



Potential of metal contamination to affect the food safety of seaweed (*Caulerpa* spp.) cultured in coastal ponds in Sulawesi, Indonesia

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ABSTRACT

This study evaluated metal concentrations in *Caulerpa* spp. cultured in 'traditional' coastal ponds in South Sulawesi and consumed locally as food. Although *Caulerpa* spp. are a rich source of supplemental dietary nutrients, like many macroalgal species they are also capable of bioaccumulating potentially toxic metals. We measured the metal concentrations of *Caulerpa* spp. from several locations in South Sulawesi to determine (1) whether cultivated *Caulerpa* spp. posed a potential risk to consumers, (2) whether *Caulerpa* spp. from cultivated ponds had different metal content that varied between localities and (3) whether there was any evidence for increased concentrations of heavy metals in *Caulerpa* spp. cultivated in ponds with known acid sulfate soils (ASS). Of the metals studied only As (0.7 mg kg⁻¹) and Pb (0.35 mg kg⁻¹) were recorded at concentrations approaching the national food safety (BSN) limits of Indonesia (1.0 and 0.5 mg kg⁻¹ respectively). Locality differences were observed between samples that could be explained by the background geography of sites. There was some indication that ASS conditions could lead to elevated levels of heavy metals. Consequently, we propose that the potential acidity of pond soil is considered when cultivating *Caulerpa*.

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1. Introduction

Seaweeds of the genus *Caulerpa* are used as food in many Southeast Asian countries including Indonesia, Malaysia, the Philippines, Singapore, Vietnam and Taiwan (Aguilar-Santos and Doty, 1968; Hong et al., 2007; Nagappan and Vairappan, 2014; Nguyen et al., 2011; Putra et al., 2013a; Trono, 1999) as well as in most Pacific Island countries (Morris et al., 2014; Paul et al., 2013; Pickering, 2006). Trono (1999) notes that *Caulerpa* may provide an important source of vitamins and minerals in local diets. The most commonly consumed species are *C. lentillifera* and *C. racemosa* (Aguilar-Santos and Doty, 1968; Anonymous, 2013). In the Philippines, *C. racemosa* is eaten as a salad vegetable, dipped in vinegar, or mixed with tomatoes, onions and vinegar (Aguilar-Santos and Doty, 1968). In some parts of Indonesia, *C. lentillifera* and *C. racemosa* are consumed fresh as a salad vegetable (Putra et al., 2013a).

Although *Caulerpa* is generally regarded as a nutritious food source, various authors have noted compositional differences between different sites and studies (e.g. Nguyen et al., 2011), and in some cases these differences can be attributed to seasonal differences in composition (Renaud and Luong-Van, 2006). In general, *Caulerpa* species have high moisture content (82–94%), are low in lipid (0.9–3.1% dry weight basis): reported protein content varies widely, from 3.6 up to 19.4% dry weight basis (Kumar et al., 2011; Kumari et al., 2010; Matanjun et al., 2009; Nagappan and Vairappan, 2014; Nguyen et al., 2011; Ratana-arporn and Chirapart, 2006). *Caulerpa* is high in several vitamins and minerals, including iron, calcium and magnesium and iodine (Kumar et al., 2011; Matanjun et al., 2009; Nagappan and Vairappan, 2014; Nguyen et al., 2011; Paul et al., 2013; Ratana-arporn and Chirapart, 2006). Other nutritional benefits from consumption of *Caulerpa* may arise from their relatively high levels of polyunsaturated fatty acids, vitamins and antioxidants (Kumar et al., 2011; Matanjun et al., 2009; Paul et al., 2013; Saito et al., 2010).

Caulerpa used as food is sourced both from the wild and from pond culture. *C. lentillifera* and *C. racemosa* typically grow on inshore coral reefs and reef flats, from which they are collected

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(Aguilar-Santos and Doty, 1968). Both species, but particularly *C. lentillifera*, are cultivated in high salinity coastal ponds in Australia (Mirosch, 2013), Indonesia (Putra et al., 2013a), Japan (Ito and Kamijo, 2012; Kudaka et al., 2008; Saito et al., 2010), the Philippines (Llana, 1991; Trono, 1999; Trono and Ganzon-Fortes, 1988), Taiwan (Nguyen et al., 2011), and Vietnam (Anonymous, 2013). In Indonesia *Caulerpa* is known as ‘lawi-lawi’ in Sulawesi and ‘latoh’ in Java (Putra et al., 2013a). Lawi-lawi cultured in ponds in South Sulawesi comprises at least three forms of *Caulerpa*: *C. lentillifera* (local name ‘bulaeng’), *C. racemosa* (local name ‘bu’ne’) and *C. racemosa* var. *laetivirens* (local name ‘lipang’). However, these three forms of *Caulerpa* can only accurately be identified to species level using molecular techniques (N. Paul, pers. comm. 2015) which were not available for this study. Because the three local forms are cultured, marketed and consumed together, and because of the difficulty in accurately identifying *Caulerpa* to species level, this study did not differentiate the three forms/species.

In addition to their use as food, *Caulerpa* species have been investigated as one option for removing nutrients in recirculation production systems and in shrimp ponds (Chaitanawisuti et al., 2011; Paul and de Nys, 2008; Ratana-arporn and Chirapart, 2006). Co-culturing shrimp with *Caulerpa* has been shown to improve pond water quality, presumably through the uptake of nitrogenous wastes, and also to reduce the number of bacteria adhering to shrimp gills and eyestalks (Hamano et al., 2006). In one experiment, *Caulerpa*-shrimp co-culture was associated with higher survival of shrimp affected by yellow-head disease (Tsutsui et al., 2012).

As part of a project to develop diversified production options for coastal aquaculture farmers in Indonesia (Putra et al., 2013b), we undertook *Caulerpa* culture trials with local farmers in Laikang, Takalar district, South Sulawesi, Indonesia (Putra et al., 2013a). These trials successfully demonstrated that *Caulerpa* could be grown in high salinity coastal ponds, and that profitability of *Caulerpa* culture was equal to, or greater than, shrimp culture (Putra et al., 2013a). Consequently, *Caulerpa* farming has been adopted by coastal pond farmers in Takalar district, who sell to local (South Sulawesi) wet markets (Putra et al., 2013a). The objective of the present study was to evaluate food safety aspects of cultured *Caulerpa*, with particular respect to metals content.

Indonesia has around 6.7 million hectares of soils categorised as acid sulfate (Klepper et al., 1990) and an estimated 35% of this area has been converted to ponds, particularly in coastal areas (Mustafa and Sammut, 2010a). Oxidation of pyrite in acid sulfate soils may cause severe acidification ($\text{pH} < 4.0$) which in turn increases the concentration of dissolved metals, including aluminium, iron and manganese (Mustafa and Sammut, 2010b; Sammut and Hanafi, 2002). Seaweeds generally are capable bioaccumulators of metals, including heavy metals (Paul et al., 2013) and macroalgae in coastal ponds have been shown to accumulate very high levels of metals, including toxic heavy metals, because of the increased bioavailability of these metals at low pH values (Gosavi et al., 2004). Recognising this capability, *Caulerpa* spp. have been investigated as a means of removing heavy metals from contaminated waters (Pavasant et al., 2006). This study quantified the levels of metals in the tissue of *Caulerpa* grown in coastal ponds in South Sulawesi, as well as comparative samples from coastal islands, to ensure that concentrations of metals in the seaweed met the required standards for human consumption.

2. Materials and methods

2.1. Study site

The ponds used in this study are ‘traditional’ coastal ponds, called ‘tambak’ in Indonesia. Traditional ponds were originally

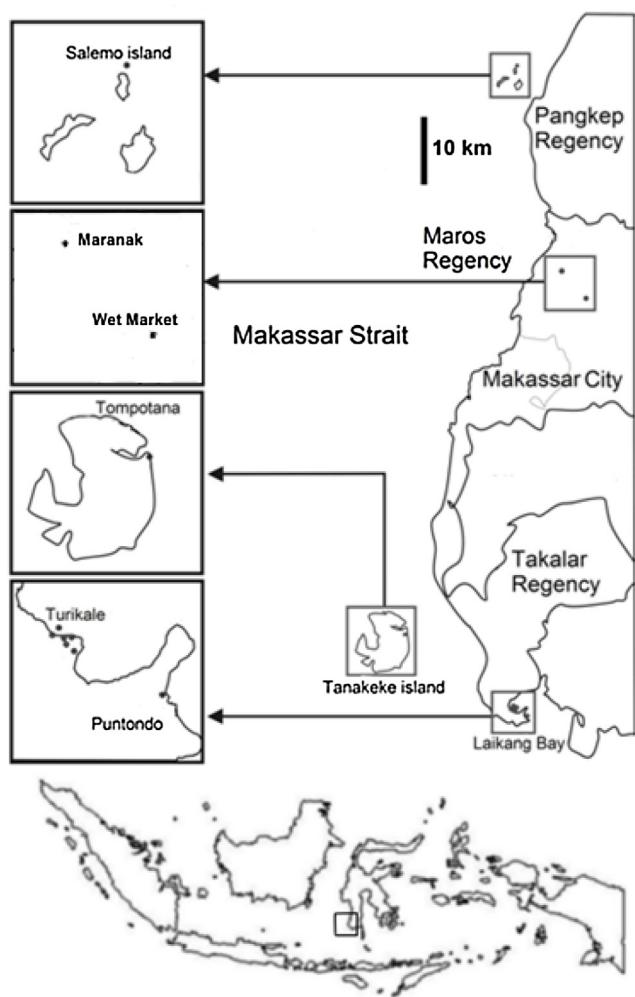


Fig. 1. Location of *Caulerpa* sampling sites. The square in the lower map indicates the regional location of South Sulawesi within the Indonesian archipelago. The scale bar indicates distance on the main map (right) while boxes on the left are enlargements of the sample locations.

developed in Java as early as the 15th century to culture milkfish *Chanos chanos*, but since the 1980’s most farmers have focussed more on shrimp production due to strong international demand and Indonesian government support for shrimp as an export commodity (Rimmer et al., 2013). ‘Traditional’ ponds are differentiated from ‘modern’ pond systems, with the latter based on technologies originating in Taiwan for more intensive monoculture of shrimp (Davies and Afshar, 1993; Muluk and Bailey, 1996). Typically, traditional pond systems use simple and low-cost technologies. Water is added to or released from the ponds based on tidal patterns, and pumps are not used. Little or no feed or artificial fertiliser is added to the ponds to increase productivity. Farm labour is largely household-based and unskilled. There are few interventions in pond management until the ponds are harvested (Davies and Afshar, 1993; Muluk and Bailey, 1996). In the case of the ponds used in this study, there was no treatment of the substrate at the time of construction, or subsequently, to reduce soil acidity.

For this study, samples of *Caulerpa* and sediment were collected from 6 coastal ponds in Laikang Village, Mangarabombang Subdistrict, Takalar Regency (Fig. 1) in July 2012. Comparative samples of *Caulerpa* were obtained from Laikang Bay adjacent to the culture pond site, from Salemo and Tanakeke Islands, and from the Maros city wet market (Table 1). These latter sites were selected because they regularly provide *Caulerpa* to wet markets in Makassar and

Table 1Sources of *Caulerpa* samples used in this study.

Site	Habitat	Habit	No. of <i>Caulerpa</i> samples	No. of subsamples analysed
Laikang	Pond	Cultured	5	12
Laikang	Pond	Cultured	5	12
Laikang	Pond	Cultured	5	11
Laikang	Pond	Cultured	5	5
Laikang	Pond	Cultured	5	6
Laikang	Pond	Cultured	5	5
Laikang	Laikang Bay	Wild	1	1
Maranak	Pond (transplanted)	Cultured	2	5
Salemo Island	Salemo Island	(unknown)	1	2
Tanakeke Island	Tanakeke Island	Wild	1	7
Maros wet market	(unknown)	(unknown)	1	2

surrounding areas for local consumption. Sediment samples were not taken from the bay and market sample sites.

An additional sample of *Caulerpa* was transferred from the Laikang ponds to a pond at the Research Institute for Coastal Aquaculture Maros (RICA Maros) Maranak Research Station (Fig. 1) to assess whether culture in known acid sulfate sediments affected the metals content of the seaweed. Approximately 1 kg of *Caulerpa* was divided and placed at two depths: on the pond bottom, and in plastic trays at 50 cm depth. Two separate introductions of *Caulerpa* were made to this pond because the first one quickly died. The second introduction was harvested after two weeks in the pond. Sediment was also sampled from this pond.

2.2. Pond sediment and water quality sampling

A hand coring device was used to extract sediment at 4 locations within each pond, at sites adjacent to stands of *Caulerpa* sp. *In-situ* measurements and sediment sampling were conducted on extracted cores at depths of 0–0.2 and 0.2–0.4 m. Sediment quality variables measured *in situ* were: pH_F (field pH) using a pH-meter, pH_{FOX} (field pH after oxidation with 30% H₂O₂) and redox potential (Ahern et al., 2004). Bulk sediment samples were combined to provide one sample for each pond.

Bulk sediment samples were stored in plastic bags and transported to the RICA Maros soil laboratory at Maros, South Sulawesi, on ice as recommended by Ahern et al. (2004). The soil samples were oven dried at 80–85 °C for 48 h, disaggregated in a porcelain mortar and pestle, and sieved through 2.0 and 0.5 mm hole sieves prior to analysis.

Sediment quality variables analysed in the laboratory included pH_{KCl} (pH of the KCl extract), pH_{OX} (pH of KCl extract after oxidation with H₂O₂), pyrite (Ahern et al., 1998b), organic carbon by Walkley and Black method, Kjedhal-N method, PO₄ by the Bray 1 method (Sulaeman et al., 2005), extractable Fe and Al with a Genesys 10S UV-vis spectrophotometer (Thermo Scientific, Massachusetts, USA) using the colorimetric methods described in Menon (1973), and texture (expressed as percent sand, silt and clay) by the hydrometer method (Agus et al., 2006).

Pond water quality measurement and pond water sampling was conducted at locations adjacent to the sites where *Caulerpa* and sediment samples were taken. Water quality variables measured *in situ* were: temperature, salinity, pH and dissolved oxygen, using a Hydrolab® Surveyor 4 (Hydrolab, Washington, USA) with a Min-iSonde water quality multiprobe.

Water samples were taken for analysis using acid-washed polypropylene sample bottles and preserved following American Public Health Association guidelines (APHA, 2005). Water quality variables analysed at the RICA Maros laboratory were: NH₃ (phenate method), NO₂ (Griess reaction), NO₃ (cadmium reduction method), PO₄ (ascorbic acid method), Fe (phenanthroline method), and total organic matter (TOM) following the methodologies of

Menon (1973), Grasshoff (1976), Parsons et al. (1984) and APHA (2005).

2.3. Seaweed metals analysis

All *Caulerpa* samples, comprising approximately 1 kg wet weight of seaweed, were rinsed in pond water, weighed and packed in individual plastic bags before being transported to the laboratory in ice-cooled styrofoam containers. Oven-dried (105 °C) and ground seaweed samples were further divided into subsamples for analysis. The *Caulerpa* samples were analysed for the total tissue metal concentrations of Al, As, B, Ba, Be, Ca, Cd, Cu, Cr, Fe, Hg, K, Mg, Mn, Na, Ni, Sb, Sn, Sr, Ti, V, Zn by the commercial testing laboratory PT. ALS Indonesia, Bogor, following standard methods. For analysis by ICP-AES, samples were acid digested using concentrated HNO₃ and H₂O₂. Following this initial digestion, samples were acid refluxed in HCl, filtered and diluted prior to analysis (USEPA Method 3050B). Samples were analysed by ICP-AES (ICP-AES Perkin Elmer Optima 5300V, Perkin Elmer, Massachusetts, USA) following the procedures described in USEPA Method 6010B. The method detection limit was 5 mg kg⁻¹.

Analysis for Mercury (Hg) was performed using a Perkin Elmer FIMS-400Flow-Injection Mercury-Atomic Absorption Spectrometer (FIM-AAS) according to Method AS3550 and 3112 Hg-B (APHA 2005). The method detection limit was 0.5 mg kg⁻¹.

Two methods of validation were used for these analyses. A certified reference material (Metals in Soil, ERA, Colorado, USA, Catalog# 540, Lot D077-540) was analysed in parallel with the samples: all analysed metals were within the range specified on the certificate of analysis. A spike equivalent to a final concentration of 10 mg kg⁻¹ of each analyte was added to a randomly selected sample. Recoveries for the spike ranged from 92 to 109% with a mean value of 103 ± 5%.

2.4. Statistical analysis of metals

The total tissue metal concentration data were screened to remove elements that were below the detection limits of the analyses. Principal component analysis (PCA) was used to simplify and explore patterns in the data set. PCA was performed using SPSS (v.19) using varimax rotation with Kaiser normalisation on the correlation matrix. Cases with values below the detection limit were filled using the average value for that metal. However, metals with fewer than 10 cases above the detection limit (As, Be, Cd, Hg, Sb, Se, Sn, and V) were excluded from the analysis.

Table 2

Soil quality parameters sampled from ponds used for commercial *Caulerpa* culture (Laikang) and experimental culture (Maranak), originally sampled from two depths (0–20 cm, and 20–40 cm). Data shown are mean \pm standard deviation.

Parameter	Laikang	Maranak
Redox potential (mV)	-270 ± 70	-166 ± 13
pH _F	7.1 ± 0.3	6.5 ± 0.1
pH _{FOX}	4.8 ± 2.0	1.8 ± 0.2
Δ pH (pH _F – pH _{FOX})	2.2 ± 2.0	4.8 ± 0.3
pH _{KCl}	7.8 ± 0.3	7.3 ± 0.1
pH _{Ox}	3.9 ± 2.1	3.5 ± 0.01
Pyrite (%)	0.9 ± 1.5	0.5 ± 0.1
Fe (ppm)	1800 ± 1700	2510 ± 9
Al (ppm)	5.6 ± 15	130 ± 13
Organic matter (%)	15 ± 7.6	7.4 ± 1.0
Total-N (%)	0.26 ± 0.13	0.06 ± 0.02
P ₂ O ₅ (ppm)	25 ± 11	40 ± 7
Sand (%)	67 ± 9.7	69 ± 1.4
Silt (%)	29 ± 9.3	31 ± 1.4
Clay (%)	4.2 ± 2.9	0

3. Results

3.1. Pond sediment and water quality parameters

Soil data from the culture ponds in Laikang and the experimental pond at Maranak are summarised in Table 2. Redox potential for soil samples taken from the Laikang and Maranak ponds were all negative (i.e. reducing), which is to be expected from submerged soils. Table 2 shows that the content of organic matter in the soil at both Maranak and Laikang is not classified as peat soil because the organic matter content was lower than 25%. The high sand fraction of the sediment at these two locations (67–70%) suggests a soil classification of sandy loam or sand.

Comparison of soil data from the Laikang ponds showed no significant difference between samples from 0–0.2 m depth and from 0.2–0.4 m depth for all variables ($P > 0.05$). Consequently, data for the two depths were combined for each site.

Field pH (pH_F) at all sites was in the approximate range of 6–7 pH units indicating low initial acidity. After oxidation with peroxide (pH_{FOX}), the field pH generally decreased. However, only 4 sites (3 at Laikang and 1 at Maranak) demonstrated a large change in pH between the initial field pH and the peroxide oxidised pH (pH_F – pH_{FOX}). This change in pH was seen at both of the tested depths at each of these sites. The KCl extracted laboratory pH levels (pH_{KCl}) also showed a low initial acidity with most sites having a pH_{KCl} between 7 and 8 pH units. Unlike the field results, laboratory results showed a much greater variation in pH levels after oxidation with many samples indicating pH levels of 3 or less. Comparison of the pH data with the measured organic carbon and iron concentrations showed that the samples that developed the lowest pH levels either had the highest organic carbon concentrations, higher iron concentrations or both. The two samples with the lowest pH_{KCl} levels also had the highest concentrations of pyrite.

Elevated pyrite levels are the main characteristic of acid sulfate soils. A slightly lower content of pyrite was found in the soil surface rather than at a depth of 0.2–0.4 m in Laikang. The content of pyrite in Maranak ponds were similar at both soil depths. The lower content of pyrite at the surface is probably a result of natural remediation processes that causes the oxidation of pyrite to be greater at the surface than deeper in the soil.

While there were some differences in the composition of the sediment from the two main sampling locations, the main physical and chemical parameters of the overlying water were similar (Table 3).

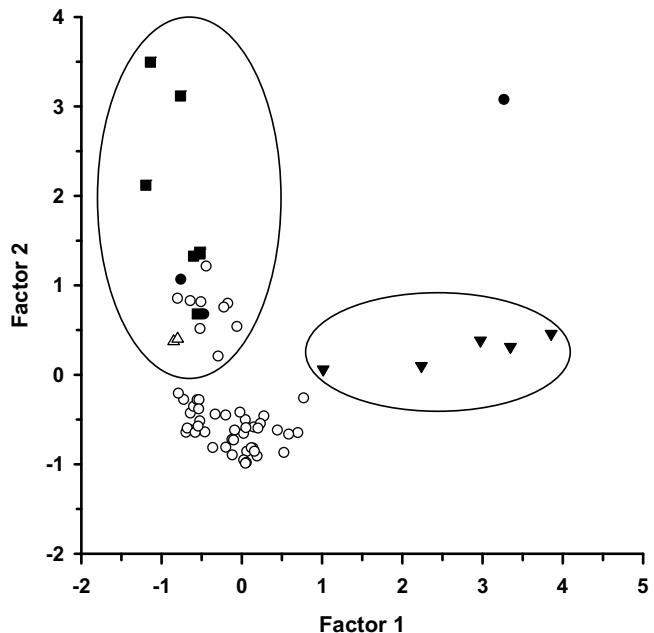


Fig. 2. PCA score plot for the first 2 factors derived from *Caulerpa* tissue metal concentrations.

Sites shown are ● – Maros City Market, ○ – Laikang Ponds, ▼ – Maranak Pond, △ – Salemo Island, ■ – Tanakeke Island.

3.2. Seaweed metals analysis

Concentrations of the toxic metals Cd, Hg, Pb and As were generally at or below the detection limits of the method and where not used in the subsequent PCA as only metals with 10 or more results were included for that analysis. Of these elements only two appeared in concentrations above the method detection limit. The maximum As concentration (from 3 results) was 22 mg kg^{-1} dry weight and the maximum Pb concentration (from 5 results) was 11 mg kg^{-1} dry weight. On a wet weight basis these dry weight concentrations were equivalent to approximately 0.70 mg kg^{-1} (As) and 0.35 mg kg^{-1} (Pb) respectively.

All site data that met the inclusion criteria were factored using PCA to explore any underlying patterns. The majority of samples formed a central group with two small clusters forming identifiable groups separate from the core group (see Fig. 2). Table 4 shows that three factors explained 76% of the total variation in the data. Table 5 shows that the PCA produced a relatively simple structure with elements strongly correlated (numbers shown in bold) with only one of the component factors. Component 1 is dominated by the transition metals (Fe, Ni, Mn, Zn, Cu) and Al. Component 2 is dominated by Ca with a lesser contribution from Sr, and Component 3 represents the univalent metals potassium and sodium. For comparison, Table 5 also shows the mean concentrations of the metals used in this analysis. Samples from Maranak (see ▼, Fig. 2) are clearly identifiable as the group with the highest loading on Component 1 corresponding to a greater contribution due to the presence of transition metals and heavy metals (e.g. Zn, Cu). Samples from Tanakeke Island (■) and Salemo Island (△), together with 2 samples from Maros Wet market (●) and 9 samples from Laikang (○) form a separate group from the main cluster along Component 2. This separation corresponds to increased concentrations of Ca along this axis (Table 5). With the exception of one isolated sample from the Maros wet market the remainder of the samples tended to cluster together. Examination of the raw data (not shown) showed that this isolated sample had concentrations of Mn and Fe an order of magnitude higher than all other samples.

Table 3

Water quality data from ponds used for commercial *Caulerpa* culture (Laikang) and experimental culture (Maranak).

Parameter	Laikang		Maranak	
	Average	Range	Average	Range
Temperature (°C)	31.7	28.7–35.0	29.0	27.7–30.3
Salinity	31.7	29–37	34.0	28–49
pH	7.9	7.4–8.4	7.3	7.0–7.9
Dissolved oxygen (mg L ⁻¹)	5.0	3.1–7.0	3.8	3.0–4.4

Table 4

Rotated Variance Matrix using Varimax Rotation with Kaiser Normalisation. The solution converged in 4 iterations.

Component	Eigenvalue	% Variance	Cumulative%
1	6.2	41.5	41.5
2	3.2	21.5	63.0
3	2.0	13.2	76.2

4. Discussion

This study was primarily concerned with the food safety aspects of *Caulerpa* cultured in coastal ponds. The appropriate limits for heavy metals in 'fruits, vegetables, seaweed and cereals' in Indonesia are set out in the National Standards Agency (Badan Standardisasi Nasional – BSN) document ICS 67.220.20 'Batas maksimum cemaran logam berat dalam pangan' ('Maximum limits for heavy metals contamination in food'). These maxima, plus those obtained for the present study for the same metals, are listed in Table 6. The Codex Standard for contaminants and toxins in food and feed (FAO/WHO, 1995) has no specific limits for seaweed, so for comparison we have used limits for 'leafy vegetables' and tinned food products (Table 6).

For three of the heavy metals that are covered in the Indonesian (BSN) limits (Cd, Hg and Sn) concentrations found in this study were below the limits of detection (Table 6). It is possible that our results for Hg understate the actual concentration due to the potential for volatilization during the drying procedure. However volatilization losses under the conditions we employed have been estimated to be no more than 10% and this should not affect the interpretation of our results (Hojdová et al., 2015).

Only As and Pb were detected in isolated samples above the method detection limit (Table 6). The national limit allowed in food stipulated by Indonesian law is 1 mg kg⁻¹ for As and 0.5 mg kg⁻¹ for Pb. On a wet weight basis no samples exceeded these limits (Table 6). International limits for Pb are lower: 0.3 mg.kg⁻¹ for 'leafy vegetables' (FAO/WHO, 1995), but only 3 sub-samples slightly exceeded this Pb limit (0.32–0.35 mg kg⁻¹).

However, our results indicate that there is potential for toxic metals to accumulate to higher levels than were found in this study, if *Caulerpa* is cultivated in strongly acidic sulfate soils. The dominant acidic ion in soils is aluminium (Boyd, 1998) and aluminium levels were much higher at the Maranak site than at Laikang (Table 2). In addition the Maranak site had higher levels of iron than Laikang but pyrite (FeS₂) levels at the Maranak site were lower than at the Laikang site (Table 2). These observations may be associated with the higher levels of organic matter found in the Laikang soil samples, which reduces iron (Fe₃⁺ to Fe₂⁺) and other substances via microbial metabolism (Boyd, 1998). While the data in Table 2 suggest some acidity in the soil at Laikang, the pH was not low enough to be of concern using established assessment criteria (Ahern et al., 1998a). The pH after oxidation of soil samples (pH_{FOX}) indicates that the Maranak site already has potential acid sulfate soil conditions but the Laikang site does not using the criteria described by Dent (1986). The overall potential for the field sites that we sampled that are currently producing *Caulerpa* to develop acid sulfate soil symptoms is correspondingly low.

How sites were related by the metal content of the *Caulerpa* is demonstrated in Table 5 and Fig. 2. There is clear separation of the samples taken from Laikang and transplanted and cultivated at the Maranak research station. As outlined above, several of the sediment samples from the Maranak site could be classified as acid sulfate. The results suggest that *Caulerpa* cultivated in waters overlying potentially acid sulfate affected sediments will absorb metals that have been mobilised by the lower pH reducing conditions found in these sediments. This is indicated in Fig. 2 by the displacement of these samples to the right along Factor 1 compared to the main cluster of samples. The results in Table 5 show that while there is a strong correlation with all of the metals composing Factor 1, Al, Fe and Mn had tissue concentrations 2–3 orders of magnitude greater, possibly reflecting the relative concentrations of these metals in the sediment. Table 2 only has limited data for Al and Fe, but these laboratory extractions show that these metals were potentially available to *Caulerpa* under suitable conditions in the field. Increases in the porewater concentrations of these metals

Table 5

Rotated Component Matrix and mean concentration for metals found in *Caulerpa* from all sites used in the PCA Numbers in bold show correlations greater than 0.6.

Metal	Component			Mean concentration (mg kg ⁻¹)	Standard deviation
	1	2	3		
Fe	0.957	-0.026	0.041	2800	1970
Ni	0.931	0.198	0.105	8.9	4.9
Mn	0.905	0.244	0.166	2226	6827
Zn	0.900	0.094	-0.040	12.7	6.2
Al	0.875	-0.001	0.017	2200	1150
Ba	0.763	-0.161	0.121	28.7	8.8
B	0.665	-0.145	0.263	40.1	9.8
Cu	0.641	0.297	0.074	14.6	11.5
Ca	-0.051	0.919	0.062	15700	12200
Sr	-0.055	0.910	0.088	230	209
Ti	0.473	0.722	0.101	20.4	18.0
Cr	0.539	0.691	-0.051	8.7	3.6
Mg	0.278	-0.510	0.435	14800	4450
Na	0.132	-0.043	0.953	133000	28500
K	0.037	0.204	0.853	7030	1440

Table 6

National (BSN ICS 67.220.20) and international (Codex standard 193-1995) food safety limits for As, Cd, Hg and Pb in seaweed or vegetable products, the maxima found in this study for the same metals and limit of resolution (LOR) for the respective analyses. Dry weight data from this study converted to wet weight based on a conversion factor of 3.2% dry weight for *Caulerpa*.

Metal	BSN limit (mg kg ⁻¹)	Codex limit (mg kg ⁻¹)	Maximum this study (mg kg ⁻¹)	LOR (mg kg ⁻¹)
Arsenic (As)	1.0 ¹	NA	0.7	0.16
Cadmium (Cd)	0.2 ¹	0.2 ³	0 (ND)	0.16
Mercury (Hg)	0.03 ¹	NA	0 (ND)	0.016
Lead (Pb)	0.5 ¹	0.3 ³	0.35	0.16
Tin (Sn)	40 ²	250 ⁴	0 (ND)	0.16

Notes:

BSN limit for 'fruits, vegetables, seaweed and cereals'.

BSN limit for 'food product (milk, baby food)'.

Codex limit for leafy vegetables.

Codex limit for canned products.

is an indicator of acid sulfate soil conditions, generally signalling an increase in acidity and the release of other potentially toxic metals which then become more available for bio accumulation.

Another clear trend of secondary importance is indicated by the separation of samples on Factor 2. This appears to be driven mostly by the tissue concentration of Ca as it has the strongest correlation with this axis and the largest concentration. The interesting trend here is that this separation appears to correlate with the degree of oceanic influence, or conversely the closeness of samples to dry land/soils. Tanakeke Island is approximately 10 km from the coast of the south-west peninsula of Sulawesi island and Salemo Island is 5 km from the coast to the north of Makassar (Fig. 1). Included in this cluster are 9 samples (marked ○) from the Laikang samples, and these were all collected from Puntondo located at the mouth of Laikang Bay (Fig. 1). The bulk of the samples in the main cluster were collected in the more enclosed and sheltered waters of Laikang Bay.

In South Sulawesi, *Caulerpa* is mostly consumed as a side dish. Questioning of local villagers indicated that the normal consumption is less than 500 g wet weight per household per day, although no accurate measurements of consumption have been carried out. *Caulerpa* may not be eaten every day unless it is purchased daily, because the quality of the seaweed deteriorates rapidly due to bacterial action, even with refrigeration (Kudaka et al., 2008). Based on this presumed pattern of consumption, the relatively low levels of heavy metals found in this study, consumption of *Caulerpa* from ponds in South Sulawesi does not pose a human health hazard. However, *Caulerpa* cultured in strongly acid soils may accumulate higher levels of heavy metals. Consequently, we propose that the potential acidity of pond soil is considered when cultivating *Caulerpa*.

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