plots show that it is not easy to generalise about the sensitivity of different organism types (acute LC50 range from 15 to 15,000 µg.L), with bivalves and amphipods being among both the most and least sensitive species.

Based on the available 96-h LC50 values for the 54 organisms and using data for juveniles in preference to adults, a SSD calculation resulted in a 95% species protection concentration (95%PC) against acute toxicity of 38 µg Cu/L (Figure A7.2). While combining these data may not appear that logical, it was considered appropriate given the large variations in species lifecycles, which were not reported for most studies. If the SSDs were calculated based on data for either adults or juveniles, the acute 95%PC would be 48 µg/. L for 96-h LC50 values for adults (n=42) and 33 µg/L for juveniles (n=20).

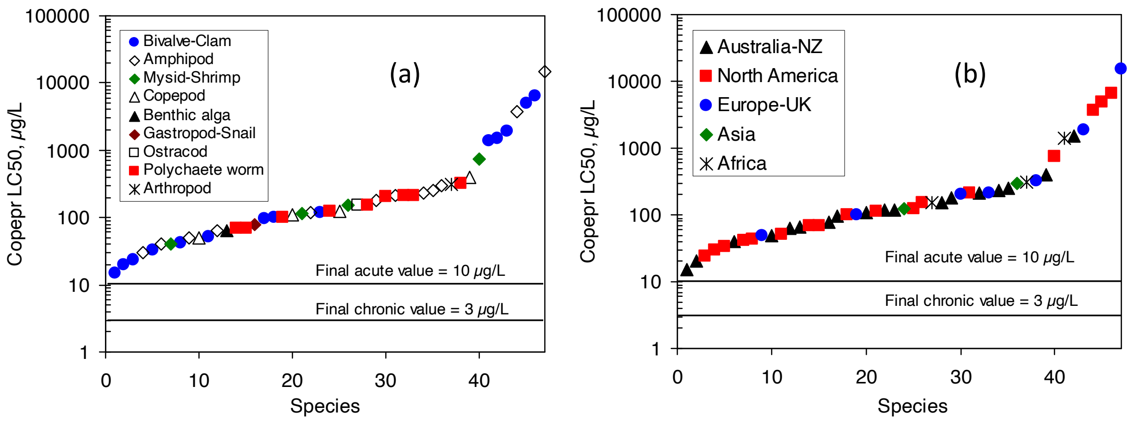

Figure A7.1. Cumulative frequency plot of LC50 values for benthic organisms used to calculate a WQG for porewater copper: (a) organism type and (b) species location (from Simpson et al., 2011)

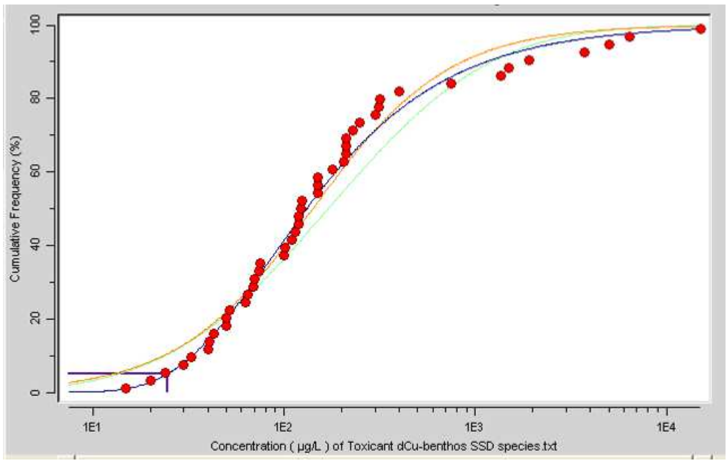

Figure A7.2. SSD calculation of final acute value LC50 (FAV-LC50) for benthic organisms exposed to copper in seawater

As benthic organisms are not always amenable to water-only exposures due to their preference to bury in sediments, it is not surprising that there are few studies that reported sub-lethal endpoints from water-only exposure tests. Significant and potentially confounding factors that should be considered when water-only effects data for benthic organisms are used include the potential for increased stress to the organism as a result of not having a sediment substrate in which it can burrow, and also the removal of possible dietary exposure routes that exist for organisms that ingest sediment particles (Strom et al., 2011). There were only 6 benthic species for which sub-lethal effects thresholds were reported (e.g. for gravidity, reproduction, growth). It is not considered appropriate to use such small data sets in SSD calculations, and it is generally recommended that a minimum of 8 species is used and preferably 10 or more. In many environments, contaminant exposures vary considerably (Angel et al., 2010; Burton et al., 2000). Organisms are able to avoid continuous exposures to high levels of contaminants (Eriksson Wiklund et al., 2006; Lopes et al., 2004). Consequently it may be appropriate to also consider chronic effects that include lethality occurring as a result of exposure periods of 10 days, as longer continuous exposures are unlikely to occur. While none of the data sets for sub-lethal or chronic effects appear ideal for guideline derivation, they may provide useful estimations. Based on the sub-lethal effects data for just the 6 species, the calculated 95%PC was 9.2 µg/L. Using the 10-day effects data for adults (LC50 values, n=20), the 95%PC value was 13 µg/L.

Water quality guidelines are traditionally based on chronic no observable effect concentrations (NOECs) (ANZECC/ARMCANZ, 2000) rather than acute or chronic data. To convert from acute LC50s to chronic NOECs, a factor or 10 is usually applied, a factor of 5 to convert chronic E(L)C50s to chronic NOECs, and a factor of 2.5 to convert LOECs (ANZECC/ARMCANZ, 2000). From the various studies (Table S1), it was difficult to derive a consistent set of ACRs as the endpoints and exposure periods for lethal and sub-lethal endpoints varied considerably. In the present study, lethality occurring within 7 days was considered to be an acute endpoint, with the chronic endpoints including sub-lethal effects or lethality occurring for exposures of 10 days (or greater) if sub-lethal endpoints were not available. There were 11 species for which ACRs could be derived and the geometric mean of the 11 ACRs was 3.2. The geometric mean of the ratios of adult/juvenile and 96-h/10-h LC50s were 2.3 and 3.2, respectively. The USEPA (2003e) draft ambient water criteria for copper in saltwater used an ACR of 3.23 for guideline derivation.

To derive a 95% species protection guideline for porewater copper, two approaches were considered: (i) the SSD-calculated 95%PC for 96-h LC50 values of 38 µg/L (n=54) divided by a factor of 10 to give a value of 3.8 µg/L; and (ii) the SSD calculated 95%PC for EC50 values of 9.2 µg/L (n=6) divided by the ACR of 3.2 to give a value of 2.9 µg Cu/L. While both approaches have strong and weak points, the outcomes were quite similar and a final chronic value (FCV) and guideline for porewater copper of 3 µg/L seems very appropriate. This value can be compared to the 95% PC of 1.3 µg/L derived as the WQG using data for 26 species

(ANZECC/ARMCANZ, 2000), which includes data for all water-column marine organisms, including fish and embryo/larval life-stages of benthic species.

A7.3 Guideline Value Based on Acute Toxicity of Copper to Benthic Organisms

The toxicity of copper in sediments may occur as a result of exposure through either dissolved or dietary exposure routes. For the dissolved phase, the exposure may be via pore water, burrow water, or overlying water. The dietary exposure route may include both living (e.g. algae, other benthos) and non-living (e.g. organic detritus, sediments) sources of particulate copper. The organism's feeding selectivity and physiology (gut passage time, metal assimilation efficiency from solid) will determine which of these phases contributes the greatest to the copper exposure.

The bioavailability of sediment-bound copper can be measured through its ability to be assimilated by the organism and may be influenced by the solid-phase speciation, i.e. copper binding with particulate sulfide, organic carbon, and iron and manganese (oxy)hydroxide phases. Accurately determining the forms of particulate copper is not possible using any existing wet-chemical or other techniques, e.g. spectrometry. At best, estimates of certain forms of copper can be made, the most commonly used approach involving variations in wet chemical extractions, to loosely classify the copper in weakly to strongly bound fractions that may or may not have ecological relevance. Naturally occurring copper is often highly mineralised and will be less bioavailable to benthic organisms compared to anthropogenic copper, which may be extractable using dilute acids (e.g. 1 M HCl). In sediments that have appreciable concentrations of reactive sulfide (e.g. AVS), the reaction between AVS and copper results in the formation of insoluble copper sulfide phases (e.g. CuS, Cu_2S) (Simpson et al., 2000; US EPA, 2005). Likewise, sediments with higher concentrations of particulate organic carbon (POC) generally bind copper more strongly than those with less POC (Simpson and Batley, 2007).

Relationships between AVS, 1 M HCl-extractable metals, POC, dissolved metals and toxicity to benthic organisms have been utilised to make predictions of the bioavailability and toxicity of metals in sediments (US EPA, 2005). However, for copper, the insolubility of copper sulfide phases in 1 M HCl severely limits this approach (Simpson et al., 1998). Other factors that are known to influence the partitioning of copper between the dissolved and particulate phases are particle size (i.e. surface area) and the concentrations and speciation of iron and manganese (Simpson and Batley, 2003).

Sediment-water partitioning relationships, which express the relative portion of copper associated with dissolved and solid phases (K_d (in L/kg) = [Sediment-Cu, mg/kg]/[Water-Cu, mg/L]), should be examined when considering which exposure pathways will be the greatest contributor to copper toxicity (Simpson, 2005). Dissolved copper is expected to be the most bioavailable form of copper to any organisms, and if the sediment-associated pore water or burrow water (or overlying water in closed systems such as toxicity tests) has dissolved copper concentrations above the water-only effects thresholds, effects would be expected in the whole sediments from the dissolved exposure. Conversely, when dissolved copper concentrations in sediment-associated waters are below these effects concentrations, any toxic effects attributable to copper are likely to be from the dietary exposure route.

A7.3.1 DERIVATION OF COPPER GUIDELINE VALUES FOR SEDIMENTS

Species sensitivity distributions (SSDs) can be used to calculate concentrations of chemicals that are protective of effects to specified percentages of species, e.g. 95% protection concentration (PC 95) (Campbell et al., 2000). Hypothetically, provided adequate data exist, it is possible to derive an SSD-based SQGV for copper that accounts for the differences in sediment properties (Figure A7.3) (Simpson and Batley, 2007). If sediment properties such as AVS, TOC, Fe/Mn modify copper effects thresholds for most organisms in the same manner (or to a comparable degree), then it may also be feasible to normalise SSDs for different sediment types to sediment properties. This type of SSD-approach would require effects data, spanning ranges of species that encompass all major exposure pathways, and all sediment types for which the guidelines may be applied. It is not known whether procedures for normalising for sediment properties would be successful and the SSD-approach may be appropriate for only a few sediment types.

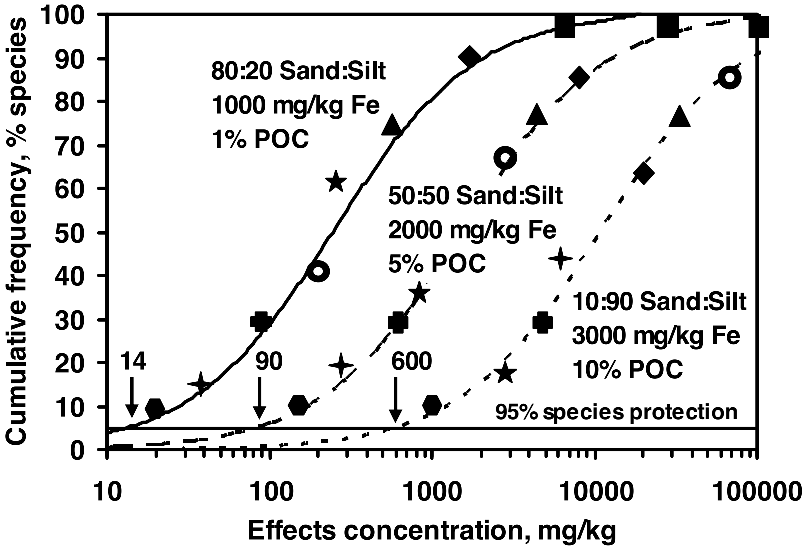

Figure A7.3. Conceptual SSDs for toxic effects in sediments with varying properties. Symbols represent different classes of sediment-dwelling biota, e.g. bacteria, algae, mysids, amphipods, bivalves, polychaete worms, snails, and crabs (from Simpson and Batley, 2007).

A7.3.2 COPPER PARTITIONING IN COPPER-SPIKED SEDIMENTS

The following key points arise from toxicity studies using copper-spiked sediments:

- Following adequate period for equilibration, for partially oxidised sediments the K_D for partitioning of copper between the sediment and water phases generally varies from around 1×10^4 L/kg for sandy sediments to 1×10^6 L/kg for silty sediments.
- Effects thresholds can be derived in terms of a number of copper fractions, e.g. total recoverable copper, 1-M HCl extractable copper, dissolved copper, or various ‘normalised’ (%silt, %TOC, AVS) fractions. Without reasonable understanding of the copper exposure pathway(s) that contributed to the lethal effects, it is not appropriate to use these data for SSD-based calculations.
- Organic carbon appeared to be more important than AVS in controlling copper bioavailability in sediments.
- Copper associated with AVS in surface sediments is labile over longer time-frames, leading to conversion to dissolved or oxidised forms.
- Copper associated with mineralised forms has a very low lability (ability to cause exposure through dissolution or dietary exposure).

There are adequate acute effects data for silty sediments to use an SSD to derive protective concentrations for copper using the same approach as used for the water quality guidelines (Table A7.1; Simpson et al., 2011).

Table A7.1. Acute lethality effects thresholds determined for a copper-spiked silty sediment

ORGANISM	DURATION	OVERLYING WATER DISSOLVED COPPER EFFECTS GV, µg Cu/L[a]				
	DAYS	LC50[a]	LC20	LC10	LOEC	NOEC
Corophium minor[a]	$10^{n=2}$	63±4	55±2	51±6	56±6	48±8
Hyale longicornis[a]	$10^{n=2}$	47±4	26±3	20±2	33±7	17±3
Nassarius burchardi[a]	$10^{n=2}$	67±7	47±2	33±2	69±8	20±17
Nassarius burchardi[a]	20/30/40 n=1	58/43/37	ND	ND	46/26/26	30/15/15
Heloecius cordiformis[a]	10[b]	ND	ND	ND	ND	70
Melita plumulosa[b]	$10^{n=3}$	76±15	ND	ND	64	36±9
		PARTICULATE COPPER EFFECTS ENDPOINT, mg Cu/kg[a]				
Organism	**Days**	**LC50**[a]	**LC20**	**LC10**	**LOEC**	**NOEC**
Corophium minor[a]	$10^{n=2}$	1980±160	1560±10	**1260±50**	1750±250	1250±250
Hyale longicornis[a]	$10^{n=2}$	1030±20	**610±35**	ND	1000	500
Nassarius burchardi[a]	$10^{n=2}$	1720±14	1550±200	**1450±20**	1750±250	1250±250
Heloecius cordiformis[a]	10[b]	ND	ND	ND	ND	2000
Melita plumulosa[b]	$10^{n=8}$	940±30	790±30	720±30	870±30 n=2	**720±120 n=2**
Tellina deltoidalis[b]	$10^{n=2}$	1030±150	850±130	790±120	950±50	**660±60**
Spicula trigonella[b]	$10^{n=8}$	940±40	790±35	710±30	950±50	**950±50**
Nitocra spinipes[c]	$5^{n=1}$	2000	ND	ND	1000	**800**
Soletellina alba[d]	$10^{n=1}$	ND	ND	ND	**1300**	ND
Mysella anomala[d]	$10^{n=1}$	ND	ND	ND	ND	**1200**
Nephtys australiensis[d]	$10^{n=1}$	ND	ND	ND	ND	**1400**
Australoneries ehlersi[d]	$10^{n=1}$	ND	ND	ND	ND	**1300**

[a] Simpson et al (2011 mean±standard deviation for *n* tests. [b] From Strom et al. (2011). [c] From Perez-Landa and Simpson (2011). [d] From King et al. (2004). ND = not determined due to inadequate concentration-response relationships. The **bold values** are the values used in the SSD. LCx and ECx are concentrations that cause x% lethality (L) or effect (E), respectively; CL confidence limit.

The acute copper effects data for these twelve benthic invertebrates were incorporated into an SSD to derive an SQG that is applicable to specific properties of this sediment (Table A7.1). The data comprised NOEC and LOEC values derived from statistical comparisons with control responses, and LC10 values calculated from the dose–response model. The mean LC10/NOEC ratio was 1.0 ± 0.2 (±standard deviation, n = 5), indicating that for many data sets these values may be interchangeable (Simpson et al., 2011). These calculations resulted in 95%PC values of 530 mg/kg for LC10s and 510 mg/kg for NOECs. On this basis, an acute no effects threshold of 510 mg/kg appears suitable for the silty sediment. Applying an ACR of 3.2 was therefore considered as appropriate (see Simpson et al., 2011), and resulted in a chronic no effects threshold of 160 mg/kg for the silty sediment.

With ~98% <63 µm particles, these sediments were possibly more silty than the 'average' silty sediments underpinning the effects-based guideline trigger value of 65 mg Cu/kg and the ISQG-high of 270 mg/kg (ANZECC/ARMCANZ, 2000). Given that empirical effects guidelines are largely based only on acute toxicity data (albeit for co-occurring contaminants) (Long et al., 1995), then for our sediment, the value 190 mg/kg could be considered a site-specific trigger value. However, this would not be considered a reliable replacement copper SQGV for a number of reasons:

(i) the acute effects for many of the species used were due, predominantly, to dissolved copper in the overlying waters that partitioned from the copper-spiked sediments. In naturally contaminated sediments at field locations, the dissolved exposure would be significantly lower due to greater dilution by overlying waters.

(ii) the SSD calculation only applies to one specific sediment for which these tests were undertaken, and different effects thresholds would be expected for different sediment properties.

A7.3.3 NORMALISED (<63 µM/OC) COPPER EFFECTS THRESHOLDS

A large amount of 10-day lethal effects data were gathered for three benthic invertebrates, *M. plumulosa, T. deltoidalis* and *S. trigonella*, for exposures to oxic/sub-oxic sediment with differing properties, both naturally varying and through modifications by amendment of silt and TOC, pH, etc. For each of these tests, detailed measurements were made of sediment properties (AVS, TOC, particle size, pH/Eh, other metals) and of the partitioning of copper between different sediment compartments (solid phases and dissolved copper in the pore water and overlying water) during tests. When all of these data are analysed collectively, the normalisation of acute effects to the epibenthic amphipod, *M. plumulosa*, and the bivalve, *S. trigonella*, using the <63 µm copper concentration/TOC concentration, provided a suitable approach for predicting effects in sediments with varying properties (Figure A7.4). Without this normalisation, there is no possibility of predicting when toxicity may occur (Figures A7.4a, c).

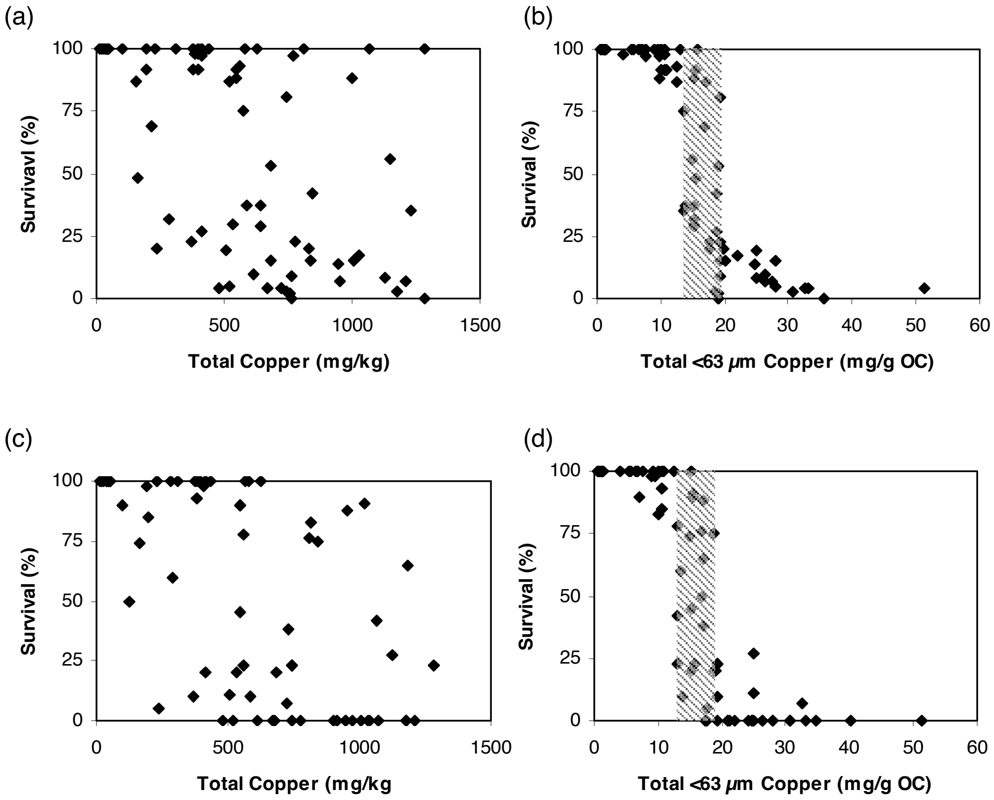

Figure A7.4. The normalisation of total copper to silt (<63 µm sediment fractions) and TOC provided a suitable approach for predicting effects in sediments with varying properties for *M. plumulosa* (a, b) and *S. trigonella* (c, d). Each data point represents the mean of three replicates (Strom, presentation at SETAC World Congress, 2008).

It is important to recognise here that the 10-day lethal effects for *S. trigonella* were due to dissolved copper exposure, while the 10-day lethal effects for *M. plumulosa* were due to both particulate (dietary) and dissolved copper exposure.

No observable effect concentrations (NOECs) were determined based on 10-day exposures to copper normalised to the <63 µm sediment fraction and organic carbon, respectively. Using these data, an SSD was created using the BurrliOZ algorithms (ANZECC/ARMCANZ, 2000) (Figure A7.5) and PC95 values (equivalent to 5HC5s) of 11 mg <63 µm Cu/g OC determined.

The PC95 value is considered to be quite conservative (protective) as:

- (i) the particulate copper was present in a highly bioavailable form (i.e. copper-spiked sub-oxic sediments) and is expected to conservatively account for any long-term transformations of mineralised and sulfidic phases into more bioavailable forms;
- (ii) the laboratory-based exposures were expected to create much higher dissolved copper exposure from the overlying water than would be expected for the same sediments in the field, i.e. where the copper released to the overlying water is diluted;
- (iii) the normalised particulate copper concentration is expected to account for dissolved and particulate copper exposure pathways for a diverse range of organisms; and
- (iv) SSD-based guidelines are considered to be quite conservative.

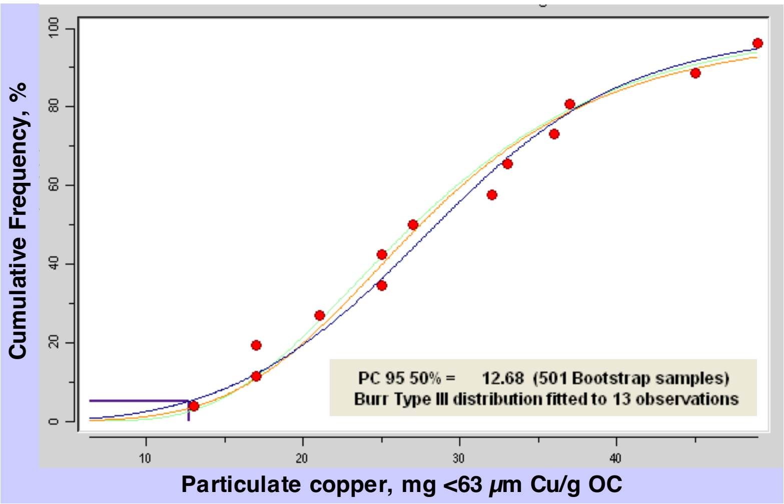

Figure A7.5. The SSD for normalised particulate copper (mg <63 µm Cu/g OC) for a range of benthic organisms in silty sediments (Simpson et al., 2008)

The use of the copper concentrations of the <63 µm sediment fraction (<63 µm Cu) normalised to the particulate organic carbon concentration of the <63 µm sediment fraction (<63 µm POC), provided the best interpretation of effects thresholds for copper in oxic/sub-oxic sediments with a wide range of particle sizes and organic carbon concentrations. Furthermore, the effects thresholds derived for oxic/sub-oxic sediments would be protective against effects from copper in sediment contain significant amounts of AVS and also for sediments containing significant amounts of highly mineralised forms of copper. AVS phases are susceptible to oxidation and there is a potential for some copper from highly mineralised forms to redistribute to more bioavailable forms.

It is suggested that the 'normalised' SSD-based acute effects threshold of 11 mg Cu/g OC (<63 µm /<63 µm OC) is used as a SQGV for copper. The significance of this normalised SQGV is demonstrated when 'conditional SQGVs' are recalculated in terms of total particulate copper in Table A7.2. For the purpose of this calculation, all the copper and OC in the sediment is assumed to be present in the <63 µm sediment fraction.

The calculation demonstrates that for sediments with greater concentrations of silt (<63 µm particles), and/or greater concentrations of fine-grained particulate organic carbon (<63 µm OC), then the copper effects threshold, below which effects will be negligible, increases, i.e. the SQGV based on total copper should increase.

Table A7.2. Calculation of conditional SQGVs for copper based on the 'normalised' SSD-based effects threshold of 11 mg Cu/g OC (<63 µm sediment/<63 µm OC)

% SILT	ACUTE SQGV FOR COPPER, mg Cu/kg [a]								
100	41	52	85	113	140	250	530	910	1130
90	41	51	83	109	135	240	500	870	1090
75	40	50	79	104	128	225	470	820	1010
50	38	47	72	93	114	200	410	700	870
25	37	43	63	80	96	160	330	560	690
10	35	40	55	67	80	130	250	430	530
2	33	37	47	56	64	100	190	310	370
% OC	0.10	0.20	0.50	0.75	1.0	2.0	5.0	8.0	10

[a] Total copper concentrations based on acute SQG of 11 mg Cu/g OC normalised to % silt and OC, and a no effect threshold of 30 mg/kg (background, non-bioavailable copper)

The conditional SQGVs consider all of the bioavailable copper to be associated with the <63 µm sediment fraction (Table A7.2). In situations where there are high copper concentrations associated with larger particle size fractions (not bioaccessible) or copper in highly mineralised forms (not bioavailable), this copper will not be expected to contribute significantly to dietary exposure and guidelines for pore waters and overlying waters should apply.

To account for this non-bioaccessible or non-bioavailable copper, the use of the conditional SQGVs based on total copper concentrations requires that an adjustment is made for this 'copper'. A suggested correction for the background copper that does not contribute significantly to the bioavailable copper exposure to benthic organisms, is to add the background copper concentration to the conditional SQGVs as shown in Table A7.2.

A7.4 Guideline Value Based on Chronic Toxicity of Copper to Benthic Organisms

Matching acute and chronic effects data for silty sediments with identical properties were produced for the amphipod, *Melita plumulosa*, the bivalve, *Tellina deltoidalis*, the copepod, *Nitocra spinipes*), the benthic alga, *Entomoneis* cf *punctulata*, and the snail, *Nassarius burchardi* (sub-chronic effects) (references by Strom, Perez-Landa, Spadaro and Simpson).

For the amphipod, *Melita plumulosa* (ACR= ~ 2), chronic effects (reproduction) were observed at normalised particulate copper concentrations of 7±1 mg Cu/g OC. For the same sediments the threshold for acute effects was 16±3 mg Cu/g OC. For *M. plumulosa* the ACR was 2.3 (Strom, 2010), and LC50s for 10-day survival = 770-850 mg/kg, 7-week gravidity = 410 mg/kg, and 7-week fertility = 320 mg/kg in copper-spiked (silty) sediments (Gale et al., 2006). Mann et al. (2009) found ACRs = 1.8-2.6 and LC50 values for 10-day survival ~ 500 mg/kg and 13-day rapid-fecundity ~250 mg/kg in copper-spiked (sandy) sediments For the bivalve, *Tellina deltoidalis* (ACR = 2.5), chronic effects (growth) were observed at normalised particulate copper concentrations of 4 and 6 mg Cu/g OC for the silty-sand and sandy copper-spiked sediments, respectively. The chronic effects threshold of 5±1 mg Cu/g OC was lower than the acute effects (lethality to similar sized bivalves) threshold of 12±2 mg Cu/g OC (Strom, 2010).

For the benthic algae, *Entomoneis* cf *punctulata* (ACR = ~2), effects to the algae occurred due to dissolved copper exposure only, but assessed for copper-spiked sediments (Strom, 2010). For the copepod, *Nitocra spinipes* (ACR = ~2): LC50s for 4-day survival ~600 mg/kg and 7-day juvenile-development ~300 mg/kg in copper-spiked (silty) sediments (Perez-Landa and Simpson, 2010). For the snail, *Nassarius burchardi* (ACR = ~1.7), LC10 values for 10-day acute lethality and 40-day sub-chronic lethality in copper-spiked silty sediments were 1470 (1340-1620) and 870 (620-1240) mg/kg, respectively (Simpson et al., 2011).

The outcome from the matching acute and chronic effects assessments was that an ACR of 3.2 appears suitable.

Using the acute effects data and the ACRs for the species *Melita plumulosa*, *Tellina deltoidalis*, *Entomoneis* cf *punctulata*, *Nitocra spinipes* and *Nassarius burchardi*, an SSD was created (Figure A7.6a). This calculation determined a 95% protection concentration of 4.8 mg <63 µm Cu/g OC. Using the all of the acute NOEC data and applying an average ACR of 2.5 to all species (*Entomoneis* cf *punctulata* not included) the 95% protection concentration was 5.1 mg <63 µm Cu/g OC (Figure A7.6b). Based on the most recent studies, this 95% protection concentration has been revised to 3.5 mg <63 µm Cu/g OC (Simpson et al., 2011; Campana et al., 2012). Chronic conditional SQGVs for total copper in sediments with a background concentration of non-bioavailable copper of 10 mg Cu/kg are shown in Table A7.3.

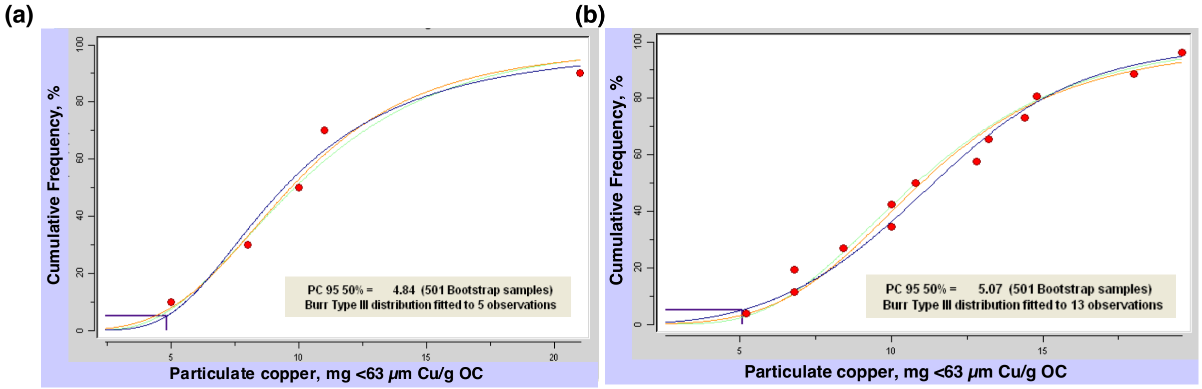

Figure A7.6. SSDs for normalised particulate copper (mg <63 µm Cu/g OC) using (a) chronic NOECs and (b) acute NOECs divided by ACR of 2.5

Table A7.3. Chronic conditional SQGVs for total copper in sediments

% SILT	CHRONIC SQGVs FOR COPPER, mg Cu/g [a]								
100	14	17	28	36	45	80	170	290	360
90	13	17	27	35	44	77	160	280	350
75	13	16	26	33	41	72	150	260	320
50	13	15	23	30	37	64	130	225	280
25	12	14	21	26	31	52	105	180	220
10	12	13	18	22	26	42	81	140	170
2	11	12	16	18	21	32	59	100	120
% OC	0.10	0.20	0.50	0.75	1.0	2.0	5.0	8.0	10

[a] Total copper concentrations based on chronic SQGV of 3.5 mg Cu/g OC normalised to % silt and OC, and a no effect threshold of 10 mg/kg (background, non-bioavailable copper)

A7.5 Validation of the Guideline Approach for Predicting Chronic Toxicity of Copper to Benthic Organisms

Further research has recently been completed on the influence of sediment properties on sub-lethal effects of copper on the reproduction of *M. plumulosa* and *N. spinipes* (Campana et al., 2012) and growth rate of *T. deltoidalis* (Campana et al., 2013). For both of these studies, the OC-normalized copper concentration in the <63 µm sediment fraction provided a single effects threshold for all sediment types. For reproduction of *M. plumulosa* and *N. spinipes*, the 10% effect concentrations (EC10s) were 5.2 and 4.8 mg <63 µm Cu g/OC (Figure 7.7). For the growth of *T. deltoidalis*, a no-effect value of 5.5 mg <63 µm Cu/g OC for was calculated (Figure 7.7). These results confirm the appropriateness of using OC-normalised copper concentration in the <63 µm sediment fraction to develop SQGVs that vary with sediment properties.

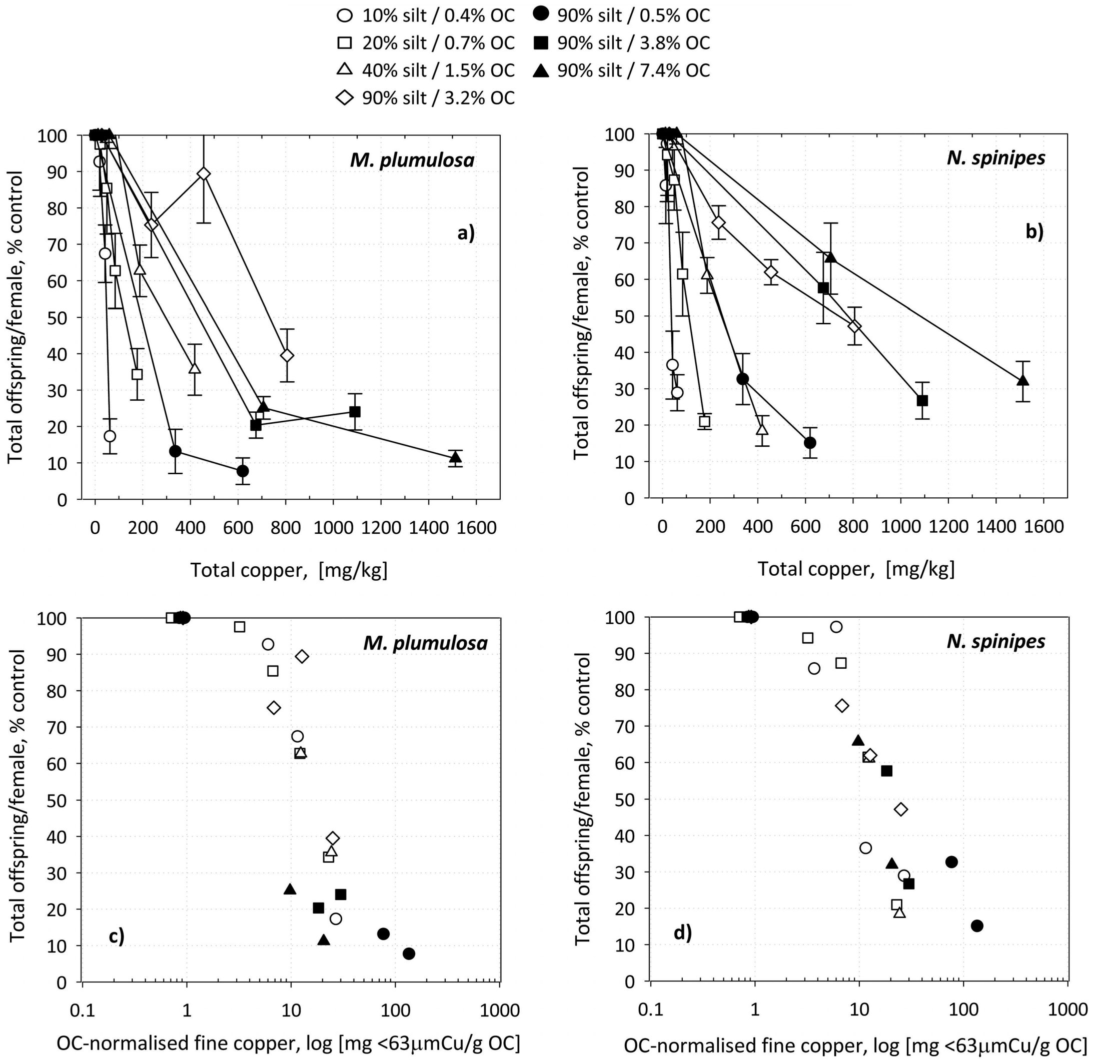

Figure A7.7. Effect (total-offspring/female) normalized to the average offspring in the corresponding control versus sediment copper concentration for exposure of *M. plumulosa* and *N. spinipes,* based on: (a, b) total copper, and (c, d) copper in <63 µm sediment/g OC (from Campana et al., 2012)

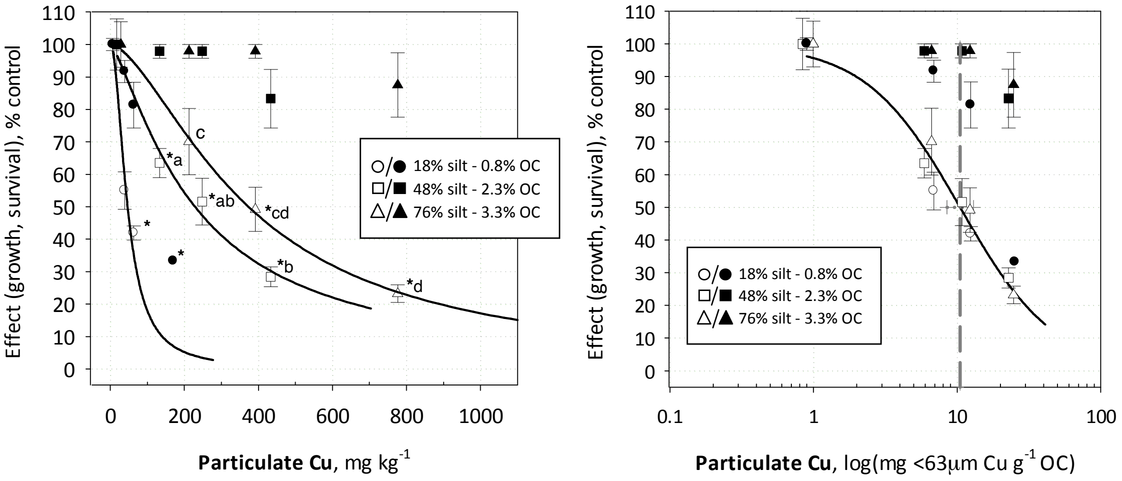

Figure A7.8. Dose-response relationships of lethal (survival - filled symbols) and sub-lethal (growth – open symbols) effects (means ± SE) to (a) particulate copper concentrations and (b) <63 µm sediment per g of organic carbon (OC). Lines represent logistic sigmoidal models applied to the growth response. * indicates significant difference from the control; means that share the same letter are not significantly different. The dashed line in (b) represents the EC50 of 10.5 mg <63 µm Cu/g OC (from Campana et al., 2013).

A7.6 Summary

The potential for modifying SQGVs for metals based on SSDs of effects data that vary in a predictable manner with changes in sediment particle-size and organic carbon appears to offer a significant improvement on the existing ‘single value’ SQGVs.

The use of metal-spiked sediments to create suitable data sets requires that the partitioning of metals between the dissolved and particulate phase is measured and deemed to be environmentally realistic before data can be used. Even with adequate equilibration, newly added metals are expected to result in greater exposure of organisms to bioavailable metals than would be expected for field-contaminated sediments with similar total metal concentrations. The metals in these sediments are expected to be highly bioavailable through both diet and partitioning to sediment pore waters and the overlying waters of the test containers.

The use of laboratory-based bioassays that do not have continuous renewal of overlying water results in greater exposure of organisms to dissolved metals than would be expected to occur for the same sediments at field locations where dilution with overlying water occurs rapidly. It is therefore important to carefully consider whether the dissolved metal exposure needs to be manipulated lower, through water renewal for example.

For sediments with significant AVS concentrations, there will be higher effects thresholds for metals such as Ag, Cd, Cu, Ni, Pb and Zn, however, AVS may not offer adequate protection for epibenthic organisms as the AVS in the surface sediments will be oxidised.

The provision of SQGs that better predict the effects of metals in sediments of varying properties and thus provide adequate protection against toxicity now appears quite achievable, as demonstrated by the present study of copper-contaminated sediments. SQGVs for copper derived from SSDs of effects data varied in a predictable manner with changes in sediment particle size and organic carbon, and were shown to offer a significant improvement on the existing ‘single value’ SQG. Adequate protection for all benthic organisms is expected to be achieved for an OC-normalised copper concentration of the <63 µm sediment fraction particulate copper concentration of 3.5 mg Cu/g OC and when dissolved copper in sediment pore waters or overlying waters is below 3 µg Cu/L. For short-term exposures, the equivalent acute guidelines are 11 mg <63 µm Cu/g OC and 9 µg Cu/L, respectively. SQGVs that vary with sediment properties can also be prepared in tabular form that both allows easy reference and can incorporate considerations of uncertainty.

The approach used for SQGV derivation incorporates a high degree of conservatism. Even with adequate equilibration, metal-spiked sediments are expected to result in greater metal exposure of organisms to bioavailable metals than would be expected for field-contaminated sediments with similar total metal concentrations. As the majority of the spiked copper was present in dilute acid-extractable metal (AEM) forms, it was seen as appropriate to apply the guidelines in Tables 7.2 and 7.3 to the AEM copper concentration. Furthermore, the use of laboratory-based bioassays that do not have continuous renewal of overlying water results in greater exposure of organisms to dissolved metals than would be expected to occur for the same sediments at field locations where dilution with overlying water occurs rapidly. Copper bioavailability will be further reduced in sediments with significant AVS concentrations, however, as AVS may be oxidised in surface sediments, it was not included as a persistent bioavailability modifying factor.

The use of metal-spiked sediments to create SSD-based guidelines for particulate metals is considered to be quite conservative (protective) as:

(i) the particulate metal exposure created using metal-spiked sediments will be present in a quite bioavailable form and is expected to conservatively account for any long term transformations of mineralised and sulfidic phases into more bioavailable forms;
(ii) the laboratory-based exposures are expected to create much higher dissolved metal exposure from the overlying water than would be expected for the same sediments in the field, i.e. where the metal released to the overlying is diluted;
(iii) the normalised particulate metal concentration may account for dissolved and particulate metal exposure pathways for a diverse range of organisms; and
(iv) SSD-based guidelines are considered to be quite conservative.

A8. References

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Glossary

GLOSSARY OF TERMS AND ACRONYMS

ACR: Acute to chronic ratio.

Acute toxicity: Effects resulting from exposure (usually short-term) over a small part of the organism's life span e.g. mortality, enzyme inhibition.

AEM: Dilute acid extractable metal (1M HCl).

*Algae: C*omparatively simple chlorophyll-bearing plants, most of which are aquatic, and microscopic in size.

Amphipod: A malacostracan crustacean of the order Amphipoda.

ANOSIM: Analysis of similarities.

ANOVA: Analysis of variance.

ANZECC: Australian and New Zealand Environment and Conservation Council.

*Aquatic ecosystem: A*ny water environment from small to large, from pond to ocean, in which plants and animals interact with the chemical and physical features of the environment.

ARMCANZ: Agriculture and Resource Management Council of Australia and New Zealand.

AVS: Acid volatile sulfides, the acid soluble sulfide concentration in an aquatic sediment.

BEDS: Biological effects database for sediment

Benthic: Referring to organisms living in or on the sediments of aquatic habitats.

Bioaccumulation: A general term describing a process by which chemical substances are accumulated by aquatic organisms from water directly or through consumption of food containing the chemicals.

Bioassay: a test used to evaluate the relative potency of a chemical by measuring its effect on a living organism relative to a control.

Bioavailable: Able to be taken up by organisms.

Biodiversity: The variety and variability of living organisms and the ecological complexes in which they occur.

Biomagnification: The result of the processes of bioaccumulation by which tissue concentrations of bioaccumulated chemicals increase as the chemical passes up through two or more trophic levels. The term implies an efficient transfer of chemicals from food to consumer so that the residue concentrations increase systematically from one trophic level to the next.

Bivalve: A mollusc with a shell in two parts, hinged together.

Chronic toxicity: Effects over a significant portion of the organism's life span e.g. effects on growth and reproduction*.*

COC: Contaminant of concern.

Community: Assemblage of organisms characterised by a distinctive combination of species occupying a common environment and interacting with one another.

Community composition: All the types of taxa present in a community.

Concentration: The quantifiable amount of a substance in water, food or sediment.

Contaminants: Biological or chemical substances or entities, not normally present in a system, capable of producing an adverse effect in a biological system, seriously injuring structure or function.

Contaminated sediment: A sediment containing chemical substances at concentrations above background concentrations and above the ANZECC/ ARMCANZ guideline values.

Control sediment: A sediment that is sufficiently free of contaminants that it will not cause effects to test organisms. Generally a control sediment will have similar physicochemical parameters as the test sediments.

Control: Part of an experimental procedure that is ideally exactly like the treated part except that it is not subject to the test conditions. It is used as a standard of comparison, to check that the outcome of the experiment is a reflection of the test conditions and not of some unknown general factor.

COPC: Contaminant of potential concern.

Copepod: A small crustaceans found in the sea and nearly every freshwater habitat; many are planktonic (drifting in sea waters), but more are benthic (living on the sediments).

DDE: dichlorodiphenyldichloroethylene.

DDT: dichlorodiphenyltrichloroethane.

Detection limit: Method detection limit is the concentration of a substance that, when processed through the complete analytical method, produces a signal that has a 99% probability of being different from the blank.

DO: Dissolved oxygen

DOC: Dissolved organic carbon

DTA: Direct toxicity assessment.

Ecogenomics: The examination of genetic (DNA) materials in environmental samples for the purpose of identifying the organisms present.

Ecotoxicology: The science dealing with the adverse effects of chemicals, physical agents and natural products on populations and communities of living organisms

EC50: The toxicant concentration that is expected to cause one or more specified effects in 50% of a group of organisms under specified conditions.

ERL: Effects range low.

EqP: Equilibrium partitioning

ERM: Effects range median.

ESB: Equilibrium sediment benchmark.

FACR: Final acute to chronic ratio.

FAV: Final acute value.

FCV: Final chronic value.

F_{OC}: Fraction of organic carbon

f-SSD: Field-based species sensitivity distribution

f-CSD: field-based community sensitivity distribution

Guideline: Numerical concentration limit or narrative statement to support and maintain a designated water use.

HC: Hazardous concentration, usually to a given percentage of species, e.g. HC5 is the concentration hazardous to 5% of species.

HOC: Hydrophobic organic contaminant.

IC50: A toxicant concentration that would cause a 50% reduction in a non-quantal measurement such as fecundity or growth.

Index (indices): Composite value(s) that can give a quick ranking to a waterbody or other ecosystem feature, derived via a formula that combines measurements of important ecosystem characteristics; typically used to rank `health' or naturalness.

Indicator: Measurement parameter or combination of parameters that can be used to assess the quality of water.

Invertebrates: Animals lacking a dorsal column of vertebrae or a notochord.

K_D: Sediment/water partition coefficient.

K_{OC}: Organic carbon based sediment/water partition coefficient.

K_{OW}: Octanol–water partition coefficient.

LC50: A toxicant concentration that is expected to be lethal to 50% of a group of organisms under specified conditions.

Level of protection: The acceptable level of change from a defined reference condition.

LOE: Line of evidence.

LOI: Loss on ignition.

LOR: Limit of reporting.

Lowest-observable-effect concentration (LOEC): The lowest tested concentration of a material (toxicant) at which organisms were adversely affected compared to control organisms.

Measurement parameter: Any parameter or variable that is measured to find something out about an ecosystem.

MDS: Multidimensional scaling.

nMDS: Non-metric multidimensional scaling.

NOAA: U.S. National Oceanic and Atmospheric Administration.`

No-observable-effect concentration (NOEC): The highest tested concentration of a material (toxicant) at which organisms were unaffected, as compared to control organisms.

NWQMS: National Water Quality Management Strategy.

OC: Organochlorine.

Organism: Any living animal or plant; anything capable of carrying on life processes.

Overlying water: The water above the sediment at a collection site or in a test chamber.

Oxidation: The combination of oxygen with a substance, or the removal of hydrogen from it, or, more generally, any reaction in which an atom loses electrons.

PAHs: Polycyclic aromatic hydrocarbons.

PCB: Polychlorinated biphenyl.

PE: High probability of effects.

PED: Polyethylene device.

POM: Polyoxymethylene.

PEL: Probable effects level.

PERMANOVA: Permutational analysis of variance.

Pesticide: Substance or mixture of substances used to kill unwanted species of plants or animals.

pH: The intensity of the acidic or basic character of a solution, defined as the negative logarithm of the hydrogen ion concentration of a solution.

Phytoplankton: Plants, usually microscopic, floating in aquatic systems.

Polychaete worm: Chiefly marine annelids possessing both sexes and having paired appendages (parapodia) bearing bristles.

Pore water: The water that occupies the space between and surrounds individual sediment particles in an aquatic sediment (often called *interstitial water*).

Pyrosequencing: A method of DNA sequencing (determining the order of nucleotides in DNA) based on real-time (quantitative) detection of pyrophosphate release on nucleotide.

QA/QC: Quality assurance/quality control.

Quality assurance (QA): The implementation of checks on the success of quality control (e.g. replicate samples, analysis of samples of known concentration).

Quality control (QC): The implementation of procedures to maximise the integrity of monitoring data (e.g. cleaning procedures, contamination avoidance, sample preservation methods).

Redox: Simultaneous (chemical) reduction and oxidation; reduction is the transfer of electrons to an atom or molecule, whereas oxidation is the removal of electrons from an atom or molecule.

Redox potential: A measure of the oxidation-reduction potential (ORP) of sediments. The redox potential is often reported as E_h (versus the normal hydrogen electrode).

Reference sediment: A sediment, generally collected near the study site, that is used to assess the affect of sediment and overlying water conditions exclusive of the material(s) (contaminants, toxicants) of interest.

Reference toxicant: A test conducted with a reference chemical (toxicant) to assess the sensitivity of the test organisms.

Reference condition: An environmental quality or condition that is defined from as many similar systems as possible (including historical data) and used as a benchmark for determining the environmental quality or condition to be achieved and/or maintained in a particular system of equivalent type.

Risk: A statistical concept defined as the expected frequency or probability of undesirable effects resulting from a specified exposure to known or potential environmental concentrations of a material, organism or condition. A material is considered safe if the risks associated with its exposure are judged to be acceptable. Estimates of risk may be expressed in absolute or relative terms. Absolute risk is the excess risk due to exposure. Relative risk is the ratio of the risk in the exposed population to the risk in the unexposed population.

Salinity: The presence of soluble salts in water or soils.

Sediment: Unconsolidated mineral and organic particulate material that has settled to the bottom of aquatic environments.

SEM: Simultaneously extracted metals

Solution concentration: Concentration of contaminants in the liquid phase.

Speciation: Measurement of different chemical forms or species of an element in a solution or solid.

Species: Generally regarded as a group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not normally breed with members of another group. (Chemical species are differing compounds of an element.)

Species richness: The number of species present (generally applied to a sample or community).

Spiked sediment: A sediment to which a material has been added for experimental purposes.

SPMD: Semi-permeable membrane device.

SPME: Solid phase microextraction fibre.

Statistical power: The ability of a statistical test to detect an effect given that the effect actually exists.

SQGV: Sediment quality guideline value (previously known as a trigger value).

SQG-High: The higher *s*ediment quality guideline value (previously the ISQG-High).

Stressors: The physical, chemical or biological factors that can cause an adverse effect on an aquatic ecosystem as measured by the condition indicators.

SSD: Species sensitivity distribution.

STU: Sediment toxic unit.

Sub-lethal: Involving a stimulus effect below the level that causes death.

Taxon (taxa): Any group of organisms considered sufficiently distinct from other such groups to be treated as a separate unit (e.g. species, genera, families).

Taxa richness: Number of taxa present.

TBT: Tributyltin.

TC: Total concentration.

TE: Threshold for effects.

TEL: Threshold effects level.

TEC: Threshold effects concentration.

TIE: Toxicity identification and evaluation.

TOC: Total organic carbon.

Trophic transfer: Transfer of accumulated contaminants from on level of the food chain to the next higher level.

Toxicant: A chemical capable of producing an adverse response (effect) in a biological system, seriously injuring structure or function or producing death. Examples include pesticides, heavy metals and biotoxins.

Toxicity: The inherent potential or capacity of a material to cause adverse effects in a living organism.

Toxicity test: The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical).

TPH: Total petroleum hydrocarbon

TPM: Total particulate metal (digestion with strong acids)

Trophic level: A notional stage in the `food chain' that transfers matter and energy through a community; primary producers, herbivores, carnivores and decomposers each occupy a different trophic level.

TV: Trigger value; the past term used for SQGV.

Uptake: A process by which materials are absorbed and incorporated into a living organism.

Whole sediment: The sediment and associated pore water that have had minimal disturbance or manipulation.

WQG: Water quality guideline.

WOE: Weight of evidence.

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ANNEXURE - 2

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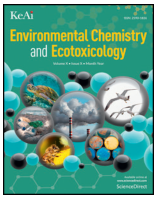

Ecological risk from heavy metals in Ennore estuary, South East coast of India

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ABSTRACT

The dynamic coastal and marine waters are frequently polluted by metals that affect the biota. Metal concentrations in the biota, water and sediments of Ennore estuary were monitored spatially over two years to understand their potential impact and ecological risk. Variations in concentrations of chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb) and nickel (Ni) were observed in water, sediment and marine organisms. The metal concentrations (μg/l) in the water were in the order of Ni (35) > Pb(16.9) > Cu(15.8) > Cr(12.5) > Cd(5.6) and sediment (μg/g) was Cr(390) > Pb(266) > Cu(162) > Ni(125) > Cd(7.6). Elevated metal levels in the ambient water and sediment tend to accumulate in the marine organisms. Average concentrations of Cr, Cu, Cd, Pb and Ni in fish *Mugil cephalus* measured as 4.6, 2.4, 2.21, 4.9 and 2.6 μg/g, 11.4, 22.1, 3.3, 8.9 and 6 μg/g in Crab *Scylla serrata*, 13, 20.8, 1.3, 7.9 and 4.8 μg/g in Oyster *Crassostrea madrasensis* and 18.8, 20.1, 1.0, 12 and 5.6 μg/g in polychaete *Eunice* spp. Distinct patterns of metal content in crab, oyster and polychaete (Cu > Cr > Pb > Ni > Cd) and in fish (Pb > Cr > Ni > Cu > Cd) are due to differences in their habitat and specific uptake and elimination capacity. Environmental quality indices (EQI) and Potential ecological risk index (PERI) were derived from the metal concentration in sediment. EQI evidenced that the Ennore estuary is highly contaminated by Cd and Pb from anthropogenic sources. PERI implied that the estuary is under considerable ecological risk from metals. Contamination and risk from metals are relatively higher during summer owing to the low influx of water and higher residential period. Metal concentrations are exceeding the guideline values in the ambient water, sediment and biota in Ennore estuary. It is concluded that long-term monitoring of metal distribution, bioaccumulation and risk assessment provides reliable data and valuable information for possible pollution management strategies in Ennore estuary.

1. Introduction

Coastal environments, especially estuaries are under serious threat due to urbanization and industrialization. Estuaries are characterized with high biological productivity, providing habitat to a plethora of organisms either during the larval stage and/or for their entire life cycle. These sensitive ecosystems are constantly under stress from various pollutants such as metals, persistent organic compounds, pesticides and hydrocarbons. Heavy metals are natural constituents of the environment, but the increase in their concentrations has been reported extensively in many coastal environments [1]. In the recent past, a great volume of scientific literature has been reviewed concerning the sources, fate and transport, toxicity, mode of action and bioaccumulation of metals in food chain organisms in the coastal realms [2]. Effluents from mining, electroplating, metallurgy, machinery, automobile, refinery, combustion of coal/fuel oil, agriculture and domestic are discharging metals into coastal waters [3, 64]. Metals tend to adsorb from water columns onto surfaces of particles and they are settled into the sediment. Metals can be accumulated in the tissues of marine organisms from the water, sediment, suspended particulate materials. Several studies have demonstrated metal contamination in seawater, sediments and bioaccumulation in coastal and estuarine ecosystems of India [4–9]. Bioaccumulation is the indication of bioavailability and it could be a biomarker of metal pollution. Bio-indicator organisms are able to bio-accumulate metals from contaminated ambient environments [10]. A review of comprehensive changes in metal contamination with changing time around India revealed that estuarine sediments from the west coast are comparatively more polluted than the east coast [11]. Ennore estuary is highly dynamic

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with geographic changes in the bar mouth and characterized by the influence of discharge from various industries and wastewater from municipal sewage. As a result availability and distribution of metals in water, sediment and biotic compartments of Ennore estuary exhibit seasonal and spatial changes. Ennore estuary has been extensively studied for its biodiversity, fishery resources, metal concentration and their biological effects on selective resident species [7,8,12–14]. However, data reported in the previous studies are patchy and are derived from discrete samplings. Hence, comprehensive monitoring of metals in Ennore estuary is necessary to record spatio-seasonal levels of metal concentrations to make policy decisions regarding environmental protection and abate metal pollution in the estuary. Priority metals viz. Cu, Cr, Cd, Pb and Ni are increasing globally in the inland and coastal waters especially regions of developing countries like India [15,16]. In view of the above facts, the present study has been undertaken to (i) monitor metal concentrations in water, sediment and tissue samples of marine organisms based on their diversified habitat like surface and bottom feeder (fishes), suspended sediment, (crabs), filter feeder (oysters) and benthic, (polychaetes), (ii) study the rate of bioaccumulation from ambient water and sediment (iii) assess environmental risk from Cr, Cu, Cd, Pb and Ni in the ecosystem through the indices like Contamination Factor (CF), Enrichment Factor (EF), Geoaccumulation Index (Igeo), Pollution Load Index (PLI) and PERI.

2. Materials and methods

2.1. Study area

Ennore estuary (13°13′54.48″N, 80°19′26.60″E) is located near the metropolitan city Chennai. Estuary is connected with Pulicat Lake in the north through Buckingham Canal and Kosasthalaiyar River in the northwest. The shore currents influence the constant accumulation of sand near the bar mouth of the estuary affecting the tidal inflow of seawater. Anthropogenic activities like industrial, thermal power plants, petrochemicals, automobiles, harbor and suburban residential areas including fishing hamlets are causing pollution in the estuary. Five sampling stations were selected in the estuary based on multiple activities in the vicinity across the main stream of estuary from the bar mouth to upstream covering ~7.5 km of distance. Bar mouth (Station1) is influenced by both freshwater run-off during monsoon and seawater during the diurnal tidal cycle. Dredging activities are being practiced in the mouth region to facilitate flow of thermal discharge from the Ennore Thermal Power Station (ETPS) to sea. Station 2 is the inlet of Buckingham Canal in the mid estuary. Fishing of the polychaete worms by groups of fishermen frequently observed in this station as an alternative livelihood. Station 3 is the mid-region of the estuary. Station 4 is influenced by the release of wastewaters from the Manali industrial area and also by municipal activities. Station 5 is the Inlet of Kosasthalaiyar River which runs for a distance of about 136 km from Vellore town and drains fresh water along with domestic wastewaters (Table 1, Fig. 1). The tidal flow is very low in both Station 4 & 5, is mostly turbid, cloudy, black colored, and may release hydrogen sulfide (H_2S) causing a foul smell. The emergence of gas bubbles was observed during field sampling which may be due to the formation of H_2S during the anaerobic decomposition of particulate matter.

2.2. Sampling and analysis

Water, sediment and marine organisms were collected fortnightly for a period of two years (December 2014 to November 2016) from the Ennore estuary. Totally 480 samples of water and sediment were collected from all the five sampling stations. Irrespective of stations, adequate samples of marine organisms were collected from the estuarine region at their habitat based on availability. Since, the selected marine organisms are not evenly distributed in all the sampling stations and sampling times. 20 numbers of each marine organism except polychaete (18 Nos.) are analyzed during the study period. These samples are categorized into four seasons viz. post-monsoon (January to March), summer (April to June), pre-monsoon (July to September) and monsoon (October to December) to determine the seasonal variations of metal concentrations and their risk. These four seasons are selected based on the influence of monsoonal runoff. Salinity was measured during the sampling using digital refractometer.

The samples were processed and analyzed for the total metal concentrations using the standard methods. Dissolved metals such as Cu, Cd, Cr, Pb and Ni in water samples were pre-concentrated following the method described by Mentasti et al. [17]. Water samples (400 ml) were filtered and acidified to pH 4.0 by the addition of ultrapure nitric acid. The samples were vigorously shaken with 2 ml of 2% ammonium pyrrolidine dithiocarbamate (APDC) and 15 ml of Methyl Isobutyl Ketone (MIBK) using an automated reciprocating mechanical shaker (Make: Recipro, Model: RS-1). The Organic layers were collected in 100 ml separating funnels and the aqueous layer were subjected for extraction of metals again. The MIBK solvent with metals were back extracted by mixing through vigorous shaking with 0.1 ml of concentrated nitric acid, subsequently 9.9 ml of deionized water and allowed to separate the layers. The aqueous layers were collected in PTFE bottles and stored in room temperature until the instrumental readings. A set of seawater spiked with metals using calibration standards were extracted for recovery measurement to validate the analytical procedure.

Surface sediment samples were collected using Van-Veen grab and transferred into polythene bags. Sediment samples were dried at 65 °C for 24 h and powdered in porcelain mortar and pestle. 0.2 g of powdered sediment samples were subjected to acid digestion in Teflon beakers with nitric, hydrofluoric and perchloric acids in a ratio of 4:1:1. Then the mixture was heated at 200 °C using a hot plate until perchloric acid evaporated as smoke. The mixture was cooled to room temperature. Again the sample was digested at 200 °C to dryness. The extracted metals from sediment samples in Teflon beaker were transferred to 0.5% nitric acid in deionized water, filtered through 0.45 μ filter and made upto 25 ml using deionized water [18].

Total organic carbon in sediment samples was analyzed by the wet oxidation method described by Walkley [19]. Briefly, 0.5 g of dried sediment sample is weighed into 500 ml of Erlenmeyer flask. Then, 10 ml of 1 N of $K_2Cr_2O_7$ was added and mixed by swirling followed by addition of 20 ml of concentrated H_2SO_4. This mixture was incubated for 20 min at room temperature, and then diluted to 200 ml in distilled water. 10 ml of

Table 1
Detail of sampling stations with geographical co-ordinates and total organic content (TOC) in sediment from Ennore estuary.

Station code	Location	Latitude	Longitude	TOC [a]
S1	Bar-mouth of Estuary	13.234247	80.329756	0.35 (0.29–0.4)
S2	Buckingham canal inlet	13.226252	80.317922	0.55 (0.48–0.63)
S3	Municipal waste water discharge point	13.215050	80.306701	1.79 (1.0–2.4)
S4	Ennore thermal station discharge point	13.203997	80.308081	0.96 (0.64–1.5)
S5	River inlet	13.184125	80.295001	0.49 (0.24–0.7)

[a] %, Mean (min–max).

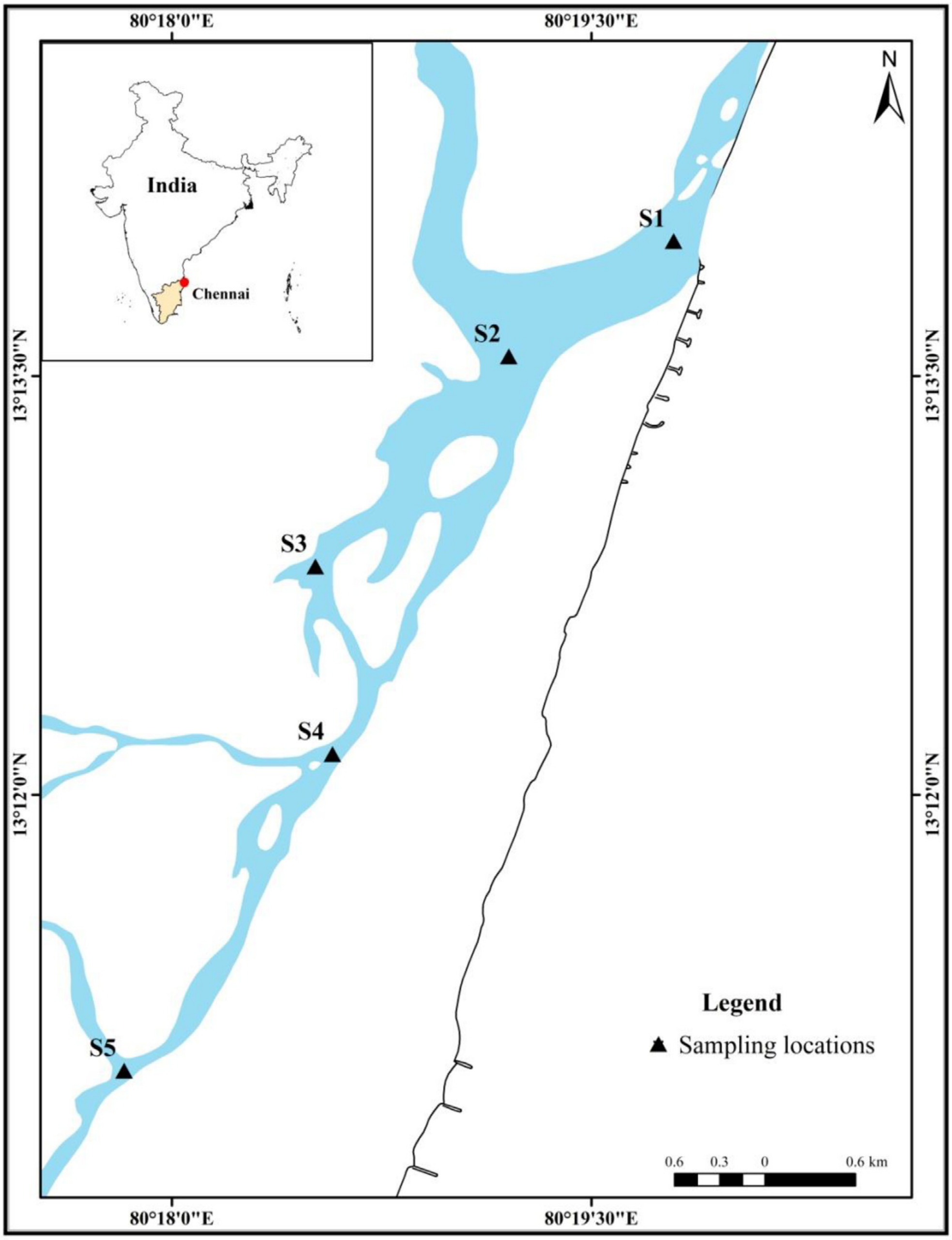


Fig. 1. Study area map showing the sampling stations in Ennore estuary, India.

phosphoric acid, 0.2 g of sodium fluoride and 15 drops of diphenylamine indicator were added into the diluted sediment mixture. The solution becomes a dark brown color and this sample was titrated with 0.5 N ferrous ammonium sulphate until the appearance of brilliant green color. The TOC in the sample was calculated using the following equation,

$$\%\text{Organic Carbon} = 10\ (1 - T/S)[1.0N(0.003)\ (100/W)].$$

where, T-Titre volume (ml) for sample, S-Titre volume (ml) for blank, 0.003-factor value converting to carbon, 1.0 N-normality of $K_2Cr_2O_7$, 10-volume (ml) of $K_2Cr_2O_7$, W-weight (g) of sediment sample.

Fish, crab, oyster and polychaete samples were cleaned with seawater, placed in clean polythene bags in an ice box to avoid spoilage and transported to the laboratory. The samples were washed with distilled water, dissected for edible portions of fish, crab, oyster, and the whole body of polychaetes were collected for further processing. The tissues of marine organisms were dried, powdered separately and 1.0 g of each tissue material was used for metal extraction. Nitric and perchloric acids were used to extract the metals in a ratio of 4:1. Extracted samples were dissolved with 10% nitric acid, filtered in Whatman No.1 filter paper and made up to 25 ml with deionized water [20].

Pre-concentrated metals in extracted samples from seawater were analyzed using ICP-OES (Make: Agilent; Model: 7500 CE). Level of detection of ICP-OES is 1 to 5 μg/l for the selected metals and samples were in the range of detection. Extracted samples of sediment and tissues of marine organisms were measured by Atomic Absorption Spectrometer (Make: Varian Model: SpectrAA 220) in flame mode. The metal concentration in extracted samples of sediments and tissues were found in the range of instrumental limit of quantification. The instrument was calibrated using known concentration of metal standards (Merck, Germany). Metal analysis was validated using the certified reference materials (CRMs) for marine sediment (PACS-3, Lot G 4169010, NRC, Canada) and fish tissue (DORM-4, Product No. 042844, NRC, Canada). The CRMs were subjected to acid digestion procedure followed for the sediment and tissue samples appropriately (Table 3).

2.3. Bioaccumulation factor (BAFs)

BAF is defined as the ratio of the metal concentration in an organism to that of the concentration in water [21]. The uptake of metals differs in each organism as they follow a passive diffusion mechanism [22]. BAFs are derived from the metal concentration in water and tissue samples for every sampling. The BAF is calculated using the formula,

$$BAF = \frac{Concentration\ of\ metal\ in\ organim}{Concentration\ of\ metal\ in\ water}$$

2.4. Biota sediment accumulation factor (BSAF)

BSAF is the net bioaccumulation of chemicals by an organism as a result of uptake from environmental sources and processes [23]. The efficiency of metal accumulation in the marine organisms was evaluated to determine BSAF using the formula [24].

$$BSAF = \frac{Concentration\ of\ metal\ in\ organim}{Concentration\ of\ metal\ in\ sediment}$$

2.5. Geochemical environmental indices

To assess the level of metal contamination in sediment, the sediment quality indices like I_{geo}, EF, CF, PLI and PERI are predominantly used and they are derived based on average shale values of metals [25].

2.5.1. Geoaccumulation index (I_{geo})

I_{geo} is used to measure the degree of metal contamination in aquatic sediments and calculated by the following formula introduced by Muller [26] to measure the degree of metal pollution in aquatic sediments [27].

$$\mathrm{I_{geo}} = \log_2 \frac{\mathrm{Cn}}{1.5 \times \mathrm{Bn}}$$

where C_n is the concentration of the metal in the sediment fraction and B_n represents the earth's crust value of the sediment [28]. The value of 1.5 is the constant for natural fluctuations in the content of a given substance in the environment with little anthropogenic influence. However, I_{geo} is not readily comparable to the other indices of metal enrichment to natural systems, which involves log function and a background multiplication of the constant [29]. Muller [26] classified the geoaccumulation index into six classes as and its contamination levels as given below,

$I_{geo} < 0$ - Unpolluted; $0 < I_{geo} < 1$ - Unpolluted to moderately polluted; $1 < I_{geo} < 2$ - Moderately polluted; $2 < I_{geo} < 3$ - Moderately to heavily polluted; $3 < I_{geo} < 4$ - Heavily polluted; $4 < I_{geo} < 5$ - Heavily to extremely polluted; $I_{geo} > 5$ - Extremely polluted.

2.5.2. Contamination factor (CF)

CF is used to demonstrate the contamination level in sediments for selected metals and it is derived by the following formula [30],

$$\mathrm{CF} = \frac{C_0}{C_n}$$

where C_0 is the mean content of metals measured from the samples and C_n is the earth crust concentration. The contamination levels could be classified into four categories [28] as follows:

Cf < 1 - Low contamination; 1 to 3 - Moderate contamination; 3 to 6 - Considerable contamination; Cf ≥ 6 - Very high contamination.

2.5.3. Enrichment factor (EF)

EF is an extensively used approach to illustrate the degree of anthropogenic pollution to establish enrichment ratios [31]. It was derived by following the method described by Quevauviller et al. [32]. In the present study, Fe was used as the reference metal using the formula,

$$EF = \frac{Cn/C_{Ref}}{Bn(Background\ conc)/BRef(Background\ conc)}$$

where C_n is the content of the examined metal found in the environment. C_{Ref} is the content of the reference metal in the examined environment. B_n (background) is the content of the examined metal in the reference environment and B_{ref} (background) is the content of the reference element in the reference environment. EF classified as given below,

$EF < 2$ - Deficiency to minimal enrichment; $2 \leq EF \leq 5$- Moderate enrichment; $5 \leq EF \leq 20$ - Significant enrichment; $20 \leq EF \leq 40$ - Very high enrichment; EF > 40 - Extremely high enrichment.

2.5.4. Pollution load index (PLI)

The degree of metal pollution in the given sediment is widely expressed as PLI and the following equation was used for deriving the PLI values.

$$\mathrm{PLI} = (Cf1 \times Cf2 \times Cf3 \times \ldots\ldots\ldots\ldots\ldots \times Cfn)^{1/n}$$

where n is the number of metals analyzed, Cf is the contamination factor (Tomlinson et al., 1980) [70].

$PLI < 1$ Unpolluted; $PLI = 1.0$ Baseline level of metals present; $PLI > 1.0$ Degradation of sediment quality.

2.6. Potential ecological risk index (PERI)

The Potential ecological risk index is used to measure the risk of a contaminant in sediment [30] PERI is defined as,

$$PERI = \sum Ef^i$$

$$Ef^i = Tf^i \times Cf^i$$

where Tr^i represents the toxic response factor for a given metal, Cf^i is the contamination factor of a selected metal and Ef^i represents the enrichment factor for a given metal. PERI is categorized to assess the level of ecological risk as given below,

$PERI < 50$ - Low ecological risk by metal pollution; $150 \leq PERI \leq 300$ -Moderate ecological risk; $300 \leq PERI \leq 600$ - Considerable ecological risk; $PERI \geq 600$ - Very high ecological risk.

3. Results

3.1. Salinity and TOC

Salinity in estuarine water ranged from 0 to 35 psu during the sampling period with minimum in mean values at >5 psu. Considering the average salinity of >5 psu in water sample collected in the estuarine waters referred as seawater. The salinity largely varied at monsoon season due to fresh water inflow from the Kosasthalaiyar River. The salinity was in the range of 20–35 psu along the estuary at summer season. Generally the trend of salinity was decreasing from bar-mouth to upstream waters in all the seasons due to influence of tidal and riverine inputs (Fig. 2). TOC content in sediment was measured in the range between 0.24 and 2.4% across the estuary. Higher mean TOC was found in sewage discharge point (S3) lower mean TOC content found at bar-mouth due to de-siltation by tidal activity, dredging and operation of fishing boats.

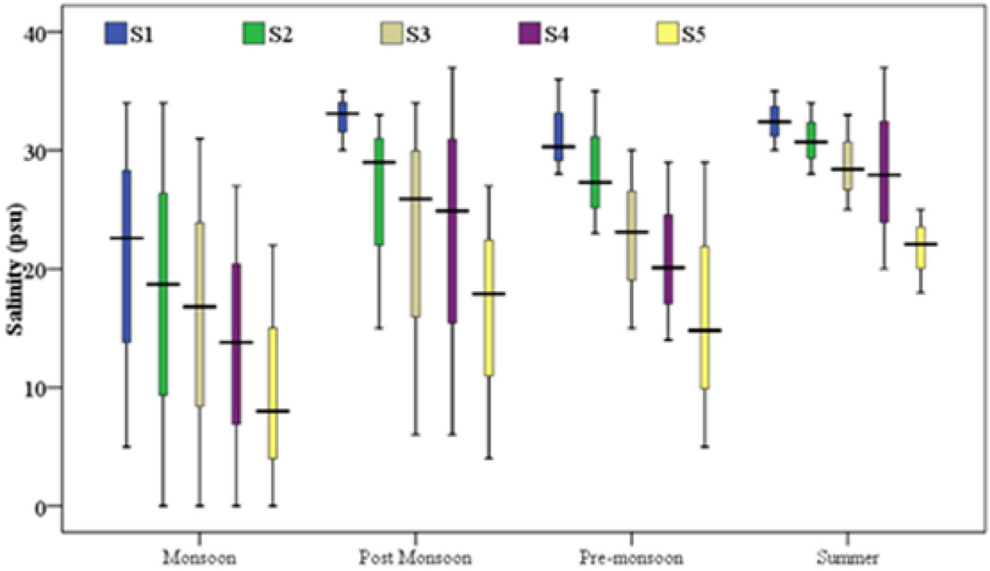


Fig. 2. Spatial and seasonal variation of salinity in seawater from Ennore estuary.

Table 2
Spatial and seasonal metal concentration in seawater and sediment samples of Ennore estuary.

Metal	Season	Seawater (μg/l)					Sediment (μg/g)				
		S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Cd	PoM	1.2 (0.1–3.7)	2.2 (0.1–8.0)	1.9 (0.1–8.7)	2.9 (0.1–8.9)	3.3 (0.1–7.8)	1.9 (0.2–4.6)	0.9 (0.2–2.4)	1.7 (0.3–3.7)	2.3 (0.3–4.7)	1 (0.3–1.6)
	Sum	1.8 (0.1–6)	2.9 (0.3–9.1)	2.5 (0.2–6.7)	2 (0.1–7.6)	2 (0.1–5.7)	2.9 (1–4.9)	4.6 (0.8–7.6)	4.5 (2.7–6.6)	3.5 (1.6–5.6)	2.8 (0.6–4.6)
	PrM	2.3 (0.2–5.1)	2.7 (0.4–6.6)	2.5 (0.4–5.4)	2.3 (0.2–5.7)	2 (0.1–5.8)	1.4 (0.4–2.7)	2.8 (1.6–4)	2.5 (0.3–4.1)	2.7 (0.4–4.8)	2.9 (1.3–5.6)
	Mon	2.5 (0.1–4.9)	2.3 (0.1–6)	2.5 (0.1–7.6)	0.6 (0.1–1.6)	1.4 (0.1–3.3)	0.4 (0.2–0.7)	0.5 (0.2–0.7)	0.8 (0.4–1.2)	0.5 (0.4–0.7)	0.9 (0.2–1.5)
Ni	PoM	6.2 (1.3–17.2)	11 (0.6–20.9)	9.7 (0.3–25.2)	7.9 (0.3–24.1)	5.5 (1.5–13.1)	27.2 (6.3–47.6)	52.8 (5.7–124.8)	35.9 (8.4–99.4)	45.2 (7.8–117.4)	57.6 (4.7–104.2)
	Sum	6.9 (0.7–15.9)	14 (1.1–35)	9.4 (0.5–24.7)	6.8 (0.2–19.6)	9.1 (0.6–17.8)	26.9 (4.6–50.6)	59.1 (14.5–94.8)	43.4 (26.4–67)	52 (28.4–93.4)	68.4 (15.4–123)
	PrM	7 (1.6–12.4)	5.5 (1.1–11.3)	6.7 (4.7–9.8)	7 (0.5–14.4)	6.6 (0.7–10.8)	19.7 (1.4–40)	35.9 (2–72.4)	48.8 (1.1–109.5)	36.9 (1–82)	36.1 (1.5–58.6)
	Mon	6.8 (3.3–11.5)	9.3 (3.9–18.9)	14.2 (2.9–32.4)	10.2 (2.2–20.1)	6.8 (1.7–14.3)	4.5 (1.3–7.9)	8.7 (2.6–16.4)	28.5 (5.5–66.7)	13.5 (5.6–32)	30.6 (2–71.8)
Pb	PoM	7.2 (1.2–18.5)	7.2 (0.8–12)	12.9 (1.3–30.7)	8.9 (0.7–29.7)	7 (0.3–21.6)	48.9 (2.1–103)	45.9 (0.5–162.5)	46.2 (1.9–102.5)	55 (4.3–135)	46 (4.4–167.5)
	Sum	6.6 (0.6–21.7)	13.1 (3.9–19.4)	3.5 (BDL-6.8)	10.3 (3.8–16.8)	2.1 (1–3.8)	59.1 (11.3–98.8)	86.1 (18.8–146.9)	85.3 (46.3–130)	103.9 (62–153.5)	115.9 (18.8–193)
	PrM	10.8 (1.9–14.4)	13 (9.7–15)	14.3 (10.9–17.9)	11 (7.3–15.2)	11.2 (1–16.6)	38 (3.6–66.3)	48.4 (18.3–73.3)	54.2 (8.9–77.8)	60.6 (6.3–104)	65 (12.8–98.9)
	Mon	1.9 (0.7–3.4)	2.9 (1.1–7.6)	4.1 (0.9–7.4)	2.5 (0.5–4.8)	5.1 (2 – 13)	7.7 (0.7–14.2)	13.7 (5–23.6)	14.1 (4.9–22.8)	19.2 (5.9–38.7)	31 (5.6–47.8)
Cr	PoM	5 (1–9.2)	7.2 (1.5–13.8)	8 (1.7–15.1)	7.1 (3.8–10.9)	5.9 (1.6–9.3)	47.7 (5.3–96.3)	115.3 (5.8–206.6)	91.2 (43.1–161.9)	88.8 (BDL-390.1)	91.3 (BDL-222.6)
	Sum	4.6 (2.6–6)	5.3 (0.6–9.1)	6.5 (3.5–9.7)	7.6 (4.5–9.6)	7.7 (3–13.2)	96.3 (30.6–183)	135.6 (70.2–169.9)	132.2 (32.6–244.3)	144.6 (38.9–224.4)	154.3 (91.7–197.9)
	PrM	3.1 (0.7–6.4)	8.9 (5.3–12.8)	7.8 (4.6–13.4)	4.6 (3–6.1)	4.2 (1.2–9.5)	37.7 (4.9–81.9)	55.7 (11–153.6)	114 (3–333.3)	84.6 (20.6–213.4)	69.9 (7.3–166)
	Mon	3.5 (2.4–6.1)	7 (1.3–15.8)	7.4 (2.4–14.6)	5.9 (2–13.5)	6.4 (1.1–12.8)	43.9 (6.1–86)	54.7 (5–100.6)	61.1 (4.9–103.6)	74.4 (5.9–163.1)	93 (5.6–143.6)
Cu	PoM	4.2 (0.2–10.5)	9.6 (0.3–19.4)	4.9 (0.8–9.1)	5.9 (1.3–12.4)	7.7 (1.4–19.9)	26.7 (7.8–57.8)	73.5 (5.8–217.5)	67.2 (4.1–205)	66.1 (BDL-234.8)	35.2 (BDL-213.8)
	Sum	1.2 (0.2–3.2)	1.5 (0.5–4.5)	1.6 (0.3–3.6)	0.9 (BDL-3.1)	0.7 (BDL-2)	22.5 (2.9–83.4)	43.8 (11.1–101.8)	26.7 (8.3–48.6)	60 (9.6–247.1)	49.3 (14.3–124.5)
	PrM	4.9 (0.3–12.3)	2.9 (0.2–6.6)	2.9 (0.3–11.1)	5 (BDL-12.3)	3.9 (BDL-11.2)	5.7 (0.3–13.8)	33 (9.3–75.1)	20.9 (11.8–46)	54.6 (12.6–170)	28.4 (7.1–57)
	Mon	7.8 (2.2–12.5)	10.2 (6–17.8)	6.2 (0.5–9.6)	5.8 (1.6–14.8)	3.4 (1.3–5.7)	20.8 (1.9–71.1)	14.7 (3.6–26.6)	14.6 (2.8–35.4)	19.6 (5.6–60.2)	78.9 (3.2–266.3)

PoM:Post Monsoon; Sum: Summer; PrM:Pre-Monsoon; Mon:Monsoon; BDL: Below detectable level.

3.2. Metal concentration in water

Spatial and seasonal concentrations of metals measured in seawater samples are presented in Table 2. The metal concentration in the estuarine water was varied spatially and seasonally. Overall concentrations of Cu, Cr, Cd, Pb, & Ni are found in the range of 0.2–19.9 μg/l, 0.6–15.8 μg/l, 0.1–9.1 μg/l, 0.3–30.7 μg/l, and 0.3–35.1 μg/l respectively in water samples. Concentrations of Ni, Pb, and Cu were higher than Cr and Cd. Higher concentrations of metals were recorded during monsoon and post-monsoon season, while lower concentrations were noted during the summer season based on maximum values (Table 2).

3.3. Metal concentration in sediment

The seasonal distribution pattern of heavy metals (Cr, Cu, Cd, Pb and Ni) in sediments is presented in Table 2. Average concentrations of metals during all the seasons in five sampling stations ranged between 64.4 and 112 μg/g with a maximum of 183 to 244.3 μg/g for total Cr; between 20.4 and 56.9 μg/g with a maximum of 83.4 to 234.8 μg/g for Cu; between 1.9 and 2.8 μg/g with a maximum of 4.9 to 5.6 μg/g for Cd; between 45.5 and 68.4 μg/g with a maximum of 103 to 187 μg/g for Pb; and between 22.2 and 53 μg/g with a maximum of 50.6 to 123 μg/g for Ni. Higher levels of metals were observed during monsoon and post-monsoon in the upstream of the estuary between station 3 and 5. Based on the average concentration of the metals the following sequential order viz., Cr (95.6) > Pb(56.7) > Cu(40.6) > Ni(42.1) > Cd(2.5) was observed.

3.4. Metal concentration in marine organisms

Average concentrations of Cr, Cu, Cd, Pb and Ni were found to be 4.6, 2.4, 2.21, 4.9 and 2.6 μg/g in fish *Mugil cephalus*, 11.4, 22.1, 3.3, 8.9 and 6 μg/g in Crab, *Scylla serrata*, 13, 20.8, 1.3, 7.9 and 4.8 μg/g in Oyster, *Crassostrea madrasensis* and 18.8, 20.1, 1.0, 12 and 5.6 μg/g in polychaete *Eunice* spp. respectively (Fig. 4). While higher metal concentrations of 45.6 μg/g for Cr, 47.2 μg/g for Cu, 5.5 μg/g for Cd, 24.2 μg/g for Pb and 18.7 μg/g for Ni were observed in crabs during monsoon and post-monsoon seasons. The metal contents in the tissues of marine organisms showed a pattern of Cu > Cr > Pb > Ni > Cd in crab, oyster and polychaete, while the trend varied in fish as Pb > Cr > Ni > Cu > Cd due to their migratory habit and they are exposed to various levels of ambient concentration compared to other resident organisms. Higher metal concentrations were recorded during monsoon, post-monsoon and summer seasons in crab, oyster and polychaetes respectively (Table 5).

3.5. Bioaccumulation factor (BAF)

BAFs of heavy metals in marine organisms collected from the Ennore estuary are presented in Fig. 3a–d. The overall average ratios

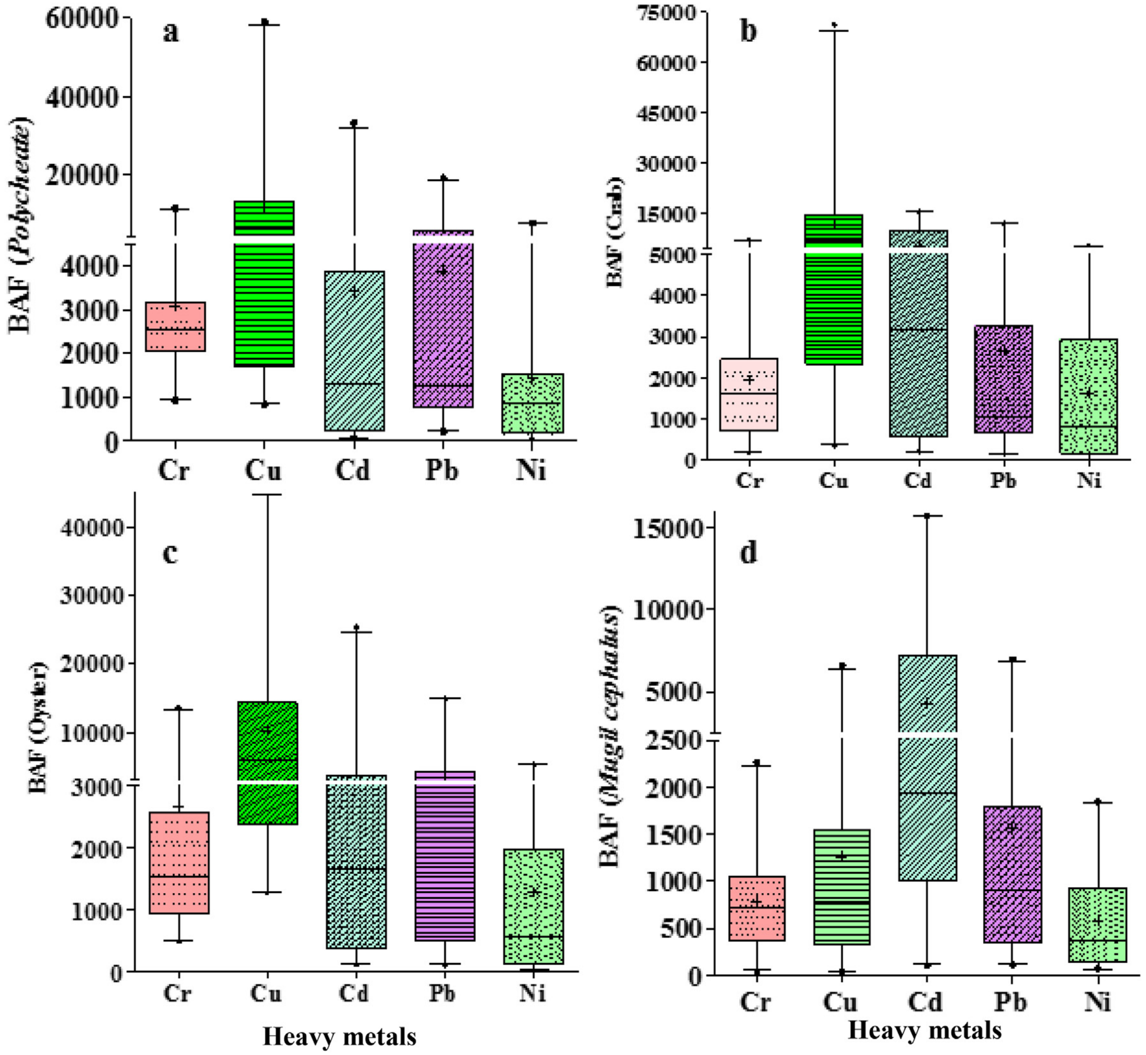


Fig. 3. a–d. Bioaccumulation of heavy metals in marine organisms from Ennore estuary.

of bioaccumulation factors in fish, polychaete, oyster and crab were 775, 3083, 2676, 1928 for Cr, 1257, 12,180, 10,203, 11,906 for Cu, 4265, 3435, 3233, 4763 for Cd, 1564, 3880, 3234, 2661 for Pb and 578, 1416, 1285, 1635 for Ni respectively. The higher accumulation of Cu was recorded in all the organisms except fish. Higher bioaccumulation factors of metals in marine organisms were observed during monsoon and post-monsoon seasons. BAF of metal in the polychaete, oyster and crab was found in the similar order of Cu > Cd > Pb > Cr > Ni whereas in fish the pattern differed as Cd > Pb > Cu > Cr > Ni. The pattern of BAF in the estuarine organisms was in the descending order viz., Crab>Polychaete>Oyster>Fish.

3.6. Biota-sediment accumulation factor (BSAF)

The BSAF of metals in the Ennore estuary is given in Fig. 4a–d. The average BSAF of metals (Cr, Cu, Cd, Pb and Ni) varied from 0.2–0.9 in fish, 0.5–1.7 in the polychaete, 0.4–1.5 in oyster and 0.4–2.4 in crab. The higher BSAF of 7.7 and 6.0 in crab and oyster respectively for Cd was recorded. Monsoon and post-monsoon seasons witnessed higher BSAFs. Similarly, metal concentrations in polychaetes and oysters were in the following order: Cu > Cd > Cr > Ni > Pb, whereas crabs and fishes were varied in the following order: Cd > Cu > Pb > Cr > Ni and Cd > Pb > Ni > Cr > Cu respectively.

3.7. Environmental quality indices

The geochemical based environmental quality indices such as I_{geo}, EF and CF for metals were shown in the following trend viz., Cd > Pb > Cu > Cr > Ni (Table 6).

3.7.1. Geoaccumulation index (I_{geo})

The seasonal variations of I_{geo} for metals like Cr, Cu, Cd, Pb and Ni ranged from 0 to 1.5, 0 to 1.9, 0.6 to 4.1, 0 to 2.7 and 0 to 0.3 respectively. I_{geo} values were found to be higher during post-monsoon and summer seasons. Based on the I_{geo} values, the sediments of Ennore estuary were heavily polluted with Cd, moderately to heavily polluted with Pb, moderately polluted by Cr & Cu and unpolluted with Ni (Table 6).

3.7.2. Contamination factor (CF)

The level of metal contamination in the sediments of Ennore estuary was classified based on CF values (Table 6). Cadmium and lead were highly contaminating, Cu and Cr were found to be considerably contaminating

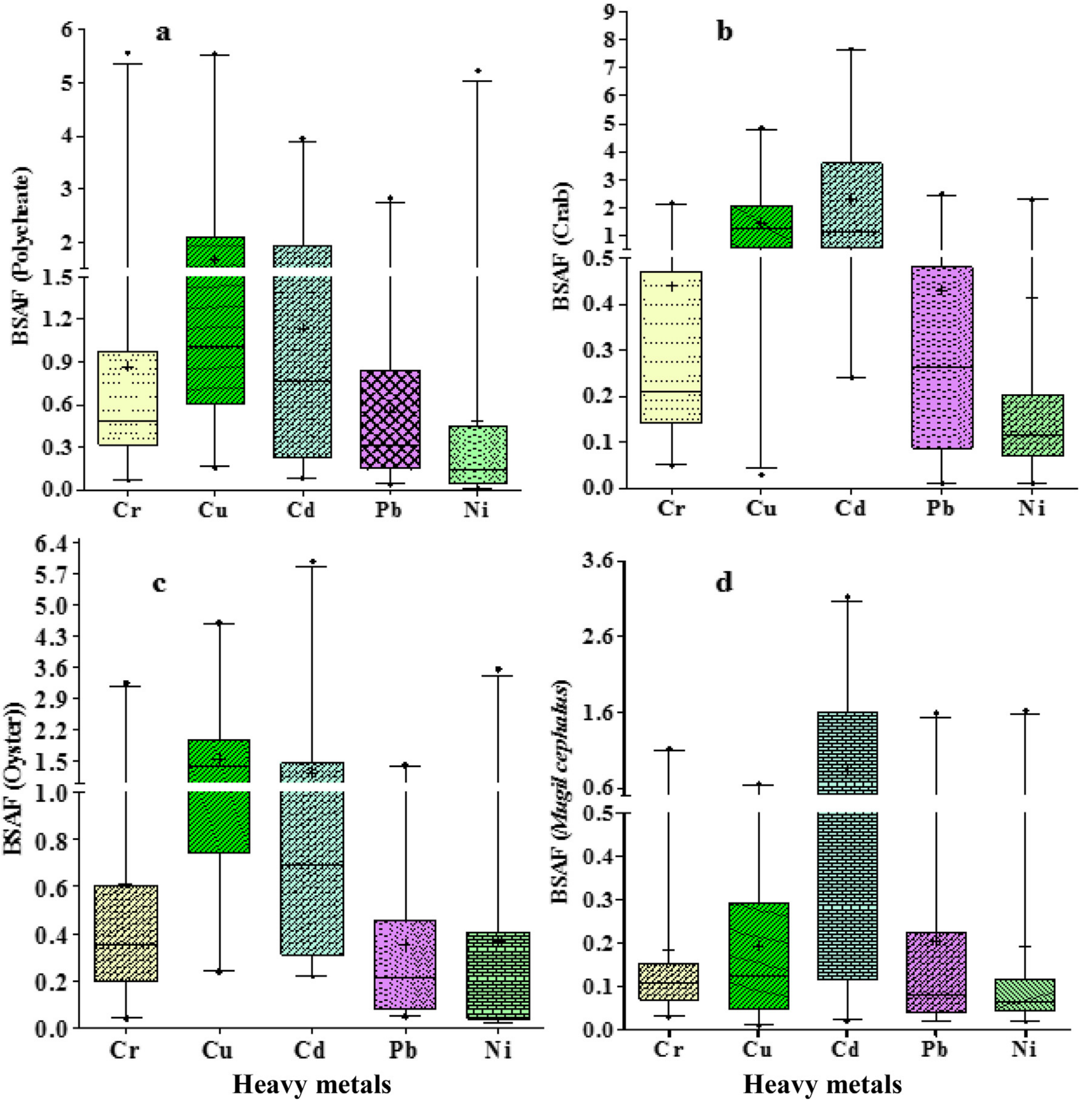


Fig. 4. a–d. Biota-sediment accumulation factors of heavy metals in marine organisms from Ennore estuary.

and Ni moderately contaminating the estuary. The CF values were found to be higher during summer and post-monsoon followed by pre-monsoon and monsoon.

3.7.3. Enrichment factor (EF)

The degree of metal enrichment in the sediment of Ennore estuary was categorized based on EF values and seasonal variations were observed (Table 6). Ennore estuary was significantly enriched with Cd and Pb, moderately enriched with Cr and Cu except during monsoon season. Higher concentrations were observed at stations 2, 3 and 5 during post-monsoon and summer seasons.

3.7.4. Pollution load index (PLI)

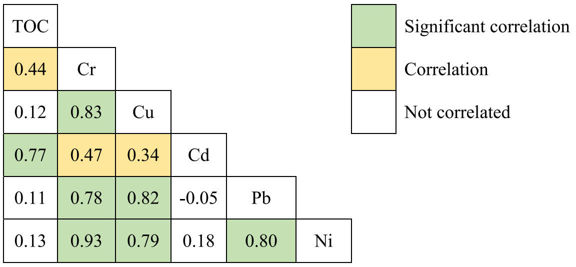
The results of PLI indicated that the Ennore estuary is polluted with heavy metals such as Cr, Cu, Cd, Pb and Ni (Table 6). The overall seasonal average indices ranged between 0.2 and 2.4 with a maximum of 3.2 recorded at station 5. Higher index values were found at sampling stations of 4 and 5 during the summer season.

3.8. Potential ecological risk index (PERI)

PERI was applied to assess the degree of risk for biological activities in the ecosystem. In this approach, a toxic factor was introduced based on abundance, uptake and sensitivity principle, i.e. the potential toxicological effect of heavy metal is proportional to abundance, uptake, and sensitivity of various metals accumulated in the sediment. The mean PERI ranged between 40.6 and 268. The higher index value of 370 and 444 at stations 2 and 3 respectively was observed during summer (Table 6).

3.9. Statistical analysis

The correlation coefficient for metal concentrations in seawater, sediment and marine organisms was performed ($p < 0.05$). No correlation with metals in seawater among the stations. Spatial concentration of metal and TOC content was analyzed for their correlation co-efficient to study the influence of discharges from different sources. Cd concentration in sediment significantly correlated with sediment TOC and Cr related with TOC content in sediment. Lack of correlation between TOC and metals (Ni, Pb and Cu) due to the TOC is discharged majorly from municipal sewage at S3 and S4. Significant correlation between the metals Cr, Cu Pb and Ni in sediment attributed to the spatial distribution and co-occurrence from various sources (Fig. 5). The cadmium concentrations in seawater significantly correlated with sediment Cu ($R^2 = 0.46$) and Ni ($R^2 = 0.36$) ($p < 0.05$). Cu concentration in water has a significant negative correlation with Cr, Cd, Pb and Ni in sediment ($p < 0.05$). Among the metal concentrations in sediment, Cr is strongly correlated with Cd, Pb and Ni ($R^2 = 0.51$, 0.65, 0.47, $p < 0.01$ respectively), whereas Cu has strong correlation with Ni ($r = 0.48$, $p < 0.05$) and Cd witnessed correlation with Pb ($r = 0.64$, $p < 0.01$). Cu concentration in water and sediments has a significant correlation with Cu and Cd content in fish respectively. Similarly, total Pb and Ni concentration in water correlated with Pb content in fish tissue. Cu and Pb concentrations in sediment were significantly related to Pb concentration in polychaete ($r = 0.71$, 0.78, $p < 0.01$). Cu and Cd concentrations between crab and polychaete were highly correlated ($r = 0.63$, 0.54; $p < 0.01$) and Pb concentration in crab was correlated with Cr, Cu and Ni concentrations of polychaete ($p < 0.05$).



TOC					
🟨 0.44	Cr				
0.12	🟩 0.83	Cu			
🟩 0.77	🟨 0.47	🟨 0.34	Cd		
0.11	🟩 0.78	🟩 0.82	-0.05	Pb	
0.13	🟩 0.93	🟩 0.79	0.18	🟩 0.80	Ni

🟩 Significant correlation
🟨 Correlation
⬜ Not correlated

Fig. 5. Correlation co-efficient ($p \leq 0.05$) between the metals and total organic carbon in sediment from Ennore estuary.

4. Discussion

Ennore estuary has been affected by coastal pollution for more than two decades and has been identified as a hotspot of metal contamination. The present study reports seasonal data on metals in seawater, sediment and marine organisms from the Ennore estuary. The bioaccumulation factors and ecological risk indices are derived from the metal concentration in the water, biota and sediment respectively. The depth of Ennore estuary varies between the sampling stations from 4 to 5 m at the bar mouth to 0.5–1 m in the river inlet. Sampling sites are located adjoining to the SIPCOT industrial complex at Manali which is influenced by the discharges from smaller channels near to the downstream end of Pulicat Lake and Kosasthalaiyar River which are drained by point and nonpoint sources. The sediment composition in Ennore estuary is sandy in the bar mouth and clay with loose texture (up to 0.2 m to 0.5 m depth) in other stations. This may be due to the discharge of large amounts of untreated municipal wastewaters with organic matter load. TOC content measured in Ennore estuary is comparable with organic content reported in the range of 0.2 to 3.3% in sediments of Halady estuary [33]. The loose clayey sediment with organic matter continuously accumulate in the sediment until flushing by freshwater inflow during monsoon floods. Heavy rainfall occurred in Chennai between November and December 2015 as a rare event. Therefore, the seasonal and spatial variations of metals in water and sediment are influenced by the source of discharges, bio-geochemistry of sediment and residential time of sediment. Higher metal concentrations in water during monsoon season are explained by the freshwater inflow in the upstream of estuary leading to enhanced redox activity, adsorption into suspended organic matter and formation of insoluble organic complexes in lesser saline waters [34]. Lack of significant correlation in spatial and seasonal distribution of metals in seawater attributed to sources, periodicity of discharges and dilutions. In contrast, metal concentrations in sediment were comparatively lesser during monsoon season and showed an increasing trend in upstream waters, which can be attributed to the freshwater inflow. Since monsoonal rainfall in the catchment areas of River causing desiltation of the surface sediment. The influence of flood on metal distribution evidenced during post-monsoon drop in Cu, Cd and Pb concentration in water and sediment. Similarly metals concetyrations were reduced after the flood in Kochi estuary [35].

Cr and Cu concentrations measured in the present study are within the range of reported concentrations in Ennore estuary. Cd, Pb and Ni in sediment are higher than reported concentrations (Table 2 and 4). The concentrations of metals in Ennore estuary are comparatively higher than that of Kalpakkam and Gulf of Kachchh coastal waters [11,36]. Similar metal concentrations were reported from Manakudi and Mahanadi estuaries of India and the Sungai Puloh mangrove ecosystem from Malaysia [37–39]. Cu, Cd, and Pb concentrations in waters from Yangtze Estuary recorded within the range of this study as 1.4, 0.033 and 0.59 μg/l respectively [40]. Metals in sediment of Yangtze Estuary was ranged from 34.4–103 μg/g of Cr, 19.7–32.1 μg/g of Cu, 0.04–0.21 μg/g of Cd, 13.7–25.8 μg/g of Pb, and 18.8–38.9 μg/g of Ni [40,41] (Table 4). Similar range of 93–206 μg/g of Cr, 30–70 μg/g of Cu, and 5–85 μg/g of Ni have been recorded in sediments from Halady estuary [33]. The higher concentrations of metals in estuarine water can be attributed to anthropogenic activities such as agriculture and industrial discharges. Perhaps, the higher concentration of metals in Ennore estuary is due to the longer residential time of sediment, lesser mobility of water and untreated sewage discharges from metropolitan Chennai. The higher correlation coefficient between metals in sediment indicates the continuous anthropogenic discharge, even distribution of suspended matters from water column, accumulation and persistence of metals. For instance, Raj and Jayaprakash [42] reported three-fold higher concentration of Cu in sediment collected from off Ennore coast. Since, Cu

Table 3
The recovery of metal concentration from certified reference materials (CRMs) of sediment and fish tissue are presented along with guidelines/permissible limits of metals in water, sediment and marine organisms.

Metals	Sediment (PACS-3)			Fish (DORM-4)			USEPA WQG[a]	Canadian SQGs[b]			Korean SQAGs[c]				Permissible limits in organisms[d]
	CV	MV	%R	CV	MV	%R		NEL	LEL	SEL	C 1	C 2	C 3	C 4	
Cr	90.6 ± 4	88.5 ± 8.3	97.7 ± 9.1	1.9 ± 0.2	2.0 ± 0.1	104.6 ± 6.3	50	< 26	26	110	≤ 112	≤ 224	≤ 991	> 991	1
Cu	326 ± 10	322.9 ± 12.3	99.1 ± 3.8	15.7 ± 0.5	15.6 ± 0.2	99.2 ± 1.4	3.1	< 16	16	110	≤ 48	≤ 228	≤ 1890	> 1890	20
Cd	2.23 ± 0.16	2.1 ± 0.2	94.6 ± 8.3	0.3 ± 0.01	0.3 ± 0.02	87.3 ± 12.8	7.9	< 0.6	0.6	10	≤ 0.4	≤ 1.84	≤ 60.9	>6.09	0.1
Pb	188 ± 7.4	184.7 ± 10.5	98.3 ± 5.6	0.4 ± 0.1	0.42 ± 0.03	99.0 ± 3.0	8.1	< 31	31	250	≤ 59	≤ 154	≤ 459	>459	0.5
Ni	39.5 ± 2.2	39.0 ± 1.9	98.6 ± 4.9	1.3 ± 0.1	1.4 ± 0.1	103.6 ± 6.9	8.2	< 16	16	75	≤ 40	≤ 87.5	≤ 330	> 330	0.5–1 [63]

CV: Certified value; MV: Measured value; %R: percentage recovery; NEL (no effect level), LEL (lowest effect level), and SEL (severe effect level); C - Category; C1: No possible sediment toxicity, C2: Possible toxic sediment, C3: relatively high toxic sediment, C4: Very high toxic sediment.

[a] USEPA [57]
[b] Persaud et al. [46].
[c] NIER [47].
[d] FAO [58].

forms complexes with the organic matter due to higher stability [43] and this fact is revealed in the present study with negative correlation observed between Cu concentration in water and other metals in sediment ($p < 0.01$). Lower concentrations observed in the present study during monsoon complements the previous reports in other estuarine sediments of the East Coast of India [5,44,45]. Sediment quality guidelines (SQGs) are derived based on metal toxicity values of benthic organisms and applied widely to assess the quality of sediment [46,47]. SQGs such as Threshold Effect Concentration (TEC), Probable Effect Concentration (PEC), Effect Range Low (ERL), and Effect Range Median (ERM) are reported for protection of the organisms. The results of the present study revealed that the metal concentration in sediment exceeded the SQGs (Table 3) indicating the effects of metal toxicity which may affect fishery potential and biodiversity of Ennore estuary. Consequently, artisanal fishermen are hard-pressed for their livelihood since most of them are presently dependent on the exploitation of polychaetes from sediment as an alternative source of income.

The higher concentration of metals in aquatic ecosystems affect biota by modifying the metabolic activities due to metal uptake through gills and skin, elimination through excretion, and storage as metallothioneins [48]. Higher levels of Cr, Cd and Pb in the ambient waters can cause physiological and genetic effects to fish due to accumulation [49]. The polychaetes, crabs and oysters were found to have higher metal content than fish which illustrates their dietary preference and exposure to contaminated sediment substratum. The seasonal variation of metal concentrations in marine organisms could result from factors such as stage in the life cycle, rate of metabolism, age, feeding habit, and ambient temperature. These factors are influenced by salinity, biogeochemical cycle and bioavailability of metals [50]. Fish *M.cephalus* in Ennore estuary was observed for higher bioaccumulation of Cr, Cd, Pb and Ni than other fish

Table 4
Comparison of metal concentration in estuarine and coastal environments throughout the world.

Place	Cr	Cu	Cd	Pb	Ni	References
Water (μg/l)						
Ennore Creek	2.01	3.53–5.35	NR	9.10–26.6	6.73–10.4	JayaPrakash et al., [7]
Romanian sector, Black sea	2.19	20.26	2.72	8.05	2.7	Jitar et al., [65]
Daya bay, China	–	2.4	0.12	0.7	–	Qiu, [52]
Sinop-Kizilirmak River, Romania	ND	15–200	13–50	25–30	81–94	Bat et al., [66]
Eastern Black Sea	ND	7.5–20.5	3	17.5–39	ND	Cevik et al., 2008
Yangtze River Estuary, China		1.4	0.033	0.591		Fan et al., [40]
Sediment (μg/g)						
Ennore creek, Tamil Nadu, India	383	102	0.51	32	35	Jayaprakash et al., [7]
Cauvery delta region, Tamil Nadu, India	45.4–153.1	10.3–39.9	NR	1.14–5.4	2.0–7.7	Dhanakumar et al., [5]
Mahanadi estuary, East Central India	NR	NR	1.45	23.89	NR	Lakshmansenthil et al., [67]
Arasalar estuary, Tamil Nadu, India	0.44	NR	3.9	0.19	0.8	Raj et al., [68]
Manakudy estuary, Tamil Nadu, India	256.9–482.1	37.35–45.87	2.69–3.17	152.25–176.88	20.14–28.91	Kumar and Edward, [37]
Romanian sector, Black sea	30.26	26.68	1.2	11.59	26.25	Jitar et al., 2014
Daya Bay, China	NR	30.6	0.28	52.7	ND	Qiu, [52]
Eastern Black Sea	5.6–56.9	4.43–122	3.8–5.9	41.7–355.1	16.1–21.4	Cevik et al., 2008
Quanzhou Bay, southeast China	37.1	19.8	0.24	42.2	10.3	Yan et al., [59]
Southwestern coastal rivers, Korea	29.1–128	3.2–68.6	0.1–0.82	11.9–79.2	11.3–44.3	Yang et al., [60]
Korean coast	NR	0.18–104	0–1.37	0.04–85.4	NR	Hwang et al., [61]
Scheldt estuary, Europe	41.6–161.4	18.6–118.4	1.51–10.24		10.8–45.5	Laing et al., [69]
Kerala Coast	NA	7.0–38.0	0–1.9	4.0–30.0	25–80	Manju et al., [62]
Yangtze River Estuary, China	34.4	19.7	0.13	25.8	NR	Fan et al., [40]
Yangtze River Estuary, China	69.5–103	14.3–32.1	0.037–0.212	13.7–23	18.8–38.9	Wang et al., [41]

NR: Not reported.

Table 5

Seasonal metal concentration in selected marine organisms (μg/g) from Ennore estuary.

Organism	Seasons	Cr	Cu	Cd	Pb	Ni
Mullet	Post Monsoon	5.6 (3.3–7.2)	3.3 (2.0–5.7)	4.0 (0.3–7.4)	3.3 (0.7–6.5)	2.4 (1.3–4.2)
	Summer	4.4 (2.3–6.1)	1.7 (0.6–2.0)	1.6 (0.3–5.0)	8.3 (0.8–21.8)	2.9 (2.2–4.2)
	Pre-monsoon	5.5 (2.6–11.4)	2.2 (0.4–3.3)	0.7 (0.1–1.4)	4.3 (1.7–10.8)	2.3 (1.1–3.6)
	Monsoon	2.7 (0.7–4.9)	2.3 (0.6–4.7)	2.3 (0.6–3.5)	3.5 (0.6–8.3)	2.8 (1.3–3.9)
Polycheate	Post Monsoon	19.2 (15.6–25.1)	26.7 (16.8–31.1)	1.3 (0.9–1.7)	7.7 (2.6–15.7)	3.5 (0.3–7.1)
	Summer	16.5 (10.8–22.5)	16.6 (9.8–20.9)	1.0 (0.4–1.5)	18.4 (13.4–22.4)	2.6 (1.4–3.7)
	Pre-monsoon	26.0 (19.0–35.4)	26.3 (13.6–41.2)	1.1 (0.7–1.5)	14.3 (12.5–16.2)	10.6 (8.6–13.1)
	Monsoon	13.6 (6.7–19.6)	10.7 (7.9–13.8)	0.7 (0.3–0.9)	7.8 (4.9–12.4)	5.9 (3.9–7.8)
Oyster	Post Monsoon	9.7 (4.4–14.1)	17.4 (8.4–30.5)	1.1 (0.3–2.3)	7.3 (3.7–11.0)	1.8 (0.5–3.2)
	Summer	10.4 (6.9–15.8)	21.3 (16.4–29.4)	1.4 (0.9–2.4)	5.4 (2.0–8.3)	4.4 (1.7–7.9)
	Pre-monsoon	12.8 (5.7–18.2)	15.3 (8.4–21.0)	1.4 (0.9–2.3)	8.9 (3.0–15.7)	6.0 (1.3–9.5)
	Monsoon	19.1 (7.0–33.3)	29.2 (17.3–44.0)	1.1 (0.4–2.2)	10.0 (4.9–16.1)	6.9 (4.6–10.3)
Crab	Post Monsoon	18.2 (4.0–45.6)	27.3 (9.0–47.0)	3.0 (1.8–5.5)	7.6 (1.5–10.4)	5.7 (0.2–10.2)
	Summer	8.6 (4.7–12.4)	18.4 (9.6–24.5)	2.8 (1.7–4.2)	6.4 (3.5–9.7)	5.9 (3.3–8.6)
	Pre-monsoon	10.8 (6.9–15.7)	32.0 (18.9–47.2)	2.0 (0.7–3.3)	16.8 (9.7–24.2)	4.6 (2.6–8.0)
	Monsoon	7.9 (2.0–16.3)	10.6 (1.8–21.6)	1.5 (0.5–3.1)	4.8 (0.5–14.2)	7.8 (1.8–18.7)

Table 6

Seasonal changes in environmental quality/risk indices (in range) of metals in Ennore estuary.

Metals	Igeo				CF				EF				PLI	PERI
	PoM	Sum	PrM	Mon	PoM	Sum	PrM	Mon	PoM	Sum	PrM	Mon		
Cr	0–1.5	0.3–0.9	0–1.3	0–0.3	0–4.3	0.3–2.7	0–3.7	0.1–3.4	0.1–4.1	0.3–2.6	0.1–3.5	0.1–1.7	PoM: 0.23–2.36	PoM: 0.52–282.6
Cu	0–1.8	0–1.9	0–1.3	0.6–1.7	0–5.2	0.1–5.5	0–3.8	0.1–1.9	0.1–5	0.2–5.2	0.1–3.6	0.1–1.5	Sum: 0.48–3.17	Sum: 5.0–444.4
Cd	1.9–3.4	3.4–4.1	2.6–3.6	0.6–1.7	0.8–15.8	0.9–25.4	1–18.8	0.7–4.9	0.7–14.9	1.8–24.1	0.9–17.8	0.6–4.7	PrM: 0.22–2.03	PrM: 0.27–325.03
Pb	1.8–2.5	1.7–2.7	1.1–1.8	0–0.7	0–8.4	0.6–9.7	0.2–5.2	0–2.4	0–7.9	0.5–9.1	0.3–4.9	0.1–2.2	Mon: 0.12–1.82	Mon: 13.04–110.0
Ni	0–0.3	0–0.3	0–0.1	0	0.1–1.8	0.4–1.7	0.1–1.6	0–1.1	0.1–1.7	0.1–1.7	0–1.5	0–1		

species from Ennore estuary, Kovalam and Cauvery delta regions of Tamil Nadu [5,13]. This evidenced from the correlation between metal concentration in water and fish ($p < 0.05$) was significant and were higher than the permissible limits. In the present study, the higher bioaccumulation of Cu, Cr Ni and Pb was recorded in polychaete, crab and oyster during monsoon, pre-monsoon and post-monsoon seasons. This could be attributed to their benthic habitat and dietary uptake from higher ambient metal in sediment [51]. The extent of accumulation depends primarily on ambient concentrations in water, their levels in prey, uptake and elimination kinetics [52]. Whenever the accumulation exceeds threshold limits in the organisms leads to health hazards [53].

In this study, BSAF of metals in polychaete and oysters were observed in the pattern of Cu > Cd > Cr > Ni > Pb indicating the common mode of uptake (Fig. 4a–d). Since these organisms are sedentary and sessile benthic inhabitants, they are inevitably exposed to contaminated sediments. The trend of BSAF in crab and fish followed the order of Cd > Cu > Pb > Cr > Ni and Cd > Pb > Ni > Cr > Cu respectively which may be attributed to differences in locomotion and feeding behavior. The BAF observed in marine organisms were similar with that of previous reports in zooplankton (Cu: 989; Cr: 129; Pb: 46.6) and fish (Cr: 10639; Cu: 13775; Cd: 1224.4; Pb: 11689; Ni: 10765) from Kalpakkam, South East coast of India [36]. The correlation coefficient between the metals (Cu, Cd and Pb), polychaete and crab are indicating similar uptake and bioaccumulation from the ambient. Metals can bio-transformed and bio-magnified in food-web from lower trophic organisms (prey) to higher trophic (predator) organisms. Metal rich ambient environment like estuary cause biomagnifications rather biodiluted to the predator or detritivores organisms due to higher metal bioaccumulation in the prey [54].

The geochemical indices I_{geo}, EF, CF, PLI and PERI were derived using the concentration of metals in sediment, earth's crust value and reference metal concentrations. These indices are applied to assess the degree of metal contamination in sediments and ecological risk from metal. Qian et al. [55] summarized the metal concentrations in the sediments from 52 selected stations of 20 countries around the globe including India and assessed the sediment quality based on the indices like enrichment factor (EF). The range of EF was higher in pre-monsoon and summer indicating a high inflow of organic sediment from land-based source. The geoaccumulation factor of Cr, Cu, Pb and Ni are higher than Kalpakkam coastal sediments (Cr: -4.41 to -1.21; Cu: -4.08 to 0.99; Pb: -2.15 to 0.27 and Ni: -2.81 to 0) except I_{geo} of Cd [36]. The results of EF revealed that the heavy metal like Cd is significantly enriched and Pb is moderately enriched in sediments of Ennore estuary. The concentrations of Cd and Pb in the present study were increased in the sediments of Ennore estuary than the previous report as reflected in the EFs. The higher EF values for Cd, Pb and Cr are mainly due to contamination from metal-based industries like electroplating and metallurgy and also from the exhaust of automobiles. The overall seasonal average of PLI values ranged between 0.2 and 2.4 with a maximum of 3.2 and higher values were found at upstream stations of the estuary during summer seasons. Similarly, PLI was reported previously from off Adyar [56] indicated pollution due to discharges from domestic and industrial wastewaters. The PERI is providing clear information on impacts of metals on the ecological system since the index is based upon the toxic response factor and contamination degree of the metals. The range of the seasonal mean of PERI is found to be 40.6 to 268 with the highest value of 444 observed in the mid estuary during summer (Table 6). These results confirmed that Ennore estuary is under moderate to considerable ecological risk from heavy metal pollution. The higher levels of PERI in the Ennore estuary are attributed to higher enrichment and contamination of non-essential heavy metals like Cd and Pb discharged from various sources through anthropogenic activities.

5. Conclusion

The data from the present study demonstrate the contamination of Ennore estuary by metals due to anthropogenic activities. Concentrations of the priority metals Cr, Cu, Cd, Pb and Ni in water, sediment and biota in the estuary are higher than other estuaries in India. The results indicate higher bio-accumulation of non-essential metals Cd and Pb in marine organisms in Ennore estuary. Higher metal contamination in Ennore estuary is posing considerable ecological risk as per the results of environmental quality indices and PERI. The long-term monitoring of metal distribution, bioaccumulation and risk assessment may provide the information for devising pollution management strategies particularly for estuaries in the region and elsewhere in general.

Author statement

P.Karthikeyan: Investigation, Data Curation, Formal analysis, Writing-Original draft; S.R.Marigoudar: Validation, Visualization, Writing - Review and Editing; D.Mohan: Conceptualization, Supervision; A. Nagarjuna: Resources, Methodology, Analysis; K.V.Sharma: Project administration, Finalization, and Editing.

Declaration of Competing Interest

Authors declare that there are no conflicts of interest

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Ecotoxicological response of potentially toxic metal (PTM) pollution in estuarine mangrove habitat of Indian Sundarban

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Contamination of potentially toxic metals (PTMs) can be detrimental to ecological processes, diversity and physiology of biological components of the surrounding habitat of estuarine and coastal environment. In our study, an inclusive approach to measure ecotoxicological parameters was followed considering spatial concentration of PTMs in the sediment system, sediment quality status, ecological risk level, bioconcentration in mangrove tissue and resulting biochemical and antioxidative response in mangroves *Avicennia alba* and *Excoecaria agallocha* in eight locations in and around Indian Sundarban. Sediments in location L4 and L8 showed highest concentration of PTMs having maximum enrichment factors, geo-accumulation indices, contamination factors and pollution load indices signifying progressively deteriorated sediment quality of the estuary and considerable ecological risk for cadmium. Significant statistical correlation observed between chlorophyll content, free radical scavenging activity, reducing ability and stress enzyme activity (peroxidase, catalase and super oxide dismutase) of mangroves with PTM concentration in respective study areas. This work will help to frame effective prediction, assessment and management policies in this extremely eco-sensitive region by envisioning the status of augmented human activities leading to considerable metal stress in the estuarine sediment and consequent ecotoxicological response as the coping up mechanisms.

Keywords. Potentially toxic metal; Sundarban; ecotoxicology; biochemical marker; estuarine health; metal pollution; biomonitoring.

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1. Introduction

Studies on ecotoxicological response to environmental stressors is an integrated biomarker based approach, which facilitate a clear understanding of the potential environmental risk and hazards in dynamic estuarine and coastal ecosystems (Maulvault *et al.* 2019). Unfortunately, there is a gap of knowledge regarding ecotoxicological study reports in tropical and subtropical regions unlike the temperate one (de Almeida Duarte *et al.* 2017). Since the inception of industrial revolution, anthropogenic activities have caused release of toxic pollutants into the environment at gradually increasing level. Pollutants like metals pose serious concern not only because they are toxic but also because they can be persistent in the ecosystem through the processes of bioaccumulation and biomagnification leading to serious impact and potential risk to various living organisms (de Almeida Duarte *et al.* 2017). Even essential metals required for metabolism could be toxic, if ingested at higher concentration and non-essential metals may cause toxicity at very low concentration (He *et al.* 2005; Crichton 2016). Potentially toxic metals (PTMs), to be precise, heavy and trace metals eventually tend to deposit into water and sediment system after they reach to coastal and estuarine areas either due to natural processes or from sources like mining, metalliferous ore smelting, industrial processing, burning of fossil fuels, wastes and sewage, fertilizers, automobiles, etc. (Yuan *et al.* 2012; Alloway 2013). Several complex multilayered factors including sediment composition and formation, grain size, hydrodynamic parameters govern these processes causing changes in degree of metal deposition in sediment over the period (Liu *et al.* 2011; Watts *et al.* 2017). Plants grown in PTM contaminated soil have evolved with highly specific as well as selective mechanism and biochemical responses to uptake, transfer and accumulate toxic metals (Lasat 2002; Verbruggen *et al.* 2009). Intracellular accumulation and sequestration of metals, exclusion or efflux into the xylem and intracellular detoxification mechanism have major role in metal tolerance, metal homeostasis and/or metal-induced oxidative stress in plants (Montargès-Pelletier *et al.* 2008). Both redox active and non-redox active metals are capable of either inducing reactive oxygen species (ROS) by Fenton and Haber–Weiss reactions or generating organellar ROS by disrupting photosynthetic and photorespiratory systems (Halliwell 2006; Rodríguez-Serrano *et al.* 2009). These might also lead to enzyme inactivation and disruption, conformational modifications, dysfunctional membrane integrity, inhibition of metabolic processes and disturbed redox homeostasis (Sharma and Dubey 2007; Sharma and Dietz 2009). Excessive toxic metal stress can dismantle the balance between ROS and the quenching properties of antioxidants (AOX) which might cause lipid peroxidation, oxidative damage, DNA breakage or cell death (Braconi *et al.* 2011; Kandziora-Ciupa *et al.* 2013).

Monitoring biochemical response of living species against these metal stressors can be efficiently used to design predictive tools in modern ecotoxicology (Skaldina and Sorvari 2017). In estuarine environment, metal biomonitors can bridge between degree of pollution of the ecosystem and resulting biochemical effects in plants for framing efficient mitigation strategies. With both enzymatic and non-enzymatic defense systems, strong antioxidant potential, metal avoidance and scavenging potential of ROS; mangroves have been established as a promising biomonitor of metal stress (Bandaranayake 2002; Yan *et al.* 2017). Their characteristic physiological activities like generation of specific proteins, activation of peroxidase–catalase–flavonoid–phenolics, and also the antioxidant feedback loop might be explored as potential biomarkers of PTM contamination in the sediment system (Duarte *et al.* 2013; Bakshi *et al.* 2019). Mangrove sediments being anoxic in nature with negative redox potential, high sulfide, iron and organic content; favour retention of metals that makes sediment a long term sink for various PTMs (Bakshi *et al.* 2017; Yan *et al.* 2017). Studies on accumulation of toxic metals in mangrove sediments thriving ecotoxicological risk have been conducted globally (Nath *et al.* 2013, 2014a, b; Bakshi *et al.* 2018).

The Sundarban delta is the world's largest single block mangrove habitat shared by India and Bangladesh. A part of it, situated on the dynamic Hooghly–Matla estuarine system in West Bengal, India, has been consistently subjected to environmental degradation due to extensive industrialization and rapid urbanization in the upstream (Banerjee *et al.* 2012a, b; Ghosh *et al.* 2016). Both natural weathering process and anthropogenic interferences have led to unwanted PTM deposition and interruption in usual ecosystem processes throughout this habitat (Antizar-Ladislao *et al.* 2015). The ecotoxicological response to this increasing environmental stress needs to be thoroughly investigated and explored as a significant indicator to enumerate the degree of pollution. Biological response of mangrove vegetation exposed to toxic metals have been mostly investigated in controlled laboratory conditions (Zhang *et al.* 2007; Huang and Wang 2010; Huang *et al.* 2010; Cheng *et al.* 2017), whereas minimal reports are available on natural mangrove population (MacFarlane 2001; Caregnato *et al.* 2008; Harish and Murugan 2011; Bakshi *et al.* 2018). In our study, the relationship between ecotoxicological risks of PTM distribution in eight randomly selected study areas in and around Indian Sundaban with consequent biotic response in two commonly found mangrove species in this region namely, *Avicennia alba* and *Excoecaria agallocha* have been studied.

2. Methodology

2.1 *Study area and sampling method*

Indian Sundarban situated at South Bengal within the imaginary Dampier and Hodges line is a vulnerable and sensitive eco-region characteristically comprised of a complex conglomeration of various confluences, tributaries, distributaries and tidal creeks. Eight sampling locations along this habitat namely, L1, L2, L3, L4, L5, L6, L7 and L8 were randomly selected to evaluate the ecotoxicological response of PTM pollution in *Avicennia alba* and *Excoecaria agallocha* (figure 1). Rhizospheric surface sediment contains Fe–Mn oxyhydroxides, clay particles and organic content which may aggregate toxic metal by adsorption onto clay particle surface or absorption into the oxides (Chaudhuri *et al.* 2014; Ghosh *et al.* 2019a, b). The ecotoxicological response of mangroves due to the accumulation of potentially toxic metals in the rhizospheric horizon could be investigated as the stress enzymes and chlorophyll content are the suitable bioindicators (Bakshi *et al.* 2018). Hence, fully grown and healthy leaves from 10 plants with similar height, health condition, light exposure and tidal amplitude at each site were collected and pooled into one sample to reduce the sampling bias as remobilization of metals could also be varied depending on physical conditions of the plants. Though several mangrove species have been reported in the sampling region, two species *Avicennia alba* and *Excoecaria agallocha* were uniformly distributed in all sampling locations and hence selected for the study. For both these species, three pooled leaf samples were collected and prepared. From each location, surface sediment samples were collected in triplicate at depth within 10–15 cm. All samples were kept in clean zip lock pouch, placed within ice box and quickly taken to the laboratory. Respective co-ordinates of all locations were designated by Trimble Juno SD hand-held GPS (supplementary table S1). Fresh leave samples were processed and analyzed immediately to avoid sample handling error as much as possible following Bakshi *et al.* (2018). Rest of the sediment and leaf samples were dried, ground and sieved through 63 μ nylon mesh and kept for further analysis.

2.2 *Physico-chemical properties of sediment samples*

pH, conductivity and oxidation reduction potential (ORP) of sediment samples were analyzed using HANNA multi-parameter probes after standardization with standard solutions for the same (Birch *et al.* 2011). Organic carbon content was determined by titrimetric method (Walkey and Black 1934).

2.3 *Analysis of PTM*

Dried and ground samples were used for acid digestion (USEPA 1997) and analyzed in Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Thermo Fisher iCAP 7400) following the method described by Bakshi *et al.* (2018) to determine the concentrations of 10 metals and trace elements namely, Aluminum (Al), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb) and Zinc (Zn). Complete data acquisition process using ThermoFisher Qtegra software was conducted in axial mode except from Al and Fe, which were performed in radial mode. The quality control of the study was ensured by running Standard Reference Materials simultaneously (SRM 1645 for sediment sample and NIST 1573a for leaf samples). Analytical accuracy, reported as recovery, was 94–106% and precision was <5% relative standard deviation for all target elements, expressed as mg/kg dry weight. Multi-element Standard Solution IV and IX (Merck, Germany) were used at different dilutions as calibration

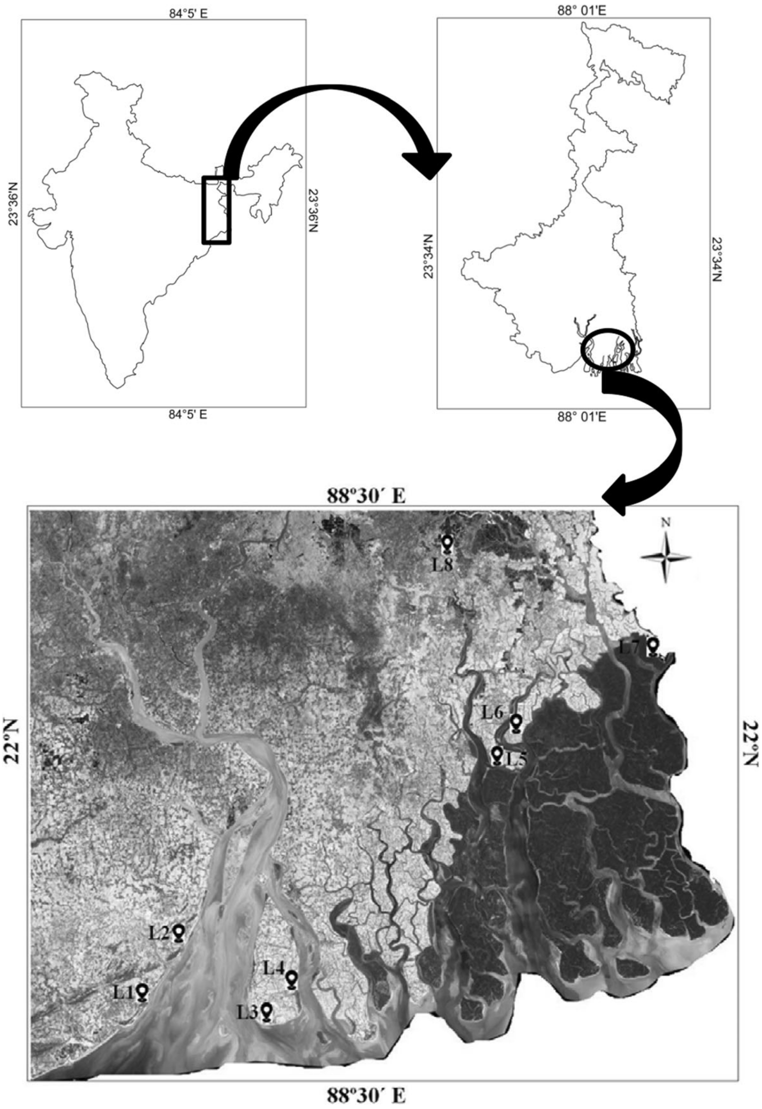


Figure 1. Map of the study locations.

standard solutions. The details of the quality control data are given in supplementary table S2.

2.4 *Ecotoxicological response*

2.4.1 *Sediment quality indices analysis*

Sediment quality indices are established indicator of metal pollution status comparing with geochemical background values in soil and sediment system. It also helps us to quantify the degree of anthropogenic interference at a specific study area (Bakshi *et al.* 2018, 2019; Ghosh *et al.* 2020). Either Wedepohl (1995) shale values and/or upper continental crust (UCC) values of Taylor and McLennan (1985) have been mostly used as geochemical background in Indian sub-continental estuarine system (Ghosh *et al.* 2016; Bakshi *et al.* 2017, 2018). In our study, we have considered UCC (Taylor and McLennan 1985) values as geochemical background as no specific geochemical background value is available for the specific region (supplementary table S3). Most commonly used indices for sediment health quality such as enrichment factor (EF), geo-accumulation index (I_{geo}), contamination factor (CF), pollution load index (PLI) have been studied. Details of calculation of these indices are provided in supplementary table S4 (Spencer and Macleod 2002; Caeiro *et al.* 2005; Birch *et al.* 2013). Sediment quality in the study locations has been enumerated by following the standard classification system based on EF (Zhang and Liu 2002; Gu *et al.* 2012;

Kumar *et al.* 2013), I_{geo} (Muller 1969), CF (Hakanson 1980) and PLI values (table S5 in supplementary materials).

2.4.2 *Potential ecological risk*

To understand the correlation between ecological risk and antioxidative response in mangroves *A. alba* and *E. agallocha,* potential ecological risk index (PERI) has been calculated for a toxic metal in a specific study area (supplementary table S4) (Gong *et al.* 2008; Guo *et al.* 2015). To identify the locations having toxicity risk, sediment quality guideline (SQG) supported by the effect range low (ERL)–effect range median (ERM) and the threshold effect level (TEL)–probable effect level (PEL) values have been applied (MacDonald *et al.* 1996; Long and MacDonald 1998) (table 1). Locations with metal concentration within TEL or ERL value indicate less adverse effects and low ecological risk. On the other hand, locations having meal concentration higher than PEL and ERM value are considered to bear greater ecological risk (Long *et al.* 1995, 1998).

Mean probable effect level quotient (mPELq) ratio has been used to express the amount of biological impact of coupled toxicity effect of the stress metals in the ecosystem (Long *et al.* 1998) as described in supplementary table S3. The sediment has been categorized based on the classification system of probability of being toxic according to the respective mPELq value (Long *et al.* 2000) (supplementary table S4).

2.4.3 *Metal bioaccumulation in mangrove*

Bioconcentration factor (BCF) of PTMs in mangrove leaves is an indicator of elemental accumulation in leaf tissues from habitat sediment and calculated as $BCF = C_{plant}/C_{sediment}$, where C_{plant} and $C_{sediment}$ are total metal concentrations (mg/kg) in plant and sediment, respectively (MacFarlane *et al.* 2007).

Table 1. *Physico-chemical properties of sediment samples.*

Location	pH	EC (μS/cm)	TOC (%)	ORP
L1	7.5 ± 0.2	1723 ± 16	0.891 ± 0.16	170 ± 27
L2	7.7 ± 0.2	981 ± 11	0.863 ± 0.07	173 ± 9
L3	7.5 ± 0.5	793 ± 31	0.847 ± 0.03	177 ± 13
L4	7.8 ± 0.1	1620 ± 21	0.916 ± 0.09	131 ± 19
L5	7.7 ± 0.5	977 ± 8	1.210 ± 0.04	85 ± 15
L6	7.8 ± 0.4	789 ± 9	0.984 ± 0.05	79 ± 11
L7	7.9 ± 0.2	791 ± 16	0.933 ± 0.02	46 ± 10
L8	7.9 ± 0.3	490 ± 24	1.440 ± 0.08	29 ± 2

2.4.4 *Biochemical response in mangroves*

Chlorophyll content (Chl *a*, Chl *b*, total Chl and Chl *a*/Chl *b*) of mangrove leaves were estimated spectrophotometrically. Calculations have been done using Arnon's equation and chlorophyll content is expressed as mg/g fresh weight (Arnon 1949).

Bound and soluble forms of polyphenols are plant derived secondary metabolites playing crucial role in natural defense system. Antioxidant property in terms of total soluble phenolics and flavonoids in leaves of *A. alba* and *E. agallocha* were assessed. The amount of total phenolics in leaf extracts was measured following Folin–Ciocalteu procedure (Singleton and Rossi 1965) and expressed as gallic acid equivalents (GAE) in mg GAE/g dry leaf. Another group of plant derived polyphenolics, flavonoids possessing antioxidant properties has reduction potential lower than alkyl peroxyl radical. Hence, flavonoids can be efficient to prevent detrimental effects of these species by inducing their inactivation. Flavonoid concentration expressed as mg Quercetin/g dry leaf is quantified using aluminium chloride (Zhishen *et al.* 1999).

The reducing ability of leaf extracts was measured following the procedure described by Oyaizu (1986) and expressed as ascorbic acid equivalent (AAE) in mg AAE/g of dry leaf. The free radical scavenging activity of leaf extracts was determined using stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl), where butylated hydroxyl toluene (BHT) was taken as positive control (Blois 1958; Yıldırım *et al.* 2001). The antioxidant activity of the extract was expressed as IC50, which was expressed as the concentration in mg of dry leaf/ml which inhibits the formation of DPPH radicals by 50%.

Soluble peroxidase (POD) activity of the leaf tissues was estimated by slightly modified method of Bashir *et al.* (2007) and Zhang *et al.* (2007) and expressed POD units/min/mg protein (Bakshi *et al.* 2018). Protein concentration of the mangrove leaves was determined using Bradford method (Bradford 1976; Kruger 2009). Catalase (CAT) activity was also measured spectrophotometrically and expressed as CAT units/min/mg protein (Chandlee and Scandalios 1984). Super-oxide dismutase (SOD) activity was analyzed using nitroblue tetrazolium (NBT) in the presence of riboflavin (Beauchamp and Fridovich 1971; Cipollini 1998) and expressed as SOD units/mg protein (Zhang *et al.* 2007). Details of all enzymatic and antioxidative analysis methods have been described in supplementary table S6.

2.5 *Statistical analysis*

All analysis was done in triplicates and the mean value was taken with the standard deviation (SD). The variance in the dataset was checked by one-way analysis of ANOVA (Bakshi *et al.* 2017, 2018). Significant statistical differences in all variables were also confirmed by Duncan's multiple range test at $p < 0.05$ in SPSS16. The Pearson's correlation coefficient significant at $p < 0.05$ and $p < 0.01$ was tested to understand the association within and among PTM concentration in sediment and mangroves, physico-chemical properties of sediment and ecotoxicological response of the plant. Similarities and relations within metal level and antioxidative response of *A. alba* and *E. agallocha* were evaluated using principal component analysis (PCA) in SPSS16 (Watts *et al.* 2017).

3. Results and discussion

3.1 *Physico-chemical properties of sediment samples*

Here, eight sampling locations in and around Indian Sundarban can be broadly classified into two categories: L1–L4 belongs to the Hooghly estuarine region and L5–L8 comes under Matla estuarine region. pH, conductivity, ORP and organic carbon content in sediment samples of location L1–L8 ranged 7.5–7.9, 490–1620 (μS/cm), 29–177 and 0.847–1.440%, respectively (table 1). The pH values at the locations close to the river Matla esuary (L5–L8) are little higher than the intertidal sediment samples of river Hooghly estuary (L1–L4), which might be due to the existing CO_2-carbonate system in the surrounding of Matla estuary (Frontier *et al.* 2008). Similarly, the Matla estuarine locations were found to have higher organic carbon (OC) values (1.44% at L8) indicating the anaerobic and reduced nature of the sediment with presence of sulfide complexes and organic matter (Bayen 2012; Bakshi *et al.* 2017) sourced from agriculture, aquaculture and domestic sectors (Banerjee *et al.* 2012a, b). Lower OC content in Gangetic estuary (0.847% at L3) reflects dynamic microbial activity and vigorous tidal cycle as well as poor adsorptionability of the organic carbon to embedded quartz grains of the recipient habitat (Rogers *et al.* 2013). This variation also goes along with the observations Banerjee *et al.* (2012a) and Dhame *et al.* (2016). The reduced nature of Matla estuarine locations (L4–L8) being closer to the denser and true mangrove habitat have resulted to lower ORP (29–85 mv) comparing to Hooghly estuary (131–177 mv) (Lyimo and Mushi 2005). Significant correlation between trace metal accumulation with organic carbon and fine sediments reported by many researchers signifies how it acts like a sink of metals (Chaudhuri *et al.* 2014; Dhame *et al.* 2016). Similar findings were also observed in our study between OC with Cd, Pb, Zn, Ni and Cr and Mn (supplementary table S7).

3.2 *Analysis of PTM*

Table 2 reflects the distribution of PTM concentration at locations L1–L8 in the extended river Hooghly and Matla estuarine region. The highest concentration of PTMs were found in L8: for Cadmium (0.212 mg/kg), Chromium (97 mg/kg), Nickle (68.1 mg/kg), Lead (35.3 mg/kg), Zinc (141.83 mg/kg) and L4: for Cobalt (12.52 mg/kg), Copper (89.33 mg/kg), Iron (53019 mg/kg), and L7: for Aluminum (51131.33 mg/kg). Location L7 was considered as the control site as it had the minimum concentration for most of the metals including Cd, Cu, Fe, Ni and Pb. The second-most deposition was observed in location L4. The major source of PTMs in this area include agricultural and aquaculture practices, human waste, port activities, tourism activities, vehicular exhaust, construction work, industrial effluent from thermal power plants, jute mills, paper mills, brick kilns, tanneries, pesticide and fertilizer factories, etc. (Ghosh *et al.* 2016; Bakshi *et al.* 2017). Kulti river lock gate, which is 35 km away from Kolkata metro city located at the North border of Dampier–Hodges line in Sundarban, receives a large section of toxic effluent, run offs from around 11516 different factories in Kolkata Megacity (Akhand *et al.* 2012). Being close to river Bidyadhari and situated on this exit point of sewage canal system in city Kolkata, location L8 is subjected to year-long deposition of high organic content loaded sediments which might cause this higher accumulation of PTMs in that location. The variation in distribution

Table 2. *Mean concentration of PTMs (mg/kg) in sediment samples in location L1–L8.*

New	L1	L2	L3	L4	L5	L6	L7	L8	ERL	ERM	TEL	PEL
Al	50144.95	49971.52	50386.71	50048.29	49940.57	50035.90	51131.33	49797.81				
SD	296.79	623.83	348.24	362.32	332.77	627.81	258.11	413.53				
Cd	0.08	0.07	0.12	0.16	0.09	0.07	0.05	0.212	1.2	9.6	0.68	4.2
SD	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.03				
Co	10.41	8.6	12.1	12.52	10.4	11.6	8.9	11.4				
SD	1.15	0.99	1.51	0.70	0.76	0.92	1.22	1.46				
Cr	71.8	51.1	61.62	77.8	83.7	75.8	60.6	97	81	370	52.3	160
SD	3.80	3.70	5.19	1.79	6.56	6.81	4.75	3.80				
Cu	72.03	70.67	81.47	89.33	73.62	66.67	48.4	84.67	34	18.7	18.7	108.2
SD	3.61	5.86	3.56	4.51	4.10	4.04	3.42	4.04				
Fe	45281.00	44805.00	48305.67	53019.00	43045.67	43747.67	34603.00	51449.33				
SD	2227.66	4412.85	3930.93	4319.50	3874.25	4565.92	1637.34	3831.36				
Mn	567.8	768.54	690.7	605.2	608.2	565.20	738.7	457.3				
SD	70.45	91.14	60.17	49.54	38.71	52.28	55.77	41.46				
Ni	50.7	41.9	49.3	54.72	59.3	54.3	32.3	68.10	20.9	51.6	15.9	42.8
SD	2.52	3.12	4.73	3.84	3.29	3.40	1.74	3.00				
Pb	19.53	23.3	21.7	24.4	23.3	19.2	15	35.30	46.7	218	30.2	112
SD	2.78	1.59	2.52	1.56	2.46	2.45	1.84	5.29				
Zn	78.03	80.3	105.20	98.3	126	115.1	118.3	141.8	150	410	124	271
SD	7.44	4.16	11.79	7.26	9.80	6.88	4.75	13.75				

and accumulation of PTMs across the study area have been governed by various geochemical, geomorphological and natural factors like variable depositional and erosional rate, coagulation–siltation–sedimentation, vigorous tidal hydrodynamics, weathering process, grain size, etc. (Antizar-Ladislao *et al.* 2015; Ghosh *et al.* 2018).

Two mangrove species *A. alba* and *E. agallocha* have been chosen to understand the PTM accumulation and consequent biotic responses. The plant *E. agallocha* showed elevated level of PTM concentration comparing to *A. alba* probably because of the difference in their uptake potential internal physiology (Bakshi *et al.* 2017). However, for both the plants maximum PTM accumulation was observed for species grown in L8 which complements the results of sediment metal distribution. The only exceptions are Al and Mn for *A. alba* and Co and Mn for *E. agallocha* (table 3). The association among the metals in sediment and plant samples can be envisaged through the Pearson correlation analysis (supplementary table S7) suggesting their common source of origin and closely related process of metal transportation, metal deposition and metal accumulation (Bastami *et al.* 2014). Negative and non-significant correlation between all studied PTMs (except of Al) and Mn indicates towards poor retention potential of Mn-oxide complexes and influence of peripheral parameters (Ray *et al.* 2006; Bhattacharya *et al.* 2015). Positive association between PTMs in both sediments and plants emphasizes on the potential of using *A. alba* and *E. agallocha* plants as a bio-monitor or indicator of PTM contamination in estuarine ecosystem of Sundarban.

3.3 *Ecotoxicological response*

3.3.1 *Sediment quality indices*

The primary investigation on ecotoxicological response of mangroves in terms of metal uptake, accumulation, translocation, tolerance, chelation or sequestration, enzymatic and non-enzymatic antioxidative mechanisms can be thrived by the sediment quality of surrounding habitat. Sediment quality indices are indicative of the metal contamination scale and human interference responsible for it, by comparing with the respective geochemical background.

In this study, Al has been selected as reference element for calculating enrichment factor (EF) which is one of the most efficient indicators to estimate the magnitude of human-induced and/or natural changes by standardizing the target PTM against the reference element having low occurrence variation (for example, Mn, Ti, Al and Fe) and categorizing them as per the standard classification system

Table 3. *Mean concentration of PTMs (mg/kg) in leaves of A. alba and E. agallocha.*

Mangrove	PTM	L1	L2	L3	L4	L5	L6	L7	L8
A. alba	Al	399.71	516.96	328.61	1005.47	253.48	221.42	248.25	505.20
	SD	11.23	6.01	15.36	13.99	7.32	3.91	8.48	6.96
	Cd	0.0040	0.0103	0.0047	0.0208	0.0047	0.0034	0.0042	0.0607
	SD	0.0008	0.0008	0.0002	0.0045	0.0005	0.0007	0.0012	0.0033
	Co	0.66	0.26	1.21	0.69	0.45	0.12	0.42	1.07
	SD	0.08	0.02	0.31	0.05	0.03	0.01	0.01	0.07
	Cr	5.76	7.77	11.04	19.09	8.68	9.59	5.26	26.53
	SD	1.60	0.54	1.98	1.13	1.60	2.08	0.38	4.06
	Cu	25.63	29.58	19.30	42.35	28.39	24.29	12.09	43.48
	SD	3.14	4.86	1.14	6.14	2.14	1.64	1.36	1.79
	Fe	149.36	186.69	156.02	344.62	141.09	102.08	115.34	364.43
	SD	23.17	10.75	10.92	21.25	14.58	25.17	20.67	18.58
	Mn	227.13	380.61	256.64	273.65	142.84	167.72	194.39	210.32
	SD	9.26	23.58	10.83	31.11	14.59	8.16	5.05	10.34
	Ni	2.57	1.34	1.97	1.64	2.61	0.07	0.19	4.13
	SD	0.60	0.09	0.34	0.05	0.57	0.00	0.13	0.60
	Pb	2.09	3.23	1.91	2.76	2.46	1.92	1.84	5.44
	SD	0.32	0.55	0.36	0.25	0.06	0.45	0.07	0.82
	Zn	15.04	30.71	14.59	13.21	19.77	13.17	6.76	35.46
	SD	1.57	3.50	5.07	1.07	1.83	2.07	2.64	2.71
E. agallocha	Al	515.51	653.83	423.81	1309.67	326.92	294.60	320.17	651.56
	SD	14.49	7.76	19.81	18.04	9.44	5.05	10.93	8.97
	Cd	0.0036	0.0094	0.0043	0.0189	0.0042	0.0031	0.0038	0.0552
	SD	0.0008	0.0008	0.0002	0.0041	0.0005	0.0006	0.0011	0.0030
	Co	0.76	0.30	1.39	0.80	0.52	0.14	0.48	1.23
	SD	0.09	0.02	0.35	0.05	0.04	0.01	0.01	0.08
	Cr	4.07	5.49	7.20	13.49	6.14	6.77	3.72	18.75
	SD	1.13	0.38	1.40	0.80	1.13	1.47	0.27	2.87
	Cu	8.27	10.35	6.75	14.82	9.94	8.50	4.23	15.22
	SD	1.10	1.70	0.40	2.15	0.75	0.58	0.48	0.63
	Fe	162.94	203.66	170.20	375.95	153.91	101.36	125.83	397.56
	SD	25.27	11.73	11.91	23.18	15.91	27.45	22.55	20.27
	Mn	86.31	144.63	97.52	103.99	53.52	63.73	73.87	79.92
	SD	3.52	8.96	4.12	11.82	5.55	3.10	1.92	3.93
	Ni	2.96	1.55	2.28	1.89	3.01	0.08	0.22	4.23
	SD	0.40	0.10	0.39	0.06	0.65	0.00	0.15	0.69
	Pb	1.64	2.54	1.50	2.17	1.93	1.51	1.45	4.27
	SD	0.25	0.43	0.29	0.20	0.05	0.36	0.06	0.64
	Zn	25.67	59.84	24.91	22.55	32.75	20.77	11.54	60.54
	SD	2.68	5.98	8.66	1.83	3.12	3.54	4.51	4.63

(supplementary table S5) (Reimann and de Carital 2000; Sutherland 2000; Gu *et al.* 2012). The highest EFs observed in L8: Cd, Cr, Cu, Ni, Pb and Zn) and L4 (Co and Fe) indicates the role of anthropogenic contribution in metal enrichment besides natural weathering processes in these two locations (Kumar 2013; Bakshi 2017). The stack columns in figure 2 have been used to compare numeric values between levels of a categorical variable for better visual understanding. As per the standard classification, two PTMs namely, Cr and Ni possessed moderate enrichment in sediment system resulting from non-crustal or non-natural weathering processes. On the other hand, PTMs like Cd, Co, Cu, Fe, Pb and Zn have significant enrichment in the studied locations. Mn is the only PTM which had highest EF in location L2 implying its enrichment to be moderate level in that location. All PTMs except Mn, showed their

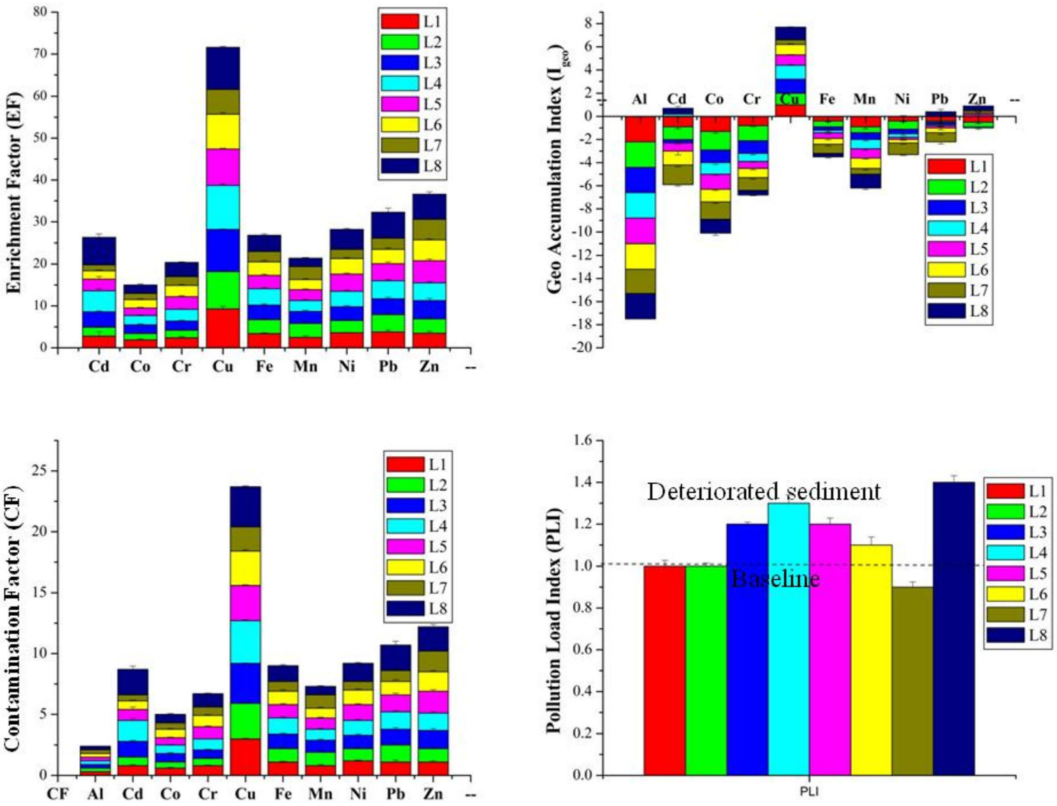


Figure 2. Sediment quality indices (enrichment factor, geo accumulation index, contamination factor, pollution load index).

lowest EFs in location L7 and L2 reflecting the non-polluted nature of these locations complementing the metal distribution data. The highest enrichment was observed for Cu among all elements.

The I_{geo} value was analyzed to classify the sediment as per seven enrichment classes based on degree of contamination (Müller 1969). Elements including Al, Co, Cr, Fe and Mn were seen to have negative I_{geo} values signifying their uncontaminated condition in all the study sites (figure 2). Cu showed positive I_{geo} values 0.4 (L7)–1.2 (L4) throughout eight locations, out of which L1, L2, L5, L6 and L7 were found to have uncontaminated to moderately contaminated level and L3, L4 and L8 expressed moderate contamination for Cu (supplementary table S5) (Müller 1969). For Cd, Ni and Pb, the uncontaminated to moderately contaminated sites were L4 and L8 (Cd); L8 (Ni and Pb); L5, L6, L7 and L8 (Zn), respectively. Derived I_{geo} values of sediment samples harmonize with the values of EF, i.e., PTMs with significant enrichment and higher EF numnbers (Cd, Cu, Ni, Pb and Zn) showed moderate contamination and positive I_{geo} values. Decipherable contamination from these PTMs might be attributed on agricultural run-off, domestic and industrial effluents, vehicular emission, weathering processes and/or related anthropogenic hindrances (Ghosh *et al.* 2016; Bakshi *et al.* 2018).

Another prominent sediment quality index, contamination has been used to express the degree of elemental contamination (Hakanson 1980). The CF values as shown in figure 2 reflects low contamination grade for Al, Cr, Co and Mn in almost every location and moderate contamination status for Cd, Fe, Ni, Pb and Zn. Location L4 was found to be considerably contaminated with Cu and also possessed highest CF values for Co and Fe. Precisely, Cu had highest CF values in all eight locations ranging from 2 to 3.5. On an average, all locations were found to have moderate level of contamination for the potentially toxic metals as per Hakanson (1980) where the calculated CF values range within 1–3. Location L8 has been identified as the most contaminated study area with reference to Cd, Cr, Ni, Pb and Zn contamination in the sediment system.

On the whole, status of PTM pollution in the sampling locations has been recognized by calculating the PLI scores (Tomlinson *et al.* 1980), which ranged from 0.9 (L7) to 1.5 (L8). The increasing order of calculated PLI is L7 > L2 > L1 > L6 > L5 > L3 > L4 > L8. As PLI values in most of the locations except of L7, lie on or above the baseline (1), it is clearly indicative of progressively deteriorated nature of the sediment quality (figure 2).

The results show consistency in the way as all sediment quality indices harmonized with each other and precisely pointed the sediment system to be moderately contaminated and progressively deteriorated. It was undoubtedly established that L4 and L8 are the most contaminated locations where the increased enrichment of the PTMs like Cd, Co, Cu, Fe, Pb and Zn might be consequence of upstream natural weathering along with amplified rapid urbanization, industrialization and developmental activities in the surrounding. In contrast, the least contaminated location is L7 (with the minimum enrichment, geo-accumulation and lowest pollution level) having no significant industries in larger surrounding except that of few brick kilns.

3.3.2 *Potential ecological risk*

The potential risk of different individual PTMs (Er) and as a whole, their comprehensive potential ecological risk index (PERI) in the sediment have been depicted in figure 3. The maximum Er for Cd, Cr, Pb and Zn were observed at L8 as 64.29, 2.28, 10.29 and 2, respectively. For Cu, it was 17.53 at L4. The range of Er for Cd was appeared to be under moderate risk class (L4 and L8), whereas Er for Cr, Cu, Pb and Zn were under low risk class.

The study locations showed a wide range of comprehensive risk factor, PERI, from 31.83 (L7) to 95.46 (L8). The risk factors imply that the overall potential ecological risk remain under low risk category. Sediment quality guidelines comprising of TEL, PEL and ERL, ERM categories can be used as an efficient tool to enumerate the degree of ecological risk posed by the PTMs (Long *et al.* 1995; Long and MacDonald 1998). Our study reveals that, despite of being at moderate risk category at location L4 and L8, in all eight sampling spots Cd concentration is lower than prescribed ERL and TEL limit (table 2). Cr concentration is lower than ERL level at all locations except of L8 and L5, whereas it is below TEL limit only at location L2. Concentration of Cu has crossed both TEL and ERL levels, but is much below the PEL and ERM limits. Ni concentrations in all eight locations have gone beyond PEL and ERL levels and even crossed the ERM limit in four locations (L4, L8, L5, L6). On the contrary, Pb concentration is above TEL and ERL limits only at location L8. Similarly, Zn concentration is also under TEL limit in all locations except L8 and L5. Overall, in location L7, PTM concentrations can be clubbed as 'low range' distribution (i.e., values within TEL or ERL limit) suggesting less adverse effects and lower ecological risk. On the other hand, PTM concentration in locations like L4 and L8 falls within 'high range' values (i.e., exceeding PEL and ERM limit) and thrives adverse effect on the ecosystem and higher ecological risk potential (Long and MacDonald 1998). This heterogeneity in the level of probable adversity level and consequent ecological risk in the study locations is also influenced by their closeness to the pollutant source, adjacent physico-chemical properties, etc.

The mean PEL quotient (mPELq) value is another significant indicator to estimate the coupled toxicity effect of the PTMs in the sediment system (Long *et al.* 1998). The calculated mPELq in eight locations ranges from 0.4 (L7) to 0.6 (L8) (figure 3). When the values are compared with the classification system prescribed by Long *et al.* (2000), it has been observed that locations L1, L2,

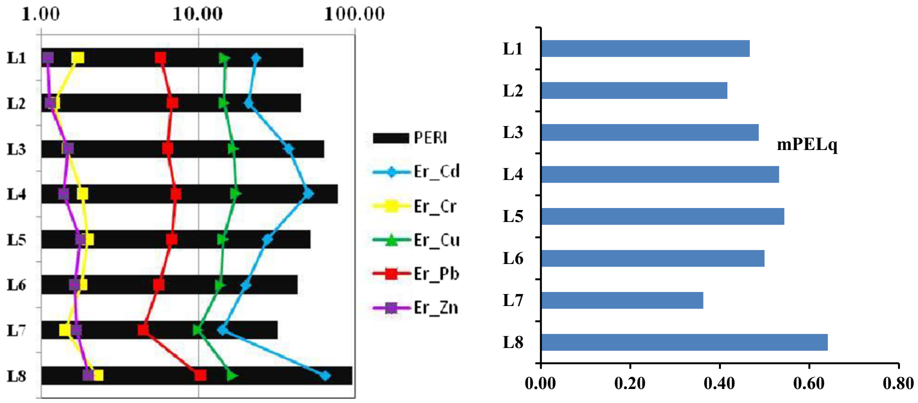


Figure 3. Ecotoxicological risk in sediment: Potential risk of individual metal and potential ecological risk index in habitat sediments, mean PEL quotient.

L3, L6 and L7 have acquired 21% probability of being toxic in terms of PTMs, whereas, for location L4, L8 and L5 probability of being toxic is 49%. Following the similar pattern as derived from the sediment quality indices, location L4 and L8 are found to pose bigger threat to the living communities as they score highest adversity and ecological risk potential values.

3.3.3 *Metal bioaccumulation in mangrove*

The ratio of PTMs in leaf to PTM concentration in sediments, defined as bioconcentration factors, is an indicator of the potential of metal bioaccumulation in plant and the interaction between plant and sediment system (Bakshi *et al.* 2018). In our study, in the case of *A. alba* and *E. agallocha*, the uppermost BCF for Cd, Cr, Cu, Fe, Ni, Pb were found in location L8, the extremely contaminated location with highest concentration of these PTMs indicating appreciable PTM bioaccumulation and uptake potential of both mangroves (figure 4). The results also complement the findings of previous researchers (MacFarlane *et al.* 2007; Chowdhury *et al.* 2015). However, in station 2, concentration of Zn in sediment was 80.3 mg/kg, but the BCF was much higher comparing the other locations. This might be due to uptake mechanisms by membrane transporters which are generally selective. In response to metal exposure, plants either undertake exclusion or efflux of metals into the xylem (xylem loading) or intracellular accumulation and sequestration. Beside efflux of metal ligands from root into xylem vessels, intracellular detoxification mechanisms potentially leading to plant metal tolerance are linked to metal homeostasis and/or metal-induced oxidative stress. The uptake and accumulation potential in plant species is also controlled by their genotype (Bakshi *et al.* 2017).

3.3.4 *Biochemical response in mangroves*

3.3.4.1 *Chlorophyll content:* Studies have shown that the physiological and metabolic processes as well as the stress tolerance of mangroves are affected by heavy metal contamination in habitat sediment (Zhang *et al.* 2007; Huang and Wang 2010). Photosynthetic system of the plants also gets disturbed by augmented metal stress (Cheng 2017). Concentration of the photosynthetic pigments chorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) in plants *A. alba* and *E. agallocha* have been depicted in figure 5(a). Plants in locations L4 and L8 (with higher PTM concentration and pollution load) showed lower concentration of Chl *a* and Chl *b* along with total chlorophyll content. Significantly negative correlation ($p < 0.01$) was established between chlorophyll content with PTMs like Cd, Cr, Cu and Pb in mangrove leaves as presented in Pearson correlation study in table 4. Photosynthetic pigments in *A. alba* and *E. agallocha* subjected to varied PTM stress have responded in a metal concentration dependent way. Here, higher chlorophyll pigment in mangrove leaves sampled from locations with lower PTM concentration (L2, L3, L5 and L6) and lower amount of pigment in the plants grown at locations having higher ecological risk and pollution load (L4, L8) reveals the PTM induced stress affecting chlorophyllase/δ-aminolevulinic acid dehydratise activity and influencing chlorophyll activation (Morsch *et al.* 2002), also supported by the findings of Huang and Wang (2010) and Cheng (2017).

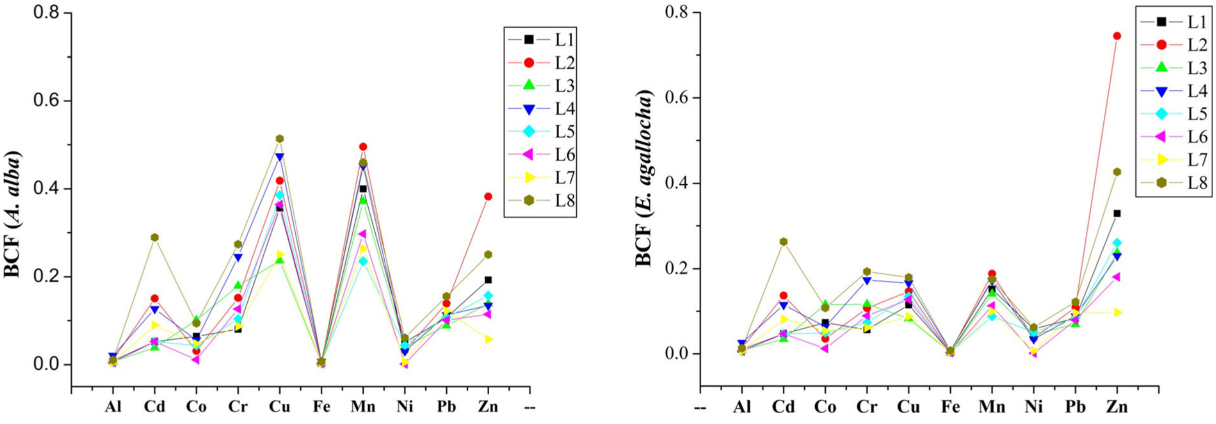


Figure 4. Bioconcentration factor in *A. alba* and *E. agallocha*.

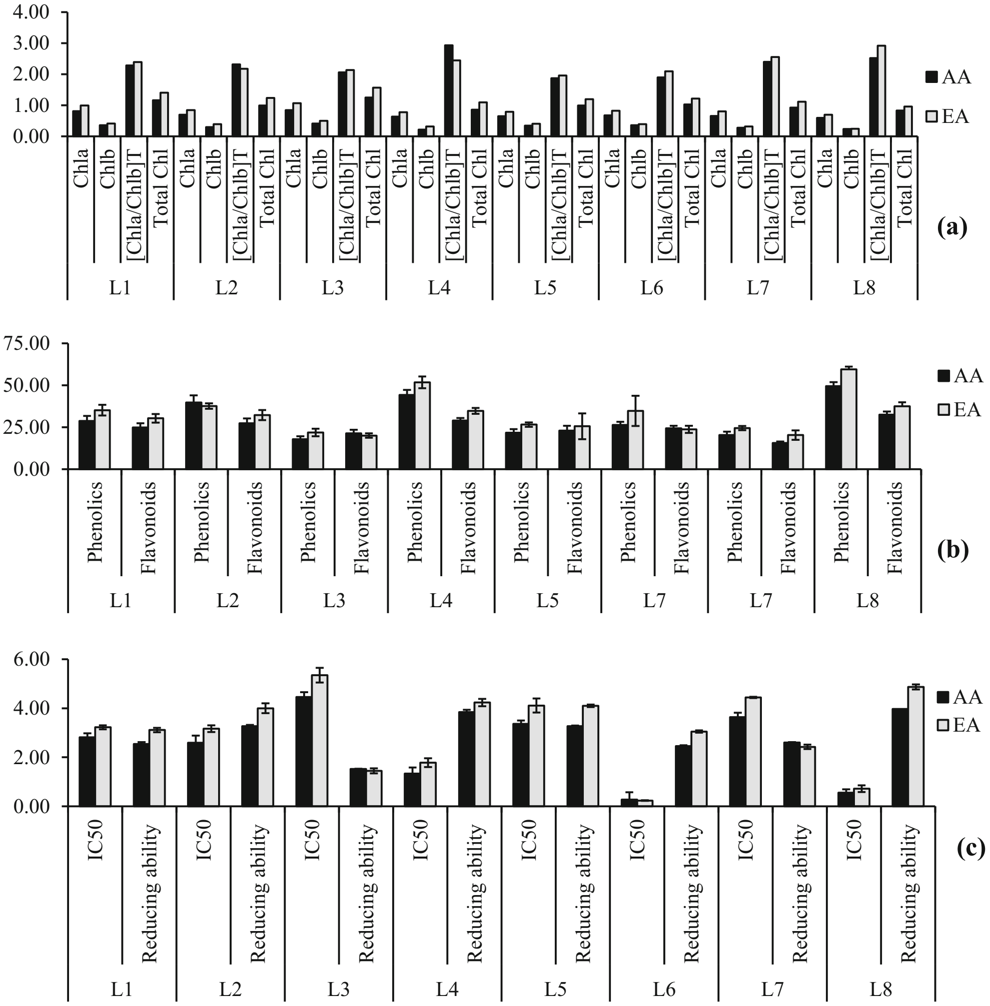


Figure 5. (**a**) Chlorophyll content, (**b**) total phenolic content (mg GAE/g dry leaf) and flavonoid content (mg Quercetin/g dry leaf) and (**c**) antioxidant activity of mangroves. AA: *A. alba*; EA: *E. agallocha*.

Table 4. *Correlation coefficients between mangrove biotic response and metal in mangroves.*

	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Chl *a*	−0.055	−0.454	0.31	−0.543	−0.690*	−0.315	−0.417	0.012	−0.584	0.054
Chl *b*	−0.348	−0.646*	0.065	−0.637*	−0.606	−0.595	−0.371	−0.108	−0.659*	−0.05
[Chl *a*/Chl *b*]T	0.578	0.625*	0.354	0.524	0.237	0.722*	0.118	0.256	0.463	0.195
Total Chl	−0.168	−0.533	0.238	−0.593	−0.689*	−0.425	−0.423	−0.023	−0.627*	0.031
Phenolics	0.722*	0.803*	0.25	0.692*	0.273	0.889*	−0.109	0.48	0.664*	0.584
Flavonoids	0.628*	0.628*	0.047	0.487	0.177	0.711*	−0.186	0.462	0.556	0.690*
IC50	−0.265	−0.534	0.244	−0.581	−0.468	−0.429	−0.076	−0.022	−0.504	−0.181
Reducing ability	0.563	0.603	−0.073	0.469	0.237	0.687*	−0.181	0.481	0.583	0.662*
Peroxidase	0.705*	0.835*	0.47	0.705*	0.127	0.918*	−0.194	0.49	0.641*	0.609
CAT	0.883*	0.625*	0.568	0.588	0.136	0.908*	−0.137	0.582	0.445	0.463
SOD	0.619	0.751*	0.222	0.569	0.062	0.815*	−0.346	0.423	0.563	0.541

*Correlation is significant at the 0.01 level.

3.3.4.2 *Total phenolics and flavonoid content:* Equilibrium between ROS and antioxidants (enzymatic and non-enzymatic) play the major role in quenching any oxidative injury resulting from any biotic or abiotic source of stress. Polyphenols and flavonoids are those in the forefront of defense mechanisms, which play the crucial role to combat environmental stress induced free radicals (Li *et al.*

2012; Dasgupta *et al.* 2015). In our study, total phenol and flavonoid content in *A. alba* and *E. agallocha* varied along with the PTM concentration in study locations. Maximum amount of total phenol (TP) concentration and flavonoids (TF) content in *A. alba* has been observed in sampling locations L4 and L8: TP: 44.27 (L4) and 49.44 mg GAE/g dry leaf (L8), TF: 28.91 (L4) and 32.48 mg Quercetin/g dry leaf (L8) as depicted in figure 5b. The similar trend was also evident in case of *E. agallocha*. Concentration of TP and TF were observed to gradually increase with the PTM content in both the mangroves, where significant positive association ($p < 0.01$) observed precisely with metals like Al, Cd, Cr, Fe, Pb and Zn (table 4). The results show their sensitivity towards these metals and also matched with studies by Lavid *et al.* (2001) and Guangqiu *et al.* (2007).

3.3.4.3 *Reducing ability and free radical scavenging activity:* Figure 5(c) represents reducing ability of mangroves ranging from 1.52 (L3) to 3.97 (L8) mg AAE/g dry leaf for *A. alba* and 1.44 (L3)–4.87 (L8) mg AAE/g dry leaf in *E. agallocha*. TP content expressed a significant positive correlation ($p < 0.001$) with reducing ability of respective mangroves. The highest reducing ability in mangrove leaves were observed in the same location (L8) having maximum phenolic content as also reported in the study by Banerjee *et al.* (2008). Table 4 represents the significant positive correlation ($p < 0.01$) of PTMs Fe and Zn in plants with their respective reducing ability. The free radical scavenging properties was assessed using the DPPH radical scavenging assay and expressed as 50% inhibition of DPPH radical (IC50). Interestingly, significant negative correlation was observed between free radical scavenging properties and corresponding reducing ability. *A. alba* and *E. agallocha* mangroves grown in location L6 were found to have lowest IC50 (0.27 and 0.23 mg/ml, respectively). Moreover, free radical scavenging activity of mangroves expressed significant correlation with PTMs like Cd, Cr, Mn and Pb.

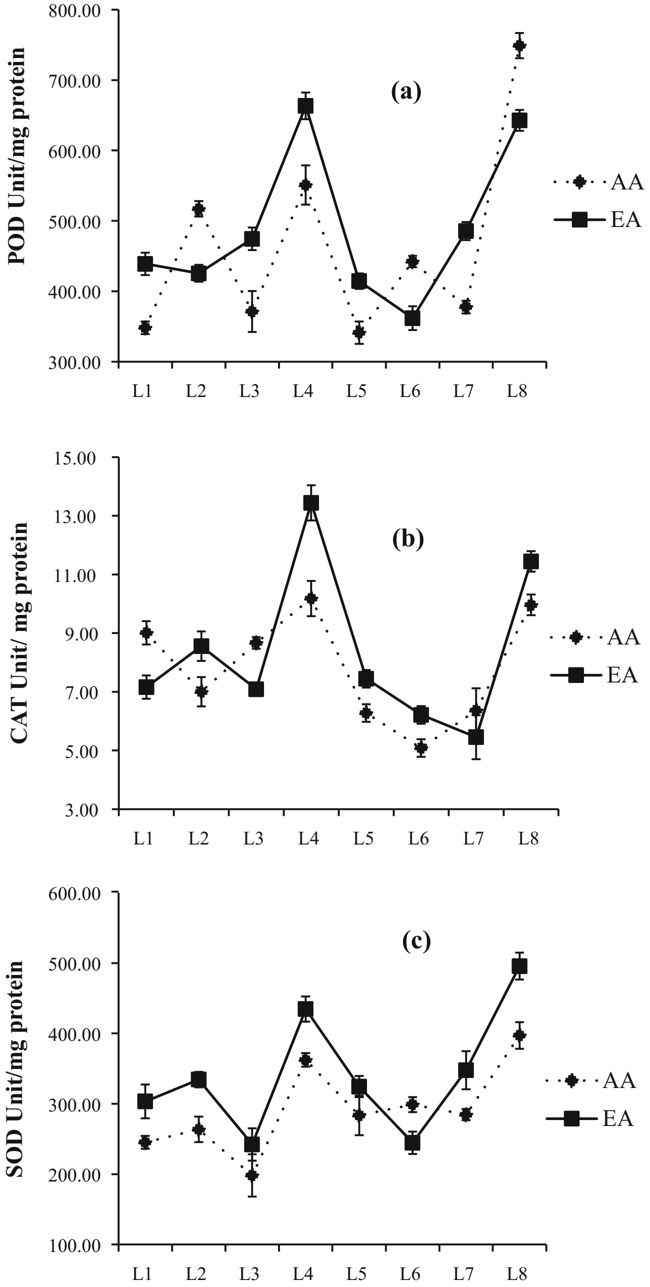


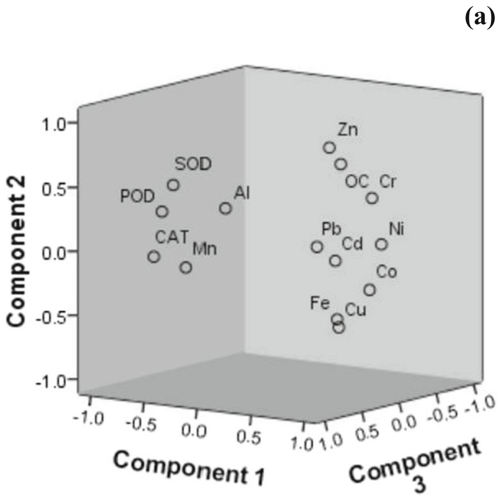
Figure 6. Enzymatic activity (**a**) peroxidase, (**b**) catalase, (**c**) super-oxide-dismutase of mangroves. AA: *A. alba*; EA: *E. agallocha*.

3.3.4.4 *Stress enzyme activity:* ROS (H_2O_2 and O_2^-) produced due to any environmental stress on plants can successfully be eradicated and modulated by antioxidant enzymes including peroxidase (POD), catalase (CAT), super-oxide dismutase (SOD) (Zhang *et al.* 2007; Doganlar and Atmaca 2011). Researchers have documented plants capable of producing excess level of ROS after experiencing toxicants and antioxidative system enabling active detoxification mechanism. Stress enzyme levels in *A. alba* and *E. agallocha* grown in eight different locations have been represented in figure 6. Guaiacol peroxidase showed varied concentration in leaves of both the species, the highest value was found at location L8 (748.89 POD unit/mg protein) and lowest value at location L1 (348.09 POD unit/protein) for *A. alba*, whereas the same was 663.33 POD unit/mg protein (L4) and 414.44 POD unit/mg protein (L5) for *E. agallocha* (figure 6a). Activation of CAT was much higher at L4 (10.18 CAT unit/mg protein) comparing with mangroves grown in other spots (figure 6b). Figure 6(c) represents the super-oxide dismutase values in leaves of *A. alba* and *E. agallocha* which was lowest at L3 (198.18 and 242.22 SOD unit/mg protein, respectively) and highest at L8 (397 and 495.56 SOD unit/mg protein, respectively). Significant positive correlation ($p < 0.01$) has been established between derived stress enzyme concentrations and PTMs like Al, Cd, Cr, Fe and Pb (table 4). The increased activity of POD, CAT and SOD were mostly observed in leaves sampled from location L4 and L8, where probability of being toxic was more than 49% as per mPELq classification. This emphasized on the formation of ROS in mangroves grown in these locations due to excess PTM stress level (Harish and Murugan 2011) as also evident from sediment quality analysis, higher PLI and ecological risk where Cd concentration was of more concern.

On being exposed to potentially toxic metals at higher concentration, plants are subjected to exclusion or efflux of metals into the xylem or intracellular accumulation and sequestration (Montargès-Pelletier *et al.* 2008). Metal homeostasis and/or metal-induced oxidative stress are significant functions for metal tolerance in plants in addition of efflux of metal ligands from root into xylem. Direct activation of reactive oxygen species (ROS) by Fenton and Haber–Weiss reactions, or indirect induction of oxidative stress, both can occur upon PTM pollution (Halliwell 2006; Bakshi *et al.* 2018). The photosynthesis and photorespiration processes also might get disturbed by organellar ROS generation due to metal exposure (Rodríguez-Serrano *et al.* 2009). PTM toxicity in plants interrupts the physiological and metabolic processes by inactivation and denaturation of enzymes, conformational modifications and disruption of membrane integrity, inhibition of photosynthesis, respiration, altered activities of several enzymes and disruption in redox homeostasis. Whereas PTMs lead to imbalance in redox homeostasis and oxidative damage, presence of certain elements like phosphorus can improve plant resistance and the ability to cope up with ambient environment by promoting growth and having synergistic or antagonistic effects on metal accumulation (Dai *et al.* 2017). This study clearly offers an insight to the variation in biochemical, antioxidantive activity and ecotoxicological response in two mangrove plants in and around Indian Sundarban, which could be correlated to the level of PTM stress. As very few researchers have documented such response in natural mangrove vegetation (MacFarlane 2002; Caregnato *et al.* 2008; Bakshi *et al.* 2018), this study shows the potential of using these ecotoxicological parameters as potential real time biomarkers to estimate PTM pollution in natural mangrove ecosystem.

3.4 *Statistical analysis*

All 10 PTMs in sediment and mangrove samples had statistical significance at 95% confidence



Component Matrix^a **(b)**

	Component		
	1	2	3
Al	-0.628	0.203	-0.152
Cd	0.792	0.014	0.399
Co	0.691	-0.310	-0.207
Cr	0.817	0.442	-0.060
Cu	0.745	-0.526	0.286
Fe	0.725	-0.466	0.278
Mn	-0.798	-0.239	0.123
Ni	0.951	0.102	0.007
Pb	0.751	0.147	0.587
Zn	0.397	0.783	-0.086
OC	0.654	0.711	0.128
POD	-0.875	0.222	0.334
CAT	-0.743	-0.075	0.632
SOD	-0.752	0.447	0.358

Extraction Method: Principal Component Analysis.
a. 3 components extracted.

Figure 7. Principle component analysis: (**a**) component plot of factors 1, 2, 3, and (**b**) component matrix.

interval as implied from one-way ANOVA. PTM distribution throughout the study region along with the ecotoxicological response against PTM stress in *A. alba* and *E. agallocha* showed wide variation (supplementary table S8). Factor analysis study has been performed in SPSS V16 to find out the interrelationship between major ecotoxicological parameters including organic content, metal concentration and stress enzymate activity (figure 7). Cumulative percentage of total variance explained as 55.869% for 1st component 72.219% for 2nd component and 82.297% for 3rd component.

4. Conclusions

Present work conducted in greater Sundarban area, has successfully compiled the ecotoxicological response in two mangrove species naturally grown in eight different locations contaminated with a varied level of potentially toxic metals sourced from both natural and anthropogenic origin. Here an inclusive approach to measure ecotoxicological parameters was followed considering distribution of potentially toxic metals in the sediment system, sediment quality status, ecological risk, bioconcentration in mangroves and their biochemical and antioxidative response. Location L4 and L8 with higher concentration of PTMs are typically characterized with higher enrichment factor, geo-accumulation index and contamination factor sourced from anthropogenic origin and natural weathering processes. The gradual deterioration in the estuarine health poses serious threat with reference to sediment quality guidelines and ecological risk category (Cd, Cu, Cr and Pb). The chlorophyll content, antioxidative activity and stress enzymatic response in both the species *A. alba* and *E. agallocha* having significant statistical correlation with elevated PTM concentration in the polluted study areas indicate towards the active detoxification capability of mangroves (Zhang *et al.* 2007). Elevated level of POD, CAT and SOD enzymes also reveal their efficient ROS scavenging activity. Study on increasing human interferences leading to toxic metal stress in sediment system and coping up mechanisms of mangroves will also help to frame effective prediction, inspection, assessment and management policies on this extremely sensitive region. Studies in natural and dynamic estuarine ecosystem facing constant and rapid changes might have many limitations. Crossing those barriers, our study explores the potential of biomonitoring PTM pollution in mangrove habitats exploiting ecotoxicological biomarkers.

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Author statement

Madhurima Bakshi: Data curation, formal analysis, investigation, validation, software, writing (original draft, review and editing). Somdeep Ghosh: Data curation, methodology, formal analysis, writing (review and editing). Shouvik Mahanty: Analysis and data curation. Tanushree Gaine: Analysis. Punarbasu Chaudhuri: Conceptualization and funding acquisition.

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2.6 TOLERANCE LIMITS FOR INLAND SURFACE WATERS SUBJECT TO POLLUTION IS : 2296 – 1982

Class A	**Drinking Water source without Conventional Treatment but after Disinfection.-** The quality of inland surface water under this category shall be such that it will be fit for human consumption without any treatment, except disinfection by approved methods. This classification is intended primarily for waters having water shed which are uninhabited and otherwise protected, which requires approved disinfection with additional treatment when necessary to remove naturally present impurities. This water is considered safe for drinking, culinary and food processing purposes.
Class B	**Outdoor Bathing.-** This water is useful for bathing. The water under proper sanitary supervision by the controlling authorities will meet accepted standards of water quality for outdoor bathing places and considered safe and satisfactory for bathing purposes.
Class C	**Drinking Water Source with Conventional Treatment Followed by Disinfection.-** This is a source of water supply for drinking, culinary and food processing purposes after it is subjected to approved treatment such as coagulation, sedimentation, filtration and disinfection, with additional treatment, if necessary, to remove naturally present impurities.
Class D	**Fish Culture and Wild Life Propagation.-** The water is fit for fish and wild life propagation.
Class E	**Irrigation, Industrial Cooling or Controlled Waste Disposal.-** This water is suitable for agriculture, industrial cooling or process water supply, fish survival etc. The waters without treatment, except for natural impurities which may be present therein, will be suitable for agricultural uses and will permit fish survival. The waters are also usable after special treatment by the users as may be needed under each particular circumstance for industrial purposes, including cooling and process water.

S.No.	**Characteristics**	**Tolerance Limit**				
		Class A	**Class B**	**Class C**	**Class D**	**Class E**
1	pH value	6.5 to 8.5	6.5 to 8.5	6.5 to 8.5	6.5 to 8.5	6.0 to 8.5
2	Dissolved oxygen, mg/L, *Min*	6	5	4	4	
3	Biochemical Oxygen Demand (5 days at 20°C), mg/L, *Max*	2	3	3		

4	Total coliform organisms, MPN/100ml, *Max*	50[†]	500[††]	5000[†††]		
5	Colour, Hazen units, *Max*	10	300	300		
6	Odour	Unobjectionable				
7	Taste	Tasteless				
8	Total dissolved solids, mg/L, *Max*	500		1500		2100
9	Total hardness (as $CaCO_3$), mg/L, *Max*	300				
10	Calcium hardness (as $CaCO_3$), mg/l, *Max*	200				
11	Magnesium (as $CaCO_3$), mg/L, *Max*	100				
12	Copper (as Cu), mg/L, *Max*	1.5		1.5		
13	Iron (as Fe), mg/L, *Max*	0.3		50		
14	Manganese (as Mn), mg/L, *Max*	0.5				
15	Chlorides (as Cl), mg/L, *Max*	250		600		600
16	Sulphates (as SO_4), mg/L, *Max*	400		400		1000
17	Nitrates (as NO_3), mg/L, *Max*	20		50		
18	Fluorides (as F), mg/L, *Max*	1.5	1.5	1.5		
19	Phenolic compounds (as C_6H_5OH), mg/L, *Max*	0.002	0.005	0.005		
20	Mercury (as Hg), mg/L, *Max*	0.001				
21	Cadmium (as Cd), mg/L, *Max*	0.01		0.01		
22	Selenium (as Se), mg/L, *Max*	0.01		0.05		
23	Arsenic (as As), mg/L, *Max*	0.05	0.2	0.2		

24	Cyanides (as CN), mg/L, *Max*	0.05	0.05	0.05		
25	Lead (as Pb), mg/L, *Max*	0.1	-	0.1		
26	Zinc (as Zn), g/L, *Max*	15	-	15		
27	Chromium (as $Cr^{6+)}$, mg/L, *Max*	0.05	0.05	0.05		
28	Anionic detergents, (as MBAS), mg/L, *Max*	0.2	1	1		
29	Polynuclear aromatic hydrocarbons (PAH), mg/L, *Max*	0.2				
30	Mineral oil, mg/L, *Max*	0.01				
31	Barium (as Ba), mg/L, *Max*	1				
32	Silver (as Ag), mg/L, *Max*	0.05				
33	Pesticides	Absent				
34	Alpha emitters, μc/ml, *Max*	10^{-9}	10^{-9}	10^{-9}	10^{-9}	10^{-9}
35	Beta emitters, μc/ml, *Max*	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}
36	Insecticides, mg/L, *Max*			Absent		
37	Oil and grease, mg/L, *Max*			0.1	0.1	
38	Free ammonia (as N) mg/L, *Max*				1.2	
39	Electrical conductance at 25°C, mhos, *Max*				1000 $x10^{-6}$	2250 x 10^{-6}
40	Free carbon dioxide (as CO_2) mg/L, *Max*				6	
41	Sodium absorption ratio *Max*					26
42	Boran (as B), mg/L, *Max*					2
43	Percent sodium					60

ANNEXURE - 5

WHO/SDE/WSH/03.04/11/Rev/1

Molybdenum in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

Rev/1: Revisions indicated with a vertical line in the left margin.

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Molybdenum in Drinking-water

Background document for development of WHO *Guidelines for Drinking-water Quality*

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Preface

One of the primary goals of the World Health Organization (WHO) and its Member States is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition will be published in 2011.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Japan, the United Kingdom and the United States of America (USA) prepared the documents for the fourth edition.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

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The current version of Molybdenum in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality*, is a revision of the background document prepared for the second edition of the Guidelines.

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Acronyms and abbreviations used in the text

DNA	deoxyribonucleic acid
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
USA	United States of America

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1. GENERAL DESCRIPTION

1.1 Physicochemical properties

The physicochemical properties of molybdenum are summarized below (Asmanguljan, 1965; Weast, 1986).

Property	*Value*
Melting point	2610 °C
Boiling point	5560 °C
Density	10.2 g/cm^3
Vapour pressure	0.133 kPa at 3102 °C
Water solubility	Insoluble

1.2 Organoleptic properties

Ammonium molybdate imparts a slightly astringent taste to water at concentrations above about 10 mg of molybdenum per litre (Asmanguljan, 1965).

1.3 Major uses

Molybdenum is used in the manufacture of special steels, in electrical contacts, spark plugs, X-ray tubes, filaments, screens and grids for radio valves, and in the production of tungsten, glass-to-metal seals, non-ferrous alloys and pigments. Molybdenum disulfide has unique properties as a lubricant additive. Molybdenum compounds are used in agriculture either for the direct treatment of seeds or in the formulation of fertilizers to prevent molybdenum deficiency (Climax Molybdenum Co., 1973; Stokinger, 1983; Weast, 1986).

1.4 Environmental fate

Molybdenum disulfide is sparingly soluble in water but is readily oxidized to give more soluble molybdates, which are stable in water in the absence of a reducing agent (Asmanguljan, 1965).

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

Human intake of airborne molybdenum is not likely to be a major exposure pathway (Chappell, 1973).

2.2 Water

Molybdenum was present in 32.7% of surface water samples from 15 major river basins in the United States of America (USA) at concentrations ranging from 2 to 1500 µg/l (mean 60 µg/l) (Kopp & Kroner, 1967; National Academy of Sciences, 1977). Levels in groundwater ranged from undetectable to 270 µg/l in another survey in the USA (Kehoe, Chalak & Largent, 1944).

In a survey of finished water supplies in the USA, concentrations ranged from undetectable to 68 µg/l (median 1.4 µg/l) (Durfor & Becker, 1964). In another survey of 380 finished water samples from across the USA, 29.9% contained measurable concentrations of molybdenum, with a mean of 85.9 µg/l and a range of 3–1024 µg/l (Kopp & Kroner, 1967). Similar results were obtained in the United Kingdom in an unpublished study that supported the Regional Heart Study (J. Fawell, personal communication, 2011). Subsequent, more modern studies, however, indicated much lower concentrations in tap water in the United Kingdom (Smedley et al., 2008) and in wells in Wisconsin, USA (Wisconsin Department of Health Services, 2010).

Levels of molybdenum in drinking-water do not usually exceed 10 µg/l (Greathouse & Osborne, 1980). However, in areas near molybdenum mining operations, the molybdenum concentration in finished water was reported to be as high as 200 µg/l. Tapwater concentrations as high as 580 µg/l have been reported in Colorado (Chappell, 1973). However, there appear to be no recent data to confirm these findings.

2.3 Food

Legumes, grains and organ meats are good food sources of molybdenum; fruits, root and stem vegetables, and muscle meats are relatively poor ones (Chappell et al., 1979; Tsongas et al., 1980).

2.4 Estimated total exposure and relative contribution of drinking-water

Molybdenum intakes in the USA range from 240 µg/day for adult men to 100 µg/day for women. Average intake is higher in those on low incomes (Tsongas et al., 1980; Pennington, Young & Wilson, 1989). In most areas, molybdenum intake via drinking-water will not exceed 20 µg/day (Greathouse & Osborne, 1980).

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

The rate of gastrointestinal absorption of molybdenum is influenced by its chemical form and the animal species. Hexavalent molybdenum is readily absorbed following oral administration, the amount absorbed being higher in non-ruminants than in ruminants (Fairhall et al., 1945; Miller et al., 1972; Kosarek, 1976). Tetravalent molybdenum is not readily absorbed (Fairhall et al., 1945). In humans, 30–70% of dietary molybdenum is absorbed from the gastrointestinal tract (Engel, Price & Miller, 1967; Robinson et al., 1973).

Following gastrointestinal absorption, molybdenum rapidly appears in the blood and most organs. Highest concentrations are found in the liver, kidneys and bones (Fairhall et al., 1945; Schroeder, Balassa & Tipton, 1970; Kosarek, 1976). Molybdenum crosses the placental barrier (Meinel et al., 1979). There is no apparent bioaccumulation of molybdenum in human tissues (Schroeder, Balassa & Tipton, 1970).

In rodents, molybdenum compounds are excreted largely in the urine and only to a small extent in faeces (Fairhall et al., 1945; Kosarek, 1976). In ponies, cattle and sheep, molybdenum excretion is generally divided between faeces and urine, owing to less complete gastrointestinal absorption (Miller et al., 1972; Cymbaluk et al., 1981; Kelleher et al., 1983). Molybdenum intake and excretion are balanced in most non-ruminant species, including humans (Schroeder, Balassa & Tipton, 1970).

4. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

4.1 Short-term exposure

Oral subchronic median lethal doses (LD_{50}s) for molybdenum(VI) oxide, calcium molybdate and ammonium molybdate in rats were 125, 101, and 330 mg of molybdenum per kilogram of body weight per day, respectively (Fairhall et al., 1945). Death occurred over a period of 8–232 days.

In animals, molybdenum interacts in a complex manner with copper and sulfate by a mechanism that is as yet unknown. Animals on copper-deficient diets are generally more susceptible to molybdenum toxicity than those on copper-adequate diets. Dietary sulfate protects non-ruminants against the symptoms of poisoning; if the animals are copper-deficient, however, it can intensify them (Gray & Daniel, 1964; Suttle, 1974).

In a study in which Holtzman rats (four per dose) were fed diets containing hydrogen molybdate at 75 or 300 mg/kg (7.5 or 30 mg of molybdenum per kilogram of body weight per day), molybdenum significantly inhibited growth and increased copper and molybdenum concentrations in liver. These effects were reduced or reversed by the addition of sulfate. An enlargement of the femorotibial joint and a thickening of the epiphysis of the femur and tibia were observed at both doses (Miller, Price & Engel, 1956). This study suggests a lowest-observed-adverse-effect level (LOAEL) of 7.5 mg of molybdenum per kilogram of body weight per day, based on body weight loss and bone deformities.

Three weanling guinea-pigs were fed a low-copper basal diet with dietary additions of 0, 200, 500, 1000 or 2000 mg of molybdenum (8, 20, 40 or 80 mg/kg of body weight per day) for 8 weeks (Arthur, 1965). An increase in molybdenum in the blood, liver and kidneys was observed with increasing dietary molybdenum levels. An increase in copper was observed in the blood and kidneys with increasing molybdenum intake; at the two highest doses, there was a decrease in liver copper concentrations.

Weanling Long-Evans rats receiving dietary sodium molybdate (50 or 80 mg of molybdenum per kilogram of body weight per day) over 5–8 weeks developed diarrhoea, whereas weight gain decreased and copper levels in the liver increased (Cox et al., 1960).

In ruminants, sulfate tends to increase the toxicity of molybdenum even in the absence of copper deficiency (Huber, Price & Engel, 1971; Suttle, 1974; Campbell et al., 1976). Molybdenum concentrations of 10 mg/kg of body weight in the ruminant diet resulted in tissue copper depletion, potentiated by dietary sulfate (Suttle, 1980).

A total of 12 male Holstein calves (three per group) received ammonium molybdate at 0, 1, 10 or 50 mg of molybdenum per litre (average daily doses of <0.01, 0.07, 0.7 or

3.7 mg of molybdenum per kilogram of body weight per day) in drinking-water for 21 days (Kincaid, 1980). No effects on growth were observed, but non-ceruloplasmin copper was significantly elevated and copper uptake from plasma into liver was less than the endogenous loss in calves receiving the highest dose. The author suggested that the minimum toxic concentration of molybdenum was between 10 and 50 mg/l, so that the no-observed-adverse-effect level (NOAEL) would be 0.07 mg/kg of body weight per day.

The effects of dietary molybdenum (1.7 g/day) were tested in four Holstein cows that were on low copper intake (Huber, Price & Engel, 1971). None of the animals showed overt signs of toxicity after 6 months. After the molybdenum intake was increased to 3.4 g/day (7 mg/kg of body weight per day), one cow developed severe diarrhoea and exhibited signs of lethargy, cessation of milk synthesis and general emaciation. When the molybdenum dose was increased to 5.1 g/day (10 mg/kg of body weight per day), two of three cows exhibited diarrhoea and emaciation. The addition of 0.26% sulfate greatly increased the severity of molybdenum toxicity. Dietary molybdenum increased the content of copper in the kidney and brain but decreased it in the liver. The kidney and spleen concentrated molybdenum to a greater degree than the liver or other organs.

4.2 Reproductive toxicity, embryotoxicity and teratogenicity

Five pairs of Charles River CD mice received 10 mg of molybdenum per litre (as molybdate) (about 1.5 mg of molybdenum per kilogram of body weight per day) in deionized drinking-water for up to 6 months (Schroeder & Mitchener, 1971). Excess fetal mortality was observed; there were 15 (of 238) dead pups in the F_1 generation and 7 (of 242) dead pups, five dead litters and one maternal death in the F_2 generation. The experiment was discontinued after the F_3 generation because of the elevated incidence of deaths of offspring and parents and infertility.

Four pregnant Cheviot ewes given diets supplemented with 50 mg of molybdenum per day (as ammonium molybdate) gave birth to four lambs, three of which exhibited ataxia (Mills & Fell, 1960). Histological examination revealed degenerative changes in the cytoarchitecture of the cerebral cortex and demyelination of the cortex and spinal cord, lesions similar to those described by other investigators as “swayback”.

The effects of dietary molybdenum on reproductive ability and pup growth during lactation were studied in Long-Evans rats fed diets containing 0.1, 2, 8 or 14 mg of molybdenum per kilogram of body weight per day and either 5 or 20 mg of copper per kilogram for 13 weeks (Jeter & Davis, 1954). The reduced number of litters at the two highest molybdenum concentrations was attributed to the apparent infertility of males in the groups concerned as a result of varying degrees of degeneration of the seminiferous tubules. Lactating mothers at the two highest doses lost less weight during lactation than females in the lower-dose groups, and there were indications that pups from mothers exposed to the highest dose of molybdenum gained less weight at weaning than other pups; these effects were probably due to reductions in milk production associated with high maternal dietary intake of molybdenum. The NOAEL was 2 mg/kg of body weight per day.

Molybdenum administered orally by capsule for 129 days to two male Holstein calves at doses between 4.1 and 7.8 mg/kg of body weight per day caused a gradual disappearance of the spermatogenic and interstitial tissue. The LOAEL was 4.1 mg/kg of body weight per day (Thomas & Moss, 1951). Female sheep fed a diet low in copper (1 mg/kg) and high in both molybdenum (25 mg/kg) and sulfate (0.53%) exhibited signs of reproductive failure (Suttle & Field, 1969).

4.3 Mutagenicity and related end-points

Ammonium molybdate was mutagenic in two of three *Escherichia coli* strains. Molybdenum(V) chloride was negative and ammonium molybdate strongly positive in the *Bacillus subtilis* rec-assay using deoxyribonucleic acid (DNA) repair-competent H17 and repair-deficient M45 strains (Nishioka, 1975). Ammonium and sodium molybdates were neither mutagenic nor recombinogenic in the *Saccharomyces cerevisiae* reverse mutation and gene conversion assays (Singh, 1983).

4.4 Carcinogenicity

Although a significantly increased incidence of lung adenomas was observed in strain A mice injected intraperitoneally with molybdenum(VI) oxide (Stoner et al., 1976), this study has no direct relevance to molybdenum intake via drinking-water. Studies suggest that molybdenum may act to prevent certain forms of cancer induced by *N*-nitroso compounds (e.g. oesophageal, forestomach and mammary gland cancer) in laboratory animals (Luo, Wei & Yang, 1983; Wei, Luo & Yang, 1985).

5. EFFECTS ON HUMANS

Molybdenum is considered to be an essential trace element in both animals and humans. Safe and adequate intake levels have been suggested for various segments of the population, namely 0.015–0.04 mg/day for infants, 0.025–0.15 mg/day for children aged 1–10 and 0.075–0.25 mg/day for all individuals above the age of 10 (National Academy of Sciences, 1989).

An infant with inborn deficiency of the molybdoenzymes sulfite oxidase and xanthine dehydrogenase exhibited abnormal distribution of urinary metabolites, neurological disorders, dislocated ocular lenses and failure to thrive (Johnson et al., 1980). A Crohn disease patient receiving total parenteral nutrition developed tachycardia, tachypnoea, severe headaches, night blindness, nausea, vomiting, central scotomas, generalized oedema, lethargy, disorientation and coma; these symptoms were attributed to dietary molybdenum deficiency resulting in impaired function of the two molybdoenzymes (Abumrad et al., 1981).

Urinary levels of molybdenum and copper and serum levels of uric acid and ceruloplasmin appeared to be affected by molybdenum levels in drinking-water over a 2-year period (Chappell et al., 1979). The low-molybdenum group consisted of 42 individuals from Denver, Colorado (USA), where the molybdenum concentration in drinking-water ranged from 1 to 50 µg/l. The high-molybdenum group consisted of 13 college students from Golden, Colorado, where the drinking-water molybdenum concentrations were equal to or greater than 200 µg/l. Plasma molybdenum levels were within the normal range among subjects in the low-molybdenum group, and no adverse health effects were observed in these subjects. Higher daily urinary molybdenum was associated with higher molybdenum intake: the mean urinary molybdenum for the Denver subjects was 87 µg/day compared with 187 µg/day for those from Golden. Higher mean serum ceruloplasmin (401 vs 30 mg/100 ml) and lower mean serum uric acid (4.4 vs 5.3 mg/100 ml) were also associated with the higher molybdenum intake. Because no adverse effects were seen in either group, this study suggested a NOAEL for molybdenum in drinking-water of 200 µg/l.

Evidence to support the suggestion that the molybdenum may have influenced serum ceruloplasmin was provided by a follow-up study of 13 students in Golden, Colorado, 2 years after the initial study. During this time, the average concentration of molybdenum in the Golden water supply decreased to 40 µg/l (Chappell et al., 1979). At this lower level of molybdenum in the drinking-water, serum molybdenum was nearly identical to the mean for the Denver residents. Serum ceruloplasmin was within the normal range of 20–35 µg/dl. Although serum uric acid values increased, this was believed to be the result of alcohol consumption. There were no significant differences in urinary copper values.

An epidemiological study involving 557 subjects in India indicated that a form of lower-limb osteoporosis may be associated with the high molybdenum content of the cereals consumed by the population (Krishnamachari & Krishnaswamy, 1974).

The results from a cross-sectional study of 400 persons in two settlements of a molybdenum-rich province of the former Soviet Union suggested that the high incidence (18–31%) of a gout-like disease was associated with high intake of molybdenum (10–15 mg/day). The disease was characterized by joint pains of the legs and hands, enlargement of the liver, disorders of the gastrointestinal tract, liver and kidney, increased blood levels of molybdenum and uric acid, increased xanthine oxidase activity, decreased blood levels of copper and increased urinary copper. An increased synthesis of the molybdoenzyme xanthine oxidase resulting from high dietary molybdenum levels was proposed as the mechanism for this disorder (Koval/skij, Jarovaja & Šmavonjan, 1961).

A cross-sectional study was conducted with 25 workers at a molybdenum smelter in Denver, Colorado, exposed to molybdenum in dust (predominantly molybdenum(VI) oxide and other soluble oxides). The calculated minimum daily body burden was 0.15 mg/kg of body weight per day. High levels of molybdenum were present in the blood of 15 workers (up to 300 µg/l) and in the urine of 12 of 14 workers (up to 11 mg/l) (Walravens et al., 1979). Mean serum ceruloplasmin and uric acid were higher for workers than controls. According to answers to medical questionnaires, six workers had upper respiratory infections in the 2 weeks prior to the questionnaire, and 15 reported joint pains, back pains, headaches, or skin or hair changes.

6. PRACTICAL CONSIDERATIONS

6.1 Analytical methods

Molybdenum can be determined by graphite furnace atomic absorption spectroscopy with a detection limit of 0.25 µg/l. Inductively coupled plasma atomic emission spectroscopy has a detection limit of 2 μg/l (American Public Health Association, 1989).

6.2 Treatment methods and performance

Molybdenum is not removed from drinking-water by normal treatment processes and appears to require specialist treatment such as ion exchange.

7. CONCLUSION

Molybdenum generally occurs at very low concentrations in drinking-water, and it is therefore not considered necessary to set a formal guideline value. For guidance purposes, a health-based value can be derived.

In a 2-year study of humans exposed via drinking-water, the NOAEL was found to be 0.2 mg/l (Chappell et al., 1979), but there are some concerns about the quality of this study. As molybdenum is an essential element, a factor of 3 was considered to be adequate to reflect intraspecies variation. This gives a health-based value of 0.07 mg/l (rounded figure), which is in the same range as that derived on the basis of the results of toxicological studies in animals and is consistent with the essential daily requirement for molybdenum.

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