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THE INFLUENCE OF HABITAT ON THE QUALITY CHARACTERISTICS OF THE GREEN MACRO ALGA *Caulerpa lentillifera* AGARDH (CAULERPACEAE, CHLOROPHYTA)

Delan GG[1,2*], Legados JA [1], Pepito AR[1], Cuñado VD[1], Rica RLV[2], Abdon HC[1], Bontia PV, Ilano AS[3] and Lamayo MHA[1]
[1*]Cebu Technological University- Carmen Campus, Cebu, Philippines, 6005
[2]Cebu Technological University- Main Campus, Cebu City, Philippines, 6000
[3] University of San Carlos- Talamban Campus, Cebu City, Philippines, 6000
[1,2*] Email address: glodelan2004@yahoo.com

ABSTRACT

The seaweed *Caulerpa lentillifera* is commonly utilized as food for human in many Asian countries. It is an efficient bio-filter, despite its ability to absorb pollutants from the habitat of sources.This study determine the influence of habitat on the quality characteristics of the green macro alga, *Caulerpa lentillifera,* taken from the different sources. Purposive sampling was used with samples taken from Bohol and Cebu islands, throughout the three (3) trials. Samples from Bohol islands were obtained from the culture ponds; while samples from Cebu islands were taken from both the culture ponds and from the wild. The sea-grapes were analyzed for the presence of heavy metals, lethality assay, bacterial count, direct pollution indicators and proximate composition. Data obtained were treated statistically using ANOVA at 5percent level of significance. Results of the study showed significant difference on heavy metals (Hg and Pb) from various seaweed sources. High concentrations of heavy metals were found in all the seaweed samples from Cebu compared with that of the Bohol samples. In terms of lethality assay (LC_{50}), the wild samples from Cebu significantly showed higher lethal values compared with that obtained from Bohol samples. As to the bacterial count (cfu/g), the wild samples from Cebu showed the lowest Aerobic Plate Count of 9.3×10^3 cfu/g. However, higher bacterial counts were obtained from Cebu samples that were taken from the two culture ponds co-cultured with milkfish. Across sites, the proximate composition of all the sea-grape samples from Cebu and Bohol also showed significant difference. In terms of *E. coli,* all the samples have shown no significant difference, with <1.0 x 10 $cfu.g^{-1}$. Regarding the indicators of direct pollution, all the samples from both islands showed low levels of direct pollutants, such as PO_4, NH_3, and NO^3. Based on the results of this study, sea-grapes showed varying quality characteristics as influenced by the habitat or sources they were obtained. As a bio-filter, the quality characteristics of the seaweed are indicators of the various pollutants found from the habitat or sources.

Keywords: Macro-alga, bio-filter, heavy metals, lethality assay, pollution indicator

INTRODUCTION

In central Philippines, such as Cebu and Bohol islands, sea-grapes or green caviar, scientifically known as *Caulerpa lentillifera* (Agardh,1837), are commonly used as food for human. It is popular as human food because of its nutritional value such as iodine, Vitamin A and C, minerals and others. It is favored by the consumers because of its soft, succulent grape like structure. However, sea-grapes are known as an efficient bio-filter which has the ability to

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accumulate contaminants from its sources. In the Visayan region, sea-grapes are locally known as "Lato", which can be obtained from the wild, cultured in ponds, in open lagoons, or even in concrete water tanks (Trono,1969).

Several studies have proven the effectiveness of the seaweeds as a bio-monitoring tool of environmental pollutants (Bryan, 1969, Melhaus et al., 1978, Sivalingam et al., 1980, Villares et al., 2001). As a bio-monitoring tool, seaweeds are used as an alternative method in determining the type of environment and the available contaminants present in a place (Rainbow, 1995). In one particular study, sea-grapes were found to contain bioactive substances that are found toxic to mouse on mouse bioassay (Doty and Aguilar Santos (1987), Ito and Hori (1989); and, Delan et al., (2007). The common symptoms of toxicity include immediate weakening, jumping, lacked of balance, gasping, and others. It was also found out that sea-grapes has a lethal potency in terms of $LC_{50} = 3.33\ g^{1}.ml^{1}$ on mouse bioassay; whilst Ito and Hori (1989) found out a lethal potency of $LC_{50} = 1g.K^{-1}$.

As a water treatment in intensive fish farms in Thailand, seaweeds particularly sea-grapes, can remove up to 90 percent of the nutrient discharge or wastes from the farms (Luning and Pang, 2003; Madacha et al, 2006).On mouse bioassay, it was found out that the grapelike structure (ramuli) was toxic to mice, while the creeping tube like structure (stolon) was not toxic (Delan et al.,2007).This result would support the study that the pollutants from the habitats can readily be absorbed by the grapelike structure (ramuli), but not the creeping tube- like structure (stolon). Since seaweeds are proven as potential bio-filter of pollutants in the environment, there is a possibility that the seaweeds consumed by man contain the pollutants that have been absorbed by the seaweeds from the source through its grape-like structure (ramuli).

Despite the above scenario, *Caulerpa lentillifera* is still very popular and are highly valued foodstuff particularly, in the central part of the Philippines, such as in Cebu and Bohol islands. This seaweed is also very popular in some Asian countries such as Japan, Malaysia, Vietnam, Thailand, Papua New Guinea and Indonesia, and in some countries where Filipino communities abound. Since sea-grapes have high commercial value as food for human, the consumers safety should be assured. This has prompted the researchers to assess the quality characteristics of the sea-grapes which may be influenced by their habitat or sources from where they were taken. Being known as an efficient bio-filter, it is expected that the quality of the sea-grapes taken from the different sources really vary depending on the type of habitat these were taken.

Results of the study can be used by the seaweed farmers as basis in choosing the area for culturing sea-grapes intended for human consumption. This is to ensure the sustainability of sea-grapes in the market for direct human consumption and food safety reasons.

MATERIALS AND METHODS

Collection of Sea-grapes Samples

The *Caulerpa lentillifera* samples were obtained from central Philippines particularly in the islands of Cebu and Bohol. Sampling was done during summer for three (3) trials in different

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sampling sites or stations. Purposive sampling was used in the study since the sampling sites were chosen based on the following criteria where the sea-grapes were taken: Sea-grapes near the industries (Cebu culture pond - sample A); Sea-grapes far from households and industries (Bohol culture pond - sample B, control); Sea-grapes near the households (Cebu culture pond - sample C); and, Sea-grapes near the households and industrial zones (Cebu wild stock - sample D).

Sample B was taken from Bohol Island as the control sample because this area is free from any industrial activities and households. The rest of the samples were taken from Cebu Island because this area is highly industrialized and is a densely populated place. The wild stock sample (sample D) was taken from the coastal areas where there are lots of households in the shoreline and an industrial zone located nearby where any waste from the households can be directly channeled to the sea. Whilst, the industrial zone comprises of activities in the welding shop, cement factory, sugar mill, paper mill, metal works and a dry docking facility in the site.

Figure 1.The study sites

The sea-grapes that were collected from these sampling sites were washed in running water to remove the mud and other adhering matters. The cleaned sea-grapes were ready to use for the following analyses of the study in triplicates per sample.

Presence of Heavy Metals

Heavy metals such as lead (Pb) and mercury (Hg) were analyzed from the sea-grapes samples. The samples were air dried and ground. About five (5) grams of a ground sample was weighed using analytical balance and placed into a 50 ml beaker. The beakers were then transferred to the fume-hood and added with 10 ml of HNO_3 for acid digestion, followed by 1 ml of H_2O_2 and heated over a hotplate at 200-250°C for 30 min. The samples were then allowed to cool, transferred to a 50 ml volumetric flask and diluted to mark. For the Hg analysis, 10ml HNO_3-$HClO_4$ (1:1) were added to each beaker followed by 5 ml of H_2SO_4 and digested in a hotplate. Samples were allowed to cool, filtered using Whatman #40 filter paper while the filtrate was placed into a 50ml volumetric flask and diluted to mark.

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An appropriate amount of samples were introduced to the Atomic Absorption Spectrophotometer (Shimadzu AA-6300) for quantitative analysis of Pb. In terms of Hg, Cold Vapor Technique was employed and the amount is measured using the mercury analyzer (Shimadzu MVU-1A).

Lethality Assay

The toxicity of the samples were measured using brine shrimp *Artemia salina* exposed to various concentrations of extracts of sea-grapes from the different habitats. Brine shrimp eggs were hatched in artificial seawater prepared by mixing 38 $g.L^{-1}$ of sea salt with pH adjusted to 8.5 using 1N NaOH, under constant aeration for 48 hours. After hatching, 10 nauplii were placed into vials containing artificial seawater added with the prepared concentrations of sample extracts (5, 25, 50, 75 and 100 %) as test solutions. The toxicity on brine shrimps was measured in terms of LC_{50}, which refers to the concentration of a substance that kills 50 percent of the population of the test organisms in a given time (MacDonald et al., 2003).

Bacterial Load

Total load of bacteria was measured using total Aerobic Plate Count (APC) and *E. coli* count following the method in Bacteriological Analytical Manual, 8th Ed.(1998). Decimal dilutions of samples were prepared, incubated, counted and the results were expressed in terms of colony forming unit (cfu g^{-1}) sample.

Direct Test for Pollution Indicator

The presence of NH_3^+, PO_4^{-3} and NO_3^- were analyzed as direct indicators of pollution in the sea-grapes from their habitat. Ammonia -N and Nitrate -N were determined through Kjeldahl using Titration Method (APHA, 1998); while the amount of phosphate was determined using Spectrophotometric method.

Proximate Composition

Sea-grapes were analyzed for the presence of water, protein, fat and ash. Water content was determined using Ohaus Moisture Determination Balance until constant weight was obtained. The total protein content was determined using standard AOAC (1990) method and calculated by multiplying Kjeldahl nitrogen by a factor of 6.25. Crude fat content was determined using the method by Bligh and Dyer (1959). Ash content was analyzed through the use of a muffle furnace set at 500- 550 ^0C for four (4) hours (AOAC, 1990).

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RESULTS AND DISCUSSION

Presence of Heavy Metals

The seaweed samples from Cebu and Bohol were analyzed for the presence of heavy metals, in terms of Hg and Pb, as shown in Table 1. The sea-grapes samples obtained from Cebu culture ponds found in close proximity to the industrial zones and households and from the wild (samples A, C and D) respectively, showed the highest level of Hg which has an average of 80.0μg^{-3} K^{-1} for the three (3) sites; while the seaweed samples obtained from Bohol (sample B) which were taken from a site without households and industries contains only about 73.4 μg^{-3} K^{-1}, is significantly lower than the 3 aforementioned Cebu sites. In terms of Pb content, the seaweed samples from Cebu similarly obtained higher levels within the three (3) sites, with sample C obtaining the highest amount of 780μg^{-3} K^{-1}, while the seaweed samples from Bohol showed the lowest level which is only 8μg^{-2} K^{-1} (Table 1).

Table1. Heavy metal accumulation in sea-grapes from Cebu and Bohol

SOURCE	Presence of Heavy Metals (Ave.)		Sampling sites
	Pb (μg^{-3} K^{-1})	Hg (μg^{-3} K-^{1})	
A Cebu	70	79.7	Culture pond with industries located nearby
B Bohol	8	73.4	Culture pond without households and industries
C Cebu	780	79.9	Culture pond with 2 sides adjacent to the households nearby
D Cebu	84	79.6	Wild stocks along the shorelines with households and industries nearby

The presence of higher heavy metals, (Hg and Pb) in the Cebu samples compared to the Bohol samples were attributed to the fact that the former source (Cebu) is a densely populated and a highly industrialized area than the latter source (Bohol) which is free from households and industries as sources of pollutants. The presence of higher Hg from the Cebu samples (Samples A, C and D) can be attributed to the sewage sludge from the industries and from the households. The existence of industries and households in a place produces the common waste due from the use of caustic soda, fossil fuel, paint, pulp and paper, batteries, dental amalgam and bactericides (Carr and Neary, 2008). Moreover, the presence of higher Pb in the samples were commonly due to the sewage discharge from the industries and households, exhaust from vehicles in the atmosphere, waste from lead ore mines, leads smelters and others. The varying and higher heavy metals accumulated from the Cebu samples only showed that sea-grapes really have the ability to accumulate contaminants from their sources and that the rate of accumulation may be dependent on the amount of pollutants available in the sites. Several studies have also proven the effectiveness of this seaweed when used as a bio-monitoring tool of environmental pollutants (Villares et al., 2001; Sivalingam et al., 1980; Melhaus et al., 1978; and Bryan, 1969). It was

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similarly proven that seaweeds can be an alternative method in determining the type of environment and the available contaminants present in a place (Rainbow, 1995; and Chaudhuri et al, (2007).

The health impact of utilizing seaweeds contaminated with heavy metals (Hg and Pb) can have adverse effects on the intellectual development of young children and also cause damage to kidney of human on long exposure (Food Safety Authority of Ireland, 2009). Lead (Pb) has the ability to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults (Commission of the European Communities, 2001). The presence of 0.01 mg/L in the water poses health significance already (ICMSF, 1986). While FAO of the United Nations and WHO (1990) have established a provisional tolerable weekly intake of Pb as 25μg /kg body weight for humans, which means equaling 1,500μg lead/week for a 60-kg person. While a high dietary intake of Hg (organic) from consumption of fish has been hypothesized to increase the risk of coronary heart disease (Salonen et al, 1995). Mercury is a latent neurotoxin compared to other metals like Pb, Cd, Cu and As (Zodape et al.,2010).

The results obtained on heavy metals were very much higher compared to the standards set by FAO of the United Nations and WHO (1990; FAO-WHO,1972) with a tolerable weekly intake for humans of only 25μg/kg body weight, and 5 μg/kg body weight for Pb and Hg, respectively. These standards would mean that a 60-kg person should consume only 25μg /kg body weight, which means equaling 1,500μg Pb/week; and 5μg Hg/week equivalent to 300μg Hg/week consumption. Based on these standards for Pb and Hg, the sea-grapes obtained from Cebu sampling sites pose some health consideration since the heavy metals present were considerably higher than the standards, in comparison with the Bohol samples. Thus, for safety reason, consumption should be in moderation so as not to exceed the standards set which is 25μg / kg and 5μg/kg body weight requirement for Pb and Hg, respectively. The pollution uptake in the sea-grapes were due to the grape- like structure or ramuli which possess the absorptive property of the seaweed making it capable of bio-accumulating heavy metals and other pollutants from the habitat.

Lethality Assay

Results on lethality assay showed that the wild samples from Cebu (D) which were taken near the households with varied industries were toxic to brine shrimp *Artemia salina*. The lethal concentrations (LC_{50}) of the wild sample from Cebu (D) resulted in about 53.33 percent mortality of the test organism compared with the seaweed samples from Bohol (B) with an LC_{50} of 16.67 percent mortality only (Figure1). Previous study on sea-grapes using mouse bioassay had proven that the toxic part of the sea-grapes was concentrated on the grapelike structure (ramuli), while the creeping tube like structure (stolon) was non- toxic to ddY mice (16-19 g) (Delan et al,2007).

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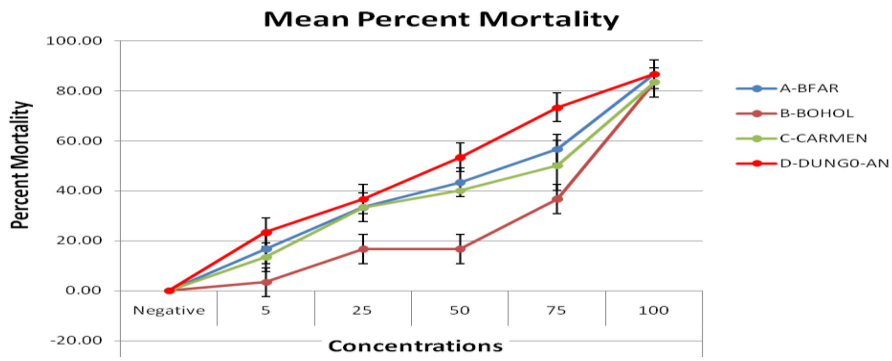

Figure 2. The LC_{50} of the Sea-grapes Samples from Different Habitats

Legend: Sample A- Culture pond with industries located nearby (BFAR)
Sample B- Culture pond far from households and industries (Bohol)
Sample C- Culture pond with 2 sides adjacent to the nearby households (Carmen-CTU)
Sample D-Wild stock with households and industries along the shorelines (Dungo-an)

In general, the LC_{50} of the sea-grape samples from Cebu (A, C and D) and Bohol (B) are highly correlated (r = o.84) with the Hg and Pb contents of sea-grapes. The samples (A, C, and D) with higher heavy metal contents (Hg and Pb) also showed higher percentage mortality on the brine shrimps, *Artemia salina.*(Table I and Figure 2, respectively). Higher toxicity effect on brine shrimps can be directly related to the lethal effects from the higher heavy metal contents of the sea-grapes in terms of Hg and Pb and other contaminants found in the sampling sites. Gajbhiye and Hirota (1990); and, Ping and Jiann (1987) reported that Pb is very toxic to brine shrimps compared to the rest of the heavy metals.

Among the Cebu samples, the wild sea-grapes (D) sample showed the highest percentage mortality on brine shrimps compared with the rest of the Cebu samples; while the sea-grapes from Bohol had the lowest percentage mortality (Figure 2). The highest lethality values from the former sample was attributed to the source pollutants from the industries which include welding shop, cement factory, sugar mill, paper mill, metal works and a dry docking facility in addition to the number of households in the coastal areas. These pollutants might have originated from these places that resulted in a higher mortality rate to the test organisms from this sample. Considering the higher amount of heavy metals that had accumulated on the sea-grapes, this might intensify the lethal effects of the samples on the test organisms (*Artemia salina*). Since studies have shown that sea-grapes can remove up to 90 percent of the nutrient discharge from an intensive fish farms in Thailand (Luning and Pang, 2003; and Madacha et al, 2006), thus, the study have shown that sea-grapes has the ability of absorbing pollutants that are available in the habitat. The absorption of pollutants by the sea-grapes from the habitat could be one of the reasons for the toxic effects on the test organisms that may also have the likelihood of affecting the safety of human being when accumulated considering its utilization as food.

Sea-grapes has only two (2) parts, the ramuli and the stolon. The former is composed of bulbs that are grapelike structure, while the latter are the creeping tube-like structure. The ability of the seaweed to accumulate pollutants from the sources occurred in the ramuli, while the stolon does not have the ability to absorb pollutants from the sources (Delan et al., 2007). The ramuli are the part of the sea-grapes which are usually picked and eaten by human, while the latter parts are usually removed prior to consumption.

Direct Test for Pollution Indicators

Generally, seaweeds are considered as bio-filter (Madacha et al, 2006) of the pollutants as a result of human activities such as industrial, agricultural and household processes. In terms of direct indicators of pollution such as ammonia (NH_3), nitrate (NO_3) and phosphate (PO_4), all the samples showed low levels of direct pollutants whether taken from Cebu or Bohol (Table 2). The seaweed samples from all the sites had obtained values lower than the standards set in U.S. EPA Standards (1986) which is not greater than 0.1 mg/L, and not greater than 10 mg/L for NH_3 and NO_3, respectively. In terms of PO_4, presence in higher amount can be felt only when the growth of marine plants and algae may occur in the environment limiting the oxygen supply of the living organisms in the habitat causing massive fish kill. When the study was conducted, there was no incident of massive fish kill in all the areas of sampling since this indicator was found very low from all the sampling sites.

Table 2. Pollution Indicators Found in Sea-grapes from Cebu and Bohol Samples

Sampling sites	NO_3	NH_3	PO_4
Cebu - A	0.32	0.05	0.12
Bohol - B	0.65	0.05	0.08
Cebu - C	1.11	0.11	0.13
Cebu - D	0.13	0.08	0.07

Proximate Composition

The sea-grapes contains nutritional value that are essential to one's health particularly iodine, Vitamin A and C, minerals and others (Food Composition Table for Use in the Philippines,1968 ; and Delan et al. 2004). Results of the present study showed that the values obtained are quite closer to the values reflected in the Food Composition Table for Use in the Philippines (1968), as shown in Table 3. This means that the proximate and or the nutritional composition of sea-grapes are not affected regardless of the sources where these were taken. The results were found to have significant difference measured through ANOVA at five (5) percent level of significance. The differences are attributed to the maturity of the sea-grapes samples when taken that had caused the variability in the proximate composition of the sea-grapes (Ito and Hori, 1989).

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Table 3. Average proximate values of sea-grapes from Cebu and Bohol Islands

SOURCES	Moisture(%)	Ash (%)	Protein (%)	Total Fat (%)
Cebu - A	90.4	4.30	0.91	0.11
Bohol - B	91.7	4.27	0.96	0.05
Cebu- C	90.1	4.37	1.48	0.38
Cebu - D	90.9	4.17	0.78	0.64
F- value	***88.85***	***71.65***	***1987.26***	***1020.82***
p-value	***1.75E-06***	***4.02E-06***	***7.93E-12***	***1.3E-10***

Bacterial Load

The sea-grape samples were analyzed of their bacterial load in terms of Aerobic Plate Count (APC) and *E. coli* expressed as colony forming unit/ sample (cfug^{-1}). The former method is used as an indicator of the level of bacteria present in a food (APHA, 1998); whilst the latter is an indicator of recent sewage or animal or human waste contamination of the food or water as this organism is commonly found in the intestines of animals or humans. The samples from Cebu that were obtained from the culture pond near the industries (sample A), and from the culture pond with two (2) sides near the households (sample C), respectively, have higher bacterial load (> 2.6 x10 ^{6} cfug^{-1}) in terms of APC. The sample which was obtained from the wild in Cebu islands (sample D) showed the lowest APC of 9.3 x 10^{3} cfug^{-1} (Table 4). Although higher bacterial load in terms of APC was found in samples from Cebu (samples A and C), higher APC count does not relate to food safety hazards (Nickelson and Finne, 1992) as these are just total counts of the number of aerobic bacteria found in the samples, not the presence of pathogenic organisms.

In terms of *E.coli*, all the sea-grape samples have obtained very low count that are all below the standard limit set by authorities which is 11-500 cfu/g sample (ICMSF, 1986). This means that the sea-grapes from all the sampling sites in Cebu and Bohol were safe for human consumption, as far as bacteriological safety is concerned.

Table 4. Microbial Load of Sea-grapes from Cebu and Bohol, Philippines (cfu g^{-1})

Bacterial Load	Cebu - A	Bohol-B	Cebu - C	Cebu-D
APC	>2.6 x 10^{6}	1.4 x 10^{5}	>2.6 x 10^{6}	9.3 x 10^{3}
E. coli	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10

CONCLUSIONS AND RECOMMENDATIONS

The place where the seaweeds were grown or cultured had made great influence on the quality characteristics of the seaweed. Since this seaweed is very popular as food for human in the Philippines, there is a need to ensure that the seaweed should be free from toxic heavy metal accumulations which bring about serious damages to the human system. As a bio-filter, culture

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sites must be situated in free or less polluted areas. Therefore, it is recommended that before the seaweed farmers will venture on sea- grapes production, they need to ascertain first the habitat that they will use so that the health of the consumers can be protected for any bioaccumulation of heavy metals for safety reason. Further studies should be done using the samples during rainy days, not just only summer time to obtain a true picture of the bio-filtering capacity of the sea-grapes in the different culture areas.

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