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Aim & Scope

CMU Journal of Science (0116-7847) is a peer-reviewed multidisciplinary journal published annually by Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. This official scientific journal of the University is accredited by the Philippine Commission on Higher Education (CHED) as category B. It publishes quality research articles, perspectives, review articles, and technical notes of researchers in the field of natural science, engineering, social science, and mathematics from local, national, and international contributors.

Editorial Policy

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The CMU Journal of Science (CMUS) is a multidisciplinary peer-reviewed scientific journal published annually by Central Mindanao University, University Town, Musuan 8710, Bukidnon, Philippines

All submitted manuscripts are reviewed and evaulated by the Editorial Board to determine whether the articles are publishable or not. To be accepted, articles must satisfy all of the following criteria.

1. The article has not been published by any journal;
2. The manuscript is recommended for publication by a minimum of two peer-reviewers;
3. The manuscript has passed the plagiarism detection test with a score of at least 85% for originality not more than 15% Similarity Index); and,
4. The manuscript has passed the grammar checker software

The CMUS adopts a double-blind peer-review process before the papers are published to ensure the quality of the publication and to avoid plagiarism.

The Editor-in-Chief, upon consultation with the Editorial Board, assigns the article to at least two reviewers.

A letter of request, together with the abstract and the Response Form, is sent to the reviewers who have the expertise on the topic. If they agree to review the article, the full paper will be sent to them.

After the review process, each reviewer will return the reviewed article and the Assessment Form indicating his/her recommendation. An aritcle recommended for deferment of publication will be considered if the comments to reviewers are complied.

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The article for submission should not have been printed previously or is not currently considered for publication in other journals. The publication of the work should be agreed by all authors and other responsible authorities involved in the work, tacitly or explicitly. If the work is accepted, its publication elsewhere in the same form, in English or any other language, including by electronic means without the written permission from the copyright-holder should not be allowed.

The article should be written in good English (American or British usage is accepted, but not a mixture of these). A soft copy of the article must be submitted in one file only. This will be emailed to journalofscience@cmu.edu.ph. Paper size is A4 (8.25 x 11.69), with an inch margin on all sides. Font style is Times New Roman; Font siaze is 12. The article should be double-spaced; with about 4,000-6,000 words in single column format, inclusive of tables and figures. Manuscripts should be encoded in Microsoft Word (atleast Word 2007) and Excel (for graphs and tables). Articles encoded using other softwares (Corel, LaTeX, Photoshop, etc.) may be accepted after consultaiton with the publication staff. Photos, maps, graphs, charts, and other illustrations shall be labeled as Figures, and tables and matrices, as Tables. These should be appropriately numbered and labeled based on their order or presentation. Figure label shall be written below the figure while table titles shall be on top of the table matix.

Introduction to the Issue

We have come up with the 2018 natural sciences issue, and we have gained a lot of lessons in the process of publishing this. Nonetheless, we are pleased to be standing the test of time through the support of our authors, dedicated reviewers, active EB members, stakeholders, and our supportive CMU administration.

The research topics published in this journal are all academically stimulating and diverse. Honestly, it was not easy to summarize with a common theme. The dominant category though is on environmental sciences, including the perspective piece on Technospheric Mining by our international based EB member Dr. Alorro. The research articles which centered on environment were the papers entitled Non-CO2 Greenhouse Gas Emissions from Field Burning of Crop residues in the Philippines: 1990-2015, Littefall Production and Litter Turnover in the Long Term Ecological Research (LTER) Sites in Southern Philippines, and Assessment of Termite Infestation in Academic Infrastructure in Central Mindanao University. After all, research outputs on the environment should be shared to everyone within and without the academic community.

The other three scientific papers focus on Food and Agriculture entitled Underutilized Senna tora (L.) Roxb-a highly potential multipurpose species for food, feed, medicine and climate resilience for the Philippines; Phytochemical and Antioxidant Activity Variation of Processed Edible ferns; and Proximate Composition of Raw and Heat-treated Seriales [Flacourtia jangomas (Lour) raeusch] Fruit. The latter was authored by the sole non-CMU affiliated author, Dr. Lynette Cimafranca.

Finally, one paper is on Phytochemical screening and in vitro antibacterial activity of Syzygium sp ("O-on") crude extract against Pseudomonas aeruginosa and Staphylococcus aureus, wherein phytochemical analysis accordingly showed that extracts of Syzygium contain a medicinally crucial bioactive compound.

Hope that this CMU Journal of Science issue will hold an exceptional place in scientific publishing as it transfers scientific knowledge to our society.

EINSTINE M. OPISO
The Editor-in-Chief

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Technospheric Mining Towards a Circular Economy

Richard Diaz Alorro, PhD
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Human activities, such as product manufacturing, mining, and ore extraction, chemical and metallurgical processing, road and building construction, municipal and household activities, and agriculture among others, have relocated and accumulated metal and material resources from geological origins to the technosphere. The technosphere is defined broadly as a material stockpile that has been established by human activities and technological processes (Johansson et al., 2013). Iron, for example, is mined and extracted from iron oxide ores from the Earth's crust, by high temperature metallurgical processes and is used to produce steel, other metal alloys, and chemical products. Iron is now present in many things we use everyday, i.e., buildings, bridges, cutting tools, bicycle, and a multitude of other materials and equipment. These materials containing iron, either active or inactive stocks, are part of the technosphere and are considered technospheric stocks or secondary sources of iron.

There are numerous other metals and material stocks of finite resources accumulated in the technosphere. But the most important technospheric stocks and the main targets of current technological and policy initiatives are the wastes and waste repositories. Industrial, municipal, metallurgical, and mining wastes pose health and environmental hazards if not managed properly. These wastes present serious storage or space problem (for disposal) and take a huge chunk of operational costs for government and companies for management and treatment. However, wastes and waste repositories from different sources are highly relevant due to the presence of valuable elements, especially the technology metals, which are considered critical and strategic based on studies by the European Commission (2011 and 2014) and the United States Department of Energy (Simandl et al., 2015). These elements include the rare earth elements (REEs), precious metals, cobalt (Co), the refractory metals particularly niobium (Nb) and tantalum (Ta), indium (In), germanium (Ge) and others. Recovering these elements from wastes and waste repositories is vital for the move towards a circular economy and sustainable development.

The extraction and recovery of metals and mineral resources from technospheric stocks, predominantly wastes and waste repositories, is called technospheric mining, in contrast to conventional mining, which targets primary ores of geologic origin. This increasingly important and significantly relevant new concept, which is geared towards waste valorisation, was introduced in the papers published by Johansson et al. (2013) and Krook and Baas (2013) and covers various areas, including urban mining, slag, tailings, waste and landfill mining, as presented in Figure 1.

The recovery of precious metals, such gold (Au), silver (Ag), platinum (Pt), and palladium (Pd) from waste electronics and electrical equipment (WEEE) is generally classified under urban mining. Slag mining is dedicated to the extraction of valuable elements from slag, a by-product or waste material generated by high temperature metallurgical processing or pyrometallurgical processing of ores or mineral concentrates. Processing of copper and nickel sulphide minerals, iron ore, tin minerals particularly cassiterite ($SnO2$), produces slag materials, which contain valuable elements. Slag may contain critical and strategic elements, such as cobalt, REEs, Ta, and Nb. Landfill mining refers to the recovery of valuable materials from landfill sites, which mostly house municipal solid wastes and other industrial rejects. Mining and mineral processing operations also generate significant amounts of wastes that can serve as valuable technospheric stocks. Wastes from these operations would include mine overburdens, mine, and beneficiation tailings. Some tailings from historical operations when extraction and processing technologies were not yet sophisticated, may contain valuable metals of concentrations higher than primary ore sources. In the Philippines, a very good example is the gold mine tailings, which can be reprocessed using new technologies to recover residual gold. Recovery of valuable elements or materials from other waste streams, such as coal fly ash (may contain REEs), steelmaking dusts, and dredged sediments, can be categorized under waste mining.

Aside from valuable elements, wastes and waste repositories can also be used as secondary sources of other materials with varied applications. For instance, coal fly ash has been known to contain aluminosilicate components, which makes it useful as an additive in cement manufacturing or the synthesis of zeolitic materials. Mine waste tailings which are rich in iron minerals can be used for iron extraction and synthesis of magnetite or other iron oxides for pigment and environmental applications. Some mine wastes are used to produce bricks for buildings and road construction purposes. Mine wastes and some industrial residues containing clays, carbonates, phosphates, and iron oxides are utilized in soil amendment/amelioration applications.

Technospheric mining can be considered as the mining of the future. It will transform the essence of mining as it will shift the focus of resource recovery from the lithosphere to the technosphere. As the grade and quality of ores or minerals, we mine from Earth's crust decrease, and as the technology required for conventional mining becomes more challenging, it is apparent that mining will adapt accordingly to the exploitation of previously extracted minerals or materials now accumulated in the technosphere. Wastes and waste repositories are abundant in the present world but the nature and the engineering used to generate them vary significantly between countries and waste types (Sapsford et al., 2017). These would have significant impacts on their repurposing and utilization for resource recovery. Some wastes may require decontamination before they can be converted to useful products or prior to valuable metal extraction. Some are regulated by legislations for waste management practices and these could vary between countries as well. The technologies required for technospheric mining also vary depending on waste types and the target material for recovery. There are existing technologies that are readily transferrable to technospheric mining, such as technologies applied for mineral processing and extractive metallurgy, but these require proper assessment on amenability and suitability. The technology readiness levels (TRLs), applied by scientists, engineers, and policy makers to evaluate the maturity of a particular process or technology, are also variable ranging from laboratory scale to commercialization levels. The development of technology tailored for a specific waste or waste repository is highly important. But of course, technospheric mining is not only about technology and scientific innovations. As with the general concept of a circular economy, the involvement of the different sectors of the society is imperative.

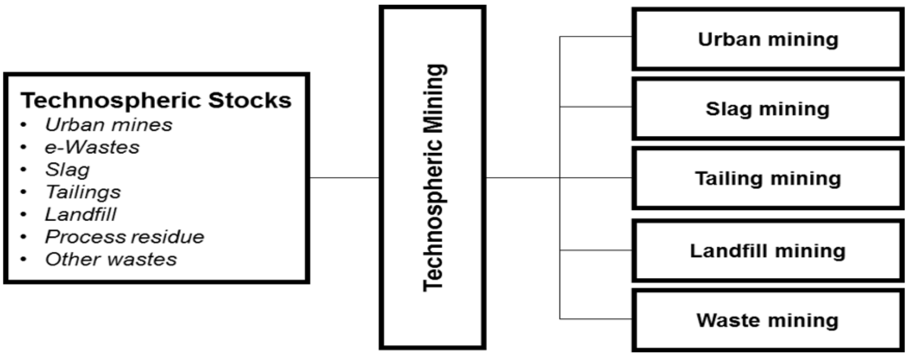

Figure 1. General concept of technospheric mining (adapted from N. Johansson et al., 2013).

Figure 2. Mine wastes are technospheric stocks and can be utilized for metal or mineral resource recovery as part of technospheric mining (photo: https://research.curtin.edu.au/industry-partners/invest/resources/mine-waste-management/)

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Central Mindanao University
Journal of Science

Non-CO_2 Greenhouse Gas Emissions from Field Burning of Crop Residues in the Philippines: 1990-2015

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ABSTRACT

About 32% of the Philippine land area is devoted for agriculture. Given this, the agriculture sector is expected to increasingly generate significant emissions of non-carbon dioxide (CO_2) greenhouse gases (GHG) and their precursors such as methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O) and nitrogen oxide (NO_x) from open burning of biomass residues from rice, corn and sugarcane farming. This study was undertaken to generate an updated yet specific inventory of contributions of provinces and regions to the total emissions of such radiative gases. Biomass residues from rice, corn and sugarcane were purposively sampled from the field and subjected to laboratory analyses to determine carbon fraction as well as carbon-nitrogen ratio values. Secondary data on annual crop production were obtained from the PSA while default values of emission factors were secured from the IPCC. Inventory of such non-CO_2 GHwG emissions covering all provinces of the Philippines were undertaken using the IPCC Tier 1 approach. Thematic maps were then generated through simple geographic information system. The study showed an average annual total emissions of 21 Gg, 550 Gg, 0.4 Gg and 15 Gg, respectively for CH_4, CO, N_2O and NO_x from 1990 to 2015 with CO constituting 94% of the emissions. Western Visayas was the top "hotspot" region with about ⅓ of the total emissions. Other "hotspot" regions include Central Luzon, Northern Mindanao and Cagayan Valley. Apart from providing technical and financial support to entice farmers to recycle crop residues, heightened monitoring and implementation of applicable environmental regulations must also be pursued to deter farmers from burning crop residues. Tax and non-tax incentives may be offered likewise to attract investors to put up processing plants utilizing crop residues primarily for power generation.

Keywords: crop residue, open burning, non-CO_2 emissions, inventory, philippines

INTRODUCTION

The total land area of the Philippines is about 30 million hectares (M ha) owwf which 9.67 M ha or about 32% is utilized for agriculture purposes (PSA, 2014). Of this, 51% and 44% were arable and permanent croplands, respectively. In 2014, palay production reached 18.97 million metric tons (MT) from a harvest area of 4.74 M ha (PSA, 2014). Significant production growths were noted in Central Luzon, Cagayan Valley, MIMAROPA, Northern Mindanao and Davao Region. In the same year, corn production reached 7.77 M MT from a harvest area of 2.61 M ha. The regions that contributed significant increment in production were Cagayan Valley, Western Visayas, ARMM, Davao Region and Northern Mindanao. On the other hand, sugarcane produced in 2013 was about 24.6 M MT harvested from an area of approximately 0.44 M ha. Expansion in harvested areas in the provinces of Kalinga, Isabela, Capiz, Cebu, Leyte and Bukidnon were observed also in 2013. Given this, the country's agriculture sector is expectedly generating every year significant amount of wastes called crop residues foremost of which are derived from rice, corn and sugarcane farming. Crop residues are the biomass left in the field after removing the economically important components such as grains and canes. It includes both the stubble left standing during the harvest process and the leaves and stems left over after threshing.

Based on estimates of Strehler and Stützle (1987), global annual crop residue production reached about 3.1 billion tons in which 60% originates from the developing world and the remaining 40% from the developed world. Specifically, sugar cane residues constitute about 11% of the world's

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agricultural waste (Crutzen & Andreae, 1990). In the Philippines, wetland rice cultivated under a moderate level of management produces 0.6 to 0.9 ton of straw per ton of grain (Ponnamperuma, 1984) while corn plantations generate 4,731.92 kg ha^{-1} of potential biomass residues (DAP, n.d.).

For various reasons, these crop residues are openly burnt in fields, which has long been a wide practice among farmers in the Philippines. Rice straw burning for one is not only a cost-effective method but it acts as an effective pest control procedure (Kadam et al., 2000; Dobermann & Fairhurst, 2002). It is also seen as a way of preparing the soil for the next crop as well as releasing nutrients contained in the residue for the next crop cycle (Gadde, et al., 2009). For sugarcane, Mendoza (2015) reported that non-burned canes slows down harvesting work by 40%. Farmers burn sugarcane trash (leaves and tops) to make harvesting easier and to prevent the trash from obstructing succeeding land operations. Besides, weedy fields are associated with the presence of snakes (Mendoza & Samson, 1999). On the other hand, post-harvest burning primarily get rid of remaining trash and tops that obstruct ratoon crop establishment or in preparing the land for new cane establishment.

Meanwhile, similar to that of rice, corn stovers are normally left in the field to dry up and decompose naturally while corn cobs are normally piled up outside processing plants after shelling for use as fuel and for other minor purposes. Only a few farmers practice burning of corn residues and it is difficult to measure how much of the corn residues are burned as no agency monitors this kind of activity. However, an estimated 489,000 tons of cobs are recoverable per year (Mendoza, 2006). The inadequacy of substitute uses for crop residues and the absence of suitable mechanization to manage increasing amounts of residue also led Asian farmers to burn crop residues more and more as a mode of disposal (Yadvinder-Singh et al., 2005 as cited in Bijay-Singh et al., 2008).

In Asia, it is estimated that 730 teragrams (Tg) are burned in a year of which 34% or 250 Tg originate from crop residue burning (Streets et al., 2003). Hao and Liu (1994) reported that the typical annual amounts of biomass burned in the Philippines is about 7.1 Tg. Based on estimates by the Industrial Technology Development Institute [ITDI, n.d.] of DOST, 250 kg of rice straw and 100 kg of rice hull are burned per ton of rice produced. This comes to a total of 5,073,880 tons of rice straw and rice hull burned every year. In terms of land area, 76% and 64% of rice and sugarcane lands, respectively are still burned (Launio et al., 2013; Mendoza & Samson, 2000).

However, the practice of field burning crop residues is usually manifested by uncontrolled and incomplete combustion of such residues which leads to the production of large amounts of greenhouse gases (GHG) such as carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4), nitrous oxide (N_2O) and nitrogen oxide (NO_x). CO_2 would not pose a serious concern though because the carbon (C) emitted into the atmosphere is re-sequestered by the vegetation during the succeeding growing season through photosynthesis. Hence, crop residue burning is not a net source of CO_2 (IPCC, 2006) and therefore need not be reported. Crop residue burning is, however, a net source of non-CO_2 and their precursors such as CH_4, N_2O, CO, and NO_x, which are released during combustion (Levine, 1995; IPCC, 2006). In fact, Romasanta et al. (2017) reported that burning of rice straw containing 10% moisture content emits 4.51 g of CH_4 and 0.069 g N_2O kg^{-1} dry weight of straw. This is equivalent to 10.04 kg of CH_4 ha^{-1} and 0.154 kg of N_2O ha^{-1} as averages for both dry and wet cropping seasons. Gadde et al. (2009) estimated that the burning of rice straw contributed 0.05%, 0.18%, and 0.56% of the total amount of greenhouse gas emissions in India, Thailand and the Philippines, respectively.

In the Second National Communication to the UNFCC, field burning of agricultural residues was reported to generate 24.42 Gg of CH_4, 512.74 Gg of CO, 0.6 Gg of N_2O and 21.56 Gg of NO_x during the year 2000 Philippine GHG inventory (DENR, n.d.; UNDP, 2011). This translates to a total of 699 Gg CO_{2e} and accounts two percent of the total agriculture GHG emissions. The year 2000 inventory would appear to have increased by 16% from the 1994 inventory, which reported a total of 581 Gg of CO_{2e} from field residue burning (DENR, 1999).

Little work has been done on the emission inventories and emission allocations of non-CO_2 GHG and their precursors from the burning of major agricultural crop residues from rice, corn, and sugarcane crops in the Philippines. In addition, these inventories were all national in scope which means that specific contributions of provinces and regions to the total national emissions were not assessed in details. Considering that its economy heavily relies on agriculture coupled with a growing population, the country needs to progressively increase agricultural productivity. As such, it is expected to increasingly generate large quantities of agricultural wastes especially from rice, corn and sugarcane farms. This would in turn result to an increase in GHG emissions. Although the Philippines contributes only a small fraction (0.27%) of the global GHG emissions (IPCC, 2007), it is still important to find ways on how to reduce emissions. Undertaking an updated and detailed

Figure 1. Field collection of rice straw samples for laboratory analysis

inventory of non-CO_2 GHG and their precursors at the regional and provincial levels could be used particularly by policymakers in national agencies like the Department of Environment and Natural Resources (DENR) and the Department of Agriculture (DA) as well as at the provincial and municipal/city government levels as basis in setting research, policy and development directions.

This study was undertaken to generate a national, regional and province-wise data on the amount of non-CO_2 GHG emissions generated from field burning of rice, corn and sugarcane residues. Specifically, this study intended to: (1) determine the "hot spot" regions and provinces in the Philippines in terms of crop residue burning, (2) propose practical measures or options to "cool" these identified hot spots and to recommend policy options to address the environmental impacts and implications resulting from the nagging practice of open burning of crop residues among farmers in the country.

METHODOLOGY

Sources of Data

This study heavily utilized secondary data from various sources. Data on region-wise annual crop production from 1990-2015 for rice and corn, and from 1990-2014 for sugarcane were obtained from the database of the Philippine Statistics Authority (PSA). Due to the unavailability of country-specific factors, other necessary data including default values of emission factors were secured from the 1996 IPCC guidelines. These consisted of values fro the following: residue to crop ratio, dry matter fraction, fraction burned in fields, fraction oxidized, and emission ration.

However, carbon fraction as well as carbon-nitrogen ratio of various crop residues were obtained through actual laboratory analysis. Seven samples per crop residue type of rice, corn and sugarcane were taken from 21 purposively selected farms (Figure 1). For practical reasons, only farms in Bukidnon were considered for sampling, which was done from November 2015 to April 2016. These samples were then brought to the laboratory at Soil and Plant Analysis Laboratory in Central Mindanao University (CMU), Musuan, Bukidnon for analysis.

Calculating Non-CO_2 Emissions from Crop Residue Field Burning

Inventory of non-CO_2 GHG emissions (such as CH_4, CO, N_2O and NO_x) covering all regions of the Philippines were undertaken using the IPCC Tier 1 method. The Tier 2 approach or higher could not be used as the Philippines has not developed yet its own country-specific default emission factors for crop residues that are openly burnt. The method involved first the estimation of total carbon released using the following equation:

$$\begin{aligned}&\textit{Total carbon released (tonnes of carbon)} = \\ &\sum_{\textit{all crop types}} \textit{annual production (tonnes of biomass per year)}, \\ &\times \textit{ the ratio of residue to crop product (fraction)}, \\ &\times \textit{ the average dry matter fraction of residue (tonnes of dry matter per tonne of biomass)}, \\ &\times \textit{ the fraction actually burned in the field}, \\ &\times \textit{ the fraction oxidized}, \\ &\times \textit{ the carbon fraction (tonnes of carbon per tonne of dry matter)}\end{aligned}$$

Values on total carbon released were then used to estimate the following:

CH_4 emissions = (carbon released)x (emission ratio)x16/12
CO emissions = (carbon released)x(emission ratio)x28/12
N_2O emissions = (carbon released)x(N/C ratio)x(emission ratio)x 44/28
NO_x emissions = (carbon released)x(N/C ratio)x(emission ratio)x 46/14

The coefficients used in the inventory as well as primary data gathered in the conduct of the study

Table 1. Coefficients used in the inventory.

Crop	Residue to Crop Ratio[a]	Dry Matter Fraction[a]	Fraction Burned in Fields[a]	Fraction Oxi-dized[a]	Carbon Fraction[b]	Nitrogen-Car-bon Ratio[b]	Emission Ratio[a]
Dry Season Rice	1.4	0.4	0.59	0.9	0.433	0.11	0.004 for CH_4; 0.06 for CO; 0.007 for N_2O; and 0.121 for NO
Wet Season Rice	1.4	0.83	0.12	0.7	0.433	0.11	
Corn	1.0	0.4	0.25	0.9	0.5264	0.12	
Sugarcane	0.8	0.9	0.25	0.9	0.5224	0.007	

[a]Source: IPCC, 1996; [b]Primary data

are presented in Table 1.

GIS Map Generation

Data on regional and provincial emissions of non-CO_2 GHG emissions were utilized in generating geographic information system (GIS)-based maps. The process required the utilization of GIS application software with base maps sourced from PhilGIS. ArcMap ver. 10.1 was used to generate the final spatial maps. Such maps were used to identify the so-called "hotspot" regions and provinces in the country. The term "hotspots" here refers to the Top 5 and 10 regions and provinces, respectively, in terms of computed mean annual emissions of non-CO_2 greenhouse gases.

Statistical Analysis

All data generated in this study were analyzed using descriptive statistics. This included trend analysis of regional variations of emissions of non-CO_2 GHG in the country.

RESULTS AMD DISCUSSION

The Volume of Crop Production, and Residue

Table 2. Region-wise estimated annual amounts of agricultural crop residue and those burned in the field, 1990-2015.

Ratio	Annual volume of Production, Gg			Annual Volume of Agricultural Crop Residue, Gg			Annual Agricultural Crop Residue Burned in the Field, Gg		
	Rice	Corn	Sugar-cane	Rice	Corn	Sugar-cane	Rice	Corn	Sugar-cane
CAR	309.1	115.9	11.7	173.1	46.3	8.4	102.1	11.6	2.1
Ilocos Region	1284.1	251.9	20.4	719.1	100.8	14.7	424.3	25.2	3.7
Cagayan Valley	1736.9	1043.4	218.7	972.7	417.4	157.4	573.9	104.3	39.4
Central Luzon	2414.5	131.0	1358.6	1352.1	52.4	978.2	797.7	13.1	244.6
CALABARZON	396.6	64.6	1953.0	222.1	25.8	1406.1	131.0	6.5	351.5
MIMAROPA	778.1	77.2	0	435.7	30.9	0	257.1	7.7	0
Bicol Region	865.0	145.1	243.0	484.4	58.0	174.9	285.8	14.5	43.7
Western Visayas	1698.9	172.3	12751.2	951.4	68.9	9180.9	561.3	17.2	2295.2
Central Visayas	242.5	176.5	2170.5	135.8	70.6	1562.8	80.1	17.6	390.7
Eastern Visayas	676.9	84.0	556.7	379.1	33.6	400.9	223.7	8.4	100.2
Zamboanga Peninsula	474.2	192.5	0.4	265.5	77.0	0.3	156.7	19.2	0.1
Northern Mindanao	514.2	911.5	2494.3	287.9	364.6	1795.9	169.9	91.2	449.0
Davao Region	415.9	215.0	430.9	232.9	86.0	310.2	137.4	21.5	77.6
SOCCKSARGEN	1002.6	1178.0	639.2	561.5	471.2	460.3	331.3	117.8	115.1
CARAGA	363.4	84.0	0	203.5	33.6	0.0	120.1	8.4	0.0
ARRM	421.0	668.8	55.2	235.7	267.5	39.7	139.1	66.9	9.9
TOTAL	13593.8	5511.8	22753.5	7612.5	2204.7	16382.5	4491.4	551.2	4095.6

Source: Philippine Statistics Authority
1 gigagram (Gg) = 1,000 metric tons

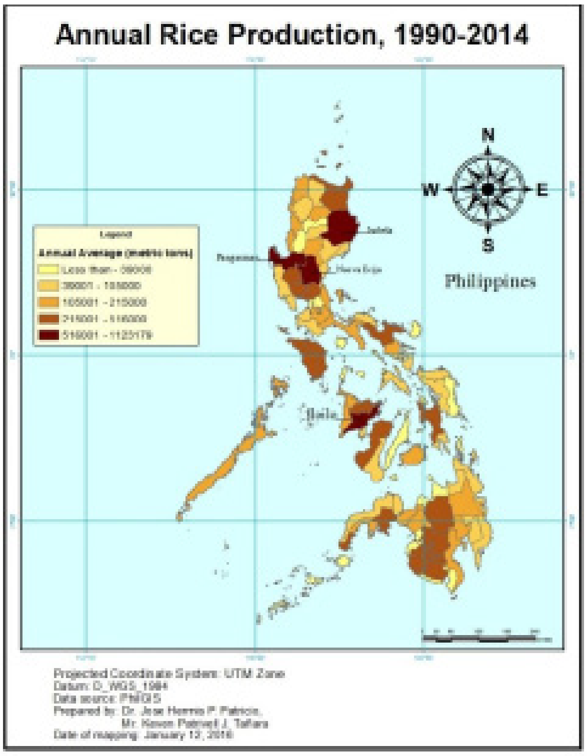

Figure 2. Mean annual rice production in the Philippines
Data sources: PSA, 2016 & PhilGIS, nd

Generation and Burned in the Field

The volume of rice production in the Philippines posted an annual mean of about 13.6 M MT (13,600 Gg) from 1990 to 2015 with Central Luzon, Cagayan Valley and Western Visayas regions topping the list (Table 2). Production of this crop yielded a 94.8% increase during this 25-year period. The most notable increases in yield are recorded in the provinces of Isabela, Pangasinan, Nueva Ecija, and Iloilo (Figure 2). This is primarily attributed to expansion in production areas, utilization of high yielding varieties, availability of irrigation water, and improved fertilizer and pest management (PSA, 2016).

Corn production at around 7,500 Gg in 2015 was higher by 35.4% as compared to 1990. Annually, the corn subsector yielded about 5,500 Gg. The corn-producing regions include

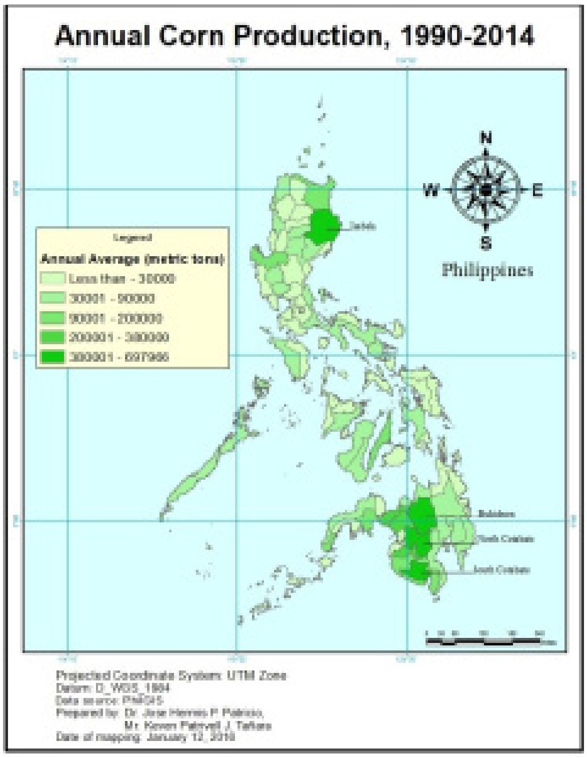

Figure 3. Mean annual corn production in the Philippines.
Data sources: PSA, 2016 & PhilGIS, nd

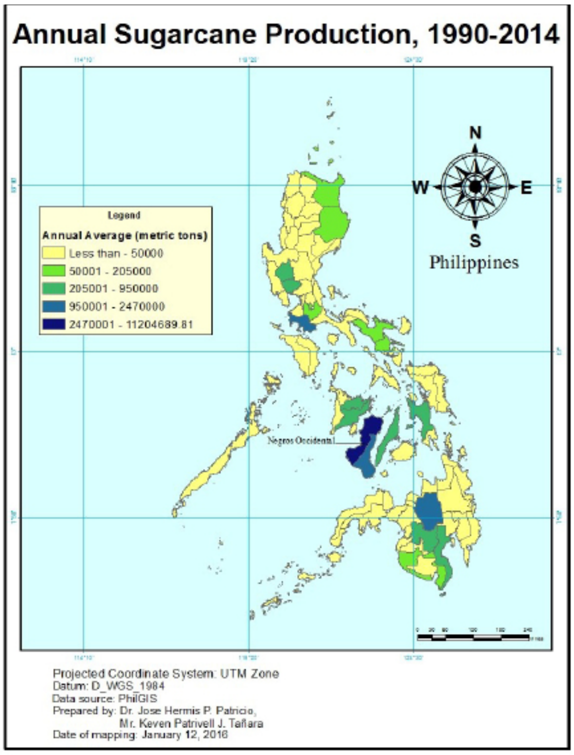

Figure 4. Mean annual sugarcane production in the Philippines.
Data sources: PSA, 2016 & PhilGIS, nd

SOCCSKSARGEN, Cagayan Valley and Northern Mindanao especially in the provinces of North and South Cotabato, Isabela, and Bukidnon as shown in Figure 3. PSA (2016) reported that increase in corn harvests was associated to sustained use of hybrid and open pollinated varieties of seeds, and favorable weather condition.

On the other hand, sugarcane production in the country rose by 22.8% from 1990 to 2015 at an annual rate of about 22,700 Gg as shown also in Table 2. The Western Visayas region had the greatest particularly the provinces of Negros Occidental and Negros Oriental (Figure 4). Other provinces like Bukidnon and Batangas also showed remarkable increases. Increase in production areas and efficient usage of fertilizer were among the reasons cited by PSA (2016).

Because of increased volume of production of these three primary agricultural crops, volume of crop residue generated and burnt consequently increased. Based on estimates of Strehler and Stützle (1987), global annual crop residue production reached about 3.1 billion tons in which 60% originates from the developing world and the remaining 40% from the developed world. This is out of the 140 billion MT of biomass that is generated globally every year from agriculture (UNEP, 2009). Annually, 7.1 Tg of crop residue is burned openly in the Philippines as reported by Streets et al. (2003).

Specifically, sugarcane residues constitute about 11% of the world's agricultural waste (Crutzen & Andreae, 1990). In the Philippines, it is estimated that about 64% of the sugarcane fields are burned before or after harvesting (Mendoza & Samson, 1999). Traditional sugarcane farmers openly burn large volumes of the crop's trash consisting of leaves and tops to hasten harvesting and the succeeding land operations. As shown in Table 2 earlier and Figure 5, sugarcane residue generation and burned in fields in the Philippines is about 16,400 Gg and 4,100 Gg, respectively or a contribution of 63% of the total in the 1990-2015 period. In terms of area planted, sugarcane is listed in the top five agricultural crops. In fact, its production area surged by 79.1%, that is, from 235,269 ha in 1990 to 421,312 in 2015 (PSA, 2016). Production is mainly focused in Western Visayas, Northern Mindanao, Southern Tagalog, and Central Luzon.

Rice is the second greatest residue-generating crop at 29%. For the assessment period, rice residue generated is on the average of about 7,600 Gg while the one burned is around 4,500 Gg. Malaysia produced around 8,750 Gg of rice straw in 2007 (John, 2013). Large volumes of straw are mostly burned during the dry season except in the northern parts of the Philippines where it is utilized as chief feed source for animals and as mulching material for vegetable crops (Villarin, et al. 1999). Rice as a residue source is expected to continuously surge given that the area utilized

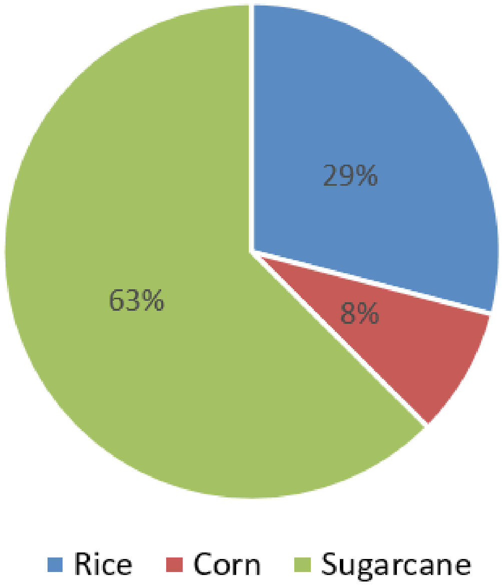

Figure 5. Contribution of different agricultural crops in residue generation.

in growing this crop globally is predicted to rise by 4.5% by 2030 (FAO, 2003). In the Philippines, wetland rice cultivated under a moderate level of management produced between 0.6 and 0.9 tonnes of straw per tonne of grain (Ponnamperuma, 1984). Mendoza and Samson (1999) estimated that about 90% of Filipino rice farmers simply burn their rice straw. Globally, emissions from rice production and burning of biomass were heavily concentrated in the group of developing countries, with 97% and 92% of world totals, respectively (Smith et al., 2007). Street et al. (2003) reported that in Asia, around 730 Tg of biomass are burned per year from both anthropogenic and natural sources.

Meanwhile, DAP (n.d.) reported that the Philippines produces 4,731.92 kg ha^{-1} of potential biomass residues from corn plantation. In this present study, corn residues in the form of stovers and cobs generated the least amount at 8% of the total volume of crop residues generated in the country annually from 1990-2015. On the average, about 2,200 Gg of corn residues were generated and 550 Gg were burned during this period. Villarin *et al.* (1999) reported that only a few farmers practice burning of corn residues which come mostly from white and yellow corn varieties. Instead of burning, most farmers just normally leave the stovers in the field to naturally decompose while corn cobs are usually piled up outside the milling plants after shelling for use primarily as fuel (Ramat, 2007).

Emissions of Non-CO_2 Greenhouse Gases

Crop residue burning is assumed to be a non-net source of CO_2 because when this gas is released into the atmosphere, it is reabsorbed in the following crop growing season. Nevertheless, burning process does not only emit CO_2 but other gases or precursors of greenhouse gases as well

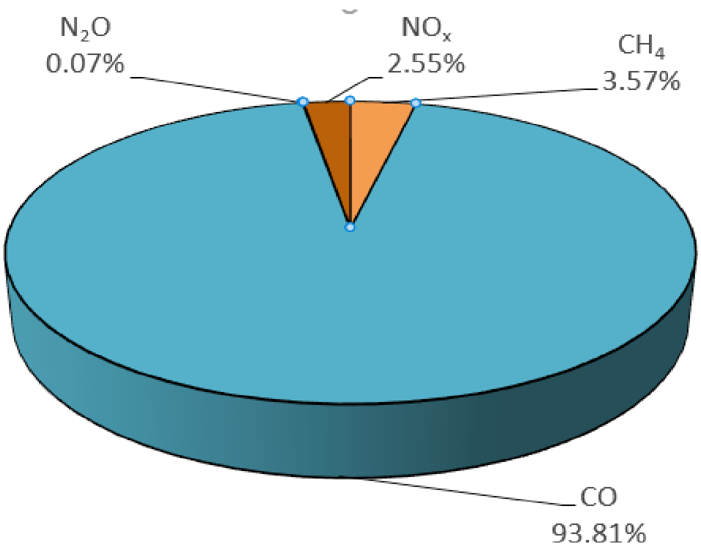

Figure 6. Percentage distribution of non-CO_2 emissions from crop residue burning

Table 3. Mean annual emissions of non-CO_2 greenhouse gases from various regions in the Philippines, 1990-2015.

Region	Emissions, Gg yr[-1]			
	CH_4	CO	N_2O	NO_x
CAR	0.2	6.5	0.01	0.2
Ilocos Region	1.0	25.0	0.02	0.9
Cagayan Valley	1.6	40.8	0.04	1.4
Central Luzon	2.3	60.4	0.05	1.8
CALABARZON	1.2	30.7	0.02	0.7
MIMAROPA	0.6	14.5	0.01	0.5
Bicol Region	0.7	19.4	0.02	0.6
Western Visayas	6.9	182.6	0.11	4.1
Central Visayas	1.2	31.2	0.02	0.7
Eastern Visayas	0.7	19.3	0.02	0.6
Zamboanga Peninsula	0.4	9.8	0.01	0.3
Northern Mindanao	1.7	44.8	0.03	1.1
Davao Region	0.5	14.0	0.01	0.4
SOCCSKSARGEN	1.3	33.4	0.03	1.0
CARAGA	0.3	7.1	0.01	0.2
ARRM	0.5	12.7	0.01	0.4
TOTAL	20.9	550.4	0.41	15.0

that arise from partial combustion of the fuel. Such farming practice therefore is an important source of carbon monoxide (CO), methane (CH_4), and nitrogen (e.g., N_2O, NO_x) species (Levine, 1994). The present study revealed that around 94% of the total amount of non-CO_2 greenhouse gases emitted through crop residue burning in the Philippines is CO (Figure 6). The remaining six percent consists of CH_4, NO_x, and N_2O emissions.

Annually, around 550 Gg of CO, 21 Gg of CH_4, 15 Gg of NOx, and 0.4 Gg of N_2O are produced in the country due to crop residue burning (Table 3). This is comparable to the amount of emissions generated for the year 2000 wherein the Philippines emitted 512.74, 24.42, 21.56 and 0.60 Gg of CH_4, CO, N_2O, and NO_x, respectively (UNDP, 2011). One-third of the emissions originate from the Western Visayas region followed by Central Luzon (11%) and Northern Mindanao (8%) as presented in Figure 7.

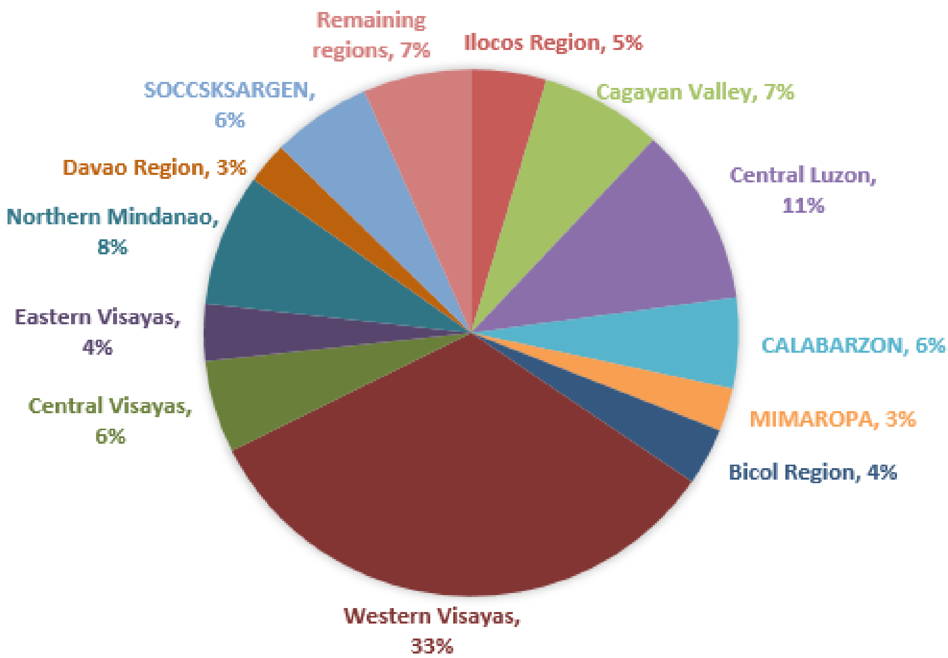

Figure 7. Regional percentage contribution to non-CO_2 emissions from crop residue burning, 1990-2015.

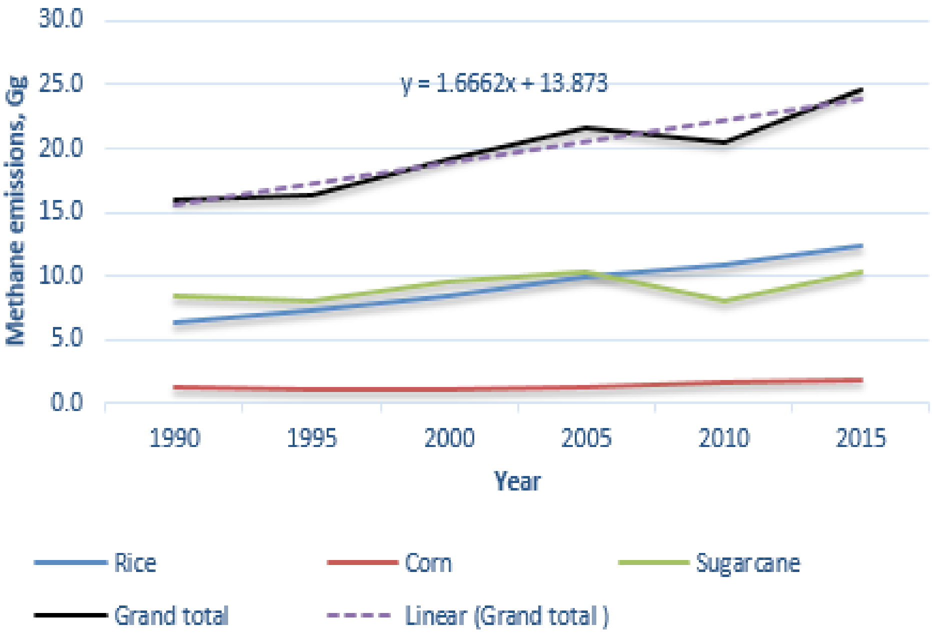

Figure 8. Methane (CH_4) emissions from crop residue burning, 1990-2015.

CAR, CARAGA, ARRM, and Zamboanga Peninsula are the regions with the least emissions from crop residue burning. Gadde et al., (2009) estimated that the burning of rice straw alone contributed 0.05%, 0.18%, and 0.56% of the total amount of greenhouse gas emissions in India, Thailand and the Philippines, respectively.

CH_4 is one of the most potent greenhouse gases with a global warming potential (GWP) 21 times greater than that of CO_2. In 2005, it was estimated that the agriculture sector contributed 33 $GtCO_{2e}$ of CH_4 per year (Smith et al., 2007). Agriculture accounts around 50% of this gas of the global anthropogenic emissions in 2005. In this study, CH_4 emissions in the country indicates an increasing trend (y = 1.6662x + 13.873). In fact, it surged by about 54% between 1990 (16

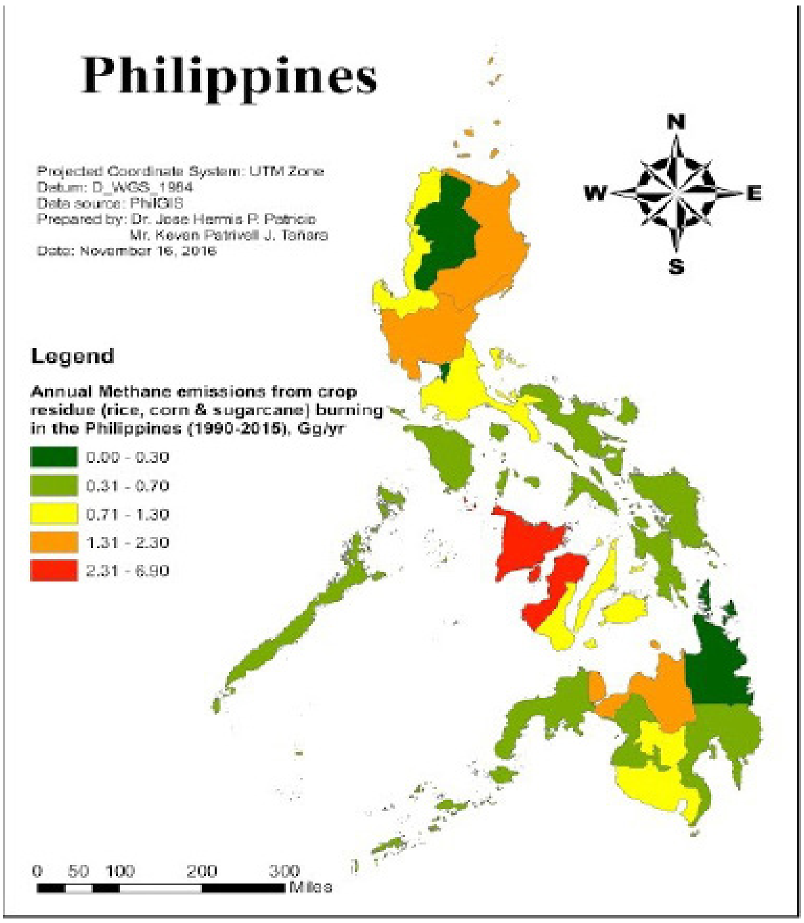

Figure 9. Provincial distribution of methane emissions from crop residue burning, 1990-2015 (Gg yr-1).

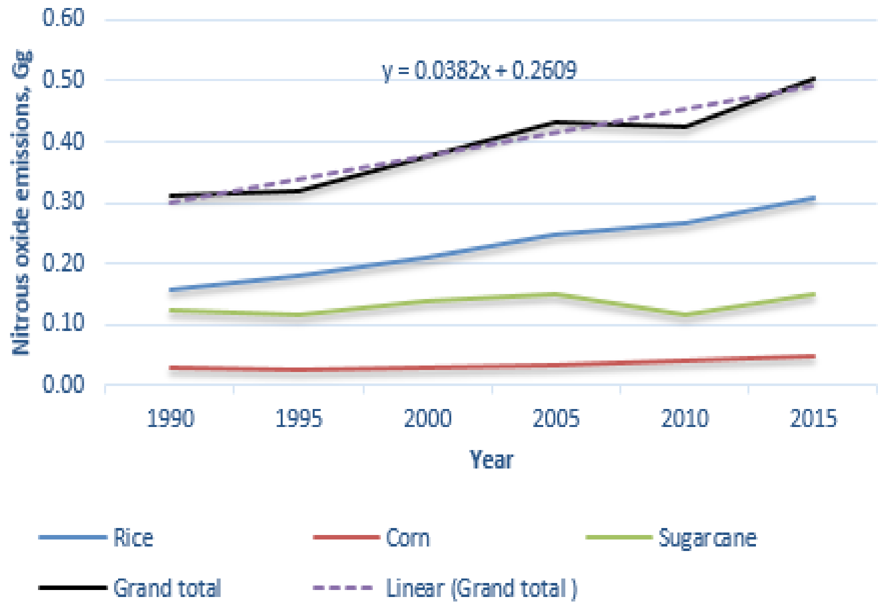

Figure 10. Carbon monoxide (CO) emissions from crop residue burning, 1990-2015.

Gg) and 2015 (24.6 Gg) as shown in Figure 8. Rice and sugarcane are the greatest contributors to CH_4 emissions in 2015 at 12.4 Gg and 10.32 Gg, respectively. Considered "hotspot" areas are the provinces of Western Visayas primarily Negros Occidental and Iloilo (Figure 9). This is primarily associated with the high volume of sugarcane production in the area.

Like CH_4, CO emissions from crop residue burning in the Philippines is moving on an upward trend (y = 43.785x + 364.57) as shown in Figure 10. CO in itself is not a greenhouse gas but a precursor in the formation of CO_2 which is the most abundant heat-trapping gas in the atmosphere causing global warming. Gupta et al. (2004) reported that a tonne of rice straw burned would produce 60 kg of CO. In this present study, CO emissions posted an

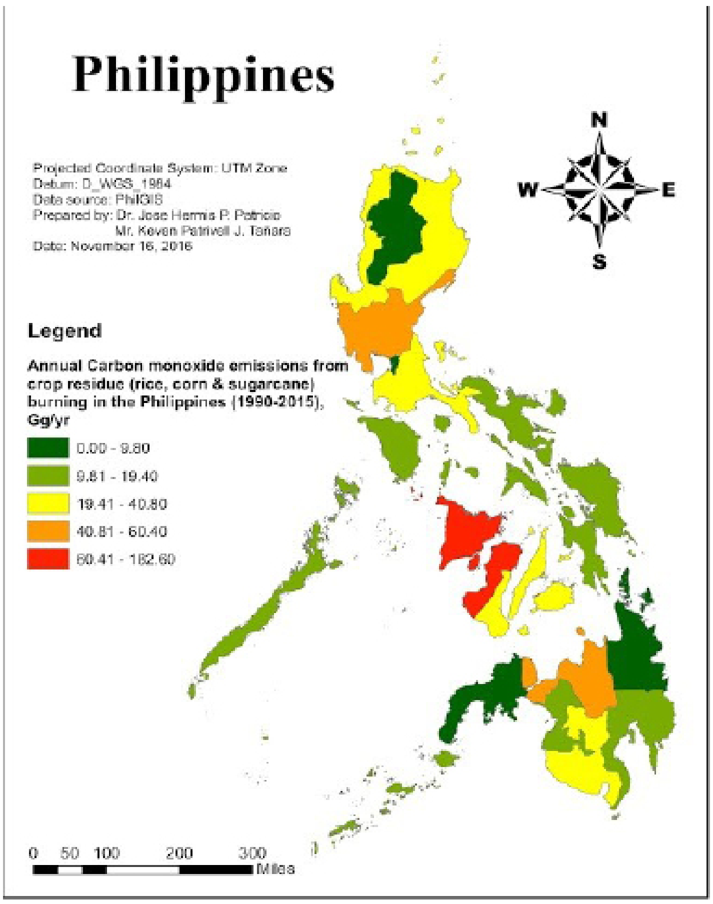

Figure 11. Provincial distribution of carbon monoxide emissions from crop residue burning, 1990-2015 (Gg yr^{-1}).

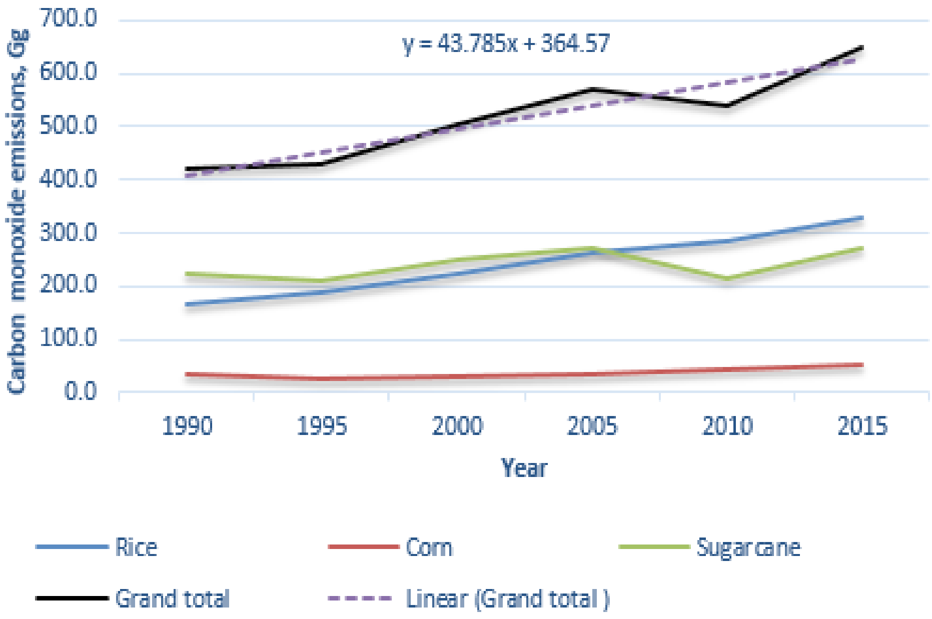

Figure 12. Nitrous oxide (N_2O) emissions from crop residue burning, 1990-2015.

increase of 54% between 1990-2015 at an average of 550 Gg yr-1. Similar to CH_4, emissions of this gas are concentrated as well in the Western Visayas region at a range of 60-183 Gg yr-1 (Figure 11).

Meanwhile, N_2O is a long-lasting, powerful GHG with GWP of 310 times to that of CO_2. Like CH_4, N_2O emissions from crop residue burning are greatly lower than CO_2. Nevertheless, the very large GWP makes N_2O a chief contributor to climate change. In this study, total N_2O emissions from crop residue burning in the country recorded an increase of 67% from 1990 to 2015 (Figure 12). With an average of 0.41 Gg yr-1, most of the N_2O emissions came from burning of rice straws and sugarcane particularly from Western Visayas, Central Luzon and Cagayan Valley regions as presented in Figure 13. The Western Visayas provinces generated an annual mean 0.6-0.11 Gg of N_2O.

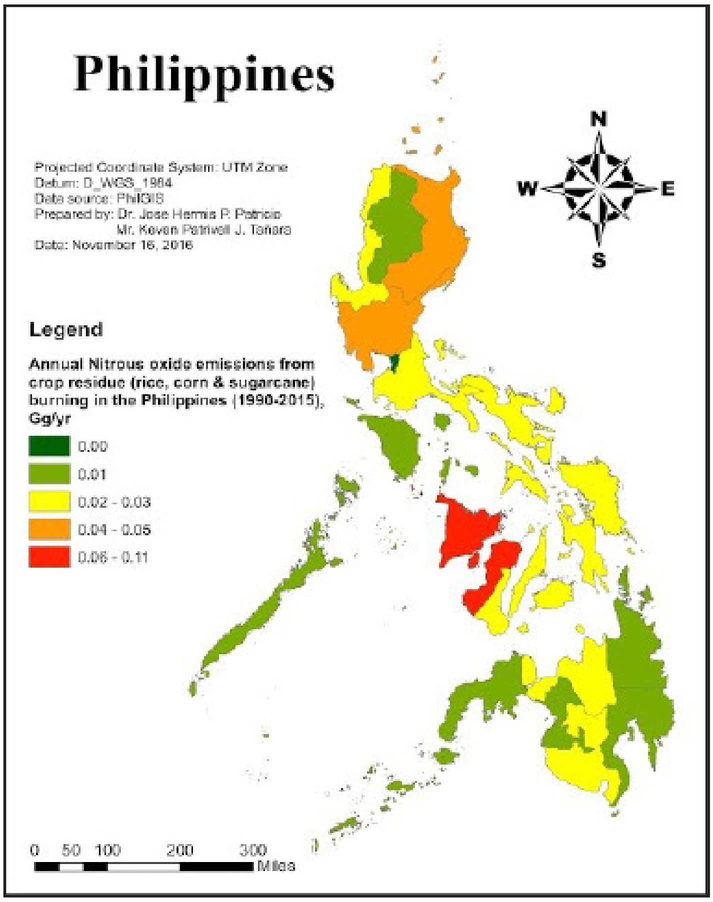

Figure 13. Provincial distribution of nitrous oxide emissions from crop residue burning, 1990-2015 (Gg yr-1).

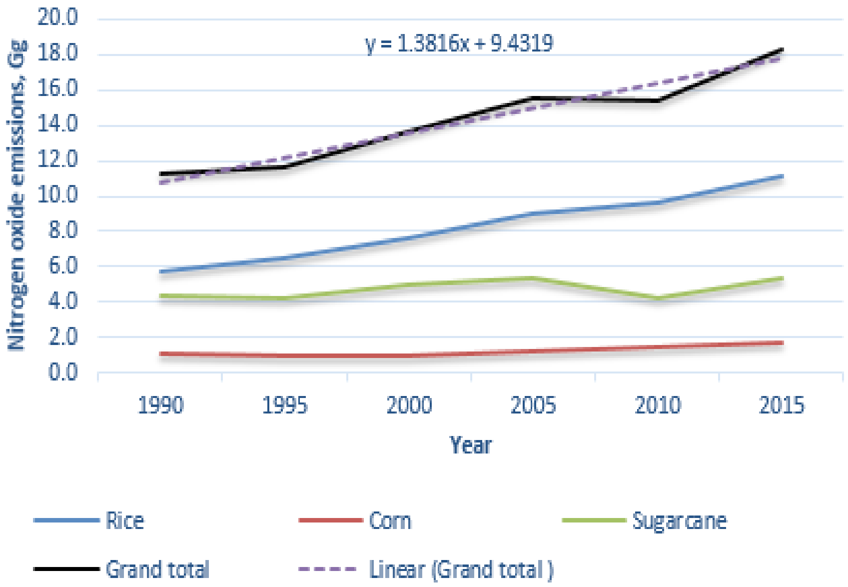

Figure 14. Nitrogen oxide (NO_x) emissions from crop residue burning, 1990-2015.

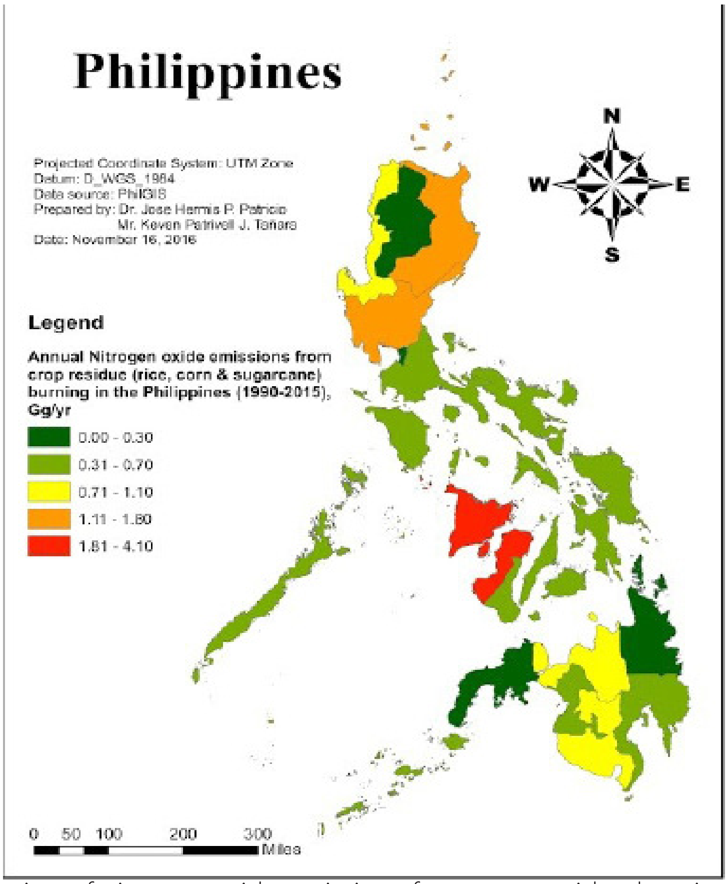

Figure 15. Provincial distribution of nitrogen oxide emissions from crop residue burning, 1990-2015 (Gg yr^{-1}).

Finally, emissions of NO_x in the country during the assessment period rose by more than 60% from 1990-2015, that is from 11.2 to 18.2 Gg per year (Figure 14). Similar to other gases, NO_x are emitted mostly from the following hotspot regions: Western Visayas, Central Luzon, Cagayan Valley, Northern Mindanao, SOCCSKSARGEN and the Ilocos Region (Figure 15). NO_x is also an important greenhouse gas that lasts 166 years in the atmosphere with a GWP of 296 times compared to CO_2 (IPCC, 2006).

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the study, the following conclusions are drawn:

1. There is an upward trend in the volume of crop residue generated and burned in the Philippines from 1990 to 2015 due to increased volume of production of rice, sugarcane and corn which is attributed to

expansion in production areas, utilization of high yielding varieties, availability of irrigation water, and improved fertilizer and pest management;
2. Sugarcane generated about 63% of the total volume of crop residue produced annually while rice and corn contributed 29% and 8%, respectively;

3. There is likewise an observed upward trend in the mean annual emissions of GHG in the Philippines from crop residue burning from 1990 to 2015 which are estimated at 21 Gg CH_4, 550 Gg CO, 0.4 Gg N_2O and 15 Gg NO_x, with CO constituting 94% of the total emissions; and

4. There is likewise an observed upward trend in the mean annual emissions of CH_4, CO, N_2O and NO_x in the Philippines from crop residue burning from 1990 to 2015 which are estimated at 21 Gg, 550 Gg, 0.4 Gg and 15 Gg, respectively with CO constituting 94% of the total emissions; and

5. Western Visayas, Central Luzon, Ilocos Region, Northern Mindanao and Cagayan Valley are considered "hotspot" regions particularly the provinces of Negros Occidental and Iloilo. However, the provinces of Isabela, Pangasinan, Nueva Ecija, North and South Cotabato, Tarlac, Cagayan, Leyte, Camarines Sur and Bukidnon are becoming "hotspot" areas as well based on 2015 data.

Based on the foregoing, the following recommendations are forwarded:

1. Future local and international research networking and collaboration need to be established to find much better sustainable technologies and overcoming logistical constraints in harnessing potential renewable energy from crop biomass residues such as production of biofuel which is the conversion of biomass to liquid fuel or as source of biopower which involves the conversion of biomass to electricity;

2. The government should consider creating investment opportunities such as provision of tax and non-tax incentives to locals and foreigners who wish to invest in processing plants that utilize crop residues particularly for power generation. This is in consonance with Republic Act 9136 (Electric Power Industry Reform Act of 2001) and Republic Act 9367 (Biofules Act of 2006). Tax incentives may include among others income tax holiday for a certain period of operation of the processing plant, and reasonable tax and duties exemptions on equipment, accessories, spare parts, and machinery. Non tax incentives may come in the form of simplified procedures in the equipment import and hiring of foreign nationals particularly for highly technical positions;

3. In tandem with industry leaders, chief executives in provinces located in "hotspot" regions such as in Western Visayas should encourage and provide the necessary policy, technical and financial support to sugarcane farmers to engage in ecological sugarcane farming where residues are conserved in the field and allowed to decompose, and where biological nitrogen-fixing sugarcane cultivars are utilized;
4. The DENR, DA and concerned local government units must pursue aggressive information, education and communication campaigns targeting farming communities to heighten their awareness on the negative environmental and health impacts associated with open burning crop residues. This should be pursued together with heightened monitoring and implementation of environmental regulations prohibiting open residue burning; and

5. There is a need to come up with more precise estimates on non-CO_2 greenhouse gas emissions from crop residue burning using the Tiers 2 and 3 methodologies of IPCC.

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Litterfall Production and Turnover in the Longer Term Ecological Research (LTER) Sites in Southern Philippines

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ABSTRACT

Forest litter is an important aspect for healthy forest ecosystems. Hence, this study monitored the litterfall production and turnover in the established two-hectare permanent plots in the three Long Term Ecological Research (LTER) Sites in Mindanao, *viz*., Mt. Apo, Mt. Hamiguitan and Mt. Musuan. Litter samples were collected from 2w0 installed traps at each site and consequently sorted to components, processed and oven-dried. Data revealed that the estimated annual litter production was highest in Mt. Musuan with 11211.5 kg ha^{-1}, followed by Mt. Apo with 6648.0 kg ha^{-1} and Mt. Hamiguitan with 4919.5 kg ha^{-1}. *Agathis philippinensis* contributed the highest litterfall production in Mt. Apo, whereas *Barringtonia racemosa* and *Artocarpus blancoi* in Mt. Hamiguitan and Mt. Musuan, respectively. Highest litter turnover was observed in Mt. Musuan (5.35%/day). Litterfall production showed to have a positive correlation with temperature for Mt. Hamiguitan and Mt. Musuan. Among the studied sites, Mt. Musuan had the highest forest primary production. Results of this study support previous reports that lowland forests have high forest productivity. Further, there is a need to plant Philippine endemic and threatened trees, such as A. *philippinensis* since these prove to have high productivity as in the case of Mt. Apo and Mt. Hamiguitan.

Keywords: Mt. Apo, Mt. Hamiguitan, Mt. Musuan, oven-dry weight, tropical forests

INTRODUCTION

Forest litterfall refers to the plant materials such as fallen leaves, branches, twigs, flowers, fruits, seeds and root parts which are shed off from trees and the amount of litterfall may be indicative of the health status and the level of forest productivity (Clark et al., 2011). Almost 80% of the net primary production is supplied back to the ecosystem by means of litterfall (Meentemeyer et al., 1982; Kassnacht & Gower, 1997). The amount and quality of litter also provide information about the dynamics of nutrient cycling in which foliar litter constitutes the major proportion of ground litter (Celentano et al., 2011). The nutrient content in litterfall gives the functional state of the forest that can be used to improve its management and production (Higuera & Martinez, 2006).

The production and amount of litterfall is affected by seasonal fluctuations, which are regulated by biological and climatological processes and factors such as topography, edaphic condition, vegetal species, age and forest density (Higuera & Martinez, 2006). Since forests are climatic formation, the amount of litterfall can be used to indicate change of climate. It has become an important parameter recently in monitoring global climate change in addition to the plant flowering and foliation used for phenological observation (Hansen et al., 2009). Tropical forests tend to sustain the ecosystem processes but the fluctuating climatic conditions relatively affect the primary productivity of the forest (Celentano et al., 2011).

The establishment of the Mindanao Long Term Ecological Research (LTER) Sites is in response to the global campaign to move towards a more sustainable world in which the health of the ecosystem and human well-beings are improved and that its ecosystem services can serve the current and future generations. Assessments and biodiversity studies on the flora and fauna of Mindanao LTER Sites were conducted. Further, forest litterfall production in these LTER Sites was considered an important aspect to look into for it provides insights on the nutrient cycling process in this ecosystem. Among the five LTER Sites in Mindanao, three of which have been monitored in 2012–2013 and 2015. The three LTER Sites viz., Mt. Apo, Mt. Hamiguitan and Mt. Musuan were monitored in 2016–2017. This is necessary in order

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to monitor and obtain reliable data on the litterfall production and turnover of said sites. Data from this research serves as baseline information regarding the litterfall production in these sites. Hence, this study was conducted to obtain additional data on its third year from the established litter traps of these mountains.

METHODOLOGY

Study sites

The establishment of the two-hectare permanent plot in Mt. Apo (MAP), Mt. Hamiguitan (MHP) and Mt. Musuan (MMP) was based on the abundance of tree specis in the sites. The MAP is situated in upper montane vegetation, whereas MHP and MMP are located in lower montane and in lowland mixed dipterocarp/agroecosystem, respectively. Distribution of the litter traps were based on the location of the identified dominant tree species, in which MAP is characterized to have evergreen species in the forest which means that their leaves shed off not at the same time, but gradually, making the forests to remain green throughout the year and this include the gymnosperm species. MMP on the other hand, has tree species which are deciduous, which means that their leaves shed off at the same time leaving the tree temporarily bare for a certain time of the year. Deciduous species tend to give higher litter production to the forest ecosystem. The selected dominant and co-dominant tree species are shown in Table 1. Among the sites, MAP has the highest elevation (1,944 masl), followed by MHP (1,044 masl) and MMP (388 masl).

Table 1: Selected dominant and co-dominant tree species tagged for litter collection in the three Mindanao LTER Sites (Quimpang et al., 2013, 2015, 2017).

Dominant Tree Species	Local Name	Family	Mindanao LTER Sites		
			MAP	MHA	MMU
1. *Agathis philippinensis* Warb	Almaciga	Araucariaceae	/	/	
2. *Alstonia scholaris* (L.) R. Br.	Dita	Apocynaceae			/
3. *Artocarpus blancoi* (Elmer) Merr.	Antipolo	Moraceae			/
4. *Barringtonia racemosa* (L.) Merr. ex DC	Malagubat	Lecythidaceae		/	
5. *Calophyllum blancoi* Planch & Triana	Bitanghol	Calophyllaceae		/	
6. *Cinnamomum mercadoi* S. Vidal	Kalingag	Lauraceae	/		
7. *Kleinhovia hospita* L.	Tan-ag	Malvaceae			/
8. *Lithocarpus apoensis (Elmer)* Rehder	Uwayan	Fagaceae	/		
9. *Melanolepis multiglandulosa (*Reinw. ex Blume) Rch. & Zoll	Alim	Euphorbiaceae			/
10. *Palaquium sp.*	Nato	Sapotaceae		/	
11. *Phyllocladus hypophyllus* Hook.f.	Tungog	Podocarpaceae	/		
12. *Senna spectabilis (D.C)* H.S. Irwin & Barnebt	Ansuan-dilaw	Leguminoseae			/
13. *Shorea polysperma* Merr.	Tangile	Dipterocarpaceae		/	
14. *Syzygium hulchinsonii* (Merr. ex C.B.Rob.) Merr.	Malatambis	Myrtaceae	/		

Lengend: (/)-Present MAP-Mt. Apo Plot MHA-Mt. Hamiguitan Plot MMP-Mt. Musuan Plot

Table 2: Forest Characteristics of the three Mindanao LTER Sites (Quimpang et al., 2013, 2015, 2017).

LTER Sites	Province[1]	Elevation (masl)[2]	Climate Type[3]	Annual Rainfall (mm)	Average Temperature[4]	Relative Humidity (%)[4]	Coordinates	Vegatation Type
Mt. Apo	North Cotabato	1,944	IV	1695	16°	90.57	6°59'47.05"N 125°15'12.18"E	Upper Montane
Mt. Hamiguitan	Davao Oriental	1,044	IV	1679	20°	95.13	6°43'58.02"N 126°9'58.32"E	Mid-montane
Mt. Musuan	Bukidnon	388	III	1887	24°	91.38	7°52'56.58"N 125°3'55.38"E	Lowland Mixed dipterocarp/ agroecosystem

1 - Google Earth 2 - GPS 3 - PAGASA (2011) 4 - HOBO ware Data Logger

The forest characteristics of the three Mindanao LTER Sites were recorded (Table 2). Temperature and relative humidity of the three sites were recorded using Hoboware Data Logger Pro v2 distributed by MicroDAQ.com, Ltd. USA.

Mt. Apo Natural Park is the highest mountain in the Philippines and can be seen in parts of Davao del Sur and Cotabato provinces. On the other hand, Mt. Hamiguitan Range Wildlife Sanctuary (MHRWS) in Davao Oriental is a protected area covering 6,834 ha and is identified as one of the Key Biodiversity Areas (KBAs) in the Philippines. It is both an ASEAN Heritage Park and a UNESCO World Heritage Site. It is located in the eastern coast of the Philippines and form the southern part of eastern Mindanao corridor (Villanueva & Mohagan, 2010). Mt. Musuan, also called Mt. Kalayo, is a prominent landmark of Central Mindanao University in Bukidnon province with a peak elevation of 646 masl.

In this study, only the dominant tree species in the three selected Mindanao LTER Sites were monitored. The most common is *Agathis philippinensis* (Almaciga) which is found in MAP and MHP. According to Higuera & Martinez (2006), litterfall of the trees that dominate the canopy has a great influence in the availability of nutrients in the ground under their tops. Five dominant and co-dominant tree species with four individuals were chosen based on the diversity of tree species. All tree species near the installed litter traps that may contribute to the collected samples were also identified.

Litterfall Collection and Processing

Quimpang et al. (2013) tagged 20 dominant tree species in each site for litter collection in the five

Figure 1. Litterfall collection and processing. A) Monthly collection; B) Collection of ground litter; C) Air-drying; D) Sorting and weighing of components; E) Oven-drying.

Mindanao LTER Sites. Litter were collected monthly from November 2016 to October 2017 from the 20 re-installed traps by handpicking and placed inside labeled collecting bags (Li et al., 2005). Following Quimpang et al. (2017), litter samples were weighed (fresh weight) using a digital weighing scale (1.0 g sensitivity), air-dried, weighed again (air dry weight) and sorted out into leaves, woody, reproductive and miscellaneous parts (i.e. dead insects, plants and others). Each sorted component was weighed again and placed in a labeled bag then oven-dried at a temperature of 70–80°C for three days or until the litter became brittle. After drying, oven-dried litter were weighed again (Fig. 1).

Analysis of Litter Turnover

Litter turnover rate was obtained by installing 0.25 m² wooden frame on the ground below the litter traps on which the ground litter (free of soil) enclosed within the wooden frame were collected. The fresh weight of collected ground litter samples were measured, air dried, weighed and oven dried. The litter turnover rate in percent per day (%/day) was calculated by dividing litterfall (g ODW/m²/day) by litter standing crop multiplied by 100 and turnover time in days by dividing litter standing crop by litterfall (Zieman et al., 1979).

This is shown below:

$$\text{Litter Turnover Rate: \% per day: } \frac{\text{LF g ODW/ m2/day}}{\text{LSC g ODW/m2}} \times 100$$

$$\text{Litter Turnover Time: \# of day: } \frac{\text{LSC g ODW/m2}}{\text{LF g ODW/m2/day}}$$

Where: LF = Litterfall
LSC = Litter standing crop

Statistical Analysis

The monthly collected litter in oven-dry weight were expressed in kilograms per hectare per month (kg/ha^1/month). Descriptive analysis was used for litter quality, data comparison and determining relationship between variables. Percentage of litter component was determined by:

% of Litter:

$$\text{Dry weight of Leaves (\%)} = \frac{\text{Total weight of dried leaves of Sp1}}{\text{Total weight of all litter components of Sp1}}$$

% of species litter:

$$\text{Sp1 \% species Litter} = \frac{\text{All dry weight of Species 1}}{\text{Total dry weight of all itter of all tree species}} \times 100$$

RESULTS AND DISCUSSION

Litter Components

The leaves account for more than 50% of the total litterfall production and therefore had contributed the highest percentage of litter component, followed by woody parts, miscellaneous parts and reproductive parts in all study sites. These findings supported the report of Cuevas & Lugo (1998) and Liu et al. (2004) which stated that leaves account as a major and most important component of the total litter and respond rapidly to climatic changes. Additionally, the portion of leaf litter commonly varies between 60 to 90% (Lisanework & Michelsen, 1994; Schrumpf et al., 2006; Zhou et al., 2006; González-Rodríguez et al., 2011). In this study, the proportion of leaf litter in relation to woody, miscellaneous and reproductive structures was high in all months (Fig. 2). The proportions of the leaves in total above ground litterfall may also provide good indication on the successional stage of tropical forests. Older forests allocate more production to fruits, flowers and seeds, and have more branch production than younger forests. This implies that Mt. Hamiguitan is a younger ecosystem as its leaf component reaches 70% of the total litterfall which supports the earlier report of Quimpang et al. (2017), whereas Mt. Apo and Mt. Musuan are implied to be older forest ecosystems.

Species Litter Contribution

Percentage litterfall production of the dominant tree species at MAP showed that *A. philippinensis* (23.18%) had the highest percentage contribution of total litter production, followed by *C. mercadoi* (21.07%), *P. hypophyllus* (19.87%), *Syzygium hutchinsonii* (Merr. ex C.B.Rob.) Merr. (19.03%) and least by *Lithocarpus apoensis* (Elmer) Rehder (16.84%). For Mt. Hamiguitan, *Barringtonia racemosa* (L.) Merr. ex DC (21.83%) had the highest percentage contribution of total litterfall production, followed by *S. polysperma* (20.14%), *A. philippinensis* (19.75%), *Palaquium* sp. (19.50%) and *C. blancoi* (18.76%), while in Mt. Musuan, *K. hospita* (22.58%) and *A. blancoi* (22.88%) had the highest percentage contribution of total litterfall production, followed by *A. scholaris* (20.89%), *M. multiglandulosa* (18.50%) and *S. spectabilis* (15.14%).

These rates of litterfall are generally positively correlated with forest productivity (Adams & Attiwill, 1991; Thomas, 1992; Madeira et al., 1995; Moroni & Smethurst, 2003). According to the studies of Bray and Gorham (1964) and Vogt et al. (1986), there was not a major difference between the trees with respect to litterfall, the significant differences depends on the climate.

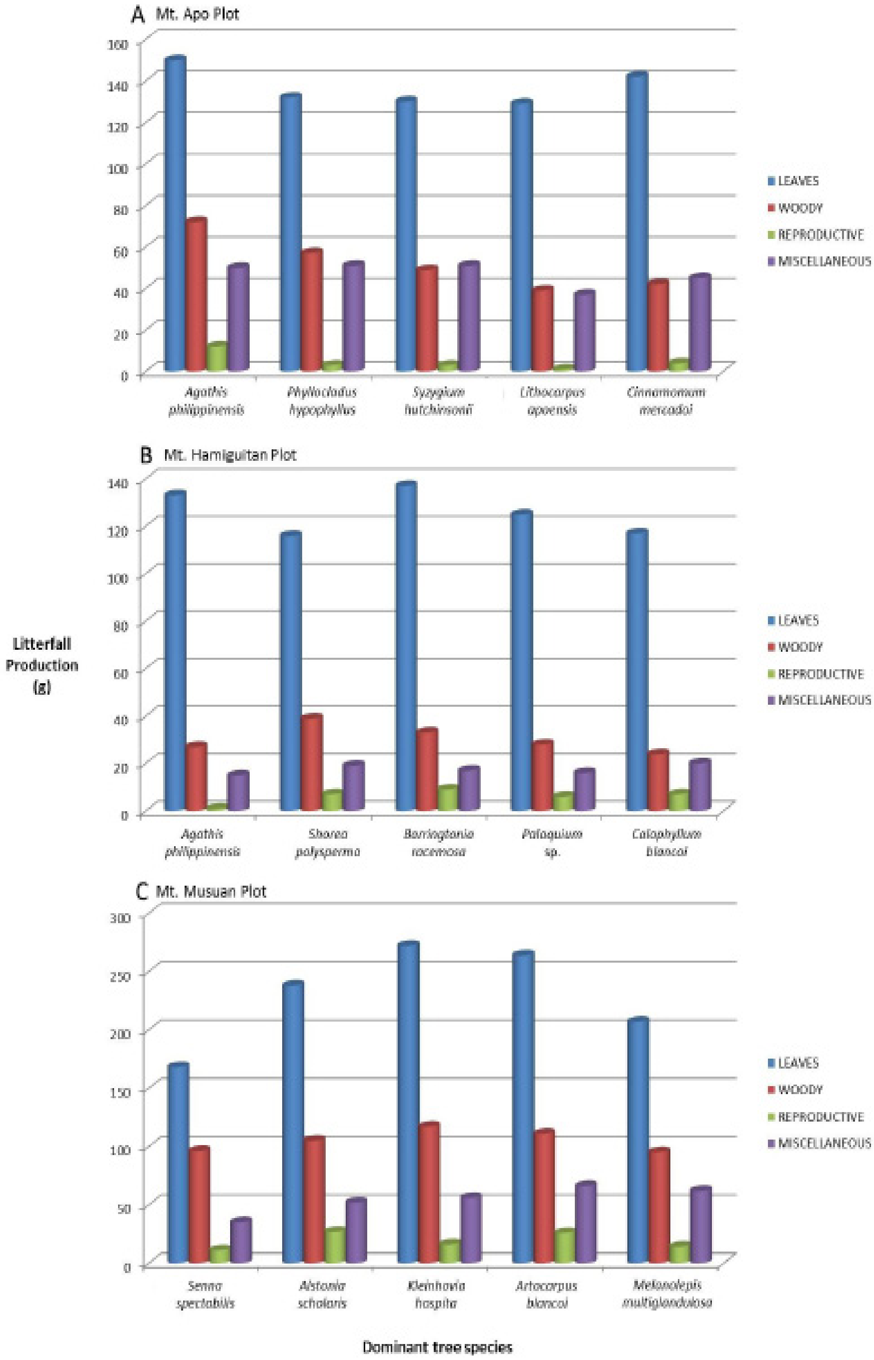

Figure 2. Litterfall production with its different components for the dominant tree species in A) Mt. Apo; B) Mt. Haimiguitan and C) Mt. Musuan plots.

Litterfall Production

At MAP, the litter production peaked on October 2017 (78.50 g) and closely uniform from February 2017 (54.95 g) to June 2017 (54.45 g). The lowest production at MAP was in May 2017 (51.40 g). At MHP, litter production peaked on July 2017 (72.25 g) and closely uniform from December 2016 (57.80 g) to June 2017 (55.35 g). The lowest production at MHP was in March 2017 (51.40 g). At MMP, the litter production peaked on June 2017 (90.65 g) and closely uniform from November 2016 (80.30 g) to February 2017 (76.25 g) and March 2017 (89.7 g) to June 2017 (90.65 g). Lowest production at MHP was in February 2017 (76.25 g) (Fig. 3). Estimated annual litter production was highest at MMP with 11211.5 kg ha^{-1} which had an estimated mean daily litter production of 30.7 kg ha^{-1}, followed by MAP with 6648.0 kg ha^{-1} which had an estimated mean daily litter production of 18.2 kg ha^{-1} and MHP with 4919.5 kg ha^{-1} which had an estimated mean daily litter production of 13.5 kg ha^{-1}. These amounts of litter production closely fall within the values obtained in other tropical forests studies as reported by Gunadi (1992) with 900 g and 400 g in the 2 sites in Indonesia and the report of Pandey et al. (2007) with 419.9 g and 547.7 g in the two forest types (plantation and forest) of northeastern India and in the study of Quimpang et al. (2013, 2015, 2017). Additionaly, these amounts of litter production might be due to the climatic type in which MAP and

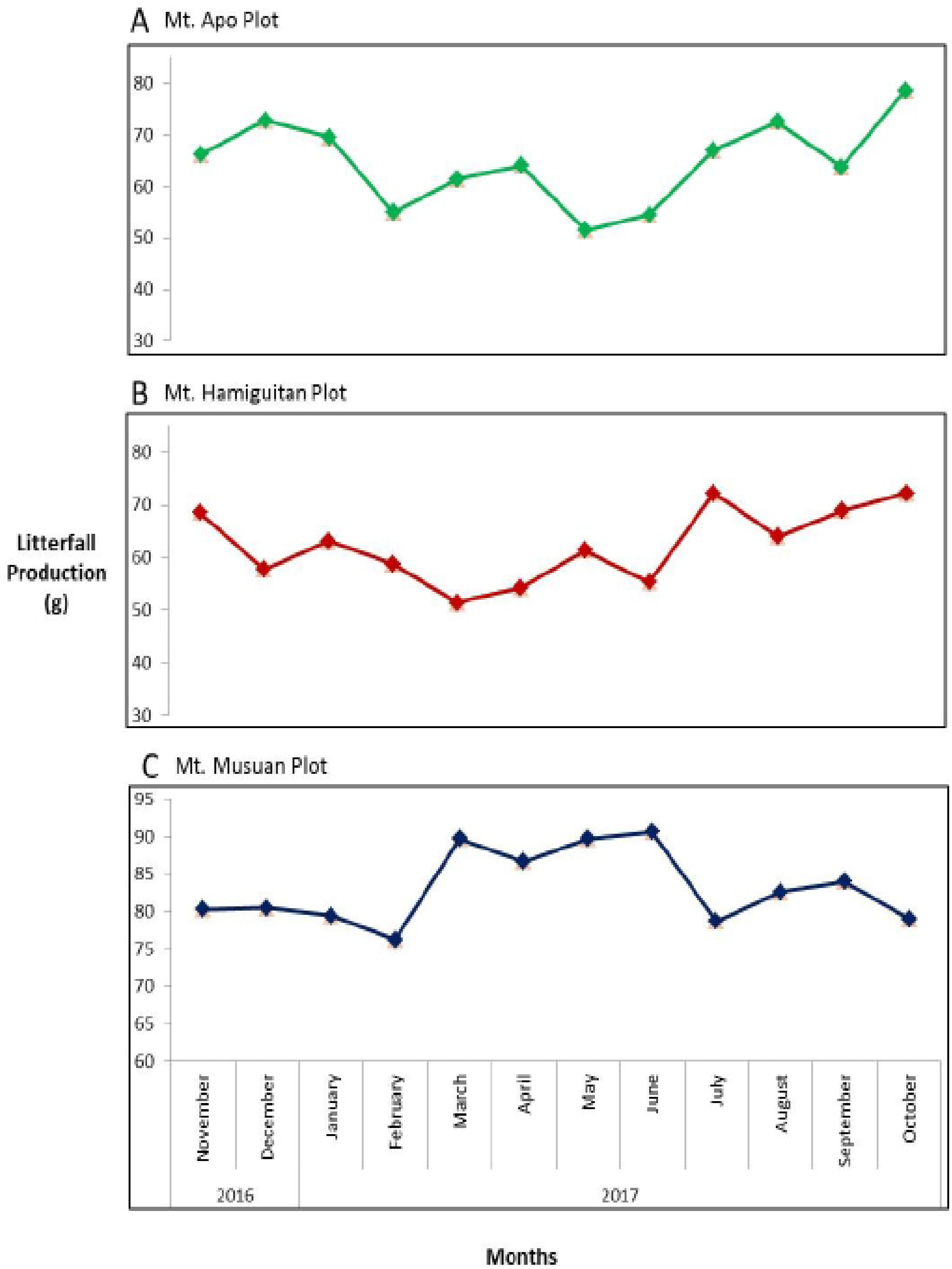

Figure 3. Patterns of monthly litterfall production of the dominant tree species in the three Mindanao LTER Sites

MHP have climatic type IV, while MMP has climatic type III (PAGASA, 2011).

As reported in several studies, the amount of litterfall may vary across different sites and biomes (Cakir & Akburak, 2017). Litterfall production was much higher in hot and wet months than the rest of the year for all studied forests by Chen et al. (1992), Tu et al. (1993), Weng *et al*. (1993) and Rawat et al. (2009). Pascal (1988) also reported that a heavy leaf litterfall occurred during the dry season in evergreen forests of Attappadi, Western Ghats, India and the generated data of Valenti et al. (2008) was affected by the season in tropical region, because litterfall production is greater during the dry season. However, Molles (2008) added that an agrosystem with the highest levels of primary production are those warm and receive large amount of precipitation, in which this present data supports this finding. In tropical montane forests, the seasonality of litterfall is generally low compared to that of tropical lowland forests (Chave et al., 2010). Recent studies supported that the litterfall production are in the peak either in dry and rainy season. Specifically, leaf aging, stomatal closure and subsequent leaf overheating, might lead to leaf shedding at the end of the dry season (Roderstein et al., 2005). As a side effect, trees are preparing for the upcoming season of highest net primary production. By contrast, the peaks during the rainy season are the result of strong winds and thunderstorms (Dawoe et al., 2010; Gonzalez-Rodriguez et al., 2011). This explains the observed increase in peaks of litter during wet months (Becker, 2015).

The tendency of litterfall to be concentrated in the cool and dry season is also related to a combination of decline in temperature and lowered soil moisture (Wang et al., 2008). However, monthly litterfall production pattern is still controlled mainly by community characteristics and environmental factors (Lu & Liu, 1988; Huebschmann et al., 1999; Pedersen & Hansen, 1999; Sundarapandian & Swamy, 1999; Kavvadias et al., 2001). Litterfall may also be affected by physical factors such as the mechanic action of wind and rain or physiological responses of the plants to environment changes (Delitti, 1998; Moraes & Prado, 1998; ICP Forests, 2004; Santiago & Mulkey, 2005) and some factors affecting litterfall amounts are also the succession stage, tree age and dominant plant or tree species (Barlow et al., 2007; Celentano et al., 2011). According to Holtgrieve et al. (1999) the annual quantity of litterfall is dependent on the proportion of foliage biomass that dies within a year, which ultimately depends on such factors as rate of leaf senescence, wind velocity, variation in canopy architecture and the tree species that make up the forest (Jackson et al., 1990).

Litter turnover

As cited by Raich et al. (2007), nutrient cycling in forestry systems is achieved when the fine litter is decomposed by soil biota, which determines forest primary productivity (Wang et al., 2008). Thus, the role of litter in plant nutrition is determined by its turnover time (Weerakkody & Parkinson, 2006). The mean average of standing litter in the five tree species in MAP was 182.72 g, in which the highest was observed in *L. apoensis* (202.29 g) and least in *S. hutchinsonii* (172.18 g). Mean average turnover rate among the five tree species was 1.00% which was highest in *A. philippinensis* (1.20%) which will decompose within 83.40 days and the least turnover rate was observed in *L. apoensis* (0.77%) which will decompose within 129.25 days. Mean turnover time for the five tree species in Mt. Hamiguitan is within 101.73 days which imply that litterfall will decompose in said number of days.

At MHP, the mean average of standing litter in the five tree species was 51.35 g, in which the highest was observed in *A. philippinensis* and *B. racemosa* (53.00 g) and least in *Palaquium* sp. (45.25 g). Mean average turnover rate among the five tree species was 2.63% which was highest in *Palaquium* sp.

Table 3: Litter turnover of the dominant tree species in the three Mindanao LTER Sites

Five dominant tree species	Standing Litter ($g\ ODW/m^2$)	Turnover Rate (%/day)	Turnover Time (Days)
1. Mt. Apo			
Agathis philippinensis	179.66	1.20	83.40
Phyllocladus hypophyllus	182.74	1.01	98.96
Syzygium hutchinsonii	172.18	1.03	97.40
Lithocarpus apoensis	202.29	0.77	129.25
Cinnamomum mercadoi	176.71	1.00	99.65
Mean	182.72	1.00	101.73
2. Mt. Hamiguitan			
Agathis philippinensis	53.00	2.51	39.80
Shorea polysperma	52.25	2.60	38.49
Barringtonia racemosa	53.00	2.78	36.02
Palaquium sp.	45.25	2.90	34.43
Calophyllum blancoi	53.25	2.37	42.12
Mean	51.35	2.63	38.17
3. Mt. Musuan			
Senna spectabilis	44.50	5.24	19.09
Alstonia scholaris	128.75	2.47	40.43
Kleinhovia hospital	51	6.81	14.68
Artocarpus blancoi	75.25	4.68	21.37
Melanolepis multiglandulosa	37.75	7.54	13.26
Mean	67.45	5.40	21.77

(2.90%) which will decompose within 34.43 days and the least in turnover rate was observed in *C. blancoi* (53.25%) which will decompose within 42.12 days. Mean turnover time for the five tree species in Mt. Hamiguitan is within 38.17 days which imply that litterfall will decompose in said number of days.

At MMP, the mean average of standing litter in the five tree species in Mt. Musuan was 67.45 g, in which the highest was observed in *A. scholaris* (128.75 g) and least in *S. spectabilis* (44.50 g). Mean average turnover rate among the five tree species was 5.40% which was highest in *M. multiglandulosa* (7.54%) which will decompose within 13.26 days and the least in turnover rate was observed in *A. scholaris* (2.47%) which will decompose within 40.43 days. Mean turnover time for the five tree species in Mt. Musuan is within 21.77 days which imply that litterfall will decompose in said number of days (Table 3).

Turnover rate is the percentage of litter standing crop to replace the litter fall every day. The higher the percentage of the turnover rate, the faster the decomposition and replacement of litterfall. Therefore, the faster the decomposition, the faster the productivity. The higher the rate of turnover time, the longer the litter turnover will stay on the ground (Quimpang et al., 2013, 2015, 2017). Litter decomposition rates are influenced by at least three general factors: the composition and activity of the decomposer community (O, organisms), the quality of the detritus (Q) and the physicochemical environment, P (Anderson and Swift, 1983). Some studies predict that lignin depresses litter turnover rates (e.g., Meentemeyer, 1978); however, Raich et al. (2007) contradicts the earlier result which found a highly significant, positive correlation between lignin contents and decay rates.

Litter decomposition has many factors to observe. As cited by Portillo-Estrada et al. (2016), soil characteristics, soil moisture (Bradford et al., 2016) and its microbial abundance and composition (Allison et al., 2013), and the species-related characteristics of litter also play an important role in the decomposition. The leaf litter may vary greatly in the leaf toughness (Gallardo & Merino, 1993), resistance to fracture (Wright & Illius, 1995), anatomical traits like leaf mass per area, and mechanical characteristics like leaf tensile strength (Cornelissen & Thompson, 1997), elemental composition (Berg & McClaugherty, 2008) and content of toxic chemicals such as terpenoids and alkaloids that are synthesized to protect against herbivory but also inhibiting soil microbes (Ormeño et al., 2009).

Relating Litter Production to Environmental Parameters

The correlations of litterfall production between temperature and relative humidity were also determined. In Mt. Apo, the patterns of litterfall production correlated with the relative humidity had a weak positive correlation of 0.13115. However, the temperature did not correlate with the litterfall production. In contrast to the correlation in MAP, the patterns of litterfall production in MHP correlated with the temperature had a weak positive correlation of 0.14617. However, the relative humidity did not correlate with the litterfall production. In MMP, the patterns of litterfall production correlated with the temperature had a strong positive correlation of 0.57478, whereas the temperature also correlated with the relative humidity had a strong positive correlation of 0.85139. However, the litterfall production did not correlate with the relative humidity (Table 4).

It was reported that positive correlation with maximum temperature in tropical species and chang-

Table 4: Correlation of litterfall production in the three Mindanao LTER Sites among the three parameters: A) Litterfall Production; B) Temperature; C) Relative Humidity

PARAMATER	Litterfall Production	Temperature	Relative Humidity
1. Mt. Apo			
Litterfall production	1		
Temperature	-0.31450	1	
Relative Humidity	0.13115	-0.25412	1
2. Mt. Hamiguitan			
Litterfall Production	1		
Temperature	0.14617	1	
Relative Humidity	-0.11849	-0.19558	1
3. Mt. Musuan			
Litterfall Production	1		
Temperature	0.57478	1	
Relative Humidity	-0.33507	0.85139	1

es in photoperiodicity can affect the flowering and bud break in plant (Cuevas & Lugo, 1998). In a regional scale, temperature and precipitation are the most important climatic factors controlling ecological processes (Liu et al., 2004) and are related to litterfall (Martins & Rodrigues, 1999; Liu et al., 2004; Cianciaruso et al., 2006). Litterfall production in this study had a positive correlation with temperature, but not with the relative humidity and rainfall. The data suggest that the climatic variables such as monthly mean and minimum temperatures and rainfall were not responsible for the patterns of monthly litterfall production in the three Mindanao LTER Sites. This supported Zhou et al. (2006) which indicated that their litterfall production in their five among the six studied communities were not significantly affected by precipitation in evergreen broadleaved forests.

A series of other studies from various ecosystems also showed no decrease with elevation (Roderstein et al., 2005; Kohler et al., 2008). However, the leaf litter production is considered dependent on temperature and thus decreases at higher elevations (Okeke and Omaliko, 1994; Zhou et al., 2006; Girardin et al., 2010). Elevation is also strongly affecting these parameters in montane ecosystems (Ensslin et al., 2015; Pabst et al., 2013; Becker et al., 2015) and is of particular importance regarding potential ecosystem shifts through climate change (Beniston, 2003). Therefore, the effect of elevation on litterfall is an important indicator for estimating future changes in ecosystem cycles (Becker et al., 2015). On the other hand, Vitousek and Sanford (1986), stated that litterfall varies considerably between ecosystems, depending on climate, tree species composition, stand structure and soil fertility.

CONCLUSIONS

Majority of the litterfall is composed of leaves, followed by woody, reproductive and miscellaneous parts. Estimated annual litter production was highest at MMP with 11211.5 kg ha^{-1} which had an estimated mean daily litter production of 30.7 kg ha^{-1}, followed by MAP with 6648.0 kg ha^{-1} which had an estimated mean daily litter production of 18.2 kg ha^{-1} and MHP with 4919.5 kg ha^{-1} which had an estimated mean daily litter production of 13.5 kg ha^{-1}. At MAP, *A. philippinensis* (almaciga) conributed the highest litterfall production, whereas at MHP was *B. racemosa* (malagubat) and at MMP was *A. blancoi* (antipolo). It is noteworthy that *A. philippinensis* is a Philippine endemic and threatened species. Highest litter turnover was observed at MMP (5.35%/day), followed by MHP (2.63%/day) and MAP (1.00%/day).

Total and leaf litterall productions were not uniform throughout the year. Total litterfall production did not correlate with changes in temperature and relative humidity. This suggests that the litterfall production changed according to other environmental factors present in the forests, but temperature showed to have a positive correlation with litterfall production or forest productivity for MHP and MMP. Correlation was also noted between litterfall production and relative humidity in MAP. Furthermore, the results of the study have contributed to understanding in litter dynamics of the dominant tree species in the three sites and could be useful for the future studies in other tropical forests.

There is therefore the need to conserve our forests moreso those that are found in lower elevations like Mt. Musuan which revealed to have high forest productivity and fast litter turnover which imply fast soil nutrient cycling. Further, this study affirms the need to plant Philippine endemic tree species such as *A. philippinensis* in the sites since they prove to have high productivity.

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Underutilized *Senna tora* (L.) Roxb. - a highly potential multipurpose species for food, feed, medicine and climate resilience for the Philippines

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ABSTRACT

There are thousands of species in the plant kingdom but less than 200 are recognized as major crops. There is even less when it comes to food crops hence food and nutritional insecurity are major concerns in either developed or developing countries. Unfortunately, manyspecies fall under the neglected and underutilized species (NUS) category. *Senna tora* (L.) Roxb. is largely utilized in some countries especially as part of their traditional medicine but is generally known as a weed in the Philippines and henceforth technically, one of the NUS. This paper reports of findings of a series of investigations from December 2009 to November 2017 of *S. tora* in Bukidnon that documented its uses as a food, feed, and medicinal crop; *in situ* and *ex situ* ecotypic diversity in the province based on its phenotypes; and potential resilience to abiotic stresses at early vegetative stage. Use of leaves, seeds and roots as food, feed and medicine were documented in 9 of 22 barangays. Percent crude protein (PCP) of seeds and leaves ranged from 12.50% to 20.48% and 16.94-39.02%, respectively. PCP of seeds (H'=0.93) and leaves (H'=0.79 and 0.81) were highly diverse based on Standardized Shannon-Weaver Diversity Index. Plant height was estimated with moderate (H'=0.67) to high (H'=0.88 and 0.93) diversity. However, all ecotypes were non-nodulating (H'=0.00). Finally, most ecotypes were more sensitive to waterlogging than to drought for 16 days at seedling stage. Three of 10 ecotypes were sensitive only to waterlogging whereas, another three ecotypes were not sensitive to both stresses.

Keywords: underutilized species, Senna tora, multipurpose species, resilient crop, Bukidnon

INTRODUCTION

Many members of the plant kingdom are considered neglected and underutilized species (NUS). NUS are species that could be crops in one country but are considered weeds and non-valuable in another. The estimated number of plant species is around 400,000 (https://www.bgci.org/policy/1521/). Christensen and Byng (2016) reported that the accepted count is around 374,000 of which 74,273 are monocots and 210,008 are eudicots. However, the Commission on Genetic Resources for Food and Agriculture (CGRFA) reports that only less than 8000 species have records of cultivation or collected for a purpose (http://www.fao.org/nr/cgrfa/cthemes/plants/en/). The CGRFA further estimates that only 30 crops serve as primary food sources, of which only five (rice, wheat, maize or corn, millet and sorghum) provide for about 60% of food energy needs of the global population.

This is unfortunate because thousands of plant species could be beneficial to agriculture, especially to address the basic needs of food, feed, fiber, fuel and pharmaceuticals. The Philippine Statistics Authority or PSA (2017) reported that farmers, fishermen and children have the highest poverty incidence among the basic sectors in the country in 2006, 2009, 2012 and 2015 (https://psa.gov.ph/poverty-press-releases). Poverty is often coupled with food and nutritional insecurity, and is particularly ironic and sad for farmers and their children.

Senna tora (L.) Roxb. is largely regarded as a leguminous weed in the Philippines (Figure 1). It is previously known as *Cassia tora* L. with variable chromosome numbers: 2n=26, 28, 52, and 56; although most papers reported 2n=2x=26 (http://

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Figure 1. *In situ* population of *Senna tora* (L.) Roxb. in a pasture land in Kibawe, Bukidnon

ccdb.tau.ac.il/Angiosperms/Leguminosae/ Senna/ Senna%20tora%20(L.)%20Roxb./). Many recent papers continue to use the name *C. tora*.

S. tora is almost ubiquitous in Bukidnon which has a variable topography and geography. Bukidnon has a total land area of 829,378 ha and is situated "between the parallels 7° 25' and 8° 38' north latitude, and the meridians 124° 03' and 125° 16' east longitude"(http://www.bukidnon.gov.ph/home/index.php/about-bukidnon/general-info/physical-feature-and-composition). Locally, *S. tora* is called "*mani-mani*" (translation: like peanut due to its leaf architecture) that is often found in pasture areas, uncultivated farmlands, and adjacent to cultivated farmlands. It has a deep tap root system and strong stem hence, is also used as a natural stake for domestic livestock (e.g. goat and cow) while grazing.

In other countries, however, it is a cultivated crop. In India, it is predominantly valued for its medicinal properties in the traditional system of Indian medicine (Pawar & D'mello, 2011). Leaves, pods, and seeds are widely used for medicinal applications (Pawar & Lalitha, 2014). The seeds are also considered alternative sources of commercial gums for their galactomannans for industrial applications (Pawar & Lalitha, 2014). Moreover, seed gums are used as thickener in foods, as well as, for non-food products such as in the manufacture of paper and textile. Hence, the viability of *S. tora* gum production in Australia was investigated (Cunningham & Walsh, 2001).

In the Philippines, *S. tora* can be considered a NUS. Hence, its local uses and phenotypic diversity in Bukidnon, crude protein concentration, and potential resilience to abiotic stresses were determined.

MATERIALS AND METHODS

Plant materials

In situ plants of *S. tora* per municipality or city were considered "ecotypes" and were arbitrarily named after each area. These were phenotyped in three separate surveys. Seeds were collected for the *ex situ* experiments done in Central Mindanao University, Musuan, Bukidnon.

Survey

Prior informed consent per municipality and barangay was secured before the conduct of the survey, interview, and sampling. Documentation of uses of *S. tora* by the locals was done through informal interviews. On December 2009 to January 2010, in situ populations of S. tora were phenotyped in 12 municipalities of Bukidnon selected based on varying geography with emphasis on micro-climate and topography: Impasug-ong, Libona, Malitbog, Sumilao, Damulog, Kadingilan, Quezon, Cabanglasan, Lantapan, San Fernando, Kalilangan, and Pangantucan (Figure 2). Three plants either at late reproductive stage or nearing senescence were randomly selected in one barangay per municipality for sampling of mature pods (and surrounding soil). These were characterized for nine traits: plant height, number of primary branches per plant, leaf area, root nodulation, number of mature pods per plant, number of seeds per pod, weight of 100 mature seeds, percent total nitrogen (PTN) of seeds, and percent crude protein (PCP) of seeds. Seed samples were submitted for PTN analysis at the Soil and Plant Analysis Laboratory (SPAL), Department of Soil Science, College of Agriculture, Central Mindanao University (CMU) using the Kjeldahl Method. PTN value was used to estimate for percent crude protein (PCP) i.e. PCP = PTN x 6.25, where 6.25 is a default constant for estimating crude protein (FAO-UN, 2003).

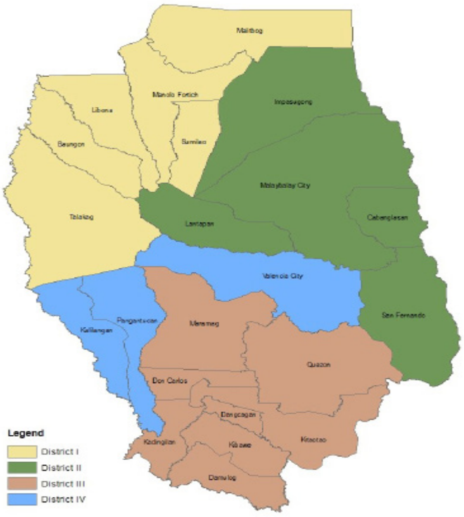


Figure 2. Map of the Province of Bukidnon
(http://www.bukidnon.gov.ph/home/index.php/cities-municipalities/republic-act-no-10184)

On September to October 2010, survey in the remaining eight municipalities and two cities of Bukidnon (Baungon, Manolo Fortich, Talakag, Don Carlos, Maramag, Dangcagan, Kibawe, Kitaotao, Malaybalay City, and Valencia City) was done. In this survey, two barangays were surveyed per municipality or city. Also, five randomly selected *S. tora* plants *in situ* per barangay were phenotyped for the same traits as in the first survey. However, number of mature pods per plant and seeds per pod, as well as, weight of 100 seeds were excluded since the *in situ* plants were mostly in their vegetative stages.

On December 2012 to January 2013, a validation survey was done in 13 municipalities and city of Bukidnon (Cabanglasan, Impasug-ong, Lantapan, Manolo Fortich, Talakag, Kibawe, Maramag, Quezon, San Fernando, Damulog, Pangantucan, Kadingilan, and Malaybalay City) that showed ecotypic diversity of *S. tora* plants based on the previous surveys. Five randomly selected *in situ* plants per barangay either at late vegetative stage or at early reproductive stage were characterized. Leaf samples were collected and submitted to SPAL for PTN analysis.

Potential resilience to abiotic stresses

On April to November 2017, 10 ecotypes of *S. tora* that showed high (32.00-71-39.02%), intermediate (29.33-29.57%) and low (18.19-22.45%) PCP of young leaves were phenotyped for potential tolerance to 16 days of drought and waterlogging stresses at early vegetative stage. This was done at the Department of Agronomy and Plant Breeding Screenhouse, College of Agriculture, CMU following a 3x10 factorial arrangement in Randomized Complete Block Design with three replications. A replication was a recycled plastic water container with a 6.50 L capacity.

Three regimes of water stress served as the main plot factors: (i) normal (provision of ~400 ml water daily), (ii) drought (provision of ~400 ml water daily then no water for 16 days starting at 13 days after transplanting or DAT until 29 DAT), and (iii) waterlogged (provision of ~400 ml water daily then increased to ~1100 ml water daily from 13 to 29 DAT). Plants were given one week to recover from stress. Plants subjected to drought were given ~400 ml water daily for seven days, whereas, water was withheld from waterlogged plants. Data were again gathered at 36 DAT. Ten *S. tora* genotypes were the subplot factors.

Plant stress tolerance was based on the arbitrary rating scale of Jamago (2017) i.e. 1 – highly sensitive (very stunted growth and highly reduced leaf area i.e. <1/2 of plant height and leaf area of normal plants), 2 – sensitive (very stunted growth and highly reduced leaf area i.e. ~1/2 of plant height and leaf area of normal plants), 3 – moderately sensitive (stunted growth and reduced leaf area i.e. ~3/4 of plant height and leaf area of normal plants), 4 – moderately tolerant (stunted growth but normal leaf area), 5 – tolerant (normal plant height but reduced leaf area), and 6 – highly tolerant (normal plant height and leaf area).

The pot experiment was transferred outside the screenhouse for the continuance of study for recovery from stress at reproductive stage until senescence, with only rainfall as source of water. Seeds were inadequate for PTN analysis per replication hence, these were bulked per treatment instead.

Statistical analyses

All data from the *in situ* phenotypic characterization were processed for the estimation of allelic richness using the Standardized Shannon-Weaver Diversity Index (SSWDI, H'). The scale of Jamago and Cortes (2012) was used to estimate the level of phenotypic diversity: H'=0.00 (invariant), H'=0.010-0.45 (low), H'=0.46-0.75 (moderate), H'=0.76-0.99 (high), and H'=1.00 (maximum variability). Data from the *ex situ* experiment were processed for Analysis of Variance (ANOVA) and Tukey's Test for treatment mean comparisons using the Statistical Tool for Agricultural Research (STAR) software (IRRI, 2014).

RESULTS AND DISCUSSION

Local uses of *S. tora*

In nine of 22 towns and cities of Bukidnon, *S. tora* was documented to be used as food, feed and medicine. Young leaves were used to relieve menstrual pain (Pangantucan), mixed with mungbean and canned fish (sardines) for viand (Impasug-ong), and used as ingredient for chicken stew or "*tinolang manok*" (Maramag). Extract from young leaves was an ingredient of herbal medicine to relieve muscle pain (Lantapan). Mature leaves were considered first aid treatment for wounds (Dangcagan) and for stomachache (Talakag). Leaves (young and mature) were mixed with corn bran for swine feed (Libona). Roots were roasted to make tea as cure for stomachache (Malitbog). Lastly, seeds were used to deworm both pigs and humans (Kibawe). In some supermarkets in Bukidnon, *S. tora* tea is sold as a slimming agent.

The medicinal potential of *S. tora* is affirmed by how it is utilized in other countries like India and China, and as evidenced by a growing number of published literature exploring or assessing its potential for several ailments. Mazunder et al. (2005) reported that the leaves and seeds are used in the Ayurvedic system of medicine to treat leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders. They added that the leaves also have some antifungal properties because of chrysophanic acid–9-anthrone (Acharya et al., 1975 as cited by Mazunder et al., 2005), whereas, the seeds have antibacterial properties due to phenolic compounds (Hatano et al., 1999 as cited by Mazunder et al., 2005). Tzeng et al. (2013) investigated in rats and reported the potential of seed extracts to alleviate high-fat diet-induced non-alcoholic fatty liver. Sreelakshmi and Abraham (2016) using Sprague Dawley pup rats reported that *C. tora* leaves could

Table 1: Phenotypic diversity estimates(H') of *S. tora* plants *in situ* in Bukidnon as per SSWDI as phenotyped on different seasons

Plant Trait	December 2009 to January 2010 (n=12)	September 2009 to October 2010 (n=10)	December 2012 to January 2013 (n=13)
Growth stage(s)	Late reproductive stage to senescence	Vegetative stage to early pod development	Late vegetative stage
Plant height (cm)	0.93^{H}	0.88^{H}	0.67^{H}
Stem diameter (cm)	-	0.64^{M}	-
Number of primary branches/plant	0.74^{M}	0.80^{H}	0.92^{H}
Number of nodes/plant	-	0.72^{M}	-
Leaf length (cm)	-	-	0.82^{H}
Leaf width (cm)	-	-	0.62^{H}
Leaf area (cm^2)	0.87^{H}	0.66^{M}	0.78^{H}
Leafiness	-	-	0.47^{H}
Root nodulation	0.00I	0.00^{I}	0.00^{I}
Number of mature podes per plant	0.87^{H}	-	-
Number of seeds per pod	0.79^{H}	-	-
Weight of 100 seeds (g)	0.85^{H}	-	-
Percent crude protein (seeds)	0.93^{H}	-	-
Percent crude protein (young leaves)	-	-	0.79^{H}
Percent crude protein (mature leaves)	-	-	0.81^{H}
Seed coat color	0.68^{M}	-	-
Seed coat texture	0.31^{L}	-	-
Seed coat glossiness	0.42^{L}	-	-
Mean H'			

H (high variability); M(moderate variability); L(low variability); I(invariant, no variant), n(number of ecotypes characterized modulate selenite cataract by enhancing antioxidant status and preventing cytoskeletal protein loss in their lenses.

Ecotypic diversity of in situ plants

There is ecotypic diversity of the natural populations of *S. tora* in Bukidnon as per SSWDI (Table 1) based on their phenotypes. In three separate assessment of *in situ* populations at different stages of growth, four common parameters were measured. Moderate to high variability were estimated for plant height, number of primary branches per plant, and leaf area. However, root nodulation was invariant since all three surveys confirmed the non-nodulating habit of *S. tora*, as reported by Allen and Allen (1976, p. xxiii) for *Cassia* species. Other quantitative plant traits phenotyped only once were estimated to have moderate to high variability. PCP of seeds, young and mature leaves were all highly variable. Three qualitative seed traits (seed coat color, texture and glossiness) were poorly or moderately variable, as these are usually consistent across environments.

H' is an estimate of the magnitude of allelic richness of a plant trait within a population or in a set of germplasm (Shannon & Weaver, 1949). Hence, the presence of adequate variation (moderate to high) could allow selection for the most desirable ecotypes based on trait(s) of interest either for the immediate promotion of *S. tora* to become a more important or popular species, or for use in a crop improvement program to further refine some ecotypes prior to wider commercial use.

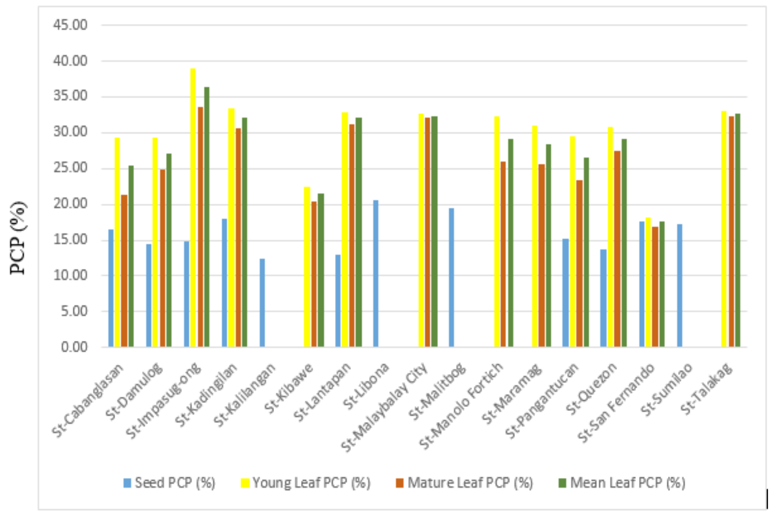
If *S. tora* is to be optimally used as food and forage crop, ecotypes that are taller, have more primary branches, leafier, with more leaflets and higher leaf PCP would be most desirable. In India, Mazumder et al. (2005) reported that *S. tora* could grow from 30.00-39.00 cm high whereas, Pawar and D'mello (2011) reported 30.00-90.00 cm range in height. In this investigation, mean height of *in situ* plants at different stages of growth ranged from 47.00-105.00 cm on December 2009 to January 2010 (late reproductive to senescence), 104.00-162.00 cm on September to October 2010 (vegetative stage to early pod development), and 13.00-38.00 cm on December 2012 to January 2013 (late vegetative stage).

Ecotypic diversity of *S. tora* in Central India was also reported by Tilwari et al. (2016) although based on molecular data. They estimated the genetic diversity of 15 accessions of *C. tora* from different agro-climatic zones of Madhya Pradesh. Fresh leaves were collected from *in situ* plants and processed for genotyping using six polymorphic RAPD primers. They reported that such accessions were genetically diverse based on the range of 0.23 – 0.65 Jaccard's similarity coefficients. *C. tora* is valued for herbal formulations hence, its genetic diversity and *in situ* conservation are critical to India.

Protein concentration of seeds and leaves of in situ plants in Bukidnon

Bulked seeds collected from *in situ* plants from each of 12 locations surveyed from December 2009 to January 2010 were analyzed for PTN used to estimate for PCP. Separate samples of young and mature leaves from *in situ* plants collected from each of 13 locations from December 2012 to January 2013 were also submitted for proximate analysis of PTN. Results are shown in Figure 2.

Mungbean (*Vigna radiata* L.) is the most common food legume in the country. Dahiya et al. (2015) reviewed various papers and summarized that



Senna tora ecotypes

Figure 3. Percent crude protein (PCP) of seed and leaf samples from i*n situ* population of *S. tora* ecotypes in Bukidnon

Table 2: Mean comparison of *S. tora* ecotypes prior to imposition of stress at 13 DAT based on Tukey's HSD

Senna tora Ecotype	Class	Seeding Height (cm)	Leaflet Length (cm)	Leaflet Width(cm)	Leaflet area (cm^2)	Number of leaves per plant	Number of leaflets per plant
St-Impasug-ong	H	10.06^{cd}	2.03^{bc}	1.19^{ab}	1.86^{bcd}	2.00^{ab}	11.61^{b}
St-Kadingilan	H	11.80^{ab}	2.21^{ab}	1.27^{ab}	2.29^{abc}	2.17^{ab}	12.61a^{b}
St-Lantapan	H	9.61^{cd}	2.04^{b}	1.16^{ab}	1.80^{cd}	2.00^{ab}	11.69^{b}
St-Malaybalay City	H	10.58^{bc}	2.23^{ab}	1.24^{ab}	2.12^{abc}	2.11^{ab}	12.44^{ab}
St-Talakag	H	8.92^{d}	1.76^{c}	1.12^{b}	1.57^{d}	1.97^{b}	11.67^{b}
St-Cabanglasan	I	11.04^{bc}	2.30^{ab}	1.26^{ab}	2.35^{ab}	2.14^{ab}	12.53^{ab}
St-Damulog	I	12.63^{a}	2.34^{a}	1.31^{a}	2.40^{a}	2.28^{ab}	13.22^{ab}
St-Pangantucan	I	11.75^{ab}	2.12^{ab}	1.24^{ab}	2.22^{abc}	2.33^{a}	13.92^{a}
St-Kibawe	L	10.32^{bcd}	2.13^{ab}	1.19^{ab}	1.98^{abcd}	2.06^{ab}	11.92^{b}
St-San Fernando	L	10.76^{bc}	2.19^{ab}	1.24^{ab}	2.08^{abcd}	2.19^{ab}	12.24^{ab}
Probability Value (p)		<0.0001	<0.0001	0.0213	<0.0001	0.0082	0.0082
CV (%)		9.48	8.61	8.81	16.48	10.61	10.25

PCP(percent crude protein), H(high PCP), I(intermediate PCP), L(low PCP), DAT(days after transplanting). Means within columns with a common letter are not significantly different based on Tukey's HSD at 0.05 level of probability.

on average, PCP of mungbean seeds was 23.80% with a range of 14.60 to 32.60%. In contrast, PCP of *S. tora* seeds collected *in situ* from Bukidnon only had 12.40-20.46%. St-Libona recorded the highest numerical estimate at 20.46%. Pawar and D'mello (2011) reported that in India, seed protein concentration of *S. tora* was about 23.20%. However, PCP of young and mature leaves of *in situ* plants of Bukidnon ecotypes were higher at 18.18-39.02% and 16.94-33.68%, respectively. St-Impasug-ong had the highest numerical PCP estimates for young and mature leaves.

Either the seeds or leaves of *S. tora* would make it a valuable protein crop for food or feed especially that it grows ubiquitously in the province, and perhaps in the whole country. Some locals have indeed utilized young leaves as vegetable and leaves (regardless of age) as feed for household livestock (swine). However, *in situ* populations of *S. tora* are often left undisturbed because it may be unpalatable when eaten raw by grazing animals. According to Mazumder et al. (2005), its leaves although smooth are slightly bitter.

Potential resilience to drought and waterlogging stresses at vegetative stage

The *S. tora* ecotypes were phenotyped before the imposition of stress at 13 DAT, immediately after stress (29 DAT) and after one week of recovery from stress (36 DAT). Table 2 shows data for six traits at 13 DAT. Ecotypes highly differed for seedling height; leaflet length, width and area; number of leaves; and number of leaflets as per ANOVA. Grown *ex situ* under Musuan conditions, results confirm that phenotypic variation among the ecotypes as observed *in situ* was not just due to differences in environment but genetic as well.

After 16 days of stress (29 DAT), means of ecotypes for plant height, leaflet length, leaflet width, leaflet area, number of leaves per plant, and plant

Table 3: Means of plant traits of 10 ecotypes under each water regime at 29 DAT (after 16 days of stress imposition).

Trait	Normal	Drought	Waterlogged
Plant height (cm)	20.48^{a}	17.88^{b}	14.45^{c}
Leaflet length (cm)	3.27^{a}	2.91^{b}	1.95^{c}
Leaflet witdth (cm)	1.84^{a}	1.62^{b}	1.08^{c}
Leaflet area (cm^2)	6.24^{a}	4.87^{b}	2.13^{c}
Number of leaves per plant	5.40^{a}	4.80^{b}	3.40^{c}
Plant stress tolerance	5.00^{a}	2.37^{b}	0.77^{c}

Means within columns with a common letter are not significantly different based on Tukey's HSD at 0.05 level of probability

Table 4: Interaction between water regimes and ecotypes for number of leaflets per plant at 29 DAT

Senna tora Ecotype	NUMBER OF LEAFLETS PER PLANT		
	Normal	Drought	Waterlogged
St-Cabanglasan	35.50$^{ab\ x}$	31.00$^{a\ x}$	23.20$^{a\ y}$
St-Damulog	35.50$^{ab\ x}$	28.20$^{a\ y}$	18.60$^{a\ z}$
St-Impasug-ong	27.60$^{bc\ x}$	28.20$^{a\ x}$	20.10$^{a\ y}$
St-Kadingilan	34.30$^{abc\ x}$	30.60$^{a\ xy}$	26.30$^{a\ y}$
St-Kibawe	34.00$^{abc\ x}$	29.80$^{a\ x}$	21.30$^{a\ y}$
St-Lantapan	24.80$^{c\ xy}$	27.20$^{a\ x}$	20.20$^{a\ y}$
St-Malaybalay City	27.40$^{bc\ x}$	30.40$^{a\ x}$	19.60$^{a\ y}$
St-Pangantucan	43.90$^{a\ x}$	28.90$^{a\ y}$	27.00$^{a\ y}$
St-Talakag	28.20$^{bc\ x}$	25.80$^{a\ xy}$	19.40$^{a\ y}$
St-San Fernando	27.40$^{bc\ x}$	25.10$^{a\ x}$	23.80$^{a\ x}$
P value (Stress, A)	<0.0001		
P value (Ecotype, B)	<0.0001		
P value (AxB)	0.0322		
Coefficient of variation (%)	14.25		

Means within the same column with common letters (a,b or c) and within the same row with common letters(x,y or z) are not significantly different based on Tukey's HSD at 0.05 level of probability

stress tolerance under each water regime showed similar patterns of variation (Table 3). Those under normal conditions had the highest values, then those under drought, with waterlogged plants showing the least. In general, ecotypes were affected, and therefore, susceptible to both drought and waterlogging at early vegetative stage for 16 days. However, ecotypes were mostly affected by waterlogging. Ecotypes under drought were moderately sensitive (2.37) whereas, waterlogged plants were highly sensitive (0.77) based on the scale of 1.00 for highly sensitive and 6.00 for highly tolerant (Jamago, 2017).

S. tora ecotypes across water regimes at 29 DAT also differed for the above traits (data table no longer shown). St-Pangantucan was tallest (21.30 cm), had the widest leaflets (1.73 cm), the biggest leaflets based on leaf area (5.85 cm^2), and the most leaf count per plant (5.70). St-Kadingilan had the longest leaflets (3.11 cm). In contrast, St-Talakag was the shortest and had the least leaf count per plant. St-Lantapan had the shortest and narrowest leaflets, and consequently the smallest leaflets. Nonetheless, means of plant stress tolerance were comparable among ecotypes, i.e. from 2.22 to 3.33 that are qualified as sensitive to moderately sensitive.

On the other hand, number of leaflets per plant at 29 DAT showed significant interaction between the two factors (Table 4). Plants have varied and complex responses to water stress which can be combinations of stress avoidance and tolerance (Chaves et al., 2002). Reducing the number of photosynthetic organs that require an adequate amount of water can be one of the adaptive changes to keep the water balance right. Results suggest that St-Pangantucan was comparably sensitive to both stresses whereas, St-Damulog was sensitive to both stresses but more sensitive to waterlogging. Five ecotypes (St-Cabanglasan, St-Impasug-ong, St-Kadingilan, St-Kibawe, St-Malaybalay City, and St-Talakag) were tolerant to drought but sensitive to waterlogging. Lastly, St-Lantapan and St-San Fernando were tolerant to both stresses having comparable number of leaflets per plant with those under normal conditions. These two ecotypes were 'average performers' under each water regime as they recorded average measurements and showed consistent average performance in normal, drought and waterlogged conditions.

At 36 DAT, ecotypes under drought were

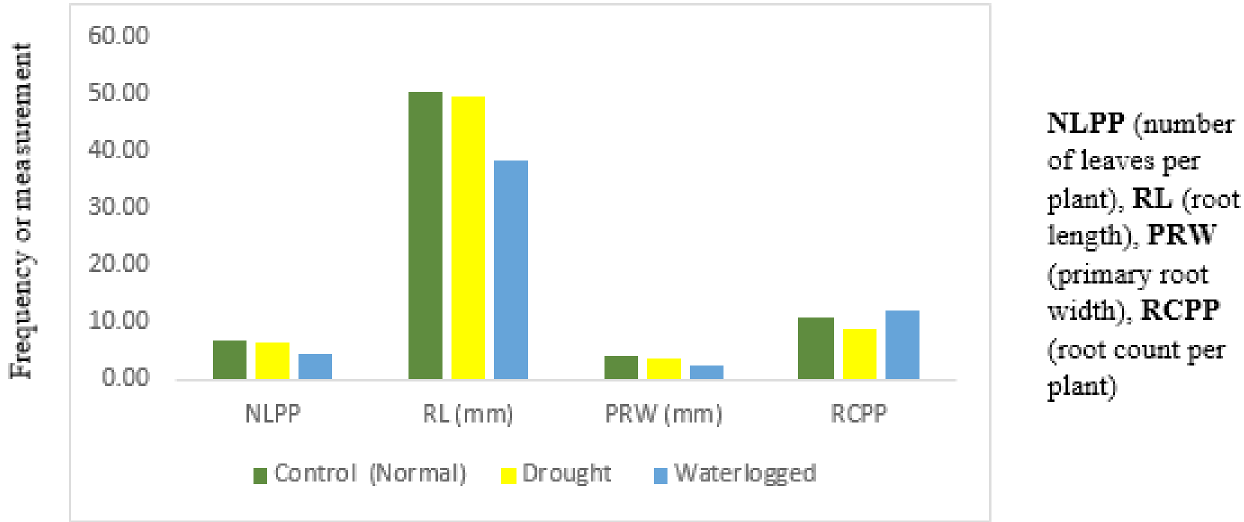


Figure 4. Means of plant traits of 10 ecotypes under each water regime at 36 DAT

able to recover and developed comparable number of leaves, root length and root width as the normal plants (Figure 4). Waterlogged plants, however, had comparable root count as the normal plants because of the development of adventitious roots above the soil. These adventitious roots allowed the ecotypes to either minimize hypoxia or prevent anoxia, which are mechanisms for tolerance against excessive moisture at the root zone.

At 36 DAT after one week of recovery from stress, two plants per replication were sampled to characterize root traits. Root length and root count per plant varied among the water regimes and among the phenotypes as per ANOVA, but interaction was not significant (Table 5). Ecotypes had significantly reduced root length after

Table 5: Mean root length and root count of ecotypes under each water regime, and across water regimes at 36 DAT

Senna tora Ecotype	ROOT LENGTH (cm)				ROOT COUNT PER PLANT			
	N	D	W	Mean	N	D	W	Mean
St-Cabanglasan	50.67	43.58	39.42	44.56[ab]	13.00	8.80	13.20	11.70[ab]
St-Damulog	55.83	55.08	41.58	50.83[ab]	12.20	6.50	14.30	11.00[ab]
St-Impasug-ong	53.42	49.75	42.42	48.53[ab]	10.20	7.80	10.30	9.40[ab]
St-Kadingilan	48.33	48.92	44.08	47.11[ab]	10.30	8.70	15.50	11.50[ab]
St-Kibawe	60.58	45.33	37.58	47.83[ab]	11.70	8.70	11.20	10.50[ab]
St-Lantapan	50.25	56.83	35.00	47.36[ab]	9.70	10.50	14.00	11.40[ab]
St-Malaybalay City	40.58	56.25	40.33	45.72[ab]	10.50	9.70	9.50	9.90[ab]
St-Pangantucan	60.75	54.92	47.24	54.31[a]	14.30	9.50	14.20	12.70[a]
St-San Fernando	45.38	46.00	33.42	41.60[bc]	9.70	10.50	9.30	9.80[ab]
St-Talakag	38.83	39.25	22.33	33.47[c]	8.20	8.50	9.00	8.60[b]
Mean	50.46[a]	49.59[a]	38.34[b]		10.90[a]	8.90[b]	12.10[a]	
P value (Stress, A)				<0.0001				0.0001
P values (Ecotypes, B)				<0.0001				0.0485
P value (AxB)				0.1420				0.1527
CV(%)				15.14				23.98

DAT (days after transplanting, N(normal water conditions), D(drought), W(waterlogged).
Means in the same column with the same letter are not significantly different as per Tukey's Test at 0.05 level of probability

waterlogging (38.34 cm), but those in normal conditions (50.46 cm) and subjected to drought (49.59 cm) were comparable.

These results suggest that ecotypes were more sensitive to waterlogging and may have potential tolerance to drought at early vegetative stage. For ecotypes across water regimes, longest roots were recorded for St-Pangantucan (54.31 cm) whereas, the shortest roots were by St-Talakag (33.47 cm). However, these ecotypes were comparable with other ecotypes. For root count per plant, waterlogged plants had the highest root count (12.10) due to presence of adventitious roots but was comparable to plants under normal conditions (10.90). Those that suffered drought had the least with 8.90. Among the ecotypes across water regimes, St-Pangantucan had the highest root count (12.70) whereas, St-Talakag had the least (8.60).

Ecotypes subjected to drought that have comparable performance with plants under normal conditions may be considered water spenders as part of their drought avoidance mechanism. They could optimize water uptake, grow more roots, longer roots, more root hairs, or enhance hygrotropism. King et al. (2009) reported that in Salvia officinalis, lateral roots were developed in the upper part of the root system in response to five days of hypoxia to survive. Ayi et al. (2016) concluded that in Alternathera philoxeroides, its adventitious roots developed upon submergence were capable of harvesting oxygen from ambient water. Consequently, this allowed the plant to reduce the detrimental effect of oxygen deficiency, enabling the efficient use of carbohydrates, and delaying plant senescence. Ho et al. (2005) and Pinheiro et al. (2005) discussed that crops or genotypes with more capability to survive drought stress are those that proliferate their roots deeper in the soil.

At 36 DAT, plant height showed significant interaction at 36 DAT (Table 6). Based on Tukey's test of means on recovery in plant height, St-Kadingilan and St-Lantapan were tolerant to both stresses. These two had comparable height under normal conditions and after one week recovery from drought and waterlogging stresses, suggesting tolerance based on the resumption of normal vertical growth through cell division and elongation. St-Impasug-ong, St-Malaybalay City, St-Cabanglasan, and St-San Fernando were drought-tolerant but waterlogging-sensitive. St-Talakag was sensitive to both stresses, whereas,

Table 6: Interaction of 10 S. tora ecotypes and water stresses of plant height and leaflet count per/plant at 36 DAT

Senna tora Ecotype	Plant Height (cm)		
	Normal	Drought	Waterlogged
St-Cabanglasan	29.13$^{abc\ x}$	25.03$^{a\ x}$	16.17$^{a\ y}$
St-Impasug-ong	23.02$^{bcd\ x}$	24.09$^{a\ x}$	14.14$^{a\ y}$
St-Kadingilan	25.70$^{abcd\ x}$	24.64$^{a\ x}$	22.17$^{a\ x}$
St-Kibawe	28.20$^{abcd\ x}$	22.40$^{ab\ y}$	16.15$^{a\ z}$
St-Lantapan	19.30$^{d\ xy}$	22.96$^{ab\ x}$	14.72$^{a\ y}$
St-Malaybalay City	22.50$^{bcd\ x}$	26.71$^{a\ x}$	14.86$^{a\ y}$
St-Pangantucan	33.29$^{a\ x}$	25.32$^{a\ y}$	19.00$^{a\ z}$
St-Damulog	29.68$^{ab\ x}$	23.23$^{ab\ y}$	17.02$^{a\ z}$
St-San Fernando	20.09$^{cd\ xy}$	22.94$^{ab\ x}$	15.38$^{a\ y}$
St-Talakag	21.62$^{bcd\ x}$	14.50$^{b\ y}$	13.15$^{a\ y}$
P value (Stress, A)	<0.0001		
P value (Ecotype, B)	<0.0001		
P value (AxB)	0.0205		
Coefficient of Variation (%)	15.87		

Means within column with common letters (a, b, or c) and within row with common letter (x, y, or z) are not significantly different based on Tukey's HSD at 0.05 level of probability.

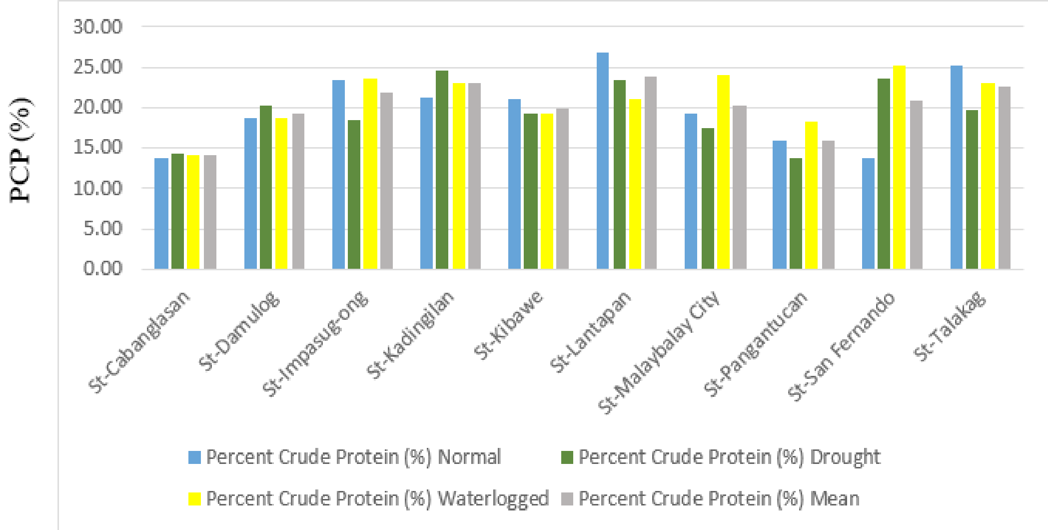


Figure 5. Percent crude protein (PCP) of seeds of 10 S. tora ecotypes subjected to drought and waterlogging for 16 days at vegetative stage

St-Pangantucan, St-Damulog, and St-Kibawe were sensitive to drought but more sensitive to waterlogging.

Resilience of S. tora ecotypes stressed at vegetative stage assessed through recovery is shown in other measured traits that did not vary as per ANOVA. Days to flowering did not differ among water regimes, among ecotypes and no interaction was detected. Days to flowering ranged from 154.37 (drought) to 155.81 d (waterlogged). Plants under normal condition flowered at 155.03 d. Among ecotypes, days to flowering ranged from 148.56 d (St-Kadingilan) to 161.58 d (St-Damulog). Similarly, duration of flowering did not differ for each factor and interaction was not significant. Ecotypes flowered for about a month. Means ranged from 31.23 d (drought) to 32.99 d (waterlogged). Plants under normal condition flowered for 32.13 d. Among ecotypes, duration of flowering ranged from 29.20 d (St-Damulog) to 34.67 d (St-Talakag).

At maturity, seeds were inadequate for analysis of PCP per replication and were not statistically analyzed. However, Figure 5 shows the PCP estimates of bulked seeds per ecotype per water regime. Under the water regimes, mean seed PCP (SPCP) of 10 ecotypes ranged from 19.49% (drought) to 21.08% (waterlogged). Plants under normal conditions had 19.92%. Numerically, St-San Fernando (25.30%) and St-Kadingilan (24.66%) had the highest SPCP after 16 days of waterlogging and drought at vegetative stage, respectively. Among ecotypes across water regimes, mean SPCP ranged from 14.09% (St-Cabanglasan) to 23.77% (St-Lantapan). Similarly, Pant et al. (2014) who subjected plantlets of two Cassia species to temperature stress from 30oC to 44oC for 16 h, reported an increase in the total protein of C. tora at 42oC. Accordingly, this tolerance to increased temperature was probably due to an increase in various osmolytes and an efficient antioxidant system. In this study however, the SPCP estimates still need to be validated using adequate seed samples.

CONCLUSIONS AND RECOMMENDATIONS

Results of these investigations show the phenotypic diversity of S. tora in Bukidnon both in situ and ex situ, and its potential to be also promoted as a food, feed, and medicinal crop in the country. A thorough and wider survey could probably record more uses of this species by the locals. Nonetheless, selection can be made to identify the most desirable ecotypes either for immediate use to commercialize or for further refinement in breeding programs.

However, additional biochemical studies are needed to ascertain its importance for food, feed, and medicine so that it could be well-utilized like in other countries. Its value and importance could increase especially in the midst of a changing climate. In general, results suggest that some ecotypes of S. tora when exposed to drought or waterlogging stress at early vegetative stage for 16 days have some mechanisms of tolerance. Such stress tolerance mechanisms include development of adventitious roots and growth recovery after when normal moisture conditions are restored. Perhaps, it can also become a cover crop encouraged and endorsed for uncultivated farmlands and other marginal areas.

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Research Articles

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Phytochemical screening and *in vitro* antibacterial activity of Syzygium sp.("O-on") crude extracts against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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ABSTRACT

Identification and subsequent isolation of anti-dengue active compounds in medicinal plants are potentially useful for developing antiviral chemical analogues. This study investigated the potential use of aqueous, methanolic, and ethanolic extracts of Syzygium sp. "O-on" against bacterial co-infection of Staphylococcus aureus and Pseudomonas aeruginosa in dengue. Taxonomic identification of Syzygium sp. "O-on" collected from Mindanao signifies possible new species through their distribution, ecology and conservation status. Phytochemical analysis showed that crude aqueous and organic solvent extracts contain medicinally important bioactive compound. Highest antimicrobial potentials were observed for the methanolic extracts of Syzygium sp. which inhibited 62.5 and 51.16% of the tested microorganisms, respectively, with higher activity against antibiotic-resistant bacteria (83.3%). We suggest that the Syzygium sp. could be potentially exploited to control the behaviors of this pathogen as well as for its utilization as a lead compound in screening for anti-dengue agents based on new antimicrobial targets.

Keywords: anti-dengue, natural products, Pseudomonas aeruginosa, Staphylococcus aureus

INTRODUCTION

Dengue is the most common mosquito-borne viral infection in humans with almost half of the world's population at risk of infection (Ferguson et al. , 2015; Rasool et al., 2011; Sessions et al., 2009). The contemporary worldwide distribution of the risk of dengue virus infection and its public health burden are poorly known (Bhatt et al., 2013). In the Philippines, the Department of Health (DOH) has recorded 131,827 dengue cases from January 1 – December 2, 2017 (Medina, 2018). A total of 13,898 dengue cases were reported nationwide from January 1 to February 17, 2018, this is 33.89% lowered compared to the same time period last year (21, 024), mostly in the region of Region IVA (21.64%), NCR (18.83%), Region III (17.18%), Region I (6.71%), and Region VII (5.89%) (Dengue Disease Surveillance Report, 2018). Although, there are currently no licensed vaccines or specific therapeutics that can stop its rapid emergence and global spread, the Philippine government hopes that a cost-effective vaccine will soon be registered.

Accordingly, this viral infection can be adversely affected by bacterial coinfection (Rice et al., 2012; Palacios et al., 2009; Purcell et al., 2002). However, bacterial coinfection can be easily overlooked in dengue-endemic or -epidemic settings wherein, identification of concurrent bacterial infection in dengue patients would be pivotal for triggering timely antibiotic therapy within the usual context of supportive management (See et al., 2013). Status for vaccine development has been described and emphasized that the only alternative available today to control the disease is through the control of its vector Aedes aegypti (Maria, 2002). Plants and plant-derived products are part of the health-care system with large source of natural antioxidants that might serve as leads for the development of novel drugs. Medicinal plants are known for their potent antioxidant property as they contain bioactive compounds such as carotenoids, benzoic acid, cinnamic acid, folic acid, phenols and flavanoids (Moure et al., 2001). Since natural drugs also possesses activity against A. aegypti by their antiviral mechanism, larvicidal, mosquitocidal action and mosquito repellents property, utilization of natural products will provide current approaches for the treatment and management/prevention of

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dengue (Rasool et al., 2011; Qadir et al., 2015).

In clinical and laboratory practices, cases of dengue virus and bacterial co-infection have shown to worsen the outcome of dengue infection (Nagassar et al., 2012). The reason behind such bacterial co-infection of some human pathogenic bacteria is their ability to reach a high density due to the formation of biofilm-like populations. In microbial biofilm, bacterial cells aggregate on the surface in microcolonies and are embedded in an extracellular matrix whose composition is as variable as that of the biofilm (Moscoso et al., 2006; Davey et al., 2003). Since the occurrence of co-infection by dengue and bacterial has been underestimated, and that few reports have been published so far (See et al., 2013; Trunfio et al., 2017; Araujo et al., 2012), dengue virus and bacterial co-infection should be investigated. This study identifies and evaluates Syzygium sp. as a potential candidate that can be used against dengue. Local people from Northern Mindanao, Philippines utilized this unidentified plant locally known as "O-on" to treat dengue, however, no scientific research has been conducted. They used to harvest the small-to medium-sized trees growing as riparian elements along the banks of a stream were the stems were cut and boiled to treat dengue patients. Some locals collected the branches colored grey when dry and sell it to the market were they thought that it can also cure other diseases aside from dengue. However, due to massive collection and selling of this unknown plant, mass propagation must also be considered. Thus, in this study, characterization of this unidentified plant through morphological, ex situ propagation, phytochemical screening, and in vitro antibacterial assay were done to assess the potential used of O-on as antibacterial co-infection in dengue. Aside that it belongs to Syzygium family used as food preservative and medicinal purposes, it also represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate and gallic acid and posses great potential for pharmaceutical, cosmetic, food and agricultural application (Corteés-Rojas et al., 2014; Sritabutra et al., 2011). Clove (S. aromaticum) for example is one of the most valuable spices and found to be a potential larvicidal agent which is an interesting strategy to combat dengue, a serious health problem in Brazil and other tropical countries (Araujo et al, 2016). In addition, methanolic extract of S. cumini were catechins (759.16 ppm) and rutin (142.24 ppm) bioactive compounds were found caused an increase of platelet counts at both 400 and 800 mg/kg and an increase in leukocyte counts at 800 mg/kg (Bandiola and Corpus, 2018).

METHODOLOGY

Plant Materials

Fresh plant sample was rinsed severally with clean tap water to make it dust and debris free. Then the sample was spread evenly and dried in the shady condition for 3 to 4 days. Dried samples were ground using an electric chopper to reduced particle size and increase surface area for extraction and phytochemical screening.

Plant Identification

Leaves, stems, roots and the reproductive parts was collected for proper identification of the plant. Taxonomic Identification of the unknown species of Syzygium was done using a local taxonomic key of Co's Digital Flora (Pelser et al., 2011), the Kew Royal Botanic Garden's key to species of Syzygium (Royal Botanic Gardens Kew Seed Information Database (SID)) and Craven & Biffin (2010). For identification, phenology, distribution and habitat, and morphological comparison of Syzygium sp. with allied taxa (S. rubicundum, S. nervosum, and S. palodense) were used for characterization, (Shareef et al., 2012).

Ex Situ and Mass Propagation of the Plant

The ex situ and mass propagation of Syzygium sp. was done following the method of Lituañas and Amoroso (2006) with slight modification. Young branches of Syzygium were cut into 4 to 5 nodal segment and planted in different soil media (garden soil, sand and vermicast) under greenhouse condition.

Preparation of Plant Extracts

The dried and powdered samples (each 50g) were extracted successively with double distilled water, ethanol and methanol (each 400 ml) for 10-12 hours using a conventional method. Then the collected solutions were filtered through Whatman No-1 filter paper.

Qualitative Phytochemical Analysis

Phytochemical examinations were carried out for all the extracts, as per standard methods (Yadav & Agarwala, 2011).

Detection of carbohydrates

Few drops of Benedict's reagent was added to each portion of crude extacts and the mixture was boiled, allowed to stand for two minutes, and the formation of reddish brown precipitate indicates positive result.

Detection of phenols and tannins

About 0.5g each portion crude extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 2% $FeCl_3$ solution were added to 2ml of the fitrate, occurence of a blue-black or blue-green precipitate indicates a positive results.

Detection of flavanoids

About 0.5g of each portion was dissolved in different solvent, warmed and then filtered. Few drops of 2 % NaOH solution were added, an intense yellow color was formed which turned colorless on addition of few drops of diluted HCl acid which indicates the positive result.

Detection of saponins

One gram of each portion was boiled with 5ml of distilled water and filtered. About 3ml of distilled water was added to the filtrate shaken vigrously for about 5 minutes, the formation of stable foam was taken as an indication for the presence of saponins.

Detected of steroids and terpenoid

About 0.2g of each portion were added with 2ml of chloroform and concentrated H_2SO_4 added sidewise, the red color produced in the lower chloroform layer indicates the presence of steriods. For terpenoids, filtrate was dissolved in 2ml of chloroform and evaporated to dryness, and then 2ml of concentrated H_2SO_4, was added and heated for about 2 minutes. The grayish colour indicated the presence of terpenoids.

Dectection of alkanoids

Few quantity of each portion was stirred with 5ml of 1% aqueous HCl on water bath and was heated gently. The filtrate was treated with Wagner's reagent (iodo-potassium iodide) and the formation of reddish/redish-brown precipitate indicated the presence of alkaloids.

Assessment of Antibacterial Activity

Syzygium sp. extracts prepared from different solvents was evaluated for antibacterial activity by diffusion and broth dilution assays. To achieve the different pH values, prepared extracts were added with NaOH or HCl solution. Sterile nutrient agar (NB) plates was prepared for bacterial strains and inoculated by a spread method under aseptic conditions. Positive control for pH 5 (NB agar adjusted with HCl solution) and the negative control (sterile water adjusted with HCl solution) while for the positive control for pH 12 (NB agar adjusted with NaOH solution) and the negative control (sterile water adjusted with NaOH solution). Filter paper disc

$$ZOI = \frac{\text{Diameter of zone of inhibition} - \text{Diameter of disk}}{\text{Diameter of disk}}$$

of 5mm diameter (Whatman No. 1 filter paper) was prepared and sterilized. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (ZOI in mm) produced by the respective extract at the end of incubation period.

RESULTS AND DISCUSSION

Natural products extracted from tissues of terrestrial plants produces innumerate metabolites with distinct biological properties that make them

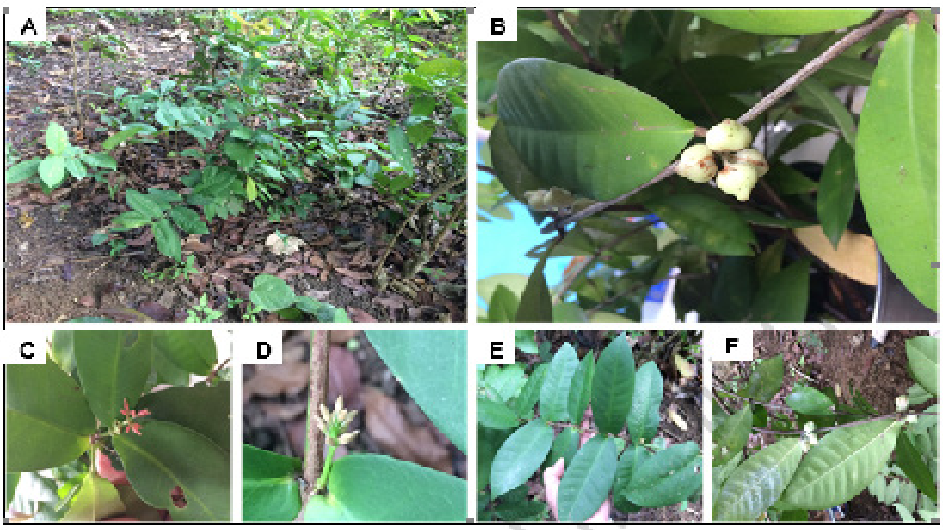

Figure 1. Morphological identification of Syzygium sp. (A) habit, growing in an undershade condition, (B) brachlet with mature fruit, (C) part of brachlet with flower, (D) part of brachlet with immature fruit, and (E&F) brachlet with immature and mature fruit respectively.

Table 1. Morphological comparison of Syzygium sp. with allied taxa (Shareef et al, 2012)

Characters	S. rubicundum	S. nervosum	S. palodense	Syzygium sp.
Bark surface	Pale, orange-brown, smooth	Greyish-white or dark brown, smooth	Pale-brown, smooth	Blackish grey, rough
Brachlets	Sharply 4-angular, pinkish-grey when dry	Terete, compressed, swollen at nodes, grayish white when dry	Sharply 4-angular, slightly winged, brownish when dry Crimson	4-angular, grey when dry
Young leaves	Rose-pink	Pink	Crimson	Crimson
Leaf lamina	Narrowly elliptic, 2.5 - 7 x 1.5 -3 cm	Elliptic-obovate, 6-27 x 3-10 cm	Elliptic to elliptic-oblong, 5.5-9.5 x 2.4 - 5.4 cm	Elliptic lanceolate 6.5 - 9.7 x 3.4 - 4.2 cm
Leaf apex	Caudate-acuminate	Obtuse nor obtusely acuminate	Caudate-accuminate	Accuminate
Leaf base	Tapering	Tapering	Obliquely-cuneate	Rounded
Leaf texture	Thinly coriaceous, dull fufuos brown to chocolate-brown beneath on drying	Thinly coriaceous, pale grey-green when dry	Coriaceous, dark-brown above and pale brown-beneath when dry	Thinly coriaceous, dark-brown above to pale-brown beneath when dry
Lateral nerves	Many, distinct, close and slightly elevated beneath, obscure above, veinlets finely reticulated	10-12 pairs on either side of midrib, distant, 8-9 mm apart, arcuate, shallowly depressed above, distinctly elevated beneath, veinlets faintly reticulated	Many, close, obscure above and distinct beneath, veinlets faintly reticulated	
Petiole Inflorescence & flower	0.5-0.6 cm long, very slender terminal or subterminal or axillary, to 7cm long. Inflorescence branches slender, flowers pedicellate, pinkish-white	1.1-2.3 cm long, slender lateral, to 14 cm long in the axil of fallen leaves; peduncle and branches terete, flowers sessile, greenish-white	0.2-0.6 cm long, thick and robust terminal or subterminal 4.5 - 9 cm long. Inflorescence branches not slender, quadrangular, flowers sessile, creamy-white	0.4-0.6 long, thick and robust laterial within nodes, fused to petiole, compund inflorescence, creamy-white
Calyx	Repand, cup sharped, 4-lobed persisten, shortly tubinate	Ovoid turbinate becoming campanulate with short obtuse lobes or nearly truncate, calyptrate petals fused, oblong, calyptras apiculate	Campanulate, lobes 4, persistent, deltoid to suborbicular	Tubular
Corolla	Petals fused, calyptrate, elliptic, calyptras without apicule	Petals fused, oblong, calyptras apiculate	Petals fused, calyptrate, calyptras without apicule, obicular to suborbicular	Petals fused oblong calyptras
Fruit	Globose, ca 1cm diam, purple	Globose to ovoid, ca. 1cm diam, purple	Subglobose to obovoid, to 2.2 x 1.8 cm, dark-purple	Globose to oxheart, 1.8 x 2.0 cm, creamy white to deep pinky

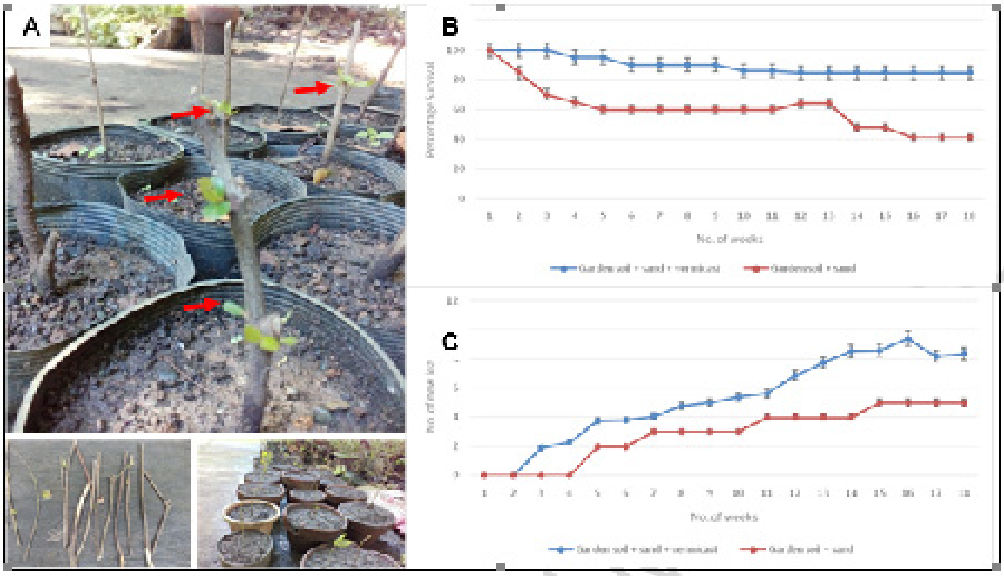

Figure 2. Propagation protocols optimization. (A) Young branches of Syzygium were cut into 4-5 nodal segments and potted in medium composed of 1:1:1 garden soil, sand, and vermicast, and (B) percentage survival of Syzygium sp. and (C) no. of new leaves formed after 18 weeks of planting under greenhouse condition observed grown through cuttings.

valuable as health products or as structural templates for drug discovery. Local people in Oroquieta City use Syzygium sp. to treat dengue, thus to investigate Syzygium sp. as potential candidate as anti-dengue lead compound, the authors collected a few specimens that were found to be similar to the new species of Syzygium (Myrtaceae) from the southern Western Ghats of Kerala, India (Shareff et al., 2012) but differ in many aspects.

Phenology, Distribution and Habitat

Syzygium sp. known as "O-on" is only from Brgy. Clarin Settlement, Oroquieta City, Misamis Occidental at ca. 18 m elevation. The present population comprises about 47 small- to medium-sized trees and numerous seedlings in its vicinity. It is growing as a riparian element along the banks of a stream. In the present locality, the members of the populations are not yet protected by the legislation of the said barangay. The authors collected a few seedlings from the type locality and are conserving at Central Mindanao University, as part of its ex-situ conservation.

Species of Syzygium are present in virtually all most of Oroquieta City, and are often important components of the biological communities, so the lack of taxonomic resolution presents a serious impediment for a better understanding of ecological processes as well as for its conservation (Fig 1 and Table 1).

Ex situ Conservation of Syzygium sp "O-on

Ex situ conservation of Syzygium sp. through stem cutting exhibited 85% survival after 18 weeks of planting in garden soil, sand, vermicast (1:1:1) potting medium under greenhouse (Fig. 2). The high percentage survival could be attributed to the high NPK content of vermicast. The sand particles provide good aeration and root growth (Lituañas & **Amoroso, 2006). However, stem cuttings planted in garden soil and sand (1:1) potting medium showed 41% survival after 18 weeks under greenhouse condition.**

In terms of number of new leaves formed, garden soil, sand, vermicast (1:1:1) potting medium revealed more new leaves formed (Fig. 3) and a higher average plant height (Fig. 3B) after 18 weeks of planting in the greenhouse. This indicates that the stem cuttings of Syzygium sp. already adapted to the greenhouse condition and growing vigorously after 18 weeks of planting (Fig.3A).

Phytochemical Analysis

Qualitative phytochemical analysis of Syzygium sp. plant extracts revealed the presence of constituents which are potential for pharmaceutical commercial development (Khoo et al., 2016). Analysis of the plant extracts revealed the presence of medically active compound as summarized in the Table 2. The results revealed the presence of medically active compounds in the plant studied. From the table, it could be seen that, carbohydrates, phenols and tannins, flavanoids, saponins, steroids, terpenoids and alkaloids were present in the extracts.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites since they possess biological properties (Singh, 2007). Accordingly, natural antioxidant mainly comes from plants in the form of phenolic compounds such

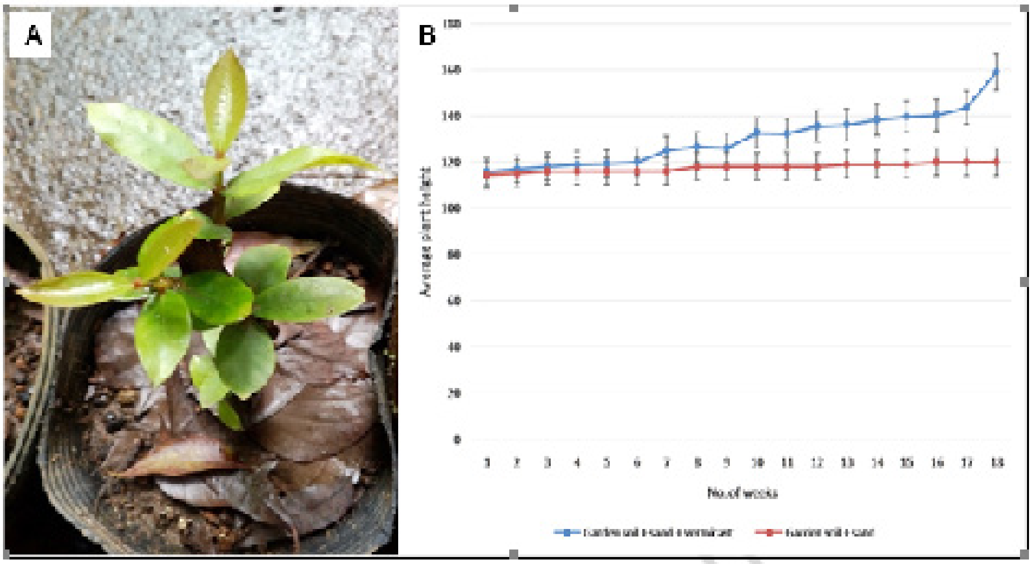

Figure 3.Syzygium adaptation condition. (A) Syzygium sp. growing vigorously after 18 weeks of planting under greenhouse condition and the (B) average plant height of Syzygium sp. after 18 weeks of planting under greenhouse condition.

as flavonoid, phenolic acids, tocopherols etc. (Zhao et al., 2014). Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection probable due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Yadav & Agarwala, 2011). Tannins bind to proline rich protein and interfere with protein synthesis and they also are effective antioxidant and show strong anticancer activities (Marjorie, 1996). The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation since it has the property of precipitating and coagulating red blood cells (Just et al., 1998). Steroids are used in the treatment of idiopathic thrombocytopenic purpura (IPT) to increase the platelet count which is mediated by auto antibodies (Shashidhara, et al., 2013) have been reported to have antibacterial properties. Alkaloids are important phytochemicals of medicinal plant and can be used as vaccine candidates for viruses since it could serve as important inhibitors to inhibit the replication of dengue virus (DENV) but need further in vitro investigations to confirm their efficacy and drug ability (Qamar et al., 2014). Based from the results obtained on the presence of phytochemical compounds in Syzygium sp. plant, it might be a valuable reservoir for bioactive constituents for substantial medicinal merit.

Antimicrobial Analysis

Since we wanted to examine the co-bacterial infection of dengue, we further tested its antimicrobial activities using varied solvent used for extraction, pH level and increasing concentration through percent inhibition (Table 3). Result showed moderate inhibition activity with the zone range of 3–5 mm since we did not obtained high purity concentration of the extracts in different solvent used. Maximum inhibition was observed against P. aeruginosa (5 mm) and minimum inhibition against S. aureus (3 mm). Among the solvent tested, methanol extracts of Syzygium sp. showed significant antimicrobial

Table 2: Phytochemical constituents of Syzygium sp.

Phytochemical Screening	Aqueous extract	Methanolic extract	Ethanolic extract
Carbohydrates	+	+	+
Phenols/Tannins	-	+	-
Flavanoids	+	+	+
Saponins	+	+	-
Steroids	+	+	+
Terpenoids	-	+	-
Alkaloids	+	+	+

(+) Positive response (-)Negative Response

Table 3: Percent inhibition of Syzygium sp. extracts against bacterial co-infection in dengue.

Treatment	Percent Inhibition (%)								
	Different Solvent			pH Level			Different Concentration		
Bacterial Strain	*aqueous*	*methanol*	*ethanol*	*pH5*	*pH7*	*pH12*	*ImM*	*10mM*	*100mM*
S. aureus	55.8	67.51	56.50	53.97	63.16	56.45	56.45	61.42	73.18
P. aeruginosa	53.69	85.08	67.51	60.24	67.15	70.45	60.45	70.85	72.35

activity against multi-drug resistant clinically isolated microorganisms. In terms of pH level, pH 12 yielded higher percent inhibition in P. aeruginosa while at pH 7 in S. aureus implying that increasing pH level inhibit microbial growth. Accordingly, P. aeruginosa outer member is sensitive when exposed with chitosan nanoparticles when increasing pH values however, increasing the pH from 7.5 to 9 abolished chitosan nanoparticles activity (Aleanizy et al., 2018). On the other hand, the optimal pH at which S. aureus can be detected at pH 6.5 and slight decrease of the probability of growth can be noticed in the pH interval of 7.0-7.5 at more stringent condition (Valero et al., 2009). Ensuring a suitable pH range is important in maintaining the growth stability of the tested microorganism. This study also demonstrated that increasing concentration of Syzygium extracts significantly yielded higher percent inhibition. The antimicrobial activity of the Syzygium may be due to tannins and other phenolic constituents that if undesirable amount can precipitate proteins and inhibit digestive enzymes and play an important role in chelating transitional metals and scavenging free radicals (Chattopadhyay et al., 1998). Although, the mechanism of the action of these plant constituents is not yet fully known, it is clear that the effectiveness of the extracts largely depends on the type of solvent used, pH level and concentration. This observation might indicate that there can be an existence of non-polar residues in the extracts which have higher both antimicrobial and phytochemical activities.

CONCLUSION

In conclusion, our results indicate that the Syzygium sp collected from Misamis Occidental have the potential to control P. aeruginosa and S. aureusgroup behaviors. Morphological characterization of Syzygium sp. compared with its allied taxa differs in several characteristics. It is similar to the new species of Syzygium (Myrtaceae) from the South Western Ghats of Kerala, India but differs from its bark surface when dry, color of the leaves when young, tabular calyx, and oblong calyptras and may represent a new species of Syzygium. Ex situ conservation also showed that cutting method have higher percent survival than using wildlings for massive propagation. Media preparation must also be considered for it propagation were garden soil, sand and vermicast (1:1:1) showed higher % survival under greenhouse condition. Phytochemical analysis also showed that crude aqueous and organic solvent extracts of Syzygium contains medicinally important bioactive compound. The antimicrobial activity of Syzygium sp. methanolic crude extracts were considerably more effective radical scavengers than those using lest polarity solvent, indicating that antioxidant or active compounds of different polarity could be present in leaves of Syzygium sp. Antimicrobial activity varies widely depending on the several factors, test medium, types of pathogens (P. aeruginosa and S. aureus), type of solvent used, pH levels and Syzygium sp concentration sensitive. Although Syzygium sp. contains bioactive compounds and appears to inhibit multiple targets could be a valuable addition to P. aeruginosa and S. aureus group-behavior inhibitors for the development of anti-virulence compounds, further studies on the efficacy of these natural antimicrobial agents in a range of food products as well as evaluation of potential interactions of antimicrobial compounds with components of food matrices must be investigated.

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Phytochemical and Antioxidant Activity Variation of Processed Edible Ferns

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ABSTRACT

The main and interaction effects on the total phenolic content (TPC), total flavonoid content (TFC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical seavenging activity of two edible ferns in various food processing methods were investigated. Factors include fern species (Diplazium eculentum and Stenochlaena palustris), and type of processing methods at various levels (blanching at 80°C for 1, 2 and 3 minutes, boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven dry at 70°C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control) were studied. Main effects were based on fern species, process and levels while, interaction effects comprise the combinations of the main effects. Results revealed that the main and interaction effects to all parameters used were significantly different. D. esculentum has a higher TPC and TFC than S. palustris. However, S. palustris attained higher DPPH radical scavenging activity than D. esculentum. Among the processing methods, oven drying attained high TPC and TFC while steaming increased the DPPH radical scavenging activity. Both of these processes were best at low levels.

Keywords: edible ferns, phenolics, flavonoids, antioxidant activity

INTRODUCTION

The Philippine archipelago is one of the most important biodiversity hotspots on earth with high amounts of endemic plant and animal species (Langenberger et al., 2006). In the Philippines, about 930 species of ferns with more than 60 species having definite or probable usages (Amoroso and Amoroso, 2003). Many species can be considered as sources of food, raw materials for handicraft manufacture, medicine, organic fertilizer, building materials, potting medium and as ornamentals (Amoroso and Amoroso, 2003). These species have been known for their antioxidant activities based on different studies of different researches. Antioxidant activity had been a help to fight against radical damages, which might result in degenerative human complications. One way to combat radical damages is to improve body antioxidant status, which could be accomplished by higher consumption of vegetables and fruits of high antioxidant content. Foods from plant origin usually contain natural antioxidants that can cleanse free radical (Amin et al., 2006). However, green leafy vegetables, such as edible ferns are not usually consumed directly, unlike fruits. Prior to its consumption, these vegetables usually undergo a heating process. Blanching, boiling, microwave and steaming are considered thermal processing and can greatly affect the plant material to cause changes in its physical state and chemical properties.

Food processing methods involve several processes such as freezing, the use of pressure, plasma and many others. These processes are usually applied to ensure the safety of the food products. Food processing methods, when applied to leafy vegetable, renders it to be more edible and less toxic but may have a drastic decrease in the antioxidant property (Oboh, 2005). While plenty of evidence flourishes on the antioxidant capacity of tropical fruits, there is lack of evidence on antioxidant and phytoconstituents existing in tropical green leafy vegetables with their phytochemicals and antioxidant capacity when the processing operation is employed. Hence, the study of two edible ferns, D. esculentum and S. palustris were conducted to evaluate the effects of the different

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methods and levels of food processing operations on the TPC, TFC and DPPH radical scavenging activity.

METHODOLOGY

Materials, Chemicals, and Equipment

Two species of ferns were used in this study namely: D. esculentum and S. palustris. The equipment used were thermometer, steamer, microwave, oven, blender, pipettor and analytical balance. The Folin-Ciocalteu reagent, quercitin, gallic acid, ascorbic acid, DPPH, aluminum chloride, sodium acetate, sodium carbonate, methanol, and ethanol used were purchased from Sigma- Aldrich®. Other materials such as glass jars, Erlenmeyer flask, test tubes, test tube racks, volumetric flask, sterilized pipette tips, 96-wells microplate, and spatula were needed. The absorbance was read using a Molecular devices® Spectra Max 250 microplate reader.

Plant Materials

The ferns were freshly picked early in the morning at the University Fernery located in the campus of Central Mindanao University, University Town, Musuan, Maramag, Bukidnon, Philippines and at Mt. Musuan fernery.

Sample Extraction

Fern samples were processed according to the types of processing methods involved with its respective levels (blanching 80°C at 1, 2 and 3 minutes; boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven dry at 70°C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control). Processed fern samples were extracted using analytical grade absolute methanol following the method of Nayak et al. (2009). The methanolic extract was prepared by homogenizing 30 grams of the fern sample with 150 mL of methanol and allowed to stand for 48 hours. The methanolic extract was filtered using Whatman no. 42 filter paper. The filtrate was then subjected to phytochemical analysis.

Phytochemical Screening Method

Determination of Total Phenolic Content (TPC)

A modified method according to Chatatikun and Chiabchalard (2013) was used for the TPC determination using the Folin- Ciocalteu method. Methanolic plant extract of 200 μL (2.5 mg/mL) was transferred to a microcentrifuge tube. Similarly, gallic acid (300 ppm) as the standard solution with different concentrations (0, 10, 20, 25, 30, 35, 40, 50 μg/mL) was transferred into a microcentrifuge tube. The extracts and standard solutions were added separately with 200 μL of 10% Folin- Ciocalteu reagent followed with 800 μL of 700 mM sodium carbonate solution. The microcentrifuge tubes were incubated at room temperature for 2 hours. After incubation, the microcentrifuge tubes were centrifuged at 11,000 rpm for 2 minutes. Then, 200 μL of the supernatant was transferred to the assigned microplate wells. The absorbance of the reaction mixture was measured at 750 nm using UV Spectrophotometer microplate reader. The total phenolic content for the methanolic plant extract was determined and was expressed as mg Gallic Acid Equivalent (GAE) per kilogram of plant sample.

Determination of Total Flavonoid Content (TFC)

The modified method according to Chatikun and Chiabcharlard (2013) was used for the TFC determination using the aluminum chloride colorimetric assay. Methanolic plant extracts of 50 μL (2.5 mg/mL) were transferred into the assigned microplate wells. Quercetin (100 ppm) dissolved in 80% methanol was used as the standard solution with different concentrations of 6.25, 12.5, 25, 50, 100 μg/mL. The standard solutions were transferred into the assigned microplate wells. 10 μL of 10% aluminum chloride solution, followed by 130 μL of 95% methanol and 10 μL of 1 M sodium acetate was added to the mixture in the microplate wells. All reagents were mixed and incubated at room temperature for 40 minutes in the dark. The absorbance of the reaction mixture was measured at 415 nm using the UV Spectrophotometer microplate reader. The total flavonoid content of the methanolic plant extract was determined and was expressed as mg Quercetin Equivalent (QE) per kilogram of plant sample.

DPPH Radical Scavenging Activity Determination

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the extract was determined using the method of Mosquera et al. (2007) as described by Amoroso et al. (2014). Briefly, 1mL of methanolic sample extract was diluted with 1mL methanol. In A 96-well template, 75 μL of the resulting solution was transferred to the assigned wells and was further diluted with 100 μL methanol. It was added with 75 μL DPPH solution (20mg/L methanol). Ascorbic acid (400ppm) and methanol served as positive and negative control, respectively. Blanks for each samples were prepared by adding 75 μL extract and 175 μL methanol. The mixture was incubated at room temperature for 30 min in the dark. The plate was shaken for 10 seconds in the microplate reader before measuring the absorbance at 517 nm. The scavenging activity was calculated using Eq. 1:

% DPPH radical scavenging activity=(Absorbance DPPH-(Absorbance DPPH+sample/Absorbance sample))/(Absorbance DPPH-Absorbance Ascorbic acid)×100

Experimental Design and Statistical Analysis

Fern species (D. esculentum and S. palustris), type of processing with respective levels (blanching 80°C at 1, 2 and 3 minutes; boiling at 4, 5 and 6 minutes; freezing at 1, 2 and 3 weeks; microwave at 30, 60 and 90 seconds; oven drying at 70°C for 1, 3 and 5 hours; steaming at 8, 9 and 10 minutes; vacuum packaging at 1, 2 and 3 weeks and fresh as control) were used as experimental factors. Data were analyzed using Statistica 6.0 through a Balanced Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference Test for the comparison between treatments to determine the main and interaction effects of the variables employed affecting the TPC, TFC and DPPH radical scavenging activity of the fern species used.

RESULTS AND DISCUSSION

Main Effects

It is known that phenolic compounds as a source of antioxidants are ubiquitous in plants that are an essential part of the human diet, and are of considerable interest due to their antioxidant properties (Balasundram et al., 2006). According to Heim et al. (2002), the antioxidant activity poses a greater contribution to the beneficial effects derived from phenolic compounds. Comparison between fern species has been reported by Amoroso et al. (2014) that D. esculentum has higher phenolic content than S. palustris. The same findings were also reported by Semwal et al. (2013) that D. esculentum showed the strongest overall antioxidant compared to other pteridophytes. These results are in agreement to the present study wherein D. esculentum showed a higher amount on the TPC, TFC, and DPPH scavenging activity (expressed as ascorbic acid equivalent) regardless of the process and levels employed in the experiment (Table 1). The lower values obtained from S. palustris suggest that the fern species has likely undergone oxidation when subjected to various processing operations and the levels applied.

Methods on the processing of ferns revealed that oven dried samples were consistently highest in TPC and TFC except for the DPPH scavenging activity as shown in Table 1. According to Capecka et al. (2005), the drying process may result to high or low levels of TPC which depends on the type of phenolic compounds present in the plant material

Table 1. Main effects on the total phenolic content, total flavonoid content and DPPH radical scavenging activity

Effects	Total Phenolic content (mg GAE/g)	Total flavonoid content (µg QE/g)	DPPH radical scavenging activity expressed as Ascorbic acid equivalent (mg AEAC/g)
Fern species			
D. esculentum	5.99±0.58[a]	1.55±0.11[a]	1.45±0.13[a]
S. palustris	5.34±0.68[b]	0.93±0.11[b]	1.33±0.08[b]
Type of food processing			
Blaching	4.16±0.39[g]	1.16±0.38[d]	1.59±0.05[c]
Boiling	4.69±0.38f	1.08±0.28e	1.63±0.05b
Freezing	3.67±1.44h	1.09±0.17e	1.19±0.05g
Microwave	5.22±0.7d	1.21±0.30cd	1.46±0.11d
Oven dried	8.49±1.51a	1.81±0.26a	1.20±0.20f
Steaming	5.12±1.71e	1.23±0.25	1.68±0.04a
Vacuum pack	6.18±1.06	1.28±0.21b	0.93±0.36
Fresh	7.74±0.32b	1.06±0.17e	1.39±0.23E
Levels			
Low	6.37±0.87a	1.29±0.19a	1.40±0,08b
Medium	5.39±0.73b	1.16±0.16b	1.35±0.
High	5.23±0.56c	1.16±0.16b	1.43±0.11a

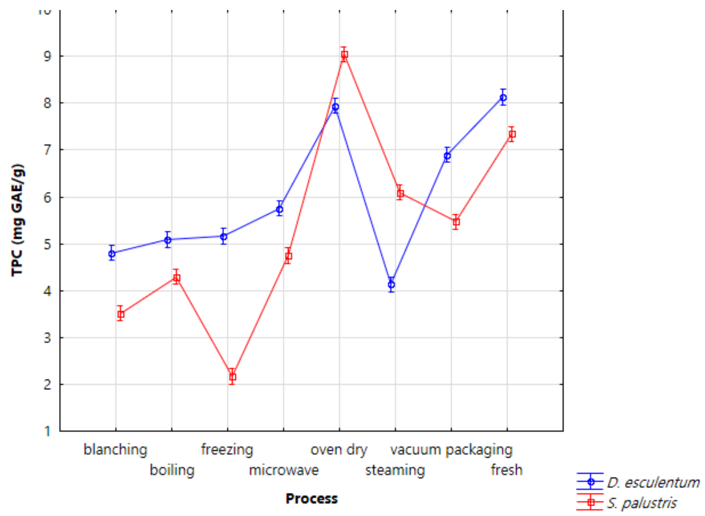
Means with the same letter are not significantly different between treatments in the column at p<0.05.

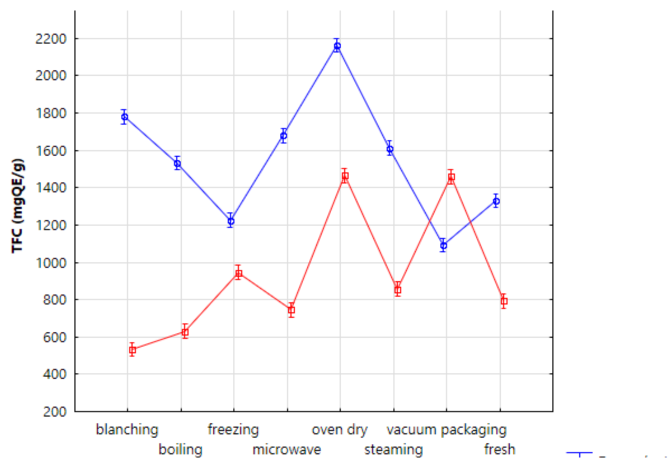
and their location in the cell. For example, related studies revealed an increase in polyphenolic content after drying which has been reported for tomatoes (Chang et al., 2006) and shiitake mushroom (Choi et al., 2006). TPC in vegetables can be affected by different drying methods as reported by Hung and Duy (2012). Specifically, free and bound forms of vegetables' TPC were determined using freeze and heat drying methods. Their findings implied that the phenolic compounds in vegetables existed primarily in free form rather than in bound form. In addition, free phenolics were significantly higher in freeze-dried vegetables, their bound phenolics were slightly lower as compared with the heat-dried vegetables. Bound phenolics were not affected by drying methods because of their association with the cell wall of vegetables.

Results also revealed that at low levels of processing, there was a significant increase in TPC and TFC values. This is in contrast to the DPPH radical scavenging activity, which favors the high levels employed in this study. Treatments at low temperature and short time might have a minimal loss of antioxidant properties as compared to higher temperature and longer time. This is speculated to be due to minimal thermal degradation and leaching into the water, resulting in solubilization of phenolic compounds (Jaiswal et al., 2011). Moreover, a significant increase in the DPPH radical scavenging activity at a high level was observed. This is due to the increased solubilization of ferulic acid sugars with both increased heating time and temperature, thus, enhancing the antioxidant activity of the commodity. Thermally processed commodity might retain or increase its total phenolics and total antioxidant activity despite the loss of vitamin C (Dewanto et al., 2002). Correspondingly, the bound phenolic content decreased as they were released from esterified and insoluble-bound forms. Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents, although disruption of cell walls would also release oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism and Haard, 1996; Dewanto et al., 2002). In addition, thermal processing at a higher temperature will deactivate these enzymes, thus preventing the loss of phenolic acids.

Interaction Effects

Interaction effects with fern species and the processing methods as factors exhibited great





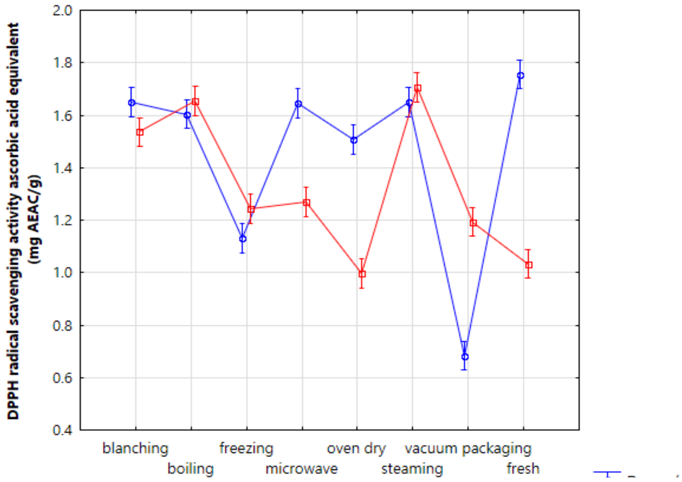


Figure 1. Interaction effects of fern species and the processing methods involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

significance to TPC, TFC, and DPPH scavenging activity as shown in Figure 1. Results revealed that there was a decrease in the total phenolic content of the D. esculentum when subjected to various processing, as compared with the fresh sample (control). A decrease in TPC values were noted in steaming (3.99 mg GAE/g sample) followed by blanching (3.32 mg GAE/g sample), boiling (3.04 mg GAE/g sample), freezing (2.97 mg GAE/g sample), microwaving (2.38 mg GAE/g sample), vacuum packaging (1.23 mg GAE/g sample) and oven-drying (0.19 mg GAE/g sample). In contrast, S. palustris exhibited a decrease of TPC with the following processing methods: freezing (5.18 mg GAE/g sample), blanching (4.19 mg GAE/g sample), boiling (3.06 mg GAE/g sample), microwaving (2.58 mg GAE/g sample), vacuum packaging (1.88 mg GAE/g sample) and steaming (1.88 mg GAE/g sample) except in oven-drying with an increase of 1.69 mg GAE/g sample.

D. esculentum showed gains and losses in TFC values with the types of processing involved. Among all types of processing operations, vacuum packaging (0.24 mg QE/g sample) and freezing (0.11 mg QE/g sample), showed a decrease in TFC compared to the fresh sample. However, an increase in TFC was observed with oven drying (0.83 mg QE/g sample), blanching (0.45 mg QE/g sample), microwaving (0.35 mg QE/g sample), steaming (0.28 mg QE/g sample) and boiling (0.20 mg QE/g sample). On the other hand, S. palustris showed a decrease on TFC with blanching (0.26 mg QE/g sample), boiling (1.16 mg QE/g) and microwaving (0.04 mg QE/g sample) but there was an increase in TFC in oven drying (0.68 mg QE/g sample), vacuum packaging (0.67 mg QE/g sample), freezing (0.16 mg QE/g sample) and steaming (0.07 mg QE/g sample) compared to the fresh sample.

The interaction of fern species and types of processing resulted to a higher TPC on S. palustris and TFC for D. esculentum both on oven dried. This increase would be associated with various reasons. According to Rhandhir et al. (2007), phenolic compounds are usually present in bound states as conjugates with sugars, fatty acids or proteins. It could be speculated that the disassociation of these complexes followed by some polymerization of the phenolic contents may be responsible for the increase on the antioxidant capacity. The increase on flavonoid content could be due to the absence of the enzymatic oxidation, caused by enzymes, which prevent the loss of the antioxidant compounds in the heat- treated plant metabolites (Dewanto et al., 2002). Microwave treatment for a short time would inactivate enzyme that would cause degradation of antioxidant properties (Ravichandran et al., 2013). The increase of antioxidant activity on microwave treatment was in agreement with the results from Dewanto et al. (2002a); Dewanto et al. (2002b). Furthermore, thermal processing increases the total antioxidant activity, which could be due to their different vitamin contents, and phenolic compounds, which can act synergistically but are then sensitive to processing.

The interaction effects between fern species and different levels of temperature also showed a significant effect on the samples as shown in Figure 2 except on TPC. Results revealed that D. esculentum showed a higher value of both TPC and TFC at a lower level while S. palustris had a higher value of antioxidant activity at low levels similar to that of D. esculentum.

Temperature is an important factor for antioxidant stability (Ravichandran et al., 2013). Results showed that at low levels, high antioxidant properties was observed in both fern species. The results suggest that the loss in water-soluble antioxidant was minimal. On the contrary, prolong thermal treatment resulted to decrease in antioxidant activity in both plant species suggesting that there was a loss of antioxidant enzyme activity that could also be accompanied by loss of bioactive compounds.

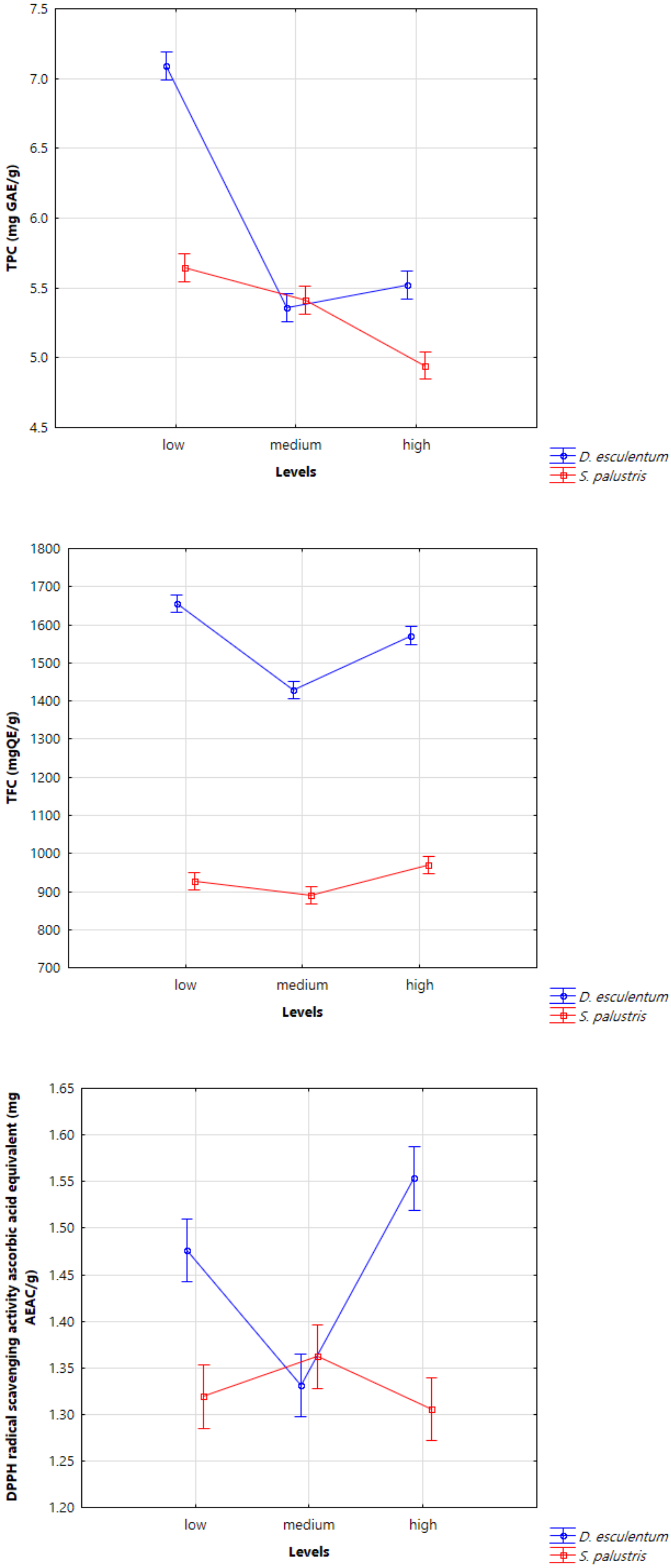
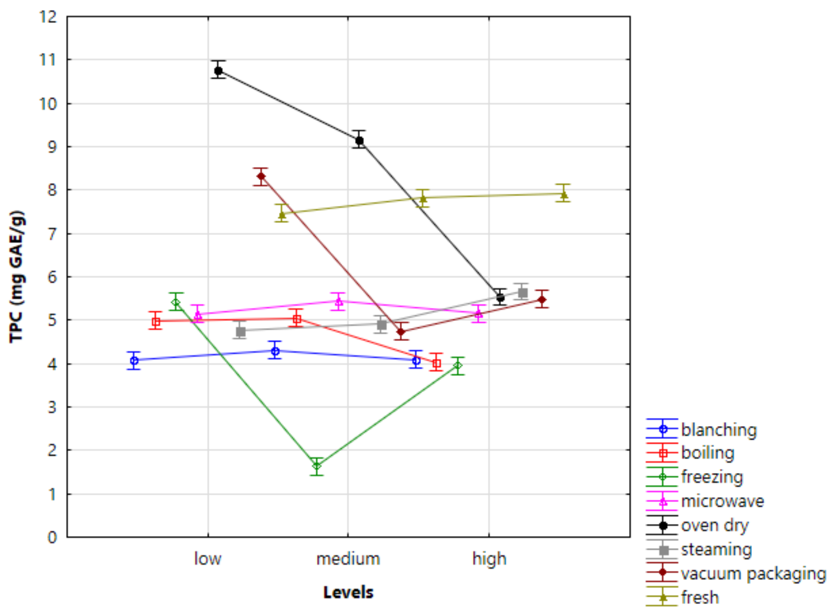


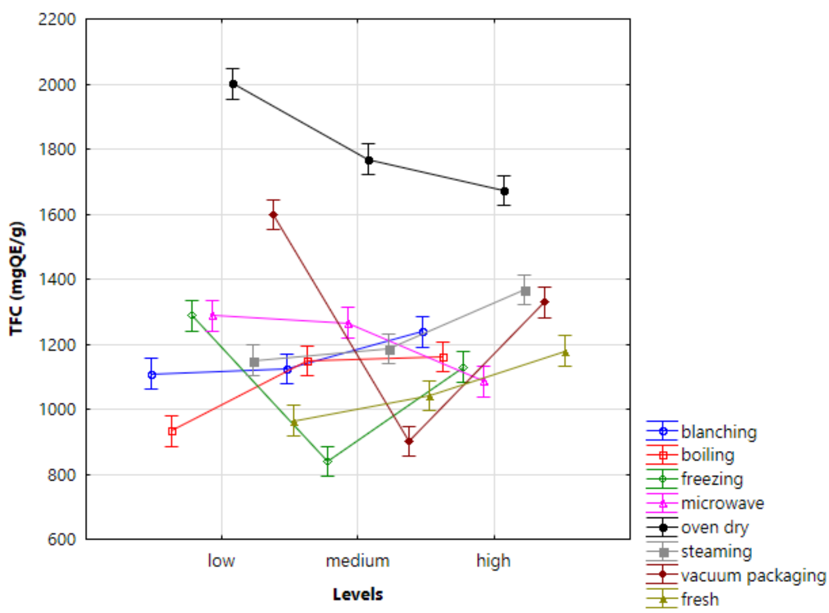
Figure 2. Interaction effects of fern species and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

The interaction between the type of processing methods and levels showed that among the processes and levels, there was an increase in both TPC and TFC as observed in the oven-dry treatment at a low level (Figure 3). Similarly, an increase in antioxidant activity was observed on microwave treatment at a high level. This is mainly due to the collapse of the intercellular spaces of the plant which will eventually liberate phenolic compounds (Hoissain et al., 2010).

Phenolic compounds are antioxidants and are subject to oxidation during storage and processing of foods (Titchenal and Dobbs, 2004). The major contribution to the antioxidant activities of plant foods is related to their content of polyphenols. Therefore, it is important to consider the effect of each treatment applied in the process upon retaining the antioxidant properties of the plant material (Jaiswal et al., 2011). Phenolic compounds are usually susceptible to different factors during the extraction process. For example, using a low temperature for drying yields the highest amount of phenolic content. However, increasing the temperature decreased the values of the phenolic content of the sample. At higher temperature, certain phenolics may decompose or react with other plant components (Jaiswal et al., 2011).

The interaction effect between processing methods and different levels of temperature also gives a significant effect on the plant sample. Results showed that among processing operations, oven drying at low level showed higher values to both TPC and TFC. While steaming at medium level revealed a higher value of DPPH radical scavenging activity. The





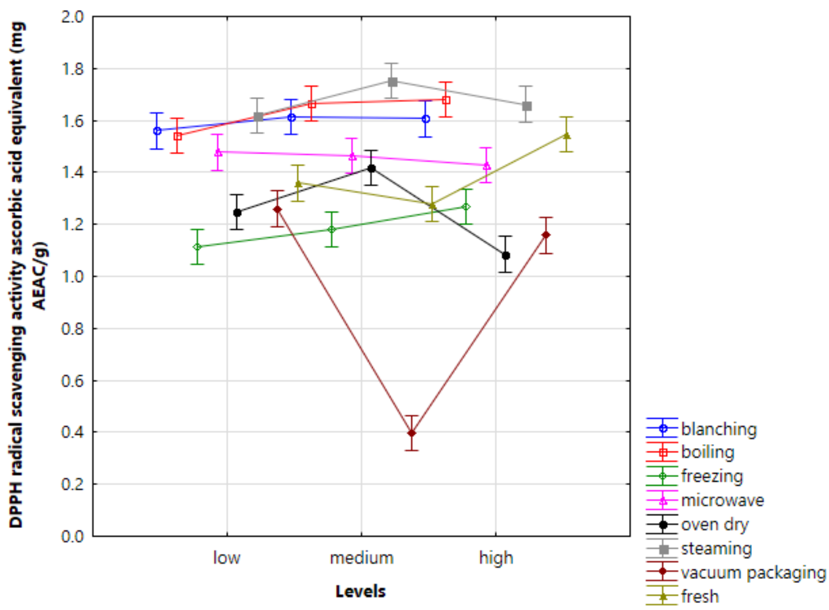
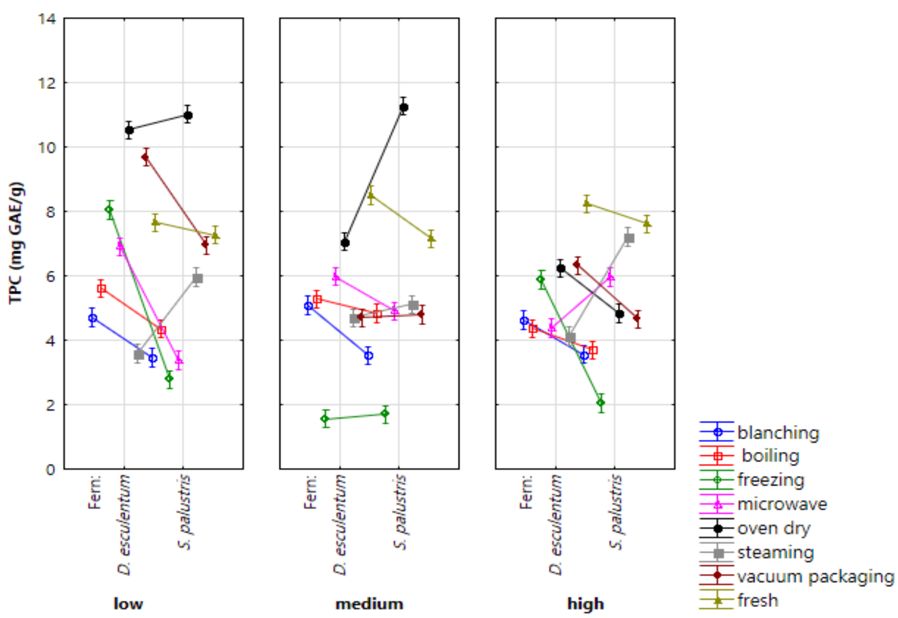


Figure 3. Interaction effects on the types of processing methods and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

increase of antioxidant activity at medium level is in agreement to the study of Ravichandran et al. (2013), wherein the longer time of processing yields an increase in the antioxidant activity. The increase in antioxidant activities of treated samples shows that the antioxidant activity depends on the other polyphenols, which could have been increased during the design of treatments.

Figure 4 shows the interaction between the three factors that include: fern species, type of processing methods and the levels. These three factors greatly affect the antioxidant properties of the fern samples. The noticeable effect of gains and losses of antioxidants was observed. Results revealed that S. palustris exhibited highest TPC when subjected to oven drying at a medium level of temperature D. esculentum on the other hand, showed the highest TFC content at high levels of oven drying while high values for DPPH radical scavenging activity when subjected to steaming at a medium level. In this case, D. esculentum is more prone to thermal degradation than that of the S. palustris. Since phenolic compounds are unstable at high temperature, thus could be easily degraded. This increase of TPC is mainly due to the drying at a medium temperature because at favorable temperature results a high yield of phenolics (Miean and Mohamed, 2001).



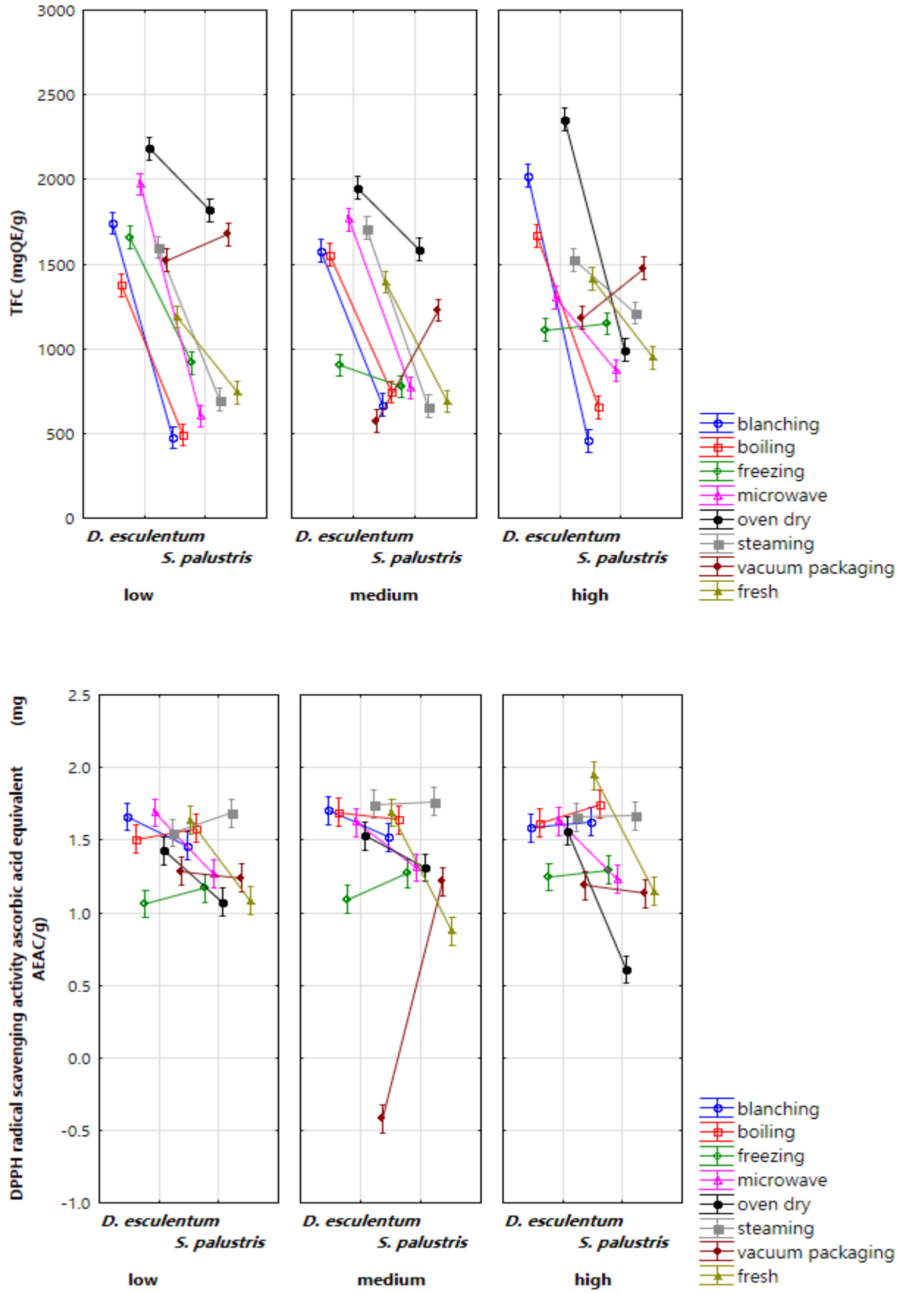


Figure 4. Interaction effects on the fern species, types of processing methods and levels involved affecting the a. TPC b. TFC and c. DPPH radical scavenging activity

Results of the study also revealed that S. palustris has high antioxidant activity on microwave treatment at a high level. The result suggests that at a high temperature of microwave treatment, there was an increase in the antioxidant activity and produced many useful metabolites that include antioxidants. These useful metabolites are considered to be a high specific metal chelating activity (Chai et al., 2012), which are likely not prone to immediate degradation.

CONCLUSIONS

The main effects revealed that D. esculentum showed a higher antioxidant property compared to S. palustris. TFC values were affected to processing methods used with an increase during oven-drying Similarly, DPPH radical scavenging activity showed the highest activity in steaming.

Interaction effects based on species and process revealed that among the combined processes, an increase of TPC was observed to S. palustris when subjected to oven drying while considering an increase in TFC was also accounted for D. esculentum when subjected to oven drying DPPH radical scavenging activity showed that D. esculentum increased in most processing operations, except for freezing at low and medium levels, and for vacuum packaging at high level. For S. palustris, a decrease in radical scavenging activity was observed when oven dried at a high level but exhibited a significant increase in most processing operations.

RECOMMENDATIONS

The researchers would like to recommend oven drying and steaming as processing methods on ferns, but other factors or combinations of food applications such as pressure as a factor should be taken into account. It is also recommended that prolong storage of sample and processing under higher temperature are not advisable.

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Central Mindanao University
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Research Articles

Assessment of Termite Infestation in Academic Infrastructure at Central Mindanao University (CMU)

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ABSTRACT

Knowing the pest species first is necessary for the formulation of pest control method. In this study an assessment of termite infestation in the academic buildings was conducted. All wooden materials both furniture and structural member were inspected for signs of termite infestation. Soldiers of termites found infesting wooden structures were collected for identification. The data gathered during actual inspections include moisture content of infested wood, coatings (painted or not), type of wood in service (furniture or structural member) and materials used (lumber or plywood). A regression analysis was run to determine if the mentioned factors or combination thereof can best explain the rate of infestation. A total of 28 buildings were inspected. All of the buildings showed signs of termite infestation. Pieces of furniture were mostly infested with drywood termites while structural member were infested mostly by subterranean termite. However, most of the buildings have empty tunnels with no active termite population. There were only 3 species of subterranean termites collected and identified viz., Macrotermes gilvus Hagen, Coptotermes gestroi Wasmann and Microcerotermes losbañosensis Oshima. Regression analysis showed that none of the factors or combinations thereof can explain the variation in the extent of damage caused by termites.
Keywords: edible ferns, phenolics, flavonoids, antioxidant activity.

Keywords: drywood termites, subterranean termites, termite infestation

INTRODUCTION

Wood as a basic structural material is susceptible to wood destroying insect attack such as termites (Indrayani et al. 2014). The attack may result to significant structural damages leading to early replacement and reduction of service life wooden structure (Acda, 2004a). The world-wide economic impact figure of termites is uncertain, but the control cost for termite pests in the United States was estimated at $1.5 billion annually in 1994 (Su & Scheffrahn, 2000). In the Philippines, no data were available on total financial losses however Yudin (2002) estimated the annual damage cost to about 8 to 10 million dollars.

There are more than 2,600 described termite species in the world (Kambhampati & Eggleton, 2000). A total of 54 species were known to occur in the Philippine encompassing 18 genera (Snyder & Francia, 1960). Within these genera, only three families are with economic importance and associated with damage to wood products and timber structures, viz., Rhinotermitidae, Termitidae and Kalotermitidae. Six species are considered serious structural pests, viz., Coptotermes gestroi, Nasutitermes luzonicus, Macrotermes gilvus, Microcerotermes losbañosensis, Cryptotermes cynocephalus and C. dudleyi (Snyder & Francia, 1960; Valino, 1967; Acda, 2004a).

Central Mindanao University (CMU) in Maramag, Bukidnon is located at the heart of Mindanao Island, Philippines where the climate is generally warm with significant amount of rainfall throughout the year. The campus is surrounded with diverse species of trees (ornamental and timber producing) and aside from its plantation forest the university was also able to preserve a substantial area of natural forest. This environment is suitable for termite colonies to thrive. Thus, a survey of termite infestation is important since wooden material is common component in buildings especially classroom and office furniture. This study provides information on the economically important species infesting academic buildings and the extent of

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Table 1. *Termite's damage rating modified from Rojo (2017)*

Rating	Definition	Qualitative rating
0	There is the presence of signs (fecal pellets, tunnels) but no apparent termite damage	With signs
1	<20% of the wooded structure were damaged	Low
2	20 - <40% of the furniture were damaged	Moderate
3	>40% of the furniture were damaged	Heavy

damage.

METHODOLOGY

Survey and Termite Collection

The methods used by Indrayani et al. (2014) were followed in this study with some modifications. 100 percent survey were conducted which involved visual inspection in all academic buildings, offices, and dormitories within the campus. Infestation was determined by the presence of damaged wooden parts, fecal pellets, tunnels, and termites. The damage was classified following the procedure described by Rojo (2017) (Table 1). Ratings of damage were based on actual inspection on furniture, structural members and other wooden materials in all buildings. Termites especially soldier caste were collected and placed in a vial with 90% ethanol for identification. The head of the soldier termite were compared to available pictures and keys in the internet for identification. The moisture content of the wood was determined using a handheld digital wood moisture meter (FA507) developed by the Forest Products Research and Development Institute (FPRDI).

Data analysis

A linear regression analysis was employed to determine which factors or combination of factors (types of wood in service either furniture or structural member, material used either pure lumber or with plywood, coatings and wood moisture content) can best explain the variation in damage intensity.

RESULTS AND DISCUSSION

Termite Infestation

Out of 262 wooden structures in 28 buildings inspected 170 were structural member, and the rest were furniture. Signs of both subterranean and drywood termites were observed which includes presence of dead termites, damaged wood with galleries lined with mud, and presence of mud tubes (tunnels). During actual inspection, mud tubes were observed on walls and flooring. However, when inspected some tunnels were already empty. An empty tunnel may simply mean that they may have abandoned this particular tunnel to move to other sections of the building to find accessible food. Unless chemical control was applied however in this study information about application of such control method is not available.

Mud tubes are made of "carton material," a mixture of fecal matter and partially digested wood (Woodrow et al., 1999), this protects termite from dry weather, direct sun light, low humidity and predation (Manzoor & Mir, 2010). For drywood termites, the noticeable sign of infestation is the presence of a pile of fecal pellets. Locally this group of termites is called "unos" in Tagalog or "agay-ay" in Visaya. These pellets are expelled from the gallery system through small holes in the infested wood surface (Grace, 2009).

Termite Species that Invaded Buildings and Furniture

There were 3 species of subterranean termites found infesting wooden structures in CMU buildings i.e. the mound building termite *Macrotermes gilvus* Hagen, Los Baños termite *Microcerotermes losbañosensis* Oshima and the Asian subterranean termite *Coptotermes gestroi* Wasmann. These species were classified as economically important termites in the Philippines considering the degree of damage it brought to wooden structures (Snyder & Francia, 1960; Valino, 1967; Acda, 2004a).

M. *gilvus* was found to be abundant in CMU. It is the most common termites observed infesting wooden walls and other structural member, and according to Acda (2003), it is a menace to farming operation. Tunnels were observed mostly from the flooring then on the walls. It is also the dominant termite observed foraging on plant debris in the open fields. This dominance was due to its aggressive agonistic behavior (Rojo & Acda, 2016). In addition, the species is relatively bigger with an average length and width of head of major soldier 3.43mm and 3.17 mm respectively (Neoh & Lee,

2009) perhaps the biggest among the destructive termites in the country. This species was ranked by Acda (2004a) as the second most destructive termites in the Philippines, however, in this study since it is the frequently encountered termite in academic buildings it can be ranked as no. 1. The estimated foraging populations ranged from .25 to .46 million per colony which can cover an area of *124 - 350* m^2 (Acda 2004b). M. gilvus is a voracious feeder capable of 1.32 to 2.36 kg per month wood consumption rate (Acda, 2004b). However, most of the feeding occurred at lower portion of the building (1st floor). Aside from its size the species can be identified easily by observing the morphological feature of the soldier caste as describe by Dahlan *et al.* (2014) Dorsal head shape is almost rectangular, the ratio between the length and width of the head capsule is greater than 1.50 with thick sword like mandible without teeth.

C. gestroi was only observed in one building, specifically at the CMU hospital attacking medicine cabinets, paper-based medicine containers, ceilings and other structural members. The low occurrence of this species can be attributed to the aggressive agonistic behavior of the bigger M. *gilvus* as discussed in Rojo and Acda (2016). Unlike *M. gilvus* which is usually confined in the lower part of the building, *C. gestroi* was found nesting (satellite nest) in the roof frame. The genus *Coptotermes* is a large group of subterranean termites (Li et al., 2010). However out of the estimated 69 named species, only 21 taxa have solid evidence, 44 names have uncertain status and the remaining should be synonymized (Chouvenc et al., 2016). In the Philippines, C *gestroi* is previously known as C. vastator Light or Philippine milk termite until the recent molecular study showed that C. vastator is a junior synonym of C. *gestroi* (Yeap *et al.*, 2014). This termite is an important structural pest reported from Asia, Pacific Islands, North America, Caribbean islands, South America, and Indian Ocean islands (Li *et al.*, 2013). In the Philippines, Acda (2003) classified this species as the most destructive subterranean termite. The termite can take advantage of cracks on cement floors, foundations or splits in treated or naturally durable timber to reach softer or untreated wood (Valino 1967; Acda 2004a). A feeding rate test showed that C. *gestroi* wood consumption rate range from .24 mg to .55 mg per termite for 21 days in jWPA-test, and 0.63--0.73 mg in MWBT-test (Sornnuwat *et al.*, 1995). C. *gestroi* can easily be distinguished from other destructive termites in the Philippines by observing the tear-drop shaped soldier's head as described by Scheffrahn & Su (2000).

M. losbañosensis was also found infesting wooden structures although in one academic building only. Acda (2004a) considered the species as the least destructive among economically important subterranean termites in the Philippines. The same author further stressed that *M. losbañosensis* is relatively smaller and are often used by farmers as chicken feeds. Similar with C. gestroi secondary nest can also be built in beams or walls of houses. The species can easily be recognized by observing

the Soldier's rectangular head and long horn like mandibles (Valino 1967; Acda, 2004a). In addition, the tunnels were narrower compared to other termites.

Drywood termites

Signs of drywood termite infestation were observed on furniture, window jambs, door jambs, and other wooden materials. Unlike subterranean termites were tunnels (Figure 1A) are conspicuous in drywood termites the only sign of infestation is the presence of fecal pellets (Figure 1B). Moreover, the galleries in infested wood in subterranean termites were lined with muds (Figure 1C) and contrarily clean in drywood termites (Figure 1D).

Although no specimens were collected for identification, there are only two species of drywood termites in the Philippines i.e. Cryptotermes dudleyi Banks and Cryptotermes cyanocephalus Light (Valino 1967; Acda 2004a). Furthermore, the variation in shape and size of the fecal pellets collected showed a hint that there are two species of drywood termites infesting wooden structures in CMU academic buildings (Rojo, 2018).

Generally, damage severity was moderate, inflicting less than 40% damage to wooden structure. Heavy infestations were observed in the furniture found in CMU Hospital, College of Arts and Sciences (CAS) building, Psychological Testing and Guidance Center and in structural member like moldings and walls in the buildings of the College of Veterinary Medicine, Accountancy and Business Management, Agriculture and Catleya Ladies Dormitory. Although the infestation was only observed in few furniture and structural members but through time when left uncontrolled especially for subterranean termite the damage can increase significantly considering the habit of subterranean termite. As observed severe damage occurred in older buildings as enumerated above where wooden materials are common. However, the building that should be given priority for termite control program is the university hospital because of the presence of C. gestroi tagged as the most destructive termites in the Philippines (Valino 1967; Acda, 2004a).

A regression analysis was carried out to determine which factor or combination of factors (coatings, type of wood in service, wood MC) can best explain the rate of damage. As observed termite infestation occurred in both furniture and structural member wood, either painted or not and at wood MC ranging from 6 to 16% MC. As shown in Table 2, none of the variables can significantly explain the rate of infestation.

The use of paint (coating) as a barrier against termites is not thoroughly investigated (Lewis 2003). However, Oi et al. (2014) argued that paint may block the entry of termites but according to Lewis (2002) this can breach through time when the paint degrades. Rojo (2017) observed that among the classroom's furniture only the wooden stool was not infested with drywood termites, the author argued that the simple design of stool provides no entry point for the drywood termites.

Although subterranean termites prefer wood with high MC (> 100%) (Gautam & Henderson, 2011), in this study, actual survey showed subterranean termites were able to inflict more than 50% damage to a wooden structure with 12% MC. On the other hand, drywood termites prefer wood with 10% MC (Gouge et. al, 2009). This observation suggests that termite infestation can occur regardless of wood MC especially for subterranean termite, materials used (lumber or plywood), types of wood in service (furniture or structural member).

Furniture were mostly infested with drywood termites while subterranean termites were most commonly observed infesting structural member

Table 2
Four variable regression coefficients with standard errors and tests of significance

Model	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	T	Sig.
(Constant)	.792	5.812		.136	.892
types of wood in service	-.392	2.797	-.011	-.140	.889
Material used	-.218	2.712	-.006	-.081	.936
Wood MC	.812	.590	.590	.095	.169
Coating	6.790	3.557	3.557	.132	.057

Dependent variable: % Damage

like walls. Drywood termites were also observed infesting door and window jambs. The colony size of subterranean termites may reach to more than .5M (Acda 2004b, Acda 2007) thus causing severe damage in shorter period compared to drywood termites in which colony size only reaches a few hundred to a few thousand individuals (Grace, 2009).

CONCLUSION

All academic buildings in Central Mindanao University were infested with termites although the damage was generally moderate (ranged of % termite damage = 20% to 40%). Signs of termite infestation either drywood or subterranean termites were observed in furniture and structural members regardless of moisture content and coatings. There were three subterranean termites collected i.e. *M. gilvus*, *C. gestroi*, *M. losbañosensis*, and two species of drywood termites under the genera *Cryptotermes*. Statistical analysis showed that coatings (painted or not), materials used (plywood or pure lumber) and wood MC or combination thereof cannot explain the rate of infestation. Termite control program should prioritize the university hospital because of the presence of *C. gestroi*.

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Proximate Composition of Raw and Heat-treated *Seriales* [*Flacourtia jangomas* (Lour) Raeusch] Fruit

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ABSTRACT

Seriales fruit is an underutilized wild fruit in the Philippines with promising food production potential, however the usual drawback of processing is the change in the composition or properties of the crop or product. Hence, the study aimed to determine and compare the proximate composition of raw and heat-treated *seriales* fruits. Heat-treatment involves blanching, steaming, boiling and microwave-cooking, which were analyzed for its moisture content, ash content, crude fat, crude fiber and crude protein following standard methods. Results revealed that heat-treatment employed cause a significant difference (p<0.05) on proximate composition of the fruit except for ash and crude fat. High moisture content was identified to boiled and steamed fruits, and the lowest was observed in microwave-cooked *seriales*. Crude protein and fiber were revealed as having increased values in comparison with the raw *seriales* fruit.

Keywords: Seriales, Flacourtia jangomas, proximate composition, heat-treatment methods

INTRODUCTION

Flacourtia jangomas belongs to the Flacourtiaceae family with varying names such as coffee plum, East Indian plum, Indian plum, and Manila cherry among others (Lim 2013), depending on the place where it is wildly distributed. It is one of the wild fruits in the Philippines and is known as Seriales/Ceriales in the Visayan and Mindanao regions. It is considered one among other less-known commodities in the Philippines. It is an important fruit crop in some parts of Southeast Asia, since it is known for food and medicine purposes (Neeharika and Pandey, 2013). The medicinal capability has been attributed to the significantly important components of the commodity. Studies made by Parvin et al. (2011) have shown that Flacourtiaceae contains bioactive compounds. Ripe fruit specifically contain alkaloids, flavonoid, phenolic compounds and tannins which proves its antioxidant potential (Neeharika and Pandey, 2013). Ara et al. (2014) reported that the fruit contains protein (0.69±0.05 gm/100 gm edible potion), fat (0.42±0.03 gm/100 gm edible portion) and fiber (0.92±0.10 gm/100 gm edible portion) which may be contributory to human need of these significantly important components. Moreover, a 100 gram edible portion of the fruit may contribute a total of 128.1 Kcal of energy (Ara et al. (2014). If unutilized, these biologically and nutritionally important compounds will just be put to waste. In order to maximize the utilization of these important compounds, processing is the best option.

There are few studies on processed seriales. Cimafranca and Dizon (2018) reported the potential of the fruit in wine production. In terms of processing methods, one among the factors affecting the sensory quality of the seriales ready-to-drink beverage is blanching time (Cimafranca and Dizon 2017). This is because processing, specifically with the use of heat not only influences the sensory quality of processed products, often than not it leads to degradation and losses of nutritionally valuable components of the fruit. Microwave heating for example reduced dietary fiber of apple, oat bran and corn (Chang and Morris, 1990). Blanching on the other hand decreases the protein and fat content of ripe banana (Musa acuminata colla)(Taiwo and Adeyemi, 2009). Results on some commodities increase instead such as in artichoke, specifically the protein and lipids content. Mepba et al. (2007) reported that sun-drying on amaranths (Amaranthus hybridus), fluted pumpkin (Talfaria accidentalis), gnectum vegetable (Gnectum africana), vine spinach (Basella alba), bush okro (Corchorus olitorus), slippery vine (Asystacia gangetica)

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and cocoyam leaves (Colocasia esculenta) resulted in moisture loss (35.6%) with insignificant ($p>0.05$) increases of protein, lipid, crude fiber and total ash. Generally, the composition or properties of the crop or product changes after heat exposure. Hence, the study aimed to determine and compare the proximate composition of raw and heat-treated seriales fruits.

METHODS

Experimental Materials

Seriales fruits were obtained from the rural areas of Baybay City, Leyte. Good quality fruits were sorted and chosen. Only the good quality ripe fruits were used, where ripeness was characterized by the purplish-red color of the skin. The sorted good quality fruits were then washed thoroughly and then drained.

Heat treatments

Heat treatment methods were adopted from the report of Ahmed and Ali (2013) as follows:

Blanching. Distilled water (1000 mL) was poured into a stainless steel vessel and heated at boiling temperature (100˚C). Pre-weighed whole seriales fruits of approximately 200 grams were immersed in the boiling water for three (3) minutes. The fruits were then drained on a stainless sieve until cold (air cooling) for approximately 22-30 minutes.

Steaming. Distilled water (1000 mL) was heated to boiling temperature using a stainless steel steamer. Meanwhile, pre-weighed whole seriales fruits of approximately 200 g were placed in the steamer basket. Then, the steam basket with seriales was placed on top of the boiling water, covered with a lid, and steam for six (6) minutes. After heating, the steamer basket was taken out from the heat and allowed to cool down similar with blanching.

Boiling. Into a stainless steel vessel, 1000 mL of water (distilled) was poured, which was then covered with a lid and heated to boiling. Upon boiling, the vessel was opened and the seriales (200g whole) fruits were submerged into the boiling water. The lid was replaced back into the cooking vessel, and the fruits were boiled for six (6) minutes. The samples were then taken out quickly from the heat and drained on a stainless sieve, air cooled for approximately 22-30 minutes.

Microwave-cooking. Whole seriales fruits (200g) were placed in a glass beaker (Pyrex 1000mL capacity), and 10 mL of distilled water was added to prevent the fruit from being burned during cooking. The fruits were then heated using the microwave oven for three (3) minutes and 30 seconds. Then, the samples were drained on a stainless sieve until cold, similar to blanching.

Proximate Analysis

Heat-treated samples were analyzed for its moisture content, crude ash, crude fat, crude fiber and crude protein (based on dry weight) at the Analytical Service Laboratory, Animal and Dairy Science Cluster, University of the Philippines at Los Banos, Laguna. The analyses were carried out in triplicate following the method of AOAC (2000). The nitrogen-free extract (%NFE) was computed based on equation (1).

$$\% NFE = \% DM - (\% CL + \% CP + \% Ash + \% CF) \quad \text{(Eq. 1)}$$

Where:

DM = dry matter
CL = crude lipid
CP = crude protein
CF = crude fiber

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) using Statistica version 10, and those that came out significant were analyzed using Tukey's with multiple range significant difference (LSD) test ($p<0.05$).

RESULTS AND DISCUSSION

The results of the proximate analysis revealed a significant difference among values except for the ash and fat contents (Table 1).

% Moisture content (% MC). The moisture content of all *seriales* samples that have undergone different cooking processes are significantly varied ($p<0.01$). Initially, the % MC of raw fruit was 72.13%. This amount is a little higher compared to F. jangomas fruit from other countries, e.g. 66.86 % MC gathered from Indian plum of Bangladesh (Ara et al. 2014), and 65.27 % from Soh mlum of India (Seal et al. 2014). Among the fruits treated, high moisture content was characteristically identified to boiled and steamed fruits, and the lowest %MC were observed in microwaved-cooked seriales. The significant increases in moisture content in steaming and boiling processes, with respective percent increments of 1.96 and 2.48 (relative to raw fruit), was due to the leaching of water-soluble nutrients during the process (Rickman et al. 2007 as cited by Ahmed and

Ali 2013).

Ash content (%). The ash content is the residue after ignition of a sample and is taken to represent the inorganic constituents of the food (Dublecz 2011). In the study, the different cooking condition employed did not significantly affect the ash content (%), which ranges from 0.77 to 0.85 (Table 1). The result obtained is in agreement with the findings of Ara et al. (2014) on Bangladesh Indian plum (0.72 %). Both, however, were found to be in disagreement with the results gathered by Dubey and Pandey (2013) as well as with Seal et al. (2014), where the result of the aforesaid authors were higher (2 % and 1.20 %, respectively).

Crude protein content (%). The statistical analysis for crude protein (%) content has shown to be significant at p<0.05 (Table 1). The range of values within treatments is 0.86 to 1.31 % which is more or less the same with chico, pomelo and papaya from Batangas (Untalan et al. 2015). The lowest value of 0.86 % crude protein was obtained from untreated seriales fruit and the rest of the treatments have considerably higher protein content than the raw, thereby establishing the fact that heat treatment causes an increase in protein content of the commodity. The increase in protein content after cooking observed in this study was in disagreement with the findings of Alajaji and El-Adawy (2006) and Chukwu et al. (2015). These authors discussed instead of the reduction of amino acids after cooking. The reduction results from the leaching of free amino acids. They further stated that boiling specifically has deleterious effects on protein content of the R. vomitoria leaves. The study made by Lewu et al. (2009), and Wang et al. (2010) on taro cocoyam leaves, and beans and chickpeas, respectively, were in line with the experimental result on crude protein content enhancement of this study. Lewu et al. (2009) cited an explanation from Onu et al. (2001) stating that increase in levels of protein after cooking is probably due to the breakdown of tannin that forms complexes with protein, making the latter unavailable.

The available protein determined in this study was of the same quantity with mango (0.979 % dry matter), and a little lower in banana (2.2 %) (Mohapatra et al. 2010). The data suggest that seriales fruit is not an excellent source of protein. In comparison with other studies on F. jangomas, it was determined that the crude protein content (raw) is known to be of lower amount as compared to the findings of Kermasha et al. (1987) and Hosain et al. (2011), who gathered values of 3.9 and 6.16 %, respectively. On the other hand, the findings of this study are in concurrent with the data gathered for Bangladesh Indian plum in the study made by Ara et al. (2014). The multiple comparison difference test following Tukey's HSD for the aforesaid variable revealed that the different cooking conditions employed does not make the blanched, steamed and boiled seriales different with that of the untreated (raw) seriales (Table 1).

Table 1. Proximate composition of ripe *seriales* (F. *jangomas*) fruit as affected by different heat treatment methods

TREATMENTS	PROXIMATE COMPOSITION					
	Moisture content (%)	Ash content (%)	Crude Protein (%)	Crude Fiber (%)	Crude Fat content (%)	Nitrogen-free extract (%)
Raw	72.13 ± 0.16^{a}	0.81 ± 0.02^{a}	0.86 ± 0.01^{a}	1.02 ± 0.07^{b}	0.06 ± 0.03^{a}	25.12 ± 0.18^{a}
Blanched	72.51 ± 0.42^{a}	0.78 ± 0.06^{a}	0.91 ± 0.09^{a}	5.76 ± 0.69^{a}	0.14 ± 0.02^{a}	19.90 ± 0.82^{b}
Steamed	74.09 ± 0.26^{ab}	0.85 ± 0.04^{a}	1.08 ± 0.09^{ab}	4.49 ± 0.43^{a}	0.15 ± 0.01^{a}	19.34 ± 0.77^{bc}
Boiled	74.61 ± 1.00^{b}	0.77 ± 0.01^{a}	1.31 ± 0.11^{b}	6.67 ± 0.76^{a}	0.15 ± 0.03^{a}	16.49 ± 0.41^{c}
Microwaved	69.85 ± 0.57^{a}	0.91 ± 0.05^{a}	1.09 ± 0.09^{ab}	6.63 ± 0.63^{a}	0.11 ± 0.01^{a}	21.41 ± 1.04^{b}

Values are means of triplicate determination ± standard error (SE) (% w/w)
Mean values followed by different letter superscripts in the same column are significantly ($p<0.05$) different according to Tukeys (HSD) test.

Crude fiber (%). The crude fiber content analysis was likewise statistically significant at $p<0.05$. Puupponen-Pimia et al. (2013) cited that thermal processes such as steaming and cooking might change the properties of dietary fiber in many ways. She reported that the results of dietary fiber content she reviewed from various studies could either increase or decrease. In this experiment, the heat treatment caused an increase in the crude fiber content. This result was in agreement with the study on chickpea seeds (Alajaji and El-Adawy 2006) and some vegetables (Puupponen-Pimia et al. 2013). The latter discussed that the reason for the increase in dietary fiber content was most probably due to the washing out of soluble components and small molecules, such as free sugars, leading to the concentration of fiber components in the processed material. Moreover, the said author added that processing might disrupt the cells resulting in better extraction of fiber components. Furthermore, enzymatic action was also mentioned as another factor that might have caused the release of fiber components from the insoluble cell wall matrix leading to increasing the soluble fiber contents of the sample.

Although the effect of heat treatment may be significant on other properties, Tukey's test revealed that these variables except for microwaved-cooked fruit, aren't significantly different from each other in terms of its fiber content. The maximum amount of fiber (6.673±% crude) was found in the seriales fruit that has been microwaved-cooked, while the least was found on the raw fruit (Table 1). The comparison of the raw fruit with the other fruits revealed that seriales fruit has more or less similar crude fiber content with avocado, jackfruit, Malay apple, tangerine, longan, and Java apple. In comparison with F. jangomas fruit studied from previous studies, it was found out that the data gathered in this study are higher than the results on paniala (as it is locally known) in Bangladesh according to Ara et al. 2014 (0.92 %), and lower (9.6 %) than the results obtained by Dubey and Pandey (2013). Also, it was gathered that the fruit was of lower fiber content (1.02 %) in comparison with mango (3.70 %) (Mamiro et al. 2007) and papaya (1.88 %) (Oloyede 2015), but this plainly suggests that the fruit can input to the dietary fiber requirement for consumption.

Fat content (%). Fat content analyses of the samples were not statistically significant ($p \geq 0.05$) (Table 1). The % crude fat ranges from 0.06 to 0.15 (Table 1), which reveals that these values are not different from each other. It is noticeable though that the fat content of the seriales fruit was low, and are even lower as compared with the values obtained from previous studies that gathered ranges of 0.42 to 0.8 % (Ara et al. 2014 and Dubey and Pandey 2013). The 0.06 % (dry weight basis) crude fat of raw seriales is too low and can be comparable with other tropical fruits with almost no fat content (less than 1 %) such as mangosteen, tamarind, balimbin, pomelo and java apple among others (Jahan et al. 2014).

Nitrogen Free Extract (NFE)(%). The second largest component in seriales fruit is nitrogen-free extracts (NFE), which mainly consists of a heterogeneous mixture of all components such as sugars, fructans, starch, pectins, organic acids and pigments (Dublecz, 2011). In fruits, variations are observed as a result of geographic, climatic and seasonal variations (Adepoju et al, 2012, Iorgyer et al., 2009). In this study on the other hand where seriales fruits were pre-treatment with heat, it was observed that pre-treatments cause significant variation in the NFE of the fruit. Table 1 reveals that the % NFE of fresh seriales was 25.12, while heat treated samples range from 16.49 to 21.41, the latter being significantly lower than the raw. Among the heat treatments applied, steaming and boiling caused the highest reduction of NFE. Similarly, Ndidi et al. (2014) observed that boiling significantly reduced NFE in Bambara groundnuts. Iorgyer et al. (2009) further observed that the reductions in NFE increased with an increase in boiling period. The decrease in proximate components with boiling could be due to the leaching of nutrients into the boiling water. Meanwhile, blanched, steamed and microwave-cooked seriales fruits were revealed not significantly different from each other, but are significantly different from the raw fruit.

CONCLUSION

The proximate analysis revealed that heat treatment caused a significant difference in the aforementioned parameters, except ash content and crude fat.

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