Antioxidant and antibacterial activity of solid-liquid and enzyme-assisted extraction of phenolic compound from three species of tropical *Sargassum*

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Antioxidant and antibacterial activity of solid-liquid and enzyme-assisted extraction of phenolic compound from three species of tropical *Sargassum*

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Abstract. *Sargassum* has been well acknowledged for the potential natural product of its phlorotannins. Solid-liquid extraction (SLE) is the most common method used to extract them. However, this method has some drawbacks such as low yield and toxic. An alternative ecofriendly method has been proposed, i.e. enzyme-assisted extraction (EAE), proven to be more efficient. The aim is to compare the efficiency of SLE and EAE concerning their extraction yield, total phenolic content and antioxidant activity. *S. aquifolium*, *S. ilicifolium* and *S. polycystum* were extracted using water, methanol, methanol 50%, and ethanol 75% and enzymes (Viscozyme and Protamex). Total phenolic content (TPC) was analyzed by Folin-Ciocalteu and antioxidant activity via DPPH and FRAP analysis. This study implied that bioactivity of *Sargassum* extracted with enzymes is better compared to the one using organic solvents.

Keywords: Conventional extraction, alternative extraction, brown algae, bioactivities, polyphenols

1. Introduction

As an archipelagic country located in the tropical zone and the coral triangle, Indonesia has been extensively known for its remarkable diversity of marine organisms. One of them is marine seaweed. There are three groups of marine seaweed that can be found throughout Indonesian water, namely, green, red and brown seaweed. For decades, the green and red seaweed, particularly from the genera *Kappaphycus* and *Gracillaria*, have been cultivated for their carrageenan and agar [1–3].

Marine seaweed is also distinguished by its polysaccharides [4,5] but also by its biologically active compounds that have been studied and proven to have promising potential for the nutraceutical, pharmaceutical and cosmeceutical industries [6–10]. Hence, these marine plants have attracted a lot attention during the exploration of alternative natural products. The natural products derived from seaweed offer a sustainable resource with an infinite application.

Among the other three, brown seaweed from the class of Phaeophyceae is one of marine plants that has not been fully explored and exploited in Indonesia despite of their abundance and their remarkable potential [11–17]. A number of previous studies have reported that polysaccharides and bioactive compounds from the brown seaweed showed an encouraging potency as source of natural product with

their antimicrobial [18], antioxidant[11,19], antifouling [20,21], antiviral [22,23], anti-proliferative [24–26], anti-inflammatory [27], tyrosinase inhibition [28] activities and many other. This potency can be further applied in foods, medicines and cosmetics in order to reduce the application of chemical products or even to fully substitute them in the future [29–33].

There are five genera of brown seaweed widely distributed in Indonesian waters which four of them are the most species-rich, namely *Dictyota*, *Padina*, *Turbinaria* and *Sargassum* with 111 species in total [34]. These four genera have not been optimally exploited; however, *Sargassum* is the most extensively studied among them concerning its alginates and bioactive compound especially the phenolic compounds also known as phlorotannins. The phlorotannin in which are mostly present in the brown seaweed [35,36] are known for its strong antioxidant activity [37–43]. Nevertheless, it also shows promising effects against cancer, allergy, diabetes, inflammation and viral and microbial infection [36]. Basically, this compound is produced as one of the components composing the cell wall of brown seaweed [35]. However, it also serves as an induced defense system as phlorotannin has plastic responses to the environmental factors for examples nutrient availability, light, ultraviolet radiation, temperature, salinity and the intensity of herbivory [44–47].

Due to the various health beneficial performed by phlorotannin, various extractants are used in order to release this compound from the algal matrix. Solid-liquid extraction (SLE) is one of the most used methods in extracting the phlorotannin of brown seaweed [48–53]. This method relies on the organic solvents during the process such as hexane, ethyl acetate, ethanol, methanol, etc. [52]. The polysaccharides' complexity of marine seaweed often becomes the hindrance during the application of SLE. Thus, it reduces the extraction efficiency causing to the low yield of extraction with only 8% to 30% of the dry material [54]. It is crucial to apply an extraction method that could enhance the target compound, improve the bioactivity, time-saving, and favorable for human and environmental. These are the necessary requirements in order to generate a 'Green concepts' as in recent trend [55].

Enzyme-assisted extraction (EAE) has been widely mentioned as a potential alternative method since it offers an eco-friendly approach, an improvement in the quantity of target compound and an enhancement in the bioactivity [56]. The advance point of EAE compared to SLE method lies on its ability to degrade or disrupt the cell walls and membranes facilitating the release of target compound [57]. The EAE method has shown to improve the extraction yields and to enhance the recovery of bioactive compound in marine seaweed [56,58–62]. Nevertheless, this method has not been extensively applied in extracting the bioactive compound from Indonesian *Sargassum.*

Consequently, the objective of this study is to evaluate the efficiency of EAE in comparison to the SLE method in extracting bioactive compound of *Sargassum* sp., particularly phenolic compounds. Later, the phenolic content and the antioxidant activity *of* Sargassum extracts from both methods will be evaluated. In additional, tyrosinase and biofilm inhibition activity of the EAE extracts will also be analyzed in order to acknowledge another possible potential from this extracts. Finally, the enzymatic extracts of *Sargassum* will be characterized using the Fourier-Transform Infra-Red (FTIR) spectroscopy. This characterization will serve as a preliminary analysis to detect the presence of the phenolic compound, phlorotannins.

2. Material and Methods

2.1 Seaweed Collection

There were three different species of *Sargassum* collected for this study. *S. polycystum* was collected from Panjang Island, Jepara in October 2013 then *S. aquifolium* and *S.ilicifollium* were collected from Telukawur, Jepara in April 2014. Both of the locations are located in the northern region of Central Java, Indonesia.

The apical and median parts of *Sargassum* thalli were cut leaving the basal part still attached to the substrate for further regeneration, rinsed with seawater and placed in closed boxes. Samples were then transferred to the Central Laboratory of Research and Services Diponegoro University (CORES-DU). They were washed with tap water to remove the remaining epiphytes and other residual sands and

naturally dried away from the sunlight for seven days. After all samples had dried, they were all grinded and stored in a sealed plastic bag that was covered to avoid direct contact with the sunlight.

2.2 Solid-liquid extraction (SLE) of Sargassum

25 gr of algal dry material was diluted in 300 ml of four different solvents, i.e. water, methanol 50 % (v/v), pure methanol and ethanol 75 % (v/v). The filtered samples were then evaporated until dryness. The dried extract obtained was then added by 20 ml of H_2O in order to get the crude extract. Finally, the crude extracts were frozen and lyophilized for further analysis.

2.3 Enzyme-assisted extraction (EAE) of Sargassum

19.5 gram of dry material was diluted in 300 ml of water, then 5 % (of dry material) commercial enzymes were added. Viscozyme® – a carbohydrase, and Protamex® – a protease, were chosen. These enzymes were provided by Novozymes®, manufactured in Denmark. Extraction without enzyme served as contro, also called the aqueous extraction.

Sample solutions were incubated for 3 hours in 40 °C. After the filtration, the soluble samples were then frozen and lyophilized for further analysis. The residual materials were stored in -20 °C for TPC analysis to determine the insoluble phenolic compound that might not be digested by the enzymes.

2.4 Total Phenolic Content Analysis

Folin-Ciocalteu is a commonly used method for measuring the phenolic content. This assay is based on the reduction-oxidation (redox) reactions, which are usually considered to be relatively stoichiometric and on the redox potential of the phenolic hydroxyl group [63].

0.5 ml of samples or standard (Phloroglucinol) was added with 0.5 ml ethanol 95 %. 2.5 ml H_2O was then introduced to the samples or standard solution followed by 0.25 ml of Folin-Ciocalteu 50 %. All samples were agitated and left to stand for 5 minutes. In the end, 0.5 ml of 5 % of Na_2CO_3 (in 100 ml H_2O) was added. Then the optical density was read against the blank prepared at 725 nm. Phloroglucinol was a standard for the calibration curve prepared in different concentrations ranged from 0 – 100 µg/ml [64–66].

2.5 Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl or DPPH is a stable free radical. DPPH assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. DPPH show a strong absorption maximum at 517 nm. This analysis is based on modified method of Yen and Chen (1995) and Chen et al. (2008).

A series of BHA, BHT and Ascorbic Acid solution – serves as standard – in different concentration is prepared (2 – 50 µg/ml). 0.25 mM of DPPH solution is introduced to 100 µL samples solution. Samples solution of *S. muticum* hydrolysates was made in different concentration by diluting the solution stock in methanol (0 – 1000 µg/mL). Before reading the optical density at 517 nm, all samples are incubated in 40 °C for 30 minutes. Then, the percentage of inhibition is calculated by following formula:

$$\boldsymbol{I}\ (\%) = \left[{(\boldsymbol{A_C} - \boldsymbol{A_S})}/{\boldsymbol{A_C}}\right] \times \mathbf{100}$$

$\boldsymbol{I}$ (%) = Inhibition (express in %)
$\boldsymbol{A_C}$ = Absorbance of control
$\boldsymbol{A_S}$ = Absorbance of samples

IC_{50} of samples is determined based on the regression obtained from dose response curve. IC is defined at the concentration sufficient to obtain 50 % of a maximum scavenging capacity.

2.6 FRAP (Ferric Reducing Antioxidant Power)

This method is based on the reduction of a ferroin analogue, the complex of tripyridyltriazine $Fe(TPTZ)^{3+}$, to the intensely bluish colour complex of $Fe(TPTZ)^{2+}$ by the presence of antioxidants in

acidic medium. Then, the results are obtained from the absorbance at 593 nm and expressed as µm Fe^{2+} equivalents (Benzie & Strain 1996; 1999)

The reagent of TPTZ consisted of 300 mM of acetate buffer with pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) and 20 mM $FeCl_3{}_6H_2O$ solution. 50 µL of *S. muticum* hydrolysates was introduced in microplate mixed with 150 µL reagent. After 15 minutes of incubation, the absorbance was read at 593 nm. The results were expressed in µM equivalent to $FeSO_4.{}_7H_2O$ calculated from the calibration curve [69].

2.7 Antibacterial Activity

Agar diffusion method [70] was chosen as method for evaluating the antibacterial activity of *Sargassum*. One gram positive bacteria – *Bacillus subtilis* – and two gram negative bacteria – *Pseudomonas aeruginosa* and *Escherichia coli* – were chosen for this test. These bacteria were prepared in liquid nutrient broth media with bacterial density 1.5 x 10^8 cfu/ml. Bacterial suspension was poured on to solidified agar media and incubated for 1 h in 37 °C.

5 mg/ml of SL extracts and hydrolysates were prepared in sterile physiological water. 20 µL of tested samples solution – hydrolysates solution or positive control (Phosphomycin, Ampicillin and Streptomycin) – was introduced to sterile paper disc. All samples were made in triplicate. Later, the impregnated sterile paper discs were placed on the agar media in accordance with samples coding made in advance. The petri dishes were then incubated at 37 °C for 48 h. Zone of inhibition established on the agar media was then measured and expressed in cm.

2.8 Statistical Analysis

All results are expressed as mean ± standard deviation (SD) with n = 3. As the design of this research was a factorial design with more than one independent variable than two ways analysis of variance (ANOVA) was chosen as statistical analysis using IBM SPSS Statistics 20. Further, the statistical difference between samples were determined via Tukey Post Hoc Test with significance level at 5% (p<0.05).

3. Results and Discussion

3.1 Comparison of SLE and EAE Extraction Yield, Total Phenolic Content, Antioxidant and Antibacterial activity of Sargassum

Two-ways analysis of variance was applied to determine whether there was a significant effect or not from several independent factors towards the dependant variable. In the case of *Sargassum* yield of extraction, total phenolic content and antioxidant activity – all were the dependant variables, different species and different type of extractants served as independent factors that would influence the dependant variables.

Statistical analysis of *Sargassum* extraction yield showed that there was a significant different between the three species extracted, i.e *S. aquifolium, S. ilicifolium* and *S. polycystum*. Different type of extractants used also gave a significant effect to the extraction yield. This result implied that the extraction yield of *Sargassum* showed an inter-individually significant effect.

As seen in Table 1, the EAE yielded higher dry material compared to the SLE. The order of samples with highest yield of enzymatic extraction was *S. polycystum* > *S. aquifolium* > *S. ilicifolium*. The highest extraction yield was shown by *S. polycystum* extracted with Protamex®, 38.1 ± 6.8 % of dry material.

Table 1. Extraction yield of *Sargassum* (% of dry material) in different method of extraction (mean ± standard deviation, n=3).

Samples	Water	MeOH	MeOH 50%	EtOH 75%	Viscozyme®	Protamex®
S. aquifolium	17.3 ± 2.2*	5.7 ± 1.8*	15.8 ± 1.5*	12.3 ± 0.4*	29.5 ± 5.4*	26.2 ± 5.8*
S. ilicifolium	14.7 ± 0.8*	3.1 ± 0.7*	7.3 ± 1.0*	7.5 ± 1.3*	27.9 ± 1.4*	21.4 ± 2.0*
S. polycystum	22.8 ± 1.1*	8.3 ± 3.2*	13.4 ± 1.9*	12.5 ± 1.9*	35.7 ± 2.6*	38.1 ± 6.8*

*Significantly different at $p<0.05$.

The total phenolic content of *Sargassum* is presented in Table 2 expressed in % of dry material. Viscozyme® extracted more phenolic content in *S. aquifolium* and *S. polycstum* with 7.0 ± 1.3 and 8.1 ± 2.6 % of dry material, respectively, compared to the organic solvents. In the contrary, the highest phenolic content of *S. ilicifolium* was obtained from the methanol 7.5 ± 2.4 % of dry material. Apparently, the efficiency between Protamex and methanol 50% (v/v) in extracting the phenolic compound were the same in all *Sargassum*. As can be seen on Table 2, they only extracted between 3 up to 5 % of phenolic content.

The antiradical activity expressed in the value of IC_{50} of *Sargassum* is presented in Table 3. *S. polycystum* only showed its antiradical activity when they were extracted with ethanol 75% and Protamex® and the best activity was shown by Protamex® with IC_{50} 1.9 ± 1.1 mg/mL. The weakest antiradical activity came from the methanolic extract of *S. aquifolium* with IC_{50} 23.8 ± 9.0 mg/mL. The three *Sargassum* extracted with Protamex® gave a better antiradical activity compared to the other extractants, it can be seen by their IC_{50} that were lower as presented on Table 3. The 75% (v/v) ethanolic extracts of *S. aquifolium* and *S. ilicifolium* showed no antiradical activity. Meanwhile, the antiradical activity of all samples were still weaker compared to the standards, i.e BHA and BHT with IC_{50} $8x10^{-3}$ ± 0.5 and 12 $x10^{-3}$ ± 4.8 mg/ml, respectively. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant that affected the antiradical activity. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant used. Based on its phenolic content, the order of samples with highest content was *S. polycystum* > *S. aquifolium* > *S. ilicifolium.* In addition, the efficiency order of extractants in extracting the phenolic compounds of *S. polycystum* was Viscozyme® > ethanol 75% (v/v) > Protamex® > methanol > methanol 50% (v/v) > water. As for *S. aquifolium*, the efficiency order was Viscozyme® > methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex® > water. Then, for *S. ilicifolium*, the efficiency order was methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex® > Viscozyme® > water.

Table 4 presents the reducing power activity also known as FRAP of *Sargassum* expressed by a concentration in µM equivalent with Fe^{2+} (µM Eq Fe^{2+}). Vitamin C was used as standard with reducing power 58.1 ± 0.7 µM Eq Fe^{2+}. The reducing power activity that was almost near as the standard was the aqueous phase of *S. polycystum* with 43.5 ± 0.7 µM Eq Fe^{2+}. In other word, this sample had the highest reducing power activity. Meanwhile, the lowest reducing power activity was shown by *S. ilicifolium* extracted with ethanol 75%, 7.9 ± 1.2 µM Eq Fe^{2+}. In addition, it appears that Viscozyme® extracts of *S. aquifolium* and *S. polycystum* had better reducing power activity compared to methanol, methanol 50%, ethanol 75% (v/v) and even Protamex®. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant that affected the reducing power activity.

As for the antibacterial activity of two methods of extraction, the tested bacteria were sensitive only to solid-liquid extraction (data not shown). In other words, the enzymatic extracts showed no sign of inhibition against the bacterial growth. It was assumed that the sugar contained in our enzymatic extracts might provoke the growth of bacteria instead of inhibiting them.

SLE has become the most used method during the exploration of marine bioactive compounds, in this case, phlorotannins. This method mostly relies on the polarity of the solvents used due to the

chemical nature of target compound [88]. In general, the process in SLE method can be categorized in three parts; (1) changing phase of the solute, in this case the target compounds, as it dissolve in the solvent, (2) its diffusion through the solvent in the pores of the solid to the outside of the particles, and (3) the transfer of the solute from the solution in contact with the particles to the main bulk of the solution [89]. The more dispersed the solute in the solid material, the more difficult the extraction will be and the extraction rate will fall. This is because the solvent will have to penetrate further in to the layer of solid material in order to reach the solute [89]. Furthermore, the efficiency of SLE highly depends on the particles size, temperature, and agitation during the process [90]. In the extraction of phenolic compounds, the storage time of dry materials and conditions, as well as the presence of interfering substances are other important factor to consider, influencing the extraction efficiency [91]. In marine seaweed, the main drawback during the extraction of phenolic compounds occurs due to the presence of complexes polysaccharides as the main component of seaweed's cell wall. Brown seaweed's phlorotannins are known to be incorporated in its cell wall [92] covalently bonded to the polysaccharides [35] and proteins [93]. Therefore, it requires a strong condition to degrade these bonds and extract the phlorotannins. The SLE method does not meet this requirement since it does not degrade such complex bonds since it works based on the solubility of target compound in to the solvents [89]. In addition, the polarity of target compound becomes the most important factor in choosing the solvent [94]. Methanol, ethanol, acetone, ethyl acetate, hexane [50,72,75,95,96] are some examples of organic solvents mostly used for phlorotannins extraction.

Phlorotannins, class of phenolic compounds synthesized only in brown seaweed [71], is one of the most studied bioactive compounds due to their wide range of bioactivities, showing a promising potential for the nutraceutical, pharmaceutical and cosmeceutical industries. As reported by previous studies, the increasing interest towards phlorotannins occurs as they have been for their antioxidant [72–77], antimicrobial [78–80], antitumor [81,82], antidiabetic [83], antiallergic [84], anti-inflammatory [85], and antiviral effects [51,86,87].

This study concerns about the possibility in applying alternative method that is not only environmentally friendly but also more efficient in extracting the phlorotannins. For that reason, enzyme-assisted extraction (EAE) is chosen as the alternative method. Additionally, the efficacy of SLE – as the conventional method, and EAE – as the alternative one, in extracting the phlorotannin from *Sargassum* were compared. For the SLE method, four different organic solvents were chosen, i.e water, methanol, methanol 50% (v/v), and ethanol 75% (v/v). Meanwhile, for the EAE method, there were two commercial enzymes, i.e. Viscozyme® – a carbohydrase, and Protamex® – a protease.

Based on the extraction yield of these two methods, the EAE yielded the dry algal material higher compared with the SLE (Table 1). As can be seen from this table, in three hours of extraction, the SLE extraction yield ranged from 3% to 15% of dry material. In the contrary to the SLE method, the extraction yield of EAE ranged from 21% to 38%. In overall, the average increase of extraction yield when using the Viscozyme® was around 62%. As for the Protamex®, the average increase of extraction was 58%. This result had shown the efficacy of EAE method in improving the extraction yield applied under the same circumstances as the SLE method. Interestingly, the efficiency of EAE in improving the extraction yield seems to be species-dependent. As can be seen on Table 1, Protamex® yielded more dry material in *S. polycystum* with 38% than Viscozyme®. In the contrary, the extraction yield of *S. aquifolium* and *S. ilicifolium* were higher in Viscozyme®, with 29% and27%, respectively. Viscozyme® was also yielded more soluble material in *S. fulvellum* (24%), *S. horneri* (30%), *S. correanum* (30%) and *S. thunbergii* (31%) than Protamex, with 17%, 27%, 21%, and 25%, respectively [97]. In *S. muticum*, the Viscozyme managed to extract 29% of soluble material [41,98]. It appears that the cellulose and the multicarbohydrase complex action of Viscozyme® were responsible for higher solid content in lyophilized extracts [98].

Table 2. Total phenolic content (TPC) of *Sargassum* (% of dry material) (mean ± standard deviation, n=3).

Samples	Water	Methanol	Methanol 50 %	Ethanol 75%	Viscozyme®	Protamex®
S. aquifolium	1.7 ± 0.0*	6.3 ± 1.5*	3.7 ± 0.2*	5.9 ± 0.4*	7.0 ± 1.3*	3.5 ± 0.0*
S. ilicifolium	2.1 ± 0.0*	7.5 ± 2.4*	5.3 ± 0.3*	5.9 ± 0.4*	4.0 ± 0.1*	4.2 ± 0.2*
S. polycystum	1.4 ± 0.0*	4.8 ± 0.3*	4.0 ± 0.3*	5.8 ± 0.6*	8.1 ± 2.6*	5.2 ± 0.1*

*Significantly different at $p<0.05$.

Table 3. Antioxidant activity of *Sargassum* expressed in IC50 (mg/ml) (mean ± standard deviation, n=3).

Samples	DPPH (IC 50 mg/ml)					
	Water	Methanol	Methanol 50%	Ethanol 75%	Viscozyme®	Protamex®
S. aquifolium	14.6 ± 5.7	23.8 ± 9.0*	22.3 ± 8.0*	n.a	n.a	5.7 ± 7.9
S. ilicifolium	n.a	17.8 ± 9.4*	3.3 ± 1.7*	n.a	6.2 ± 3.4	2.9 ± 1.7
S. polycystum	n.a	n.a	n.a	5.2 ± 5.9	n.a	1.9 ± 1.1

*Significantly different at $p<0.05$.

Table 4. Reducing activity of *Sargassum* expressed in µM Equivalent Fe^{2+} (mean ± standard deviation, n=3).

Samples	FRAP (Concentration Equivalent Fe^{2+} (µM))					
	Water	Methanol	Methanol 50%	Ethanol 75%	Viscozyme®	Protamex®
S. aquifolium	14.9 ± 1.7*	12.6 ± 2.5*	22.4 ± 4.9*	14.4 ± 0.3*	33.2 ± 2.5*	17.0 ± 0.7*
S. ilicifolium	23.7 ± 1.6*	11.1 ± 0.8*	14.9 ± 0.8*	7.9 ± 1.24*	13.4 ± 2.2*	11.7 ± 1.2*
S. polycystum	43.5 ± 1.8*	12.8 ± 3.1*	16.9 ± 2.6*	13.8 ± 1.9*	27.7 ± 4.9*	15.9 ± 3.0*

*Significantly different at $p<0.05$.

he EAE had also recovered more of phlorotannins represented by the total phenolic content. By using Viscozyme, the total phenolic content of *Sargassum* was 28% higher than using the organic solvents. Unfortunately, the phenolic content of Protamex® extracts of *Sargassum* were not in consistent with the Viscozyme® extracts. The Protamex did not seem to recover the phenolic content of *Sargassum* as they yielded lower content compared to the organic solvents, except for water. It implied that the Viscozyme® recovered more of phenolic compounds in *Sargassum* than the Protamex®. Phlorotannins are hydrophilic cell-wall-bound compounds [99] and despite of its soluble characteristic, compounds attached to the cell wall are not easily extracted using the typical extraction methods such as the SLE [56,61]. The application of Viscozyme®, as a carbohydrase, during the extraction allowed to facilitate the recovery of phenolic compound as this enzyme degrades the cell wall of seaweed mainly composed of polysaccharides. Viscozyme® has been extensively used for the recovery of phenolic compounds of terrestrial and marine plants as reported by many studies [41,65,100–102]. However, the efficiency of extraction relies on the optimum conditions applied. Selecting the appropriate hydrolytic enzymes is important to digest specific polymer bonds present in seaweed cell wall followed by the selection of suitable process conditions for maximum recovery of active compounds [56]. Combination of incubation time-temperature, pH, size of the interested molecules and agitation are mentioned to play a critical role during the process [14].

Phlorotannins have been reported to be responsible for many bioactivities, such as antioxidant, antimicrobial, antiviral and many more, as reported by previous studies [9,29,51,96]. The key of phlorotannins important bioactivities lies to its eight interconnected ring as they contain more hydroxyl groups than other tannins – hydrolysable tannin and condensed tannins [107,108]. As a consequence, they have greater antioxidant activity [107]. Hydrogen atom transfer is one of its mechanisms against the effects of unregaluted production of free radicals [36]. As performed in this study, *Sargassum* extracts – extracted with SLE and EAE method- showed their capability in scavenging the free radicals with best activity exhibited by the Protamex® extracts in spite of Viscozyme® extracted more phenolics. It suggested the presence of other molecules like polysaccharides or proteins as shown by the FTIR since the current study used crude extracts instead of the purified. Besides free radical scavenging, our results showed that the enzymatic extracts of *Sargassum* had the ferric reducing ability – a conversion of Fe^{III} to Fe^{II}. In addition, it showed quite promising potential as skin whitening antifouling coating. Such potential was based on the tyrosinase and biofilm inhibition activities of *Sargassum* enzymatic extracts.

Antioxidant activity of *S. horneri, S. fullvelum, S. correanum,* and *S. thunbergii* have been reported by Heo et al [65]. *S. correanum* contained higher phenolics when it was extracted with Viscozyme® and higher free radical scavenging activity compared to the others. Another study also showed that Viscozyme® used to extract *S. horneri* had the ability to scavenge the free radicals until 95% of inhibition [109]. Sanchez-camargo et al. [41] stated that the total phenols and antioxidant activity of *S. muticum* extracted with Alcalase® and Viscozyme® were improved compared to control – aqueous extraction. Therefore, it is obvious that enzymes used have facilitated the release of active compounds and in the same time it enhances the bioactivities especially the antioxidant – best known activity for phlorotannins.

4. Conclusion

A strong urge to explore marine bioactive compounds has brought green chemistry concept to the surface along the process. The principle of this concept is basically to apply a method that is environmentally friendly and favorable for human interest as well. In the exploration of marine bioactive compounds that can be further applied for the nutraceutical, pharmaceutical and cosmeceutical industries, the realization of this concept is crucial. Therefore, many studies have highlighted the possibilities of enzyme-assisted extraction as an alternative method that might replace the use of organic solvent to extract the active compounds.

This study has revealed that by using enzymes, the efficiency of the extraction increased compared to the solid-liquid extraction. This method not only improves the quality and quantity of extracts but it

also shows its promising potential by showing diverse bioactivities. Nevertheless, one should note that this study still works with crude extracts. Therefore, the interference of other compounds like polysaccharides and proteins should be considered. As a consequence, it is crucial to further evaluate the bioactivities of purified samples and characterize them.

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Understanding coastal processes to assist with coastal erosion management in Darwin Harbour, Northern Territory, Australia

Understanding coastal processes to assist with coastal erosion management in Darwin Harbour, Northern Territory, Australia

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Abstract. Sand transport pathways in Darwin Harbour, Northern Territory, Australia, are being investigated to assist with coastal management. Coastal erosion, which threatens public and private infrastructure, is one of the major problems along the harbour beaches. A study of sediment transport is essential to identify the challenges encountered by the stakeholders in coastal management. Darwin Harbour, located in the tropical, cyclone prone area of Australia, was, until recently, considered a near pristine estuary. A semi-diurnal macro-tidal embayment, the tidal variation in the harbour reaches up to 8 m with a mean tidal range of 3.7 m. The beach morphology consists of sandy pocket beaches between coastal cliffs, sandbars, rocky shore platforms, tidal flats and mangrove fringes. A two-dimensional depth averaged finite-element hydrodynamic model (RMA-2), coupled with a sediment transport model (RMA-11) from Resource Modelling Associates, has been used to infer the sources and the depositional areas of sand in the harbour. Grain size distributions and geochemical analysis are also used to characterize the sand and its source(s). Initial results show that the beach sand is mostly of offshore origin with small sand input from the rivers. Potential supplementary sand sources are the eroded materials from the shore platforms and the rocky cliffs. Due to the rapid development in Darwin Harbour, this study is fundamental in understanding coastal processes to support decision making in coastal management, particularly in a macro-tidal, tropical estuary.

Keywords: Darwin Harbour, macro-tidal, sand transport, coastal erosion, RMA

1. Introduction

Coastal erosion is a natural phenomenon. In fact, coast lines change continually, controlled by the interaction of the local hydrodynamics and their morphology. Coastal change is a longstanding problem that mankind has had to deal with to provide safety from flooding and to protect transportation infrastructure. Conventionally, coastal erosion is managed locally using hard engineering approaches, such as sea walls or breakwaters, which do not guarantee good outcomes and often create erosion in other areas [1, 2]. These consequences often stem from engineering decisions that only consider the immediately affected area, underestimating the processes that are occurring in the wider coastal zone.

The coastal zone is composed of diverse complex environments, shaped by coastal processes, coastal geology, variations in coastline characteristics and coastal sediment budgets [3]. A more technically and environmentally satisfactory coastal engineering design should include each element and its interaction, incorporating how they affect the whole system [1]. Coastal processes relate to physical processes such as tides, waves, currents and winds that act upon and shape the coastline, while coastal geology determines the origin, structure and characteristics of the sediments that make up the coastal region. Considering that coastal erosion essentially indicates an imbalance in the sediment supply and removal in the area, an ideal way to deal with the problem is to study the complete cycle of sedimentation in a theoretically confined coastal area called a sediment/coastal cell [3]. The boundaries of a sediment cell can be marked by features such as headlands, submarine canyons or river mouths, where the sources, transport paths and sinks of sediment occur.

The sources of coastal sediment can be examined by means of provenance indicators using mineralogical or geochemical properties. Among a number of suitable indicators of provenance, carbonate minerals and rare earth elements (REEs) are often used. In coastal sediments, carbonate mineral constituents, such as calcite and aragonite, can be used to distinguish whether a sediment is of marine or of terrestrial origin [4]. REEs are excellent in determining sediment sources because the ratios of individual REE are not easily fractionated during transport and show consistent behaviour during weathering [5].

This study examines the coastal processes in Darwin Harbour, a macrotidal estuary situated in cyclone prone tropical northern Australia. The semi-diurnal tides record the highest astronomical tide at 8 m and the smallest low tide at 0.3 m with a mean range of 3.7 m [6, 7]. A large embayment, Darwin Harbour covers the area from Charles Point in the west to Gunn Point in the east with Blackmore, Elizabeth and Howard Rivers, the major streams that flow into the harbour. Presently coastal cells have not been defined in Darwin Harbour, therefore this study was carried out within two prominent headlands of the harbour i.e. Charles Point in the west and Lee Point in the east (Figure 1).

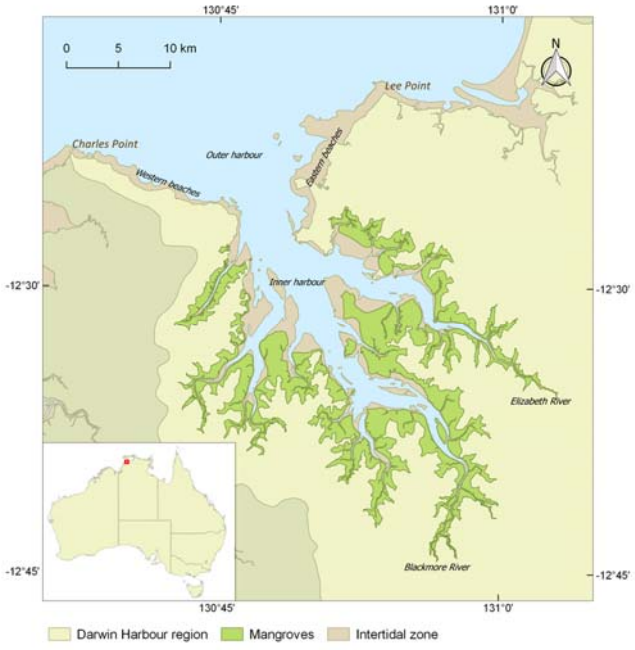

Figure 1. Darwin Harbour, the study area.

The western part of Darwin Harbour consists of mainly rocky shore platforms interspersed by sandy pocket beaches. The eastern part of Darwin Harbour comprises longer stretches of sandy beach and less extensive rock flats. The beaches are backed by either coastal cliffs or sand dunes. Mangrove forests border the intertidal areas of the inner harbour and to a lesser extent on the west and east beach areas.

Similar to other areas in the world, coastal problems encountered in Darwin Harbour arise from mixed uses of the area and conflicting concerns among the stakeholders. Previous coastal erosion studies mainly focused on the eastern part of the harbour. The studies documented coastal cliff erosion rates averaging 30 cm y^{-1} [8], while the sandy beaches experience seasonal changes in both climatic and oceanographic events [9]. Visual observation at several sites and anecdotal information indicates that the eastern beach dunes and the western beaches have also experienced substantial erosion in recent decades. Sand dynamic studies were carried out on relatively small areas in the harbour for specific purposes, e.g. shipping channels development work. Despite long term beach erosion, no study on sand dynamics, incorporating coastal processes, has been carried out for Darwin Harbour. This study is an attempt to make a start at filling this gap, and aims to contribute to understanding the role of coastal processes occurring in the area, i.e. to determine the sand pathways in the harbour, to infer the sources of beach sand, and thereby assist with coastal management in Darwin Harbour. Two potential sand sources are investigated: the inflow from offshore and the contribution of Elizabeth and Blackmore Rivers, while the potential sand sinks are the western and eastern beaches of the harbour.

2. Methods

A 2-D depth-averaged hydrodynamic (RMA-2) and sand transport modelling (RMA-11) software package from Resource Modelling Associates [10, 11] was used to simulate the hydrodynamics of the study area and to infer the sources and sinks of sand in Darwin Harbour. The simulations were run on the calibrated and validated Darwin Harbour modelling-mesh constructed by the Australian Institute of Marine Science (AIMS) based on 2012 bathymetry. Numerous surveys of tidal currents profiling by AIMS have confirmed that a 2D modelling method is valid for Darwin Harbour hydrodynamic simulations, i.e. the vertical profiles of currents are of similar magnitude and direction during the tidal cycle. In multiple AIMS projects it was also proven that the computation of bed shear gives similar values compared to a 3D model.

The model mesh consists of 10,227 elements and 21,219 nodes. The cell sizes range from 20 m^2 at the wharf area to 3,000 m^2 at the offshore boundary. The mesh was divided into three element types, each assigned with different bed roughness in Manning's 'n' values, i.e.: 1) Submerged/water area, 'n' = 0.030; 2) Mangrove area, 'n' = 0.100; and 3) Intertidal area, 'n' = 0.025. The model was run for a 12-month period, from May 2012 to April 2013, covering both the dry and the wet seasons. Tide forces and river inflow were used to run the model with a 15-minute time step.

The results from the RMA-2 hydrodynamic simulations were used as the input to simulate sand transport pathways in the harbour with RMA-11. The fine, medium and coarse sand transport were simulated using the sand transport potential method based on Van Rijn's 1984 computation, which is most suitable for sand with diameter > 0.100 mm (fine sand size and greater). The size distribution of sand used in the simulations was determined from terrestrial and marine samples from the study area. Sub samples were also taken for geochemical analysis.

In order to simulate the transport pathways in the harbour, 5 mg L^{-1} of sand was introduced from the network boundaries with no initial bed thickness in the harbour. The sand transport pathways were inferred by the bed level changes in the modelling area. Positive bed change indicates sand deposition, which shows the sand sink areas, therefore, any positive bed change in the model domain can be inferred as the sand direction from the source to the sink area. In order to distinguish between offshore and terrestrial sources area, offshore and river sand simulations were run separately. River sand simulations were carried out from Elizabeth and Blackmore Rivers (Figure. 1).

The sediment transport pathways were also inferred using the Calcium Carbonate concentration and the REE composition of the sand samples. The $CaCO_3$ concentration was determined using cold acid digestion, while REE compositions were determined using a semi-quantitative ICP-MS method [5].

3. Results and Discussion

The sand transport pathways analysis is discussed based on the beach areas depicted in Figure 2.

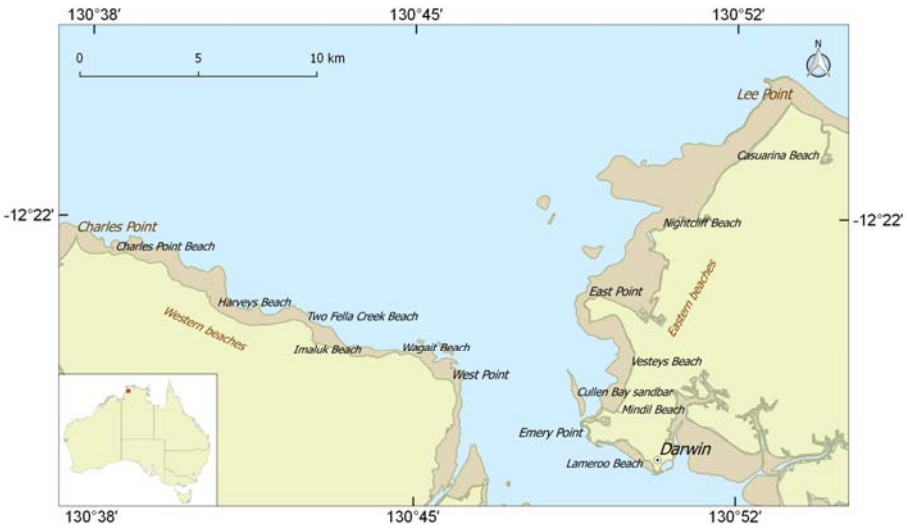

Figure 2. Beach area under study in Darwin Harbour.

3.1. Sand pathways from offshore

The simulation results showed that offshore sand deposited predominantly on the northern parts of the western and eastern beaches and decreased further into the harbour (Figure 3). The extent of deposition was governed by beach morphology, more deposition occurred in embayment areas, particularly on the lee side of the headlands due to the sheltering effect of the headlands [12]. This trend was very apparent in the western beach areas, on the lee side of Charles Point (node 99). The deposition level at this point was significantly higher compared to other beach areas. Apart from the deposition at this node, the long term deposition of offshore sand at the western and eastern beaches showed an analogous trend (Figure 4), inferring similar pathway patterns of offshore sand to the beach area.

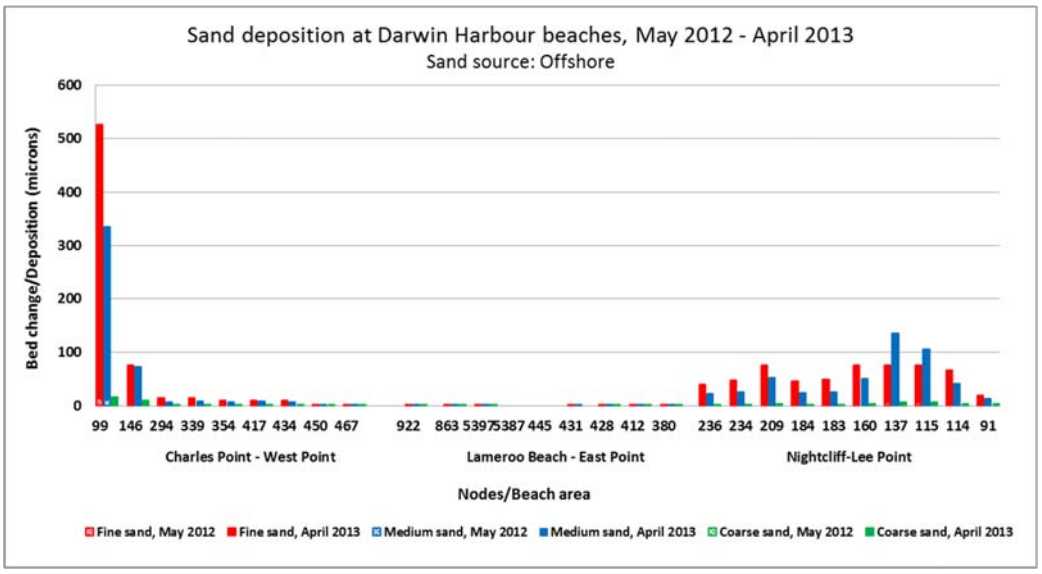

Figure 3. Offshore sand deposition on Darwin Harbour beaches.

As indicated in Figure 4, offshore sand deposition at the beaches in the eastern part of the harbour, between Lameroo Beach and East Point, was substantially lower than at the northern beaches, suggesting that East Point headland, Cullen Bay sandbar and Emery Point prevent the offshore sand from being transported to the area.

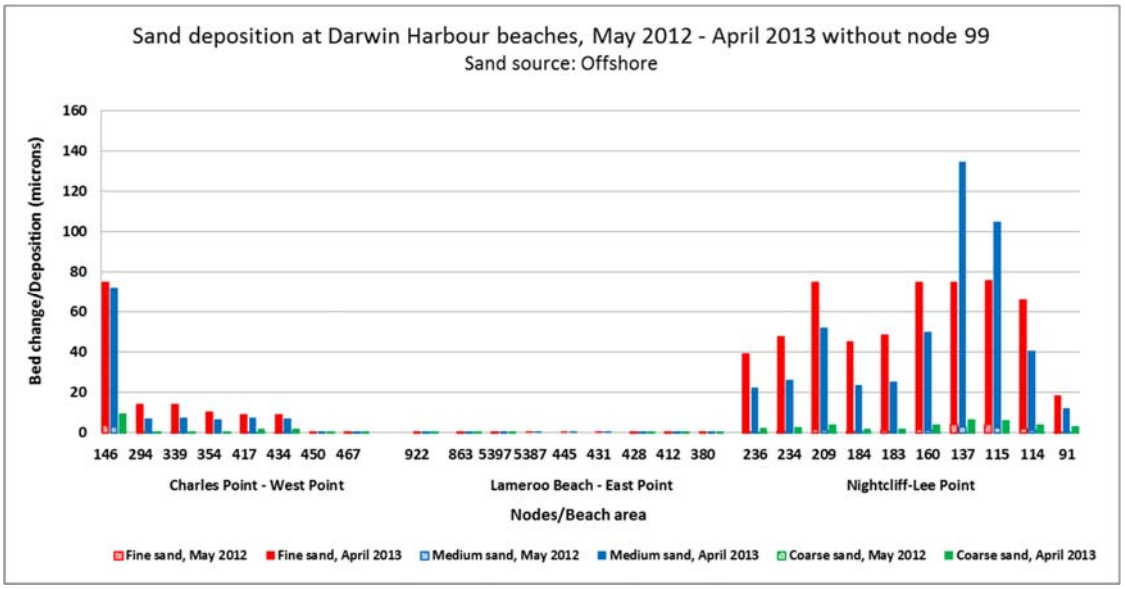

Figure 4. Offshore sand deposition on Darwin Harbour beaches excluding node 99.

3.2. Sand pathways from Rivers

The sand transport simulations showed that the contribution of river sand to the beaches in Darwin Harbour was significantly lower than offshore sand. Albeit of very small quantity, river sand deposited mostly in the embayment facing the inner harbour (Lameroo Beach, Figure 5).

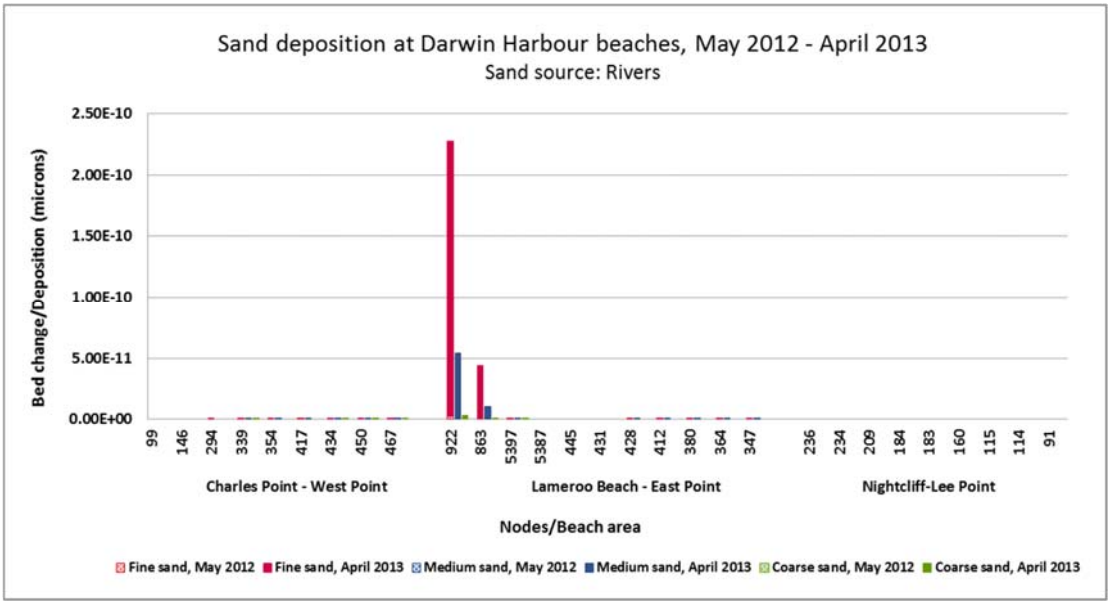

Figure 5. River sand deposition on Darwin Harbour beaches.

After passing Emery Point, the river sand was transported more to the western beaches rather than eastward, confirming that Cullen Bay sandbar and East Point constrain the sand transport pathways eastward (Figure 6).

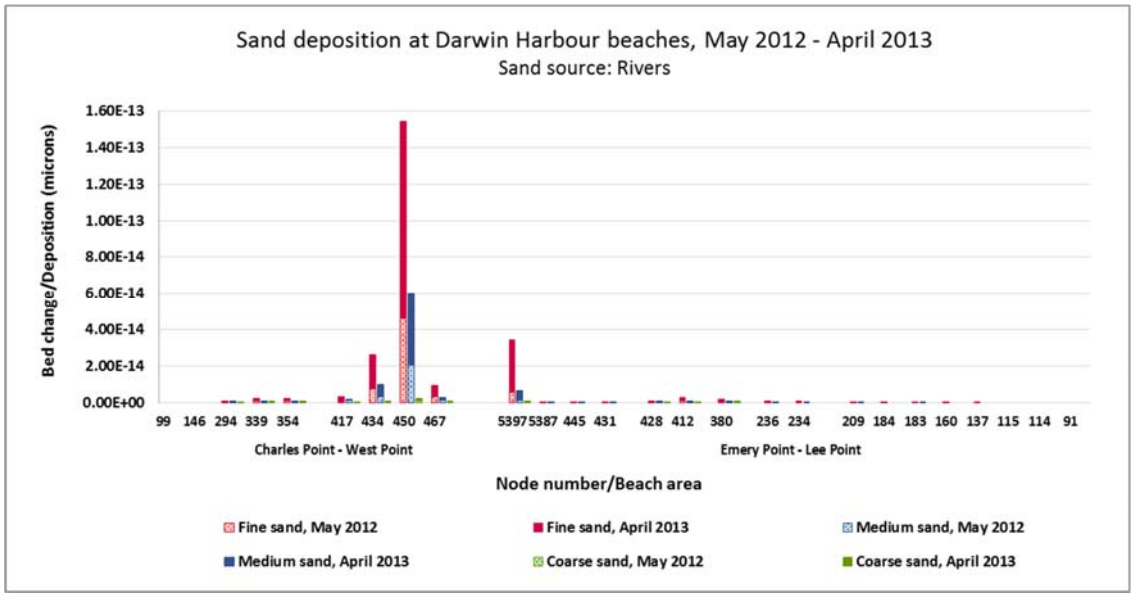

Figure 6. River sand deposition at Darwin Harbour beaches.

This minor contribution of sand from the rivers is likely due to the small drainage basin areas and consequently small river inflows into the harbour. One important characteristic of Darwin Harbour is the small catchment area relative to the waterways, i.e. about 3:1, which is smaller than other Australian harbours, for example 14:1 for Moreton Bay in Queensland and 10:1 for Port Jackson/ Sydney Harbour [13]. Other factors could be the low erodibility of soils in the catchment area or the ability of the catchment area to retain sediment. The macro-tidal nature of Darwin Harbour also overcomes the ability of river inflow to extend far to the outer harbour. The maximum recorded cumulative catchment discharge into the harbour during floods was estimated to be about 1% of the peak spring tide discharge [14].

Notwithstanding the low contribution, the simulation results showed that the sand transport pathways from rivers in Darwin Harbour were initially inclined eastward in the inner harbour and turned westward after passing the 'neck' of the harbour. These pathways are obviously influenced by the current strength and directions, while the location of the rivers, which are on the east side of the harbour, is another important factor.

3.3. The provenance of beach sand

Based on sedimentary composition, sand on Darwin Harbour beaches appears to be a mix of marine and terrigenous sources, with $CaCO_3$ concentrations in the sand samples from eastern beaches substantially higher compared to the sand samples from the western beaches (Figure 7).The carbonate content in sand from the eastern beaches is likely derived from offshore sources, in-situ sources and reworked sediment within the harbour. There are scattered hard substrates in the harbour providing carbonate sand sources from marine organisms, e.g. corals, molluscs, echinoderms and foraminifera [15] that may contribute carbonate sand to the area. The majority of foraminifers' biotopes located in the eastern part of Darwin Harbour contains foraminifera species typically found on the shallow continental shelf [6]. The fact that the local coral reefs are mostly located in the eastern part of the harbour (Lee Point and East Point), demonstrates that a more detailed study confirming the source of carbonate sand is necessary.

The low $CaCO_3$ concentration in the western beach samples shows a more terrestrial origin compared to offshore elements in the samples. Considering that the river contribution to beach sediment is relatively low, the signature may also be contributed by attrition of the local shore rock platforms, which occur extensively in the western beach area.

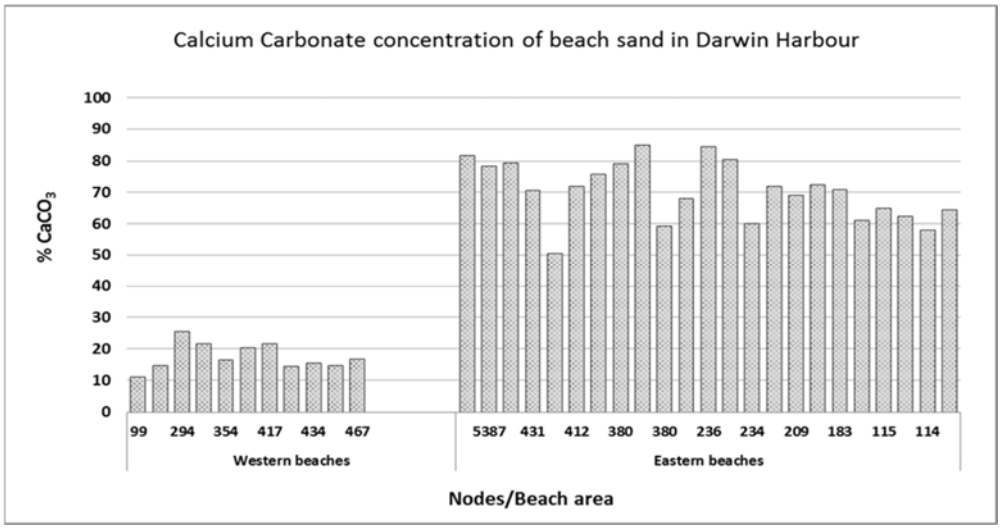

Figure 7. Calcium Carbonate concentration of beach sand samples in Darwin Harbour.

The REE composition of the beach sand samples, as depicted by a light- and heavy-REE bi-plot, shows that the eastern beach sand clusters closer to the outer harbour sand, while the western beach sand shows closer similarities with river sand (Figure 8).

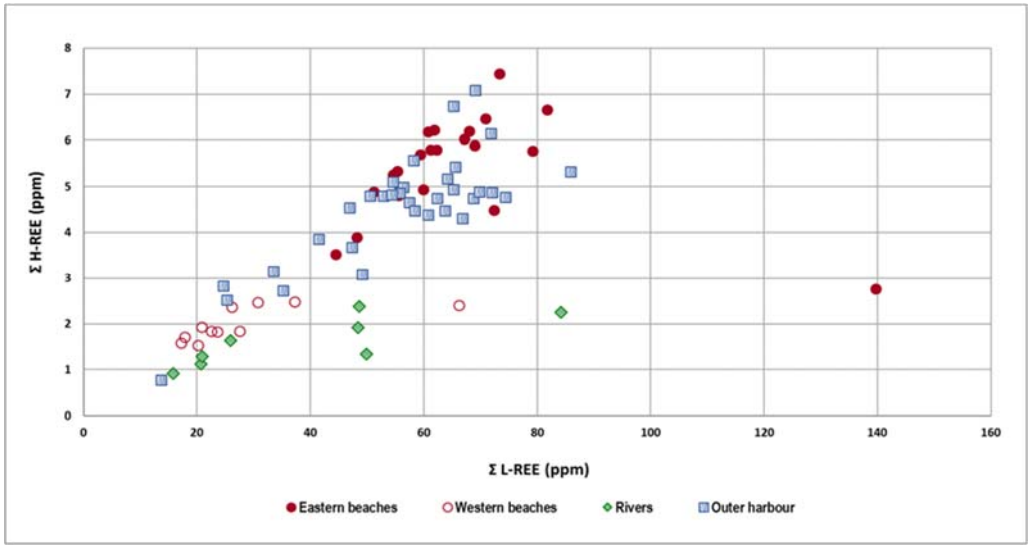

Figure 8. Light- versus Heavy-REE concentration of sand samples in Darwin Harbour.

With the assumption that outer harbour sand is largely of offshore origin, it is evident from Figure 8 that the sand in the east beach area indicates an offshore source, while the west beach sand shows a more river/terrestrial origin. Once again, as the sand transport simulations show that river input to beach sand

is low, the characteristics of the west sand may also be due to the contribution from the local bedrock formations.

Notwithstanding the high signature of an offshore sand source on the eastern beaches, considering the presence of eroding cliffs in the eastern part of Darwin Harbour [7], we cannot overlook the possibility that the rocky cliffs at the back of the beach are also a source of sand to the beach. Future study of the possibility of the rock flats and cliffs as sources of beach sand is necessary.

4. Conclusion

Two-dimensional hydrodynamic and sand transport simulations showed that beach sand in Darwin Harbour is mainly of offshore origin. On the other hand, the parallel geochemical analysis showed a slightly different pattern. The $CaCO_3$ concentration in beach sand suggests that the eastern beaches received substantially more offshore sand compared to the western beaches. Similarly, parallel Rare Earth Element analysis indicates a closer relationship between sand from eastern beaches and the outer harbour area. While the Rare Earth Element analysis shows that the western beach sand has more similarities with Elizabeth and Blackmore Rivers, a contribution from the local shore platform bedrock is possible.

Acknowledgement

The first author would like to acknowledge Charles Darwin University, the Indonesian Directorate General of Research, Technology and Higher Education (Kemenristekdikti) and the Australian Institute of Marine Science (AIMS) for supporting this research.

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Past and Future Ecosystem Change in the Coastal Zone

Past and Future Ecosystem Change in the Coastal Zone

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Abstract. The coastal zone is in a constant state of flux. Long term records of change attest to high amplitude sea level changes. Relative stability though the Late Holocene has allowed for the evolution of barrier dune systems, estuaries and coastal lakes with associated plant and faunal associations. This evolution has been interspersed with changes in the balance between climate driven changes in outflow from catchments. These interactions have been considerably disturbed through the impacts of industrialised people who have diverted and consumed water and invested in infrastructure that has impacted on river flows and the tidal prism in estuaries. This has impacted their provisioning services to humans. It has also impacted their regulating services in that development along the coastline has impacted on the resilience of the littoral zone to absorb natural climate extremes. Looking from the past we can see the pathway to the future and more easily recognise the steps needed to avoid further coastal degradation. This will increasingly need to accommodate the impacts of future climate trends, increased climate extremes and rising seas. Coastal societies would do well to identify their long term pathway to adaptation to the challenges that lie ahead and plan to invest accordingly.

Keywords. estuaries, paleolimnology, climate change, hydroecology, sediments, nutrients, salinization

1. Introduction

The coastal zone is in a constant state of change. This is most evident through the daily cycles of the tides that, particularly in the macrotidal zones of the world, inundate and then strand the marine littoral zones. This alone demands that the biological communities affected by the rise and fall of tides be adaptable to inundation and exposure, and in estuaries, exposed variously by marine waters and those flowing from the hinterland which are usually fresh to oligosaline. This balance varies seasonally with, in temperate zones, wet seasons coinciding with destructive waves regimes leading to the opening of estuary mouths, while the dry season and associated constructive wave regimes lead to mouth closure and the establishment of lagoonal conditions. The consequences are a winter of tidal and river flushing with oxygenated water and a summer with little flow, stratification, hypersalinity and de-oxygenation. These seasonal patterns are taken to extreme under multi-year (e.g. El Nino Southern Oscillation) and even multi-decadal (Inter-decadal Pacific Oscillation) phases of wet and dry conditions exacerbating or subduing the contrasts between seasons. Further the frequency of these cycles extends out to multi-

millennial scales with the highest amplitude variations coming from the substantial eustatic rises and falls in sea level documented on the Huon Peninsula [1]. These occur on account of the orbital cycles (Milankovitch) of the Earth which, in south-east South Australia [2], have left a series of fossil coastal dunes, each one hundred thousand years older than their more coastal neighbour.

Within the present high sea level stand it is it is tempting to consider that most variations in estuarine condition relate to natural climate and tidal cycles. However, there is also ongoing linear change. Once river valleys were inundated as seas reached maximum level after the last post-glacial transition the river gradients declined and waters in the new coastal zones estuaries became relatively quiescent. This allowed for the transported sediment to settle and so set in place a long extended phase of estuarine evolution that persists into the present[3]. Depending on the balance between the onshore wave environment and river flow an estuary gradually fills with sediment progressing from a wide, open estuary, to a narrow estuary in a wide delta, to an intermittently closed and open lagoon and, ultimately, to a largely closed system with coastal lakes.

A further directional change exists in the form of humanity. Human populations have grown over millennia accelerating after the industrial revolution and more so since the 1950s during the 'Great Acceleration' leading to the identification of a new geological epoch, the Anthropocene [4]. At the coast protective mangrove forests have been removed, coastlines reclaimed, harbours dredged and hard infrastructure commissioned to defend the coast against the tides and waves. Catchments have been cleared leading to increases in the volume of salts and sediments they leak, and nutrients have been mobilised and washed or leached into waterways after deliberate or inadvertent application. River flows have been held behind weirs, diverted for human consumption or regulated with hard infrastructure to allow for navigation and limit flooding. At the coast this results in the reduction of flushing flows, yet increasing loads of nutrients, metals, and sediments. Salinity levels increase owing to increased flux from inland but also due to the reduction in diluting flows[5].

Recent changes, and cyclical variations with short return times, are more familiar to people than those masked by the passing of time. However, because changes are more familiar is no reason why they are greater or have a more substantial impact on coastal ecosystems. The tendency to neglect infrequent cyclical change, or events deeper in the past, leaves society less well informed about the drivers of change and so less well armed to diagnose and treat coastal management issues. Historical evidence of environmental change is reliant on documentary or photographic evidence which tends to be patchy, or on memory which has proven to be unreliable[6]. Deep-in-time and continuous records of change are available, however, in the sediments of inundated coastal systems. Estuaries and lagoons provide a service to historical studies by archiving fossil remains of the biological, chemical and physical nature of waterways as they infill with sediment. The paleolimnological approaches that retrieve this evidence are now widely used globally and provide clear evidence of the influence of climate and people on ecosystems, including in the coastal zone.

2. Paleolimnological approaches

Sedimentary sequences accumulate offshore over long time periods. Onshore they accumulate when depressions are inundated as the still water allows sediment to settle and the anoxia in the littoral zones allow dead remains to be preserved with each deposited layer. As sea levels stabilised ~ 7000 years BP most near-shore records of change extend back to this time. Sophisticated sediment coring techniques are able to extract continuous sediment sequences[7] spanning this period and radiometric dating techniques are able to align a timeline to the extracted core. Being at the end of river catchments the sedimentation rates are often relatively large allowing for high resolution reconstruction of estuary condition through time. The physical nature of the sediment (e.g. particle size) can inform on variations in fluvial energy and so the impact of infilling[8]. Chemical analyses can inform on the source of the sediment (magnetic susceptibility, X-Ray Fluorescence) and the foundations of the food web and trophic status (stable isotopes of nitrogen and carbon) [9]. A wide range of biological remains can be extracted, including gastropods[10], ostracods [11], algal pigments [12], pollen [13], diatom algae [14] and plant

fragments [15]. These may provide evidence of changing habitat and substrate availability, the balance between tidal and inland water, salinity and nutrient status, the existence of anoxia and the nature of the light environment of the water body (see [16]). Integrated, multi-proxy investigations of sediment layers provide a means of reconstructing many elements of ever-changing nearshore ecosystems, albeit with the evidence of short time (daily to monthly) variations blended into single samples. Similar proxies may reflect onshore changes to catchments and this evidence, combined with archaeological or ethnohistoric records, can allow for responses to be attributed to particular human or climatic causes [17].

3. South-east South Australia.

The region between Brisbane and Adelaide is the most heavily settled in Australia. There is evidence for indigenous settlement since the Pleistocene and archaeological remains attesting to their activities, living sedentary, rather than nomadic lifestyles and manipulating coastal waterways, at least where resources were plentiful [18]. European settlement commenced in the 18th century and the goldrush of the 1850s brought large numbers of immigrants that saw a rapid expansion in population, much at the expense of the indigenous populations that suffered from disease and conflict. Coastal-flowing catchments were impacted by clearing and grazing and the populations of coastal margins increased dramatically from the mid-20th century and societal wealth increased. Most Australians now live in close proximity to the south-east coastline driving much landscape disturbance and ecosystem change in the near shore zones.

4. Evidence for Ecosystem Change

The first calls for action to mitigate this impact of European activities on coastal ecosystems emerged in the 1960s and 1970s and the first EPA departments was established in 1971 in Victoria [19] but as late as 1991 in NSW. National State of the Environment reporting commenced in 1996[20]. A cap was placed on the extraction of water from the nation's largest basin in 1995 and the Murray River estuary was deemed to be in ecological crisis in 2011[21]. This, however, does not reflect the timing of the impact of the expansion of settlement and associated industries such as mining and agriculture. In fact, long term records of change attest to considerable coastal change from the 1840s [22] and substantial changes in floodplain sedimentation rates and salinity from the 1880s [23].

4.1. Hydrological Change.

Rivers were impounded to retain water supplies for expanding urban populations from the 1860s such as Thorndon Park reservoir in Adelaide. Closer to the coast barrages were established at Geelong in 1840 and at the Murray mouth in 1940 to preserve freshwater resources from the impact of tidal inflows. At Geelong this immediately shifted an estuary to a freshwater lagoon [22] which was gradually colonised by *Phragmites* reedbeds. It also limited the outflow of freshwater from the Barwon River leading to increasing salinity levels in the estuary below the weir [22]. At the Murray mouth regulation constrained the tidal prism leading to the permanent freshening of Lake Alexandrina [24] but lead to rapid sedimentation at the mouth driving the formation of new islands and closing the outlet [25]. This turned the coastal lagoon into a closed system leading to hypersalinity[26], hypoxia and the collapse of critical *Ruppia* seagrass habitats [27],[28]. In the Gippsland lakes a temporary mouth was dredged for navigation leading to a permanent shift in the halo-ecology of the estuary[12]. While this lead to the death of fringing vegetation[29] increased flushing limiting the high cyanobacterial character [12]. The largest engineering feat in Australia, the Snowy River Scheme, diverted ~ 98% of the Snowy River's mean annual flow inland to drive the expansion of the irrigation industry and to generate power. Starved of freshwater supplies, Lake Curlip in the coastal floodplain shifted from a dystrophic lake to a system 50 times more saline than the pre-European baseline[30]. At Tuckean Swamp weirs reduced the tidal

prism leading to a freshening of surface waters but deliberate drainage exposed sediments leading to unprecedented acidification [31].

4.2. Pollutants

While the waters at the end of catchments are typically more enriched and saline than their upland counterparts there is clear evidence for the eutrophication of coastal systems through the 20th century (e.g. The Coorong; [26]) and, in the Gippsland Lakes, a return to the cyanobacterial conditions the prevailed before artificial opening, albeit with a novel bloom risk species emerging [12]. Coastal lakes receiving industry waste have seen nutrients rise and the onset of eutrophic conditions [32]. Mining, and industrial development in the coastal zone, are evident in high levels on heavy metals in upper sediments in the lower Barwon River estuary [33] and in Botany Bay in Sydney [34]. The Gippsland Lakes receive drainage from several catchments including the La Trobe River valley which has been long the focus of coal mines and coal-fired power stations, and a native and plantation timber pulp mill, which has lead to fears of high loads of metals including mercury into the estuary sediments and food chain.

4.3. Sedimentation

Catchment development in the Murray River basin has accelerated sediment accumulation rates in floodplain wetlands in the order of 5-30 times and up to 80-fold in the estuary [35]. In parts of the Coorong Lagoon in excess of 50 cm of sediment has accumulated in less than a century and aerial photographs attest to massive deposition of sediments around the river mouth. The barrages have maintained high water levels that have lead to the scouring of exposed littoral margins in Lake Alexandrina and accretion in sheltered zones[25]. The flux of sediments has increased in most situations impacting on the light environment and placing stress on submerged macrophyte communities.

4.4. Salinity

Some lagoons have shown an increase in salinity on account of the reduction in the flushing of freshwater (Reeves et al. 2014) or the construction of openings to the sea ([29],[12]) while others, once saline or tidal, have become relatively fresh on account of barrages restricting inflow of tidal water [33] or the redirection of freshwater into once saline coastal lagoons [26]. In the south lagoon of the Coorong the increase in salinity was from a high base, with the most notable feature being the replacement of salt tolerant thalassic species with euryhaline inland taxa of both diatoms and ostracods ([33]; [24]).

4.5. Ecological Implications

Many estuaries have been impacted by the development of agriculture and intensive industries since the arrival of European settlers. The impact of water quality change is greatest near to large populations e.g. Sydney, or at the end of larger, disturbed catchments e.g. the River Murray. Many of the ecological impacts remain unclear while others had become evident in recent decades and critical with the extended drought period that commenced in 1997. Extreme hypersalinity in the Coorong lead to a shift from a community dominated by ***Ruppia megacarpa***, through a ***Ruppia tuberosa*** ([28]) community phase, to a phytoplankton-brine shrimp state that lead to a decline in ducks and swans and their replacement by Banded Stilt (*Cladorhynchus leucocephalus*) ([24]), as well as declines in fish stocks. Rapid recent accumulation of high acid potential sediments drew an alarming acidification risk in the Murray River's lower lakes while, in the Gippsland Lakes, high river flows in 2007 lead to an unprecedented bloom of the toxic cyanobacterium *Synechococcus* [12] with impacts on seagrasses and bivalve populations. Migratory waterbird populations have declined owing to reduced habitat suitability and, possibly,

reduced food stocks, although the impact of modifications to resting sites in their Asian flyway are also implicated.

5. Management Implications

The long term record of change in south-east Australia attests to substantial modifications to coastal waterways from early in European settlement. These shifts are the result of the direct effects of the construction of barrages or the chronic release of sediments, salts and nutrients from various industrial sources and through catchment disturbance from widespread vegetation clearance. While observation and monitoring programs had lead to concerns being raised as to the health of these systems, the onset of record drought conditions rapidly brought forward substantial change in ecosystems and likely ecological regime shifts. Ecosystem managers failed to read the impending crisis and, in several situations, are now left with limited options to halt or reverse the impacts without massive intervention (e.g. [23]).

South-east Australia has been identified as a climate hotspot on account of scenarios of large declines in wet season rainfall and contraction of the winter growing season [36]. This is likely to exacerbate the declining ameliorative effect of freshwater flows into coastal systems leading to further concentration of nutrients and salts. The impact of this in the mobility and accumulation of sediment-bound metals raises issues of concern for the safety of any harvest from these waterways. High nutrients loads, coupled with warmer temperatures and higher concentrations of atmospheric CO_2 are likely to exacerbate cyanobacterial blooms [37] and expand hypoxic zones.

Australia's population is growing at a rapid rate and coastal development is increasing accordingly. There is a seeming reluctance to adequately care for the provisioning, regulating and cultural services these systems provide now, and may do in the future, in the rush to exploit them today. The long term record reveals that the post-1950 Great Acceleration, clearly evident in south-east Australia, rides on the shoulders of a century of ecosystem change wrought by early European settlers. Present practices and population growth is clearly unsustainable. Evidence for non-linear shifts in aquatic ecosystems in highly populated regions elsewhere [38] forewarn of the potential of catastrophic ecological collapses and substantial declines in aquatic resources. This also has the potential to challenge the capacity of society to deal with the risks associated with ongoing warming, a continuing drying trend and the prospects of coastal erosion and inundation with rising sea levels.

These case studies reveal that the provision of evidence of coastal change from long term sedimentary records provides clear insights unavailable from even long term monitoring programs. These provide evidence for long term trajectories of change and so can distinguish cyclical shifts from directional change. This can assist management in assessing the need for action and the level of urgency that measures need to be applied. Societies who are embarking on monitoring programs for coastal system assessment more recently could be well advised to supplement the accumulating survey data with that archived in sediments. In combination these would provide the data bank necessary to both identify slow and low frequency drivers of change and to qualify the importance of monitored variations by setting them in a longer term context.

6. Conclusion

Increasing capacity to observe changes in our environment has enabled humanity to better recognise the opportunity costs of catchment and coastal development to provide services and goods to human economies. In south-east Australia evidence is increasingly being assembled to provide a strong case that many coastal systems have been severely impacted by this development. The assembly of several records of change reaching back further into the past provides an earlier baseline and documents much change before technological advances and concern drew contemporary scientists to monitor long term change. These centennial scale records available in continuous sediment sequences reveal that substantial changes occurred in coastal waterways with years of the building of first settlements. These

changes are evident in changed assemblages of fossil biota and the physical and chemical evidence contained in sediment cores. It is clear that coastal systems have been impacted by the increased flux of sediments, nutrients, salts and metals or more than 150 years. Over this time they have been greatly impacted by hydrological change ranging from reduced flows from catchments through inland diversion, increasing or decreasing freshwater inflow or tidal penetration owing to near-mouth impoundments or freshening through the diversion of unwanted fresh floodwaters. All sites studied have changed relative to a pre-settlement baseline in one of these ways, some catastrophically.

Australia is a signatory to the Ramsar Convention on wetlands that obliges it to advise the Secretariat whenever a listed wetland has changed, is changing or is about to change. This convention also requires signatory nations to ensure wise use of all wetlands. Reference to evidence of change (e.g. [29]) from contemporary approaches questions whether Australia is fulfilling those obligations. Independent assessment of condition from long term sedimentary records (e.g. [12]) shows clearly that most wetlands have changed and that regulating, and provisioning services are being compromised. In line with the observations of Finlayson et al. [39] the government should embark on a program that assesses the present condition of its listed wetlands against more realistic, long term baselines to implement measures to avoid them passing irrevocably into degraded states.

7. References

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