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CONTENT

Biogeochemical Cycling of Carbon and Nitrogen in Rainfed Rice Production Under Conventional and Organic Rice Farming

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ABSTRACT

Dwindling carbon (C) and nitrogen (N) levels in paddy soils decreases rice production and threaten human food security globally. The efficient maintenance of C and N fluxes in soil-rice systems is a crucial prerequisite for agricultural and environmental sustainability. Herein, we examined the C and N fluxes from 63 rainfed rice paddy fields under conventional farming (CF) and organic farming (OF) systems in Thailand. The C and N fluxes were measured based on a detailed analysis of relevant influxes (fertilizer, manure, and biomass addition) and effluxes (biomass harvest and greenhouse gas emission). The results demonstrated that the harvested grain and straw contributed to the most abundant C and N effluxes for both farming systems. The CH_4 effluxes were moderate, whereas the N_2O effluxes were meager relative to their total effluxes. Stubble incorporation and animal manure addition to soil were the most extensive C influxes. However, the primary N influxes were stubble incorporation and animal manure addition for the OF system, and chemical-N fertilizers for the CF system. Net C depletions were observed in both the CF and OF systems. However, net N was depleted and accumulated in the CF and OF systems, respectively. Straw incorporation to soils could restore the net C accumulations for the CF and OF systems and elevate the net N accumulation for both systems. This study highlighted that complete straw removal has exacerbated the C and N stock in soil-rice systems, inducing insecurity for the environment and the agricultural systems. Effective straw management is a simple approach for sustaining paddy rice production.

1. INTRODUCTION

Carbon (C) and nitrogen (N) are essential macronutrients for plants; their cycling in terrestrial ecosystems is of global importance to agriculture and the environment (Xue and An, 2018). Both elements play critical roles in plant productivity and environmental sustainability through climate change mitigation via reduced greenhouse gas emission and increased carbon sequestration (Purwanto and Alam, 2020). These elements are also primary integral components of soil organic matter (SOM) (Cheng et al., 2016; Xue and An, 2018). Globally, C distribution in agricultural soils is characterized by extensive areas of low C and N levels worldwide (Zomer et al., 2017). This indisputable evidence demonstrates that global warming threatens C sequestration in soil and terrestrial systems (Arunrat et al., 2018). Therefore, there is an urgent prerequisite to implement practical measures for enhancing C and N levels in soil-plant systems for agricultural and environmental sustainability.

Rice is a primary staple food of people worldwide, especially in Asia. Maintaining soil C and N stocks in paddy rice systems is crucial for human food security and long-term food production (Li et al., 2017; Purwanto and Alam, 2020; Zhou et al., 2020). Biogeochemical cycling of C and N fluxes in a soil-rice system involves their influxes and effluxes (Atere et al., 2017; Ge et al., 2015; Liu et al., 2019). Organic fertilization and harvested residue incorporation are primary C influxes in soil systems (Mortensen et al.,

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2021; Zhou et al., 2020). Mineral and organic fertilizer utilization are the major soil N influxes (Cui et al., 2020). Both C and N effluxes from rice paddies could be related to harvested crop removal and atmospheric greenhouse gas emission (Witt et al., 2000).

A recent study in the Japanese soil-rice system revealed a net N accumulation accomplished by combined chemical and organic fertilizers as the main N influxes, with straw removal as the primary N efflux (Nguyen et al., 2020a). Conversely, the N depletion associated with the harvested rice contributed 61-68% of the total N loss, with chemical fertilization, crop residue, and animal manure incorporation causing a net N depletion (Sridevi and Venkata Ramana, 2016). Paddy rice production under anaerobic conditions could promote soil C sequestration by decreasing the SOM decomposition rate (Qiu et al., 2018) and enhancing net C and N accumulations (Liu et al., 2018).

Agricultural practices are critical factors for C and N cycling in soil-plant systems (Yadav et al., 2019). Two typical farming systems are conventional farming (CF) and organic farming (OF). Regardless of agrochemical utilization, the CF system can apply chemical and organic fertilizers, ensuring high yields from large-scale production (Arunrat et al., 2017). Much research has reported that the CF systems with high chemical-N fertilization have exacerbated nitrous oxide emissions (Rahmawati et al., 2015; Robertson et al., 2000). Conversely, OF systems supplying plant nutrients solely from organic materials may result in low crop yields (Seufert et al., 2012; Timsina, 2018). Much studies have demonstrated that organic amendment additions in the OF system could profoundly mitigate greenhouse gas emissions and promote net N accumulation in soil-rice systems (Qin et al., 2010; Rahmawati et al., 2015; Setyorini and Hartatik, 2021). The types and extents of C and N influxes are derived from the diverse agricultural practices affecting the yield, biomass, and net C and N fluxes of the soil-rice systems.

We hypothesized that CF and OF systems could have pronounced impacts on C and N cycling and their stock in soils and could cause long-term insecurity for sustainable rice production. Obtaining practical and effective rice paddy management measures that achieve C and N accumulations in soil-rice systems through proper management is an essential prerequisite to agricultural and environmental sustainability. Therefore, the objective of this study was to examine detailed measurements of the C and N fluxes in rice paddies in Thailand under CF and OF systems. The conceptual framework for this research is given Figure 1. This study should enhance understanding of how agricultural practices affect C and N fluxes, which can assist in the design of a strategy and policy to implement adaptive management for sustainable agriculture and the environment.

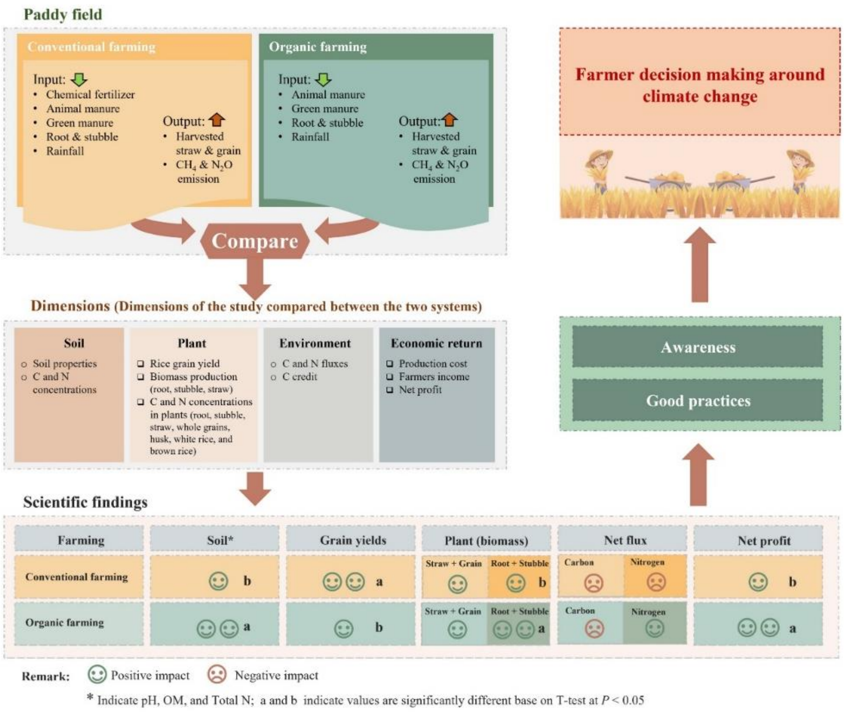

Figure 1. The conceptual framework for investigating biogeochemical cycling of carbon and nitrogen in rice production under conventional and organic farming

2. METHODOLOGY

2.1 Soil and rice sampling

This study investigated 63 pairs of soil and rice samples from Amnat Charoen Province, Thailand, where rice (*Oryza sativa*) is a major product (Figure 2). Jasmine rice, Khao Dawk Mali 105 (KDML105) variety is the most common rice variety in the studied area. This is a photosensitive rice with a typical cultivation period from mid-July to mid-December. One rice crop is grown annually. The studied area was in the rainfed zone with average annual temperature and rainfall of 27.6ºC and 1,349 mm/year, respectively (Figure 3). The limited irrigation areas allow for the cultivation of some other crops. The soil and rice samples were collected on a grid (6 km × 6 km) that covered major representative soil series (Re, Kt, Ub, and Ng) in the studied area. Detailed sample coordinates are given in Table S1. The soils in this area have been mainly associated with sandstone.

At harvest in late November to early December 2019, composite and undisturbed soil samples from each defined location were taken from the topsoil layer at 0-20 cm depth. The soil samples (n=63) were air-dried, ground, and passed through a 2 mm sieve for further physiochemical analysis. The finely ground soil materials (<0.5 mm) were prepared for analyses of organic carbon (OC), total carbon (TC), and total nitrogen (TN). The samples were preserved in plastic containers at room temperature before the chemical analyses. The undisturbed samples of soil cores were determined for bulk density.

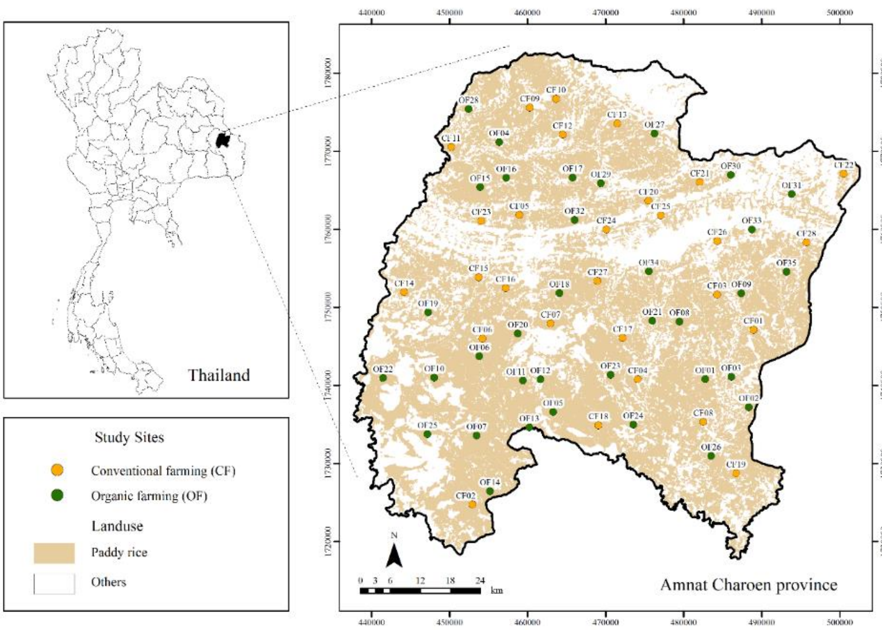

Figure 2. Sampling locations of soil and rice pairs (n=63) in Amnat Charoen Province, Thailand. Orange and green symbols indicate conventional farming (CF, n=28) and organic farming (OF, n=35), respectively.

For rice sampling and data collection, rice straw was manually cut at about 60 cm above the soil surface in the same paddy field where the soil samples were collected. This cutting height reflected the common practice of hand-harvesting by farmers. Such a height allows more stubble to remain in the field relative to machine harvesting (average cut height is 30 cm above the soil surface). Biomass samples of roots, stubble, and straw (combined panicles, leaf, and stem) were collected from a 1 m × 1 m area. The roots and stubble were carefully removed from each soil sample and thoroughly washed with surface water. The samples were rinsed with tap water on arrival at the laboratory, and deionized (DI) water was used for the last washing step. The rice grain yield was taken from an area of 2 m × 2 m and reported at 14% (w/w) moisture content. Whole grains were dehusked and polished using a small milling machine; the corresponding yields of brown and white rice grains were recorded. All biomass weights were recorded after oven-drying at 65℃ until constant weight. The plant samples were ground and preserved in plastic containers before analyses. The resultant seven rice parts (roots, stubble, straw, whole grains, husk, white rice, and brown rice) were used for the subsequent C and N measurements.

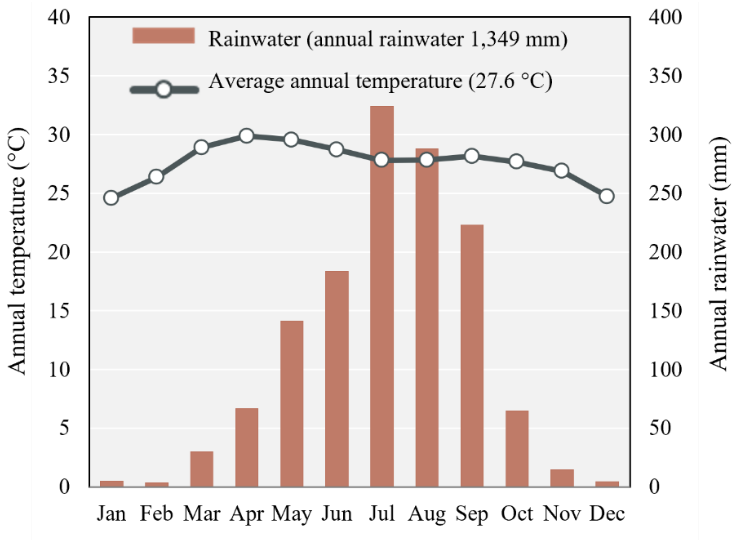

Figure 3. Average values of annual temperature and annual rainwater in Amnat Charoen Province, Thailand 2010-2019

2.2 Soil management data

The soil and fertilizer management regime for each location was obtained based on rice farmer interviews (n=63). The soil-rice management data, including the rates and types of chemical and organic fertilizers and green manure utilization applied in each area, were also collected during the interview; together these were used to calculate the C and N fluxes in the soil-rice systems. The C and N effluxes from harvested crops were calculated from the rice biomass and the C and N contents in all plant parts. Conventional farming (CF, n=28) and organic rice farming (OF, n=35) systems were observed in the studied area. The OF system was certified according to European or Thai organic standards (Organic Agriculture Certification Thailand, 2019).

Economic returns, including the production cost, the farmers income, and net profit, were also estimated for each location. The production costs in our study were calculated based on information provided by the respondents to the questionnaire on the inputs of rice cultivation, including chemical fertilizer, animal manure, and green manure utilization. Farmer income was solely from rice sales, calculated from the rice yield and price. The yield was measured at each location. The price was derived from Office of Agricultural Economics (2021). Net profit was the difference between the production cost and the rice sale income. Carbon credits were determined based on the tonnes of carbon dioxide equivalent (t CO_2e) of soil C stock and rice biomass (root and stubble incorporation) using an average price of 0.80 USD/t CO_2e (USD 1=THB 31.5) for biomass trading volume (Thailand Greenhouse Gas Management Organization, 2021).

2.3 Soil analysis

The physicochemical analyses were carried out using standard procedures, with details of the analysis described by Sparks et al. (1996). The soil texture and bulk density were determined using a hydrometer and soil core methods, respectively. The soil pH was measured using a soil-to-water ratio of 1:1. Organic matter (OM) was measured using the Walkley-Black wet oxidation method (Walkley and Black, 1934). Available phosphorus (P) was extracted using Bray-II extracting solution. The cation exchange capacity (CEC) and exchangeable bases (Ca, Mg, Na, and K) were obtained using the 1 M NH_4OAc (pH 7.0) method. The concentrations of Ca, Mg, Na, and K in the NH_4OAc extract were determined using atomic absorption spectroscopy.

2.4 Carbon and nitrogen analyses in soils and rice plants

For C and N determination in the soil and rice samples, total carbon (TC) and total nitrogen (TN) in the soil samples were measured using a dry combustion method (ISO, 1995). Approximately 10-20 mg of finely ground soil samples were weighed in tin capsules and measured using an NCS analyzer (Elementar, Flash 2000 Series, Thermo Scientific) combusted at 900°C. All seven plant parts (roots, stubble, straw, whole grains, husk, white rice, and brown rice) were measured for their TC and TN concentrations using the same procedure. Duplicates of an organic analytical standard (acetanilide OAS) were conducted to check the result accuracy of each batch (n=63). The accuracy levels for C and N in the samples was excellent at 100.15±0.25 and 100.60±0.44%, respectively.

2.5 Carbon and nitrogen stocks and fluxes in soil-rice systems

The total C and N stocks in the topsoil layer for each location were calculated by multiplying the C and N concentrations in the soil samples with their respective bulk densities and a soil depth of 20 cm (the common depth of paddy surface soils). The C stock and N stocks were reported as the total mass of the respective total C (Mg C/ha) and N (kg N/ha) in soils.

Net C flux was calculated from the difference between C influxes and effluxes. The C influxes consisted of the C concentrations from the roots and stubble and the animal and green manure applied to the soils. The C effluxes were the C concentrations from the harvested straw and grain and the CH_4 emission. Typically, the straw is completely removed from the field for sale or as cow fodder, some of which is later recycled in the form of cow manure. Rice stubble burning is rare in the studied area because farmers can only grow one rice crop annually and so there is no haste regarding land preparation for a second rice crop. The CH_4 emission was based on the IPCC Guidelines (IPCC, 2006), as shown in the equation:

$$CH_4 = t \times (SF_w \times SF_p \times SF_0) \times \frac{12}{16} \times \frac{1}{1000}$$

Where; CH_4 is the C released from methane emission for each area (mg C/ha), t is the rice cultivation period (115 days in this study), SF_w is the scaling factor to account for the differences in water regimes during the cultivation period, SF_p is the scaling factor to account for the differences in water regimes in the season before the cultivation period, and SF_0 is the scaling factor to account for differences in the types and amounts of applied organic amendments calculated as:

$$SF_0 = (1 + \sum ROA_i \times CFOA_i)^{0.59}$$

Where; ROA_i is the application rate of organic amendment i in dry weight for straw and fresh weight for other amendments (ton/ha), and $CFOA_i$ is the conversion factor for organic amendment i in terms of its relative effect relative to straw applied shortly before cultivation.

The N fluxes were calculated from the N influx and efflux differences. The N influxes consisted of the N contents from incorporating roots and stubble, fertilizer, animal, and green manure additions, and rainfall. The influxes from fertilizers, manure, and green manure were calculated using their respective application rates and N concentrations. The rainfall N was estimated from rainfall records in the studied area with the NO_3^- and NH_4^+ concentrations based on those reported by Panyakapo and Onchang (2008). The N effluxes were calculated from the N concentrations from harvested straw and grain as well as from N_2O emissions.

The direct and indirect N_2O emissions (reported as kg N/ha/year) were based on the Tier 1 IPCC Guidelines (IPCC, 2006), as shown in the equations:

$$\text{Direct } N_2O \text{ emission} = \left[(F_{SN} + F_{ON} + F_{CR} + F_{SOM}) \times EF_{1FR} \right]$$

Where; F_{SN}, F_{ON}, and F_{CR} are the annual amounts of chemical-N fertilizer, organic manure, and crop residues applied to soils, respectively (kg N/year), F_{SOM} is the annual amount of mineralized N related to C loss from soils (kg N/year), and EF_{1FR} is the emission factor for N_2O emission from N inputs to flooded rice.

The F_{SOM} value was computed as:

$$F_{SOM} = \sum \left[\Delta C_{Mineral} \times \frac{1}{R} \right] \times 1000$$

Where; $\Delta C_{Mineral}$ is the average annual loss of soil carbon for paddy rice (t C/ha/year) and R is the C-to-N ratio of the soil organic matter at each location. The calculated carbon mineralization data were derived from Arunrat et al. (2018), whose study was conducted on the same type of coarse soil texture in the same region as in the current study area.

$$\text{Inirect } N_2O \text{ emission} = [F_{SN} \times Frac_{GASF} + F_{ON} \times Frac_{GASM}] \times EF_4 + [(F_{SN} + F_{ON} + F_{CR} + F_{SOM}) \times Frac_{Leach} \times EF_5]$$

Where; $Frac_{GASF}$ and $Frac_{GASM}$ are the N fractions of the respective synthetic and applied organic N fertilizer that volatilizes as NH_3 and NO_x, whereas the EF_4 is the emission factor for N_2O emissions from atmospheric deposition of N in soils and water, $Frac_{Leach}$ is the fraction of all added N that is mineralized in managed soils in regions where leaching/runoff occurs, and EF_5 is the emission factor for N_2O emissions from N leaching and runoff. All emission factors relevant to the CH_4 and N_2O emissions applied in this study are provided in Table 1. Details of the C and N influxes from chemical fertilizers, animal, and green manures are given in Table S2.

The changes in total C and N stocks in soils were estimated under two schemes: 1) removal and incorporation of straw as:

Soil C stock change=Total C stock+n C fluxes (with and without straw incorporation)

Soil N stock change=Total N stock+n N fluxes (with and without straw incorporation),

Where; total C and N stocks are the total C and N stocks of the year i at each location and i indicates the year of projection (n=1-10).

2.6 Statistical analysis

Normality of the data was checked using the Shapiro-Wilk test. The mean differences in soil properties, the C and N contents in each rice part, soil stock and fluxes of C and N, the economic return (production cost, farmers income, net profit, and carbon credit from soil C stock and rice biomass) between the two farming systems were analyzed using a t-test. Mean differences of the C and N contents in the different rice parts were tested using an F-test and ANOVA. All statistical analyses were tested at a significance level of 0.05.

Table 1. Relevant emission factors based on IPCC Guideline (IPCC, 2006) used for calculating CH_4 and N_2O emissions in this study

Abbreviation	Description	Values and specific notes
CH_4 emission		
SF_w	Scaling factor to account for the differences in water regime during the cultivation period	0.27 for regular rainfed
SF_p	Scaling factor to account for the differences in water regime in the season before the cultivation period	0.68 for non-flooded pre-season >180 days
$CFOA_i$	Conversion factor for organic amendment i in terms of its relative effect with respect to straw applied shortly before cultivation	0.29 for straw incorporation >30days before cultivation, 0.14 for animal manure, and 0.50 for green manure
N_2O emission		
EF_{1FR}	Emission factor for N_2O emissions from N inputs to flooded rice	0.003
$\Delta C_{Mineral}$[1]	Average annual loss of soil carbon for paddy rice (t C/ha)	1.173
$Frac_{GASF}$	Fraction of synthetic fertilizer N that volatilizes as NH_3 and NO_x	0.1
$Frac_{GASM}$	Fraction of applied organic N fertilizer materials that volatilizes as NH_3 and NO_x	0.2
$Frac_{Leach}$	Fraction of all N added mineralized in managed soils in regions where leaching/runoff occurs	0.3
EF_4	Emission factor for N_2O emissions from atmospheric deposition of N on soils and water surfaces	0.01
EF_5	Emission factor for N_2O emissions from N leaching and runoff	0.0075

[1] $\Delta C_{Mineral}$ is obtained from Arunrat et al. (2018)

3. RESULTS

3.1 Soil characteristics

The physicochemical properties of the studied paddy soils (n=63) under conventional farming (CF, n=28), and organic farming (OF, n=35) systems varied substantially among the locations tested within each rice farming system (Table 2). Sandy loam was the most common soil texture in the studied area on 37 sites. The soil pH varied greatly from extremely acidic to moderately alkaline. The OM and TC contents varied from very low to slightly high, while the TN content ranged from low to high. The available P varied from very low to very high. The CEC ranged from very low to low, whereas the exchangeable Ca, exchangeable Mg, and exchangeable Na varied between very low and moderate, and exchangeable K varied between very low to very high.

Based on average data, the studied soils were sandy loam with the bulk density not affecting the penetration of plant roots (BD=1.47 g/cm). The soils were acidic (pH=5.14) with very low contents of TC (4.65 g/kg), TN (0.82 g/kg), and exchangeable Ca (1.22 $cmol_c$/kg), low contents of exchangeable Mg (0.31 $cmol_c$/kg), exchangeable Na (0.19 $cmol_c$/kg), and exchangeable K (0.07 $cmol_c$/kg), and a moderately low level of available P (9.35 mg/kg). Comparing both farming systems, the OF system soils had significantly higher pH, OM, and TN values than those in the CF system. The available P and total C contents in the soils of the OF system had higher average values than for the CF system but none were at a significant level.

Table 2. Range (minimum-maximum) and average (mean±SD) of soil properties in topsoil (0-20 cm depth) for paddy rice production from conventional farming (CF, n=28) and organic farming (OF, n=35)

Practice		pH[1] (H_2O)	OM (g/kg)	Total C (g/kg)	Total N (g/kg)	Avail.P (mg/kg)	CEC ($cmol_c$/kg)	Exch.Ca ($cmol_c$/kg)	Exch.Mg ($cmol_c$/kg)	Exch.Na ($cmol_c$/kg)	Exch.K ($cmol_c$/kg)	BD (g/cm)	Sand (%)	Silt (%)	Clay (%)
All	Min-Max	4.30-7.95	2.90-29.0	1.81-18.55	0.36-1.94	1.00-65.50	0.72-12.07	0.16-9.18	0.03-2.97	0.05-0.66	0.02-0.40	1.25-1.69	40-88	6-45	2-30
Location	Mean±SD	5.14±0.66	8.50±3.70	4.65±2.17	0.82±0.28	9.35±9.35	3.10±2.20	1.22±1.64	0.31±0.50	0.19±0.12	0.07±0.07	1.47±0.09	72±11	19±8	10±6
CF	Min-Max	4.30-6.05	2.90-12.0	1.81-7.02	0.36-0.84	1.00-24.00	0.72-12.07	0.16-7.50	0.04-2.97	0.05-0.52	0.02-0.24	1.32-1.62	40-88	6-45	5-27
	Mean±SD	4.95±0.48^b	7.34±2.13^b	4.43±1.24	0.64±0.13^b	7.16±5.16	3.43±2.58	1.37±1.70	0.31±0.55	0.19±0.12	0.06±0.06	1.49±0.08	71±12	19±10	10±5
OF	Min-Max	4.45-7.95	4.07-28.9	2.32-18.55	0.51-1.94	2.00-65.50	0.75-9.90	0.24-9.18	0.03-2.22	0.05-0.66	0.02-0.40	1.25-1.69	49-87	8-36	2-30
	Mean±SD	5.29±0.74^a	9.41±4.41^a	4.82±2.70	0.97±0.29^a	11.1±13.26	2.84±1.84	1.11±1.60	0.32±0.46	0.19±0.12	0.07±0.08	1.47±0.09	72±10	19±7	9±6
T-test		*	*	ns	*	ns	ns	ns	ns	ns	ns	-	-	-	-
CV (%)		12.76	43.63	46.69	34.23	113.13	67.54	133.68	158.40	63.38	97.59	-	-	-	-

[1]OM: Organic matter; Total C: Total Carbon; Total N: Total Nitrogen; Avail. P: Bray-II extractable P; CEC: Cation exchange capacity; Exch. Ca, Mg, Na, and K: NH_4OAc exchangeable Ca, Mg, Na, and K respectively; BD: Bulk density; CV: Coefficient of variation. Mean values with different lowercase superscripts are significantly different based on t-test at $p<0.05$.

3.2 Rice grain yield and biomass production

The rice grain yield and biomass production in different rice parts differed among the locations and farming systems (Figure 4). The rice grain yield in the CF system (3.53 ton/ha) produced a significantly higher grain yield than the OF system (3.03 ton/ha). The combined straw and grain biomass on a dry matter basis in the CF system (7.04 ton/ha) was also higher than for the OF system (6.32 ton/ha) but none of these components were significantly different from each other. Conversely, the combined root and stubble biomass in the OF system (3.26 ton/ha) was significantly more abundant than in the CF system (2.35 ton/ha). The low rice yield observed in this study was typical for this Thai fragrant rice compared to other varieties (Suwanmontri et al., 2021).

3.3 Carbon and nitrogen concentrations in rice parts

The concentrations of C and N in the different rice parts (root, stubble, straw, whole grain, husk, brown rice, and white rice) under both the CF and OF systems are presented in Figure 5. The average C concentrations in the different plant parts for each farming system were significantly different (Table S3). The average C concentrations in the whole grain (CF=404 and OF=409 g C/kg) and straw (CF=402 and OF=406 g C/kg) were significantly higher than in the stubble (CF=395 and OF=398 g C/kg) and roots (CF=334 and OF=339 g C/kg).

The average N concentrations were significantly different among the rice parts. There was more N in the whole grain (CF=10.06 and OF=10.48 g N/kg), but accumulated less in the roots (CF=6.77 and OF=7.51 g N/kg), straw (CF=5.96 and OF=6.12 g N/kg), and stubble (CF=3.98 and OF=4.26 g N/kg), respectively. The brown grain had the highest N concentration (CF=12.82 and OF=12.64 g N/kg) in the whole grain but was lowest in the husk (CF=3.56 and OF=3.70 g N/kg), indicating that rice bran contained the greatest accumulation of the N fraction in the rice plants.

3.4 Carbon and nitrogen fluxes in paddy rice systems

Since C and N are the primary plant nutrient elements for sustainable paddy rice production, the C and N flux differences (influxes and effluxes) for the CF and OF systems were measured (Table 3). Our C mass fluxes demonstrated that the current C influxes varied greatly from 0.97 to 1.63 mg C/ha/year

($\bar{x}$=2.64 mg C/ha/year), depending on the location and farming system (Table S4).

In 62 of the 63 sites (98%), the C mass fluxes showed net depletion, indicating a severe decline in soil C in these rice production systems. The C mass in the OF system (-3.05 to +0.12 mg C/ha/year, $\bar{x}$=-1.50 mg C/ha/year) was less depleted than in the CF system (-4.78 to -0.95 mg C/ha/year, $\bar{x}$=-2.37 mg C/ha/year) (Table 3). This could be primarily attributed to the considerable influxes of biomass (roots and stubble) and animal manure added to the soils in the OF systems. The rice roots and stubble incorporated into the soils contributed the most significant C influxes to paddy rice production, corresponding to 58-67% of the total C influxes. The C influxes from the roots and stubble in the OF system (0.46-2.02 mg C/ha/year, $\bar{x}$=1.21 mg C/ha/year) were significantly higher than in the CF system (0.19-1.71 mg C/ha/year, $\bar{x}$=0.84 mg C/ha/year). The C influx from animal manure in the OF system ($\bar{x}$=0.58 mg C/ha/year) was also more abundant than in the CF system ($\bar{x}$ = 0.29 mg C/ha/year), corresponding to 23-28% of the total C influxes (Table 3).

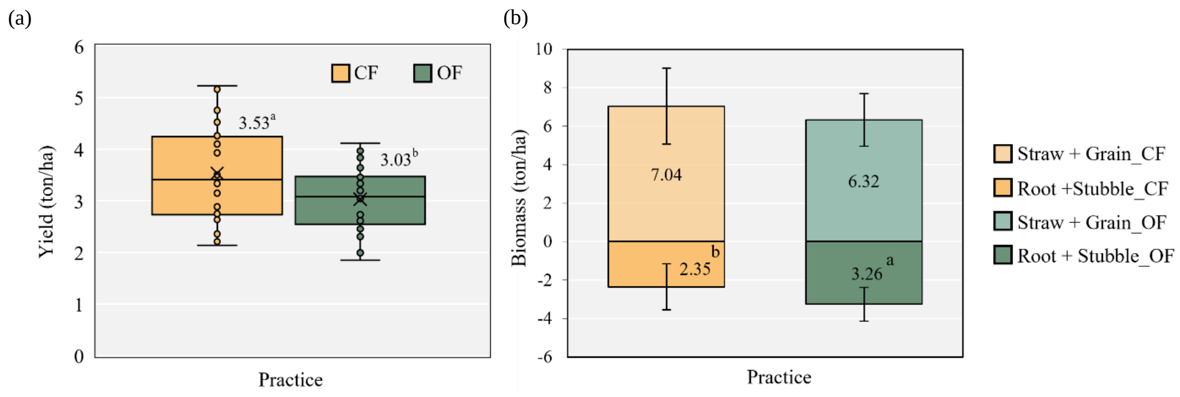

Figure 4. Rice grain yield box plot (a) and biomass production bar plot in different parts of rice plant parts (b) under conventional farming (CF, n=28) and organic farming (OF, n=35) systems. Error bars for (b) indicate standard variation of data in each system. Different lowercase superscripts indicate significant difference based on t-test at p<0.05.

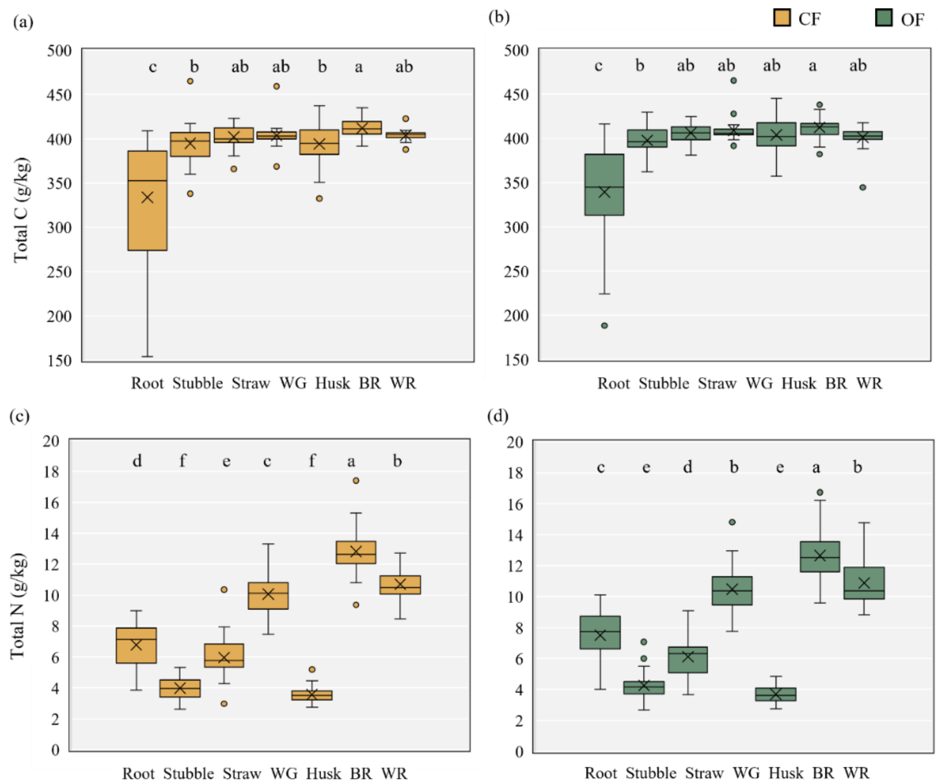

Figure 5. Concentrations of C (a, b) and N (c, d) in different rice parts under conventional farming (CF, n=28) and organic farming (OF, n=35) systems. Different lowercase letters above box plots indicate significant differences based on F-test at p<0.05. WG, BR, and WR denote whole grain, brown rice, and white rice, respectively.

Table 3. Range (minimum-maximum) and average (mean±SD) values of C and N stock in soils, and fluxes of C and N in paddy rice production under conventional farming (CF, n=28) and organic farming (OF, n=35)

Parameter	Carbon (mg C/ha/year)					Nitrogen (kg N/ha/year)				
	Conventional farming		Organic farming		T-test	Conventional farming		Organic farming		T-test
	Min-Max	Mean±SD	Min-Max	Mean±SD		Min-Max	Mean±SD	Min-Max	Mean±SD	
Soil C and N stock	5.87-22.2	13.17±3.83	6.65-54.5	14.12±7.99	ns	1,121-2,656	1,905±384[b]	1,516-5,702	2,827±842	*
Influx										
Chemical fertilizer	-	-	-	-	-	9.38-57.2	22.25±14.91[a]	0-0	0±0[b]	*
Green manure	0-1.79	0.13±0.47	0-1.79	0.31±0.68	ns	0-85.0	6.07±22.29	0-85.0	14.6±32.5	ns
Manure	0-1.34	0.29±0.34[b]	0-2.00	0.58±0.46[a]	*	0-50.3	11.4±13.11[b]	0-75.4	23.5±17.8[a]	*
Rainfall	-	-	-	-	-	0.40-0.55	0.46±0.06	0.40-0.55	0.46±0.06	ns
Roots	0.05-0.63	0.27±0.17[b]	0.16-0.79	0.45±0.15[a]	*	0.81-15.36	5.75±3.93[b]	4.52-21.3	10.2±4.27[a]	*
Stubble	0.14-1.08	0.57±0.28[b]	0.30-1.23	0.76±0.21[a]	*	1.46-12.38	5.74±2.9[b]	2.84-16.5	8.21±3.05[a]	*
Total	0.21-3.03	1.25±0.58[b]	0.83-4.04	2.10±0.84[a]	*	13.04-118.0	51.7±25.0	13.0-142.9	57.0±33.6	ns
Efflux										
Straw	2.75-0.68	0.44±1.56	0.95-2.45	1.46±0.37	ns	8.23-51.2	23.4±9.07	11.3-43.0	21.8±6.37	ns
Grain	0.68-2.11	1.27±0.38	0.57-1.75	1.11±0.26	ns	15.0-58.2	31.6±10.2	14.3-47.9	28.5±7.42	ns
CH_4 emission	0.30-1.25	0.80±0.21[b]	0.56-1.33	1.02±0.17	*	-	-	-	-	-
N_2O emission	-	-	-	-	-	0.68-2.02	1.27±0.32[b]	0.79-3.66	1.80±0.71[a]	*
Total	2.03-6.12	3.63±0.92	2.33-5.06	3.60±0.63	ns	24.3-110.5	56.3±17.45	29.2-86.1	52.1±12.0	ns
Flux difference	(-4.78)-(-0.95)	(-2.37)± 0.83[b]	(-3.05)-(+0.12)	(-1.50)± 0.80[a]	*	(-59.4)-(+56.9)	(-4.56)± 28.26	(-42.7)-(+70.8)	4.87±31.4	ns

(*) and ns in the t-test column indicate values are significantly different and not significantly different, respectively, at $p<0.05$. Mean values with different lowercase superscripts are significantly different based on t-test at $p<0.05$. The period of flux is one year.

The harvested straw and grain accounted for the most significant part of the total C effluxes (71-78%), for which the CF system (1.36-4.86 mg C/ha/year, $\bar{x}$=2.83 mg C/ha/year) was higher than for the OF system (1.52-4.20 mg C/ha/year, $\bar{x}$=2.57 mg C/ha/year). There were no significant differences in the C effluxes between the systems. The C efflux from straw in the CF and OF systems contributed 53-57% of the combined straw and grain C effluxes, indicating that considerable C from the rice straw was removed from the paddy fields and could be recycled into the soil-rice systems. The calculated CH_4 emission efflux ranged from 0.80 to 1.02 mg C/ha/year (Table S5), corresponding to 22-28% of the total C effluxes. The CH_4 efflux in the OF system ($\bar{x}$=1.02 mg C/ha/year) was significantly higher than that in the CF system ($\bar{x}$=0.80 mg C/ha/year).

The N mass fluxes revealed net accumulations in the OF system (-42.7 to +70.8 kg N/ha/year, $\bar{x}$=+4.87 kg N/ha/year) but showed net depletions in the CF system (-59.4 to +56.9 kg N/ha/year, $\bar{x}$=-4.56 kg N/ha/year), as shown in Table 3. The large variation in N mass fluxes occurred because of the large variations in the chemical and organic fertilizer inputs. There was no significant difference between the two systems. N fertilization was the most critical N influx in the CF system (9.38-57.2 kg N/ha/year, $\bar{x}$=22.25 kg N/ha/year), corresponding to 42% of the total N influxes. Conversely, the animal manures (0-75.4 kg N/ha/year, $\bar{x}$=23.5 kg N/ha/year) and root and stubble incorporation (7.36-37.8 kg N/ha/year, $\bar{x}$=18.41 kg N/ha/year) were the two main N influxes for the OF system, corresponding to 41% and 32%, respectively. The N influx from precipitation was meager, accounting for only 0.8-0.9% of the total N influxes ($\bar{x}$=0.46 kg N/ha/year, Table S6).

Straw and grain harvests were the primary N effluxes for the CF ($\bar{x}$=55.0 kg N/ha/year) and the OF ($\bar{x}$=50.3 kg N/ha/year) systems. The N efflux from grains in both systems accounted for 57-58% of the combined straw and grain N effluxes. The N efflux from N_2O emission (1.27-1.80 kg N/ha/year) was very low (2-4%) compared to the total N effluxes (Table S5). The OF system ($\bar{x}$= 1.80 kg N/ha/year) had a significantly higher N_2O emission than the CF system ($\bar{x}$=1.27 kg N/ha/year).

3.5 Estimation of changes in carbon and nitrogen stocks and fluxes

Based on the mass fluxes of the C and N influxes and effluxes for our studied locations, we estimated the changes in the C and N stocks in the next 10 years under two scenarios: (1) current practice of straw removal (Figure 6) and (2) proposed measure of straw incorporation (Figure 7). The existing soil C stock in the CF system (5.87-22.18 mg C/ha, $\bar{x}$ =13.17 mg C/ha) was not significantly different from the OF system (6.65-54.5 mg C/ha, $\bar{x}$ =14.12 mg C/ha). Conversely, the current soil N stock in the OF system (1,516-5,702 kg N/ha, $\bar{x}$ = 2,827 kg N/ha) was significantly higher than in the CF system (1,121-2,656 kg N/ha, $\bar{x}$ =1,905 kg N/ha).

In the first scenario (straw removal practice), by the year 2030, the C soil stock declined critically from 13.17 mg C/ha to -10.58 mg C/ha for the CF system and from 14.12 mg C/ha to -0.83 mg C/ha for the OF system. Most importantly, the estimated soil C stock in the CF and OF systems severely declined and was exhausted by 2026 and 2029, respectively (Figures 6 (a) and (b)).

The second scenario (entire straw incorporation; Figures 7 (a) and (b) could turn the current net C depletion into a net accumulation of +0.19 mg C/ha for the CF system and of +0.96 mg C/ha for the OF system. Therefore, the estimations of the soil C stock in the next 10 years (up to 2030) considerably increased to 15.05 mg C/ha for the CF system and to 23.73 mg C/ha for the OF system. This C stock in the straw incorporation scenario was far greater than the unchanged practice scenario without the straw incorporation (-10.58 and -0.83 mg C/ha for the CF and OF systems, respectively). The soil C concentration increased to 5.08 g C/kg for the CF system and to 8.10 g C/kg for the OF system in the next 10 years, whereas those in the current measure were exhausted.

The soil N stock in the CF system slightly decreased from 1,905 kg N/ha to 1,860 kg N/ha in the next 10 years (Figure 6 (c)). Conversely, the N stock in the OF system slightly increased from 2,827 kg N/ha to 2,876 kg N/ha with stable soil N at a low level (Figure 6 (d)). With the straw incorporation over the next 10 years, the current net N depletion ($\bar{x}$=-4.56 kg N/ha) turned into a net N accumulation ($\bar{x}$=+42.15 kg N/ha) for the CF system and significantly enhanced the N accumulation from +4.87 kg N/ha to +48.39 kg N/ha for the OF system. By 2030, the N concentration for the CF and OF systems was expected to increase to 0.79 and 1.13 g N/kg, respectively.

Notably, the estimation was linearly based on the constant net C and N fluxes and thereby had some limitations as it did not consider the consequential effects of changes in C and N stocks to C and N effluxes. However, it was worth demonstrating that simple practices of straw incorporation (typically neglected by local farmers) could result in net C accumulation along with bolstering net N accumulation in both systems.

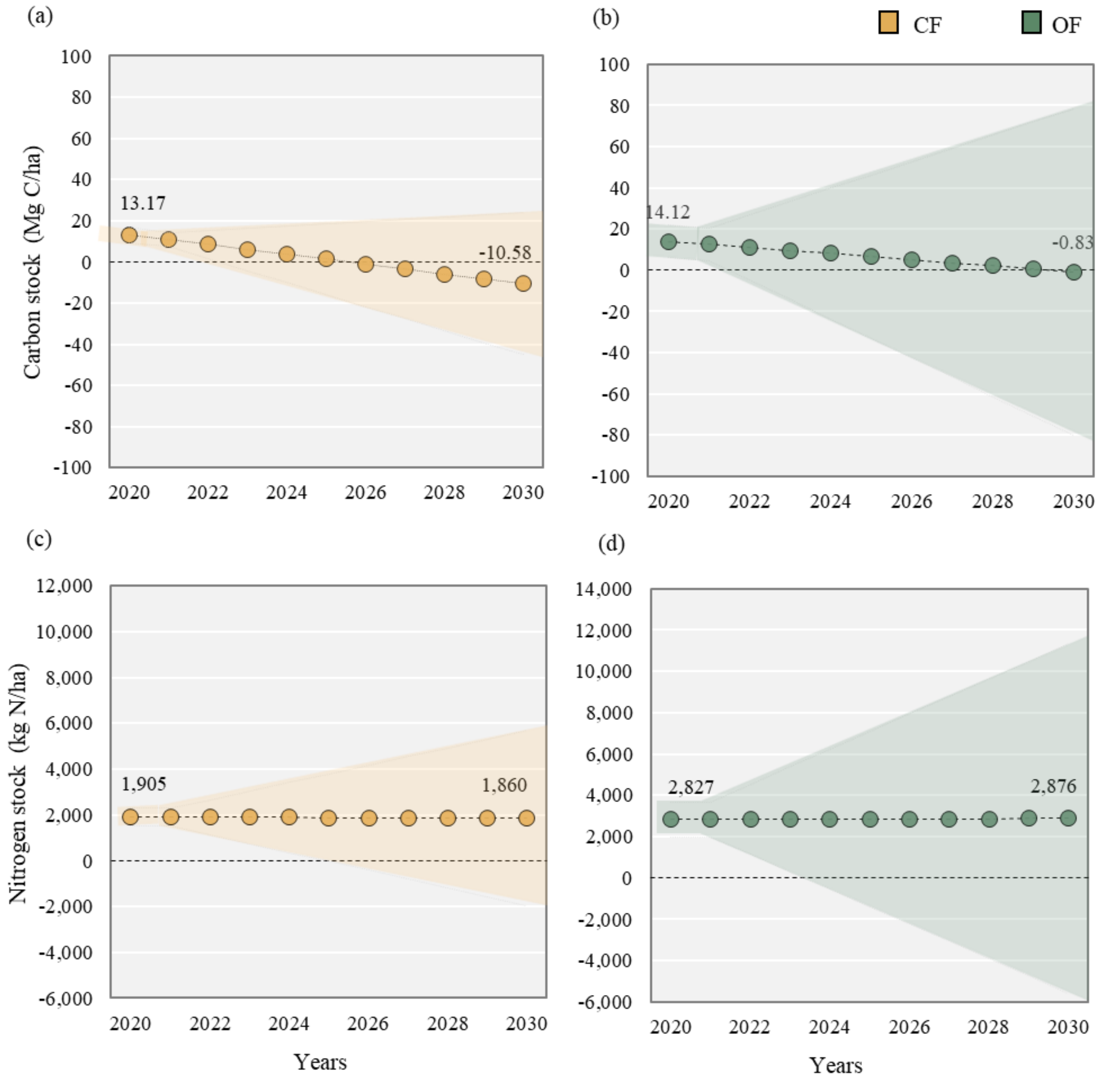

Figure 6. Estimation of changes in stocks of soil carbon (a, b) and nitrogen (c, d) under conventional farming (CF, n=28) and organic farming (OF, n=35) systems. Each data point indicates average values of soil C and N stocks with corresponding C and N fluxes. The orange and green shaded areas indicate standard deviation of entire dataset (n=63). The C fluxes were -2.37 mg C/ha for CF and -1.50 mg C/ha for OF. The N fluxes were -4.56 kg N/ha for CF and 4.87 kg N/ha for OF.

3.6 Estimation of values of rice production cost, farmers income, net profit, and carbon credits

For sustainability of rice production, the production cost, the farmers income, and net profit (Table 4) should be taken into consideration in addition to the rice grain yield. Although the CF system (3.53 ton/ha) had a significantly higher grain yield than the OF system (3.03 t/ha) (Table 3), the OF system (USD 1,729/ha) had significantly higher income from rice sales than the CF system (USD 1,458 /ha) due to the price of organic rice being higher than for regular rice of about USD 160/ton. Conversely, the CF system (USD 72/ha) had a significantly higher production cost (principally from chemical fertilizer input) than the OF system (USD 20/ha), which resulted in the net profit for the OF system (USD 1,710/ha) being significantly higher than for the CF system (USD 1,390/ha) by about USD 320/ha.

Based on the soil C stock and biomass production in our study (Table 3, Figure 4), we estimated the values of carbon credits in soil and biomass incorporation as an additional source of income. Considering the soil C stock from both systems (Table 4), the value of the carbon credits for soil C sequestration in the CF and the OF systems were USD 39/ha and USD 41/ha, respectively. The value of the carbon credits from the combined root and stubble in the CF system (USD 7/ha) was significantly lower than for the OF system (USD 10/ha). Conversely, considering straw incorporation to the soil, the carbon credit values from the straw in the CF system and OF system were respective USD 11.4/ha and USD 10.5/ha, respectively. The addition of the carbon credit for rice husk in the CF system (USD 1.8/ha) was slightly higher than for the OF system (USD 1.6/ha).

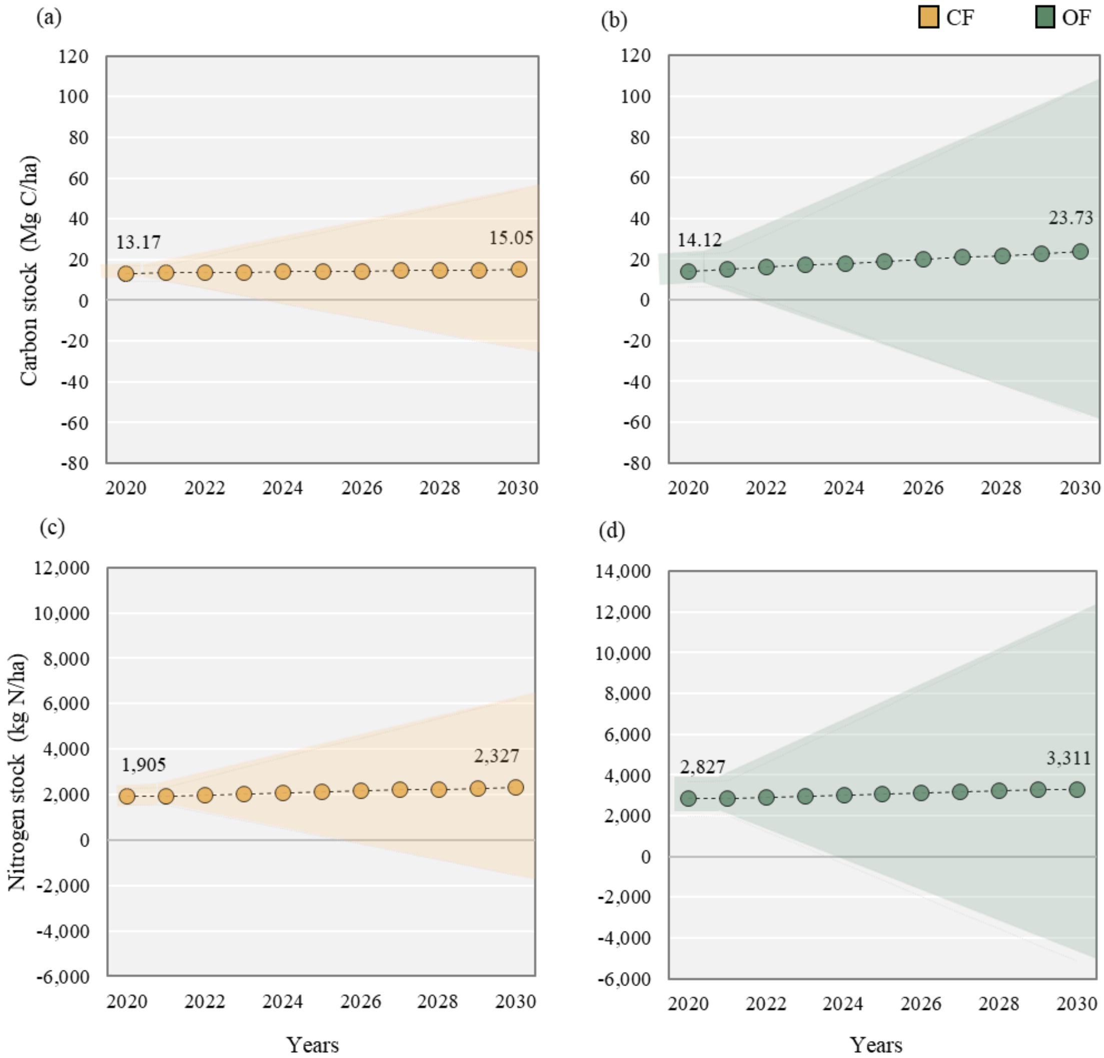

Figure 7. Estimated change in soil C (a, b) and N (c, d) stocks under both conventional (CF, n=28) and organic farming (OF, n=35) systems induced by straw incorporation. The orange and green shaded areas indicate standard deviation of data (n=63). The C fluxes were +0.19 mg C/ha for CF and +0.96 mg C/ha for OF, whereas the N fluxes were +42.15 kg N/ha for CF and +48.39 kg N/ha for OF.

Table 4. Range (minimum-maximum) and average (mean±SD) of production cost, income, net profit, and carbon credit from conventional farming (CF, n=28) and organic farming (OF, n=35), where the calculation is based on one crop season per year.

Practice		Production cost[1] (USD/ha)	Income[2] (USD/ha)	Net profit[3] (USD/ha)	Carbon credit[4] (USD/ha)			
					Soil	Root+stubble	Straw	Rice husk
CF	Min-Max	24-215	882-2,158	696-2,101	17-65	2-16	4.7-20.7	1-3
	Mean±SD	72±43[a]	1,458±389[b]	1,390±392[b]	39±11	7±4[b]	11.4±2.7	1.8±0.5[a]
OF	Min-Max	3-57	1,056-2,351	1,040-2,342	19-160	4-14	6.9-18.1	1-2
	Mean±SD	20±14[b]	1,729±345[a]	1,710±346[a]	41±23	10±3[a]	10.5±2.7	1.6±0.3[b]
T-test		*	*	*	ns	*	ns	*

[1] Production cost calculated from inputs for growing rice, including seed, chemical fertilizer, animal manure, and green manure utilization.
[2] Income is farmer income from rice sale calculated from yield and price of rice.
[3] Net profit is difference between production cost and rice sale income.
[4] Carbon credit estimated from tonnes of carbon dioxide equivalent (t CO_2e) of soil C stock and rice biomass with an average price of USD 0.80/t CO_2e.
(*) and ns in the t-test column indicate values are significantly different and not significantly different, respectively, at p<0.05. Mean values with different lowercase superscripts are significantly different based on t-test at p<0.05.

4. DISCUSSION

4.1 Effects of agricultural practices on carbon and nitrogen fluxes in paddy rice system

Agricultural practices exert a strong influence on yield as well as C and N fluxes in soil-rice systems. Several studies on farming practice impacts on the C and N fluxes and yield have reported there were variable effects on the C and N mass from a net accumulation to net depletion (Cheng et al., 2016; Cui et al., 2020; Mortensen et al., 2021; Nguyen et al., 2020a; Sridevi and Venkata Ramana, 2016; Witt et al., 2000; Yang et al., 2020). Many factors affect the influxes and effluxes of C and N, which vary among agricultural practices and sites. Manures, organic fertilizers, and harvested crop residues are documented to be the primary C influxes (Mortensen et al., 2021), but chemical and organic fertilizers and harvested crop residues are the major N influxes for arable soils (Cui et al., 2020). However, another study showed that the harvested crop removal and greenhouse gas emission could be key pools of C and N effluxes for rice paddies (Witt et al., 2000).

Nguyen et al. (2020a) revealed net N accumulation in a Japanese paddy rice system could be achieved by applying chemical N fertilizer, rice straw, and cow dung compost, with plant uptake being the primary N efflux. Nonetheless, Sridevi and Venkata Ramana (2016) showed that either chemical fertilizer and straw, or manure incorporation failed to meet the net N accumulation in Indian 25-year paddy rice cultivation. In addition to the influxes and effluxes of C and N, rice production under anaerobic conditions promoted SOM accumulation by lowering the SOM decomposition rate (Qiu et al., 2018), thereby leading to net C and N accumulations (Liu et al., 2018).

Our data demonstrated rainfed paddy rice cultivation in sandy soils under tropical environments resulted in net C depletions under both the CF and OF systems (Table 3). The CF system had a net N depletion, while the OF system had a net N accumulation. The distinct differences in the C and N fluxes were attributed to variations in the influxes and effluxes between the sites and farming systems. Collectively, harvested grain and straw biomass were the primary source of both C and N effluxes under field conditions in the current study. Conversely, the roots and stubble left in the fields and animal manure addition were the main C influxes to paddy soils. Such measures as entirely harvested straw and grains resulted in net C depletions in both farming systems. The animal and green manures dominated the N influxes for the OF system, resulting in net N accumulations. Conversely, chemical-N fertilizer and animal manure predominated the N influxes for the CF system and the net N depletions. The CH_4 emission contributed to a moderate proportion of the total C effluxes, while the N emission was paltry. The estimated CH_4 (0.80-1.02 Mg C/ha) and N_2O (1.27-1.80 kg N/ha) emission data, based on the IPCC Guideline (IPCC, 2006), were higher than the direct measurement values of CH_4 and N_2O emissions from coarse-textured paddy soils in a province nearby to the studied sites and ranged from 0.019 to 0.029 mg C/ha/season and from 0.075 to 0.088 kg N/ha/season (Malumpong et al., 2021). These variations could be attributed to the different types of soil and crop management at each location and inherent deviation reported by the IPCC Guideline (IPCC, 2006).

Paddy rice production under the OF system had significantly higher root and straw biomass than in the CF system (Figure 4) probably because the OF system supplied abundant organic materials compared to the CF system, contributing to a higher SOM concentration (Table 2). Organic materials, such as animal manure, have a circumneutral condition, which could elevate the soil pH value (Table 2). The organic amendments added to soils could compete with the reactive sites of clay minerals and Fe/Al oxyhydroxides in soils, thereby mitigating Al toxicity and promoting soil P availability (Haynes and Mokolobate, 2001). P is an essential plant nutrient that supports the increased density and length of small and lateral roots (Vejchasarn et al., 2016), resulting in more roots and stubble being left in the fields and building up soil C stocks. However, additions of organic matter to soils could promote CH_4 emission from paddy soils as observed in the current and other studies (Nguyen et al., 2020b; Zhang et al., 2018). Therefore, effective water management, such as an alternate wetting and drying cycle, should be implemented together with organic matter addition to concurrently promote C sequestration and demote CH_4 emission.

Based on the mass C flux calculation, straw recycling to soils could have the greatest potential to turn the net C depletion into net C accumulation under both farming systems. This procedure could enhance N accumulation in the soils and result in net N accumulation for the CF system. However, the critical challenge is that most rice farmers must adopt practical measures to incorporate such a large straw volume into their paddy fields. The high C-to-N ratio of the straw material (~70 in the current study), with a slow decomposition rate could induce N immobilization and hinder rice growth and yield. Rapid N immobilization occurred after straw addition to soils under both flooded and non-flooded conditions within 1-10 days. The fine soil particles served as the primary sink of the immobilized N, most of which (71-91% of the immobilized N) could be mineralized after 160 days (Said-Pullicino et al., 2014). Although it is only temporal immobilization, rice plants could experience an N deficit during the early rice-growing period. To resolve this concern, straw incorporation into the paddy rice system should be processed chemically (for example, using urea and molasses) and biologically (for example, using cellulolytic bacteria) and subsequently used as livestock fodder (Aquino et al., 2020), with any cow dung manure being returned to support paddy rice production. The combined incorporation of green manure and rice straw to soils could enhance both the soil chemical and microbiological properties and could be a promising measure for rice production, consistent with recent observations in Chinese paddy soils (Zhou et al., 2020)

Though the mass C flux calculation revealed that straw incorporation to sandy soils in the current study could cause net C accumulation and be expected to enhance the soil C concentration in the next 10 years, the estimated values in both farming systems would remain at a very low C level (5.08-7.98 g C/kg), likely due to the rapid decomposition rate of organic matter in sandy soils under tropical climates that could accelerate C loss from the soils (Puttaso et al., 2011). Recycling the straw and husk in the form of biochar with high stability (Manyà, 2012) could be another promising approach to gradually build up soil C sequestration for long-term rice production in addition to the above measure of fresh straw incorporation. Biochar addition could also decrease the leaching of several nutrients such as N, P, Ca, Mg, and Si from highly weathered soils (Aldana et al., 2021; Laird et al., 2010). Furthermore, the regular application of co-composting of animal manure with biochar and the use of biochar-based slow-release fertilizer (Hagemann et al., 2017) could be an effective strategy to resolve plant nutrient deficiency, C sequestration, and greenhouse gas emission in paddy rice production in sandy soils under tropical environments with a high organic matter decomposition rate and poor soil nutrient fertility.

4.2 Effects of rice residues on silicon addition into paddy system

Since rice is an Si-hyperaccumulator plant, its residues are Si-laden materials (Ma et al., 2006). Recycling of the Si-rich rice residues would also enhance the Si phytolith with a high available Si form compared to silicate minerals (Seyfferth and Fendorf, 2012). Si-rich agricultural residues have proved to be an effective material for mitigating As accumulation in rice grain (Leksungnoen et al., 2019; Limmer et al., 2018), which is a critical human health concern (Zhu et al., 2008). This measure is necessarily required for paddy soils to produce rice containing an As-safe level (Carey et al., 2020). Rice residues typically contain about 10% Si by weight. The incorporation of roots and stubble, straw, and husk could potentially supply Si equivalent to 0.05-0.54 ($\bar{x}$=0.29 ton Si/ha), 0.16-0.71 ($\bar{x}$=0.37 ton Si/ha), and 0.03-0.11 ton Si/ha ($\bar{x}$=0.06 ton Si/ha), respectively. Therefore, recycling all Si-rich residues (roots, stubble, straw, and husk) could enhance the Si contents by about 0.24-1.35 ton Si/ha ($\bar{x}$=0.72 ton Si/ha) for each crop cycle. The calculated Si contents from rice residues in the current study were lower than the values reported by Penido et al. (2016) for straw (0.5-1.5 ton Si/ha) and husk (0.07-0.20 ton Si/ha) from Bangladesh, Cambodia, China, and the USA. The lower calculated Si content in the straw and husk biomass in the current study may have been due to the nature of the rice cultivar growing in the study area with low yield and low biomass. The proposed measure of whole straw recycling for about 10 years with the potential to change the C and N accumulation levels could elevate the bioavailable Si level by 1.6-7.1 ton Si/ha (3.7 ton Si/ha).

4.3 Approach to sustainable rice farming practices

To make development more sustainable in the agricultural sector, the Thai government launched the first 20 year national strategy to lead the country toward security, prosperity, and sustainability. According to the current scientific findings, the OF system produced higher belowground rice residues (root and stubble) than the CF system, which enhanced soil organic carbon and increased soil health. Most importantly, increasing soil C sequestration is one of the emerging Sustainable Development Goals that have been proposed to provide urgent action to address climate change. Although the grain yield of the OF system was lower than for the CF system, the net profit of the OF system was greater than for CF system by approximately USD 320/ha. The carbon credits from the soil C stock and root and stubble incorporation for the OF system were also higher than for the CF system by about USD 5.5/ha. Clearly, organic rice farming was the more profitable and environmental friendly farming system. To build a better future, it should be highlighted as a lesson-learned and be advocated as good practice for rice farmers globally. All governments, not only in Thailand, should adopt this scientifically based, firm evidence to drive policy making, formulation, and implementation. Strategic plans, roadmaps, input subsidies, and an organic rice price guarantee that could motivate farmers to convert to or establish organic rice farms should be undertaken to put these activities into practical actions.

5. CONCLUSION

The investigation of C and N cycling in rainfed rice production under conventional and organic rice farming revealed that organic rice farming improved many soil properties, including pH, OM, total N, total C and available P compared to conventional rice farming. However, the conventional paddy rice farming provided higher rice yield than from organic rice farming by about 0.5 ton/ha. Organic farming enhanced root and stubble biomass, which promote soil carbon input. The most important C and N effluxes occurred through straw and grain harvesting. Therefore, the C mass fluxes revealed net C depletion for both the rice farming systems, whereas the N mass fluxes had a net N depletion for conventional farming and a net N accumulation for organic farming. The variation in the C and N influxes, including for the combined roots and stubble incorporation and chemical-N fertilizer and animal manure, caused differences in the net C and N fluxes for both systems. Straw incorporation to soils could potentially resolve the net C depletion and greatly elevate the net N accumulation in paddy sandy soils under a tropical environment. Recycling Si-rich residues could gradually build up plant-available Si that could benefit long-term paddy rice production with safe-As levels. Organic rice farming had higher net returns than the conventional rice farming by USD 320/ha. Overall, the scientific findings from the current study revealed that improving soil quality and achieving a higher net profit from rice production can encourage extrinsic motivated action to turn the interest into action to combat climate change and to develop sustainable soil quality.

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REFERENCES

Aldana GO, Hazlerigg C, Lopez-Capel E, Werner D. Agrochemical leaching reduction in biochar-amended tropical soils of Belize. European Journal of Soil Science 2021; 72(3):1243-55.

Aquino D, Del Barrio A, Trach NX, Hai NT, Khang DN, Toan NT, et al. Rice straw-based fodder for ruminants. In: Gummert M, Hung NV, Chivenge P, Douthwaite B, editors. Sustainable Rice Straw Management. Cham: Springer International Publishing; 2020. p. 111-29.

Arunrat N, Pumijumnong N, Hatano R. Practices sustaining soil organic matter and rice yield in a tropical monsoon region. Soil Science and Plant Nutrition 2017;63(3):274-87.

Arunrat N, Pumijumnong N, Hatano R. Predicting local-scale impact of climate change on rice yield and soil organic carbon sequestration: A case study in Roi Et Province, Northeast Thailand. Agricultural Systems 2018;164:58-70.

Atere CT, Ge T, Zhu Z, Tong C, Jones DL, Shibistova O, et al. Rice rhizodeposition and carbon stabilisation in paddy soil are regulated via drying-rewetting cycles and nitrogen fertilisation. Biology and Fertility of Soils 2017;53(4):407-17.

Carey M, Meharg C, Williams P, Marwa E, Jiujin X, Farias JG, et al. Global sourcing of low-inorganic arsenic rice grain. Exposure and Health 2020;12(4):711-9.

Cheng W, Padre AT, Sato C, Shiono H, Hattori S, Kajihara A, et al. Changes in the soil C and N contents, C decomposition and N mineralization potentials in a rice paddy after long-term application of inorganic fertilizers and organic matter. Soil Science and Plant Nutrition 2016;62(2):212-9.

Cui N, Cai M, Zhang X, Abdelhafez AA, Zhou L, Sun H, et al. Runoff loss of nitrogen and phosphorus from a rice paddy field in the east of China: Effects of long-term chemical N fertilizer and organic manure applications. Global Ecology and Conservation 2020;22:e01011.

Ge T, Liu C, Yuan H, Zhao Z, Wu X, Zhu Z, et al. Tracking the photosynthesized carbon input into soil organic carbon pools in a rice soil fertilized with nitrogen. Plant and Soil 2015;392(1):17-25.

Hagemann N, Joseph S, Schmidt HP, Kammann CI, Harter J, Borch T, et al. Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. Nature Communications 2017;8(1):Article No. 1089.

Haynes RJ, Mokolobate MS. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: A critical review of the phenomenon and the mechanisms involved. Nutrient Cycling in Agroecosystems 2001;59(1): 47-63.

Intergovernmental Panel on Climate Change (IPCC). IPCC Guidelines for National Greenhouse Gas Inventories: Volume

4. Hayama, Japan: Institute for Global Environmental Strategies; 2006.

International Organization for Standardization (ISO). ISO 10694: Soil Quality - Determination of Organic and Total Carbon after Dry Combustion (elementary analysis). Geneva, Switzerland; 1995.

Laird D, Fleming P, Wang B, Horton R, Karlen D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 2010;158(3):436-42.

Leksungnoen P, Wisawapipat W, Ketrot D, Aramrak S, Nookabkaew S, Rangkadilok N, et al. Biochar and ash derived from silicon-rich rice husk decrease inorganic arsenic species in rice grain. Science of the Total Environment 2019;684:360-70.

Li Z, Liu C, Dong Y, Chang X, Nie X, Liu L, et al. Response of soil organic carbon and nitrogen stocks to soil erosion and land use types in the loess hilly-gully region of China. Soil and Tillage Research 2017;166:1-9.

Limmer MA, Mann J, Amaral DC, Vargas R, Seyfferth AL. Silicon-rich amendments in rice paddies: Effects on arsenic uptake and biogeochemistry. Science of the Total Environment 2018;624:1360-8.

Liu Y, Ge T, Zhu Z, Liu S, Luo Y, Li Y, et al. Carbon input and allocation by rice into paddy soils: A review. Soil Biology and Biochemistry 2019;133:97-107.

Liu Y, Zang H, Ge T, Bai J, Lu S, Zhou P, et al. Intensive fertilization (N, P, K, Ca, and S) decreases organic matter decomposition in paddy soil. Applied Soil Ecology 2018;127:51-7.

Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, et al. A silicon transporter in rice. Nature 2006;440(7084): 688-91.

Malumpong C, Ruensuk N, Rossopa B, Channu C, Intarasathit W, Wongboon W, et al. Alternate Wetting and Drying (AWD) in broadcast rice (*Oryza sativa* L.) management to maintain yield, conserve water, and reduce gas emissions in Thailand. Agricultural Research 2021;10(1):116-30.

Manyà JJ. Pyrolysis for biochar purposes: A review to establish current knowledge gaps and research needs. Environmental Science and Technology 2012;46(15):7939-54.

Mortensen EØ, De Notaris C, Peixoto L, Olesen JE, Rasmussen J. Short-term cover crop carbon inputs to soil as affected by long-term cropping system management and soil fertility. Agriculture, Ecosystems and Environment 2021;311:Article No. 107339.

Nguyen TT, Sasaki Y, Kakuda K-I, Fujii H. Comparison of the nitrogen balance in paddy fields under conventional rice straw application versus cow dung compost application in mixed crop-livestock systems. Soil Science and Plant Nutrition 2020a;66(1):116-24.

Nguyen BT, Trinh NN, Bach Q-V. Methane emissions and associated microbial activities from paddy salt-affected soil as influenced by biochar and cow manure addition. Applied Soil Ecology 2020b;152:Article No. 103531.

Office of Agricultural Economics. Agriculture Commodities [Internet]. 2021 [cited 2021 March 1]. Available from: https://www.oae.go.th/view/1/Information/EN-US.

Organic Agriculture Certification Thailand. Certification Alliance Organic Standard Version 1.0. Thailand: ACT Organic Co.,Ltd.; 2019.

Panyakapo M, Onchang R. A four-year investigation on wet deposition in western Thailand. Journal of Environmental Sciences 2008;20(4):441-8.

Penido ES, Bennett AJ, Hanson TE, Seyfferth AL. Biogeochemical impacts of silicon-rich rice residue incorporation into flooded soils: Implications for rice nutrition and cycling of arsenic. Plant and Soil 2016;399(1):75-87.

Purwanto BH, Alam S. Impact of intensive agricultural management on carbon and nitrogen dynamics in the humid tropics. Soil Science and Plant Nutrition 2020;66(1):50-9.

Puttaso A, Vityakon P, Saenjan P, Trelo-ges V, Cadisch G. Relationship between residue quality, decomposition patterns, and soil organic matter accumulation in a tropical sandy soil after 13 years. Nutrient Cycling in Agroecosystems 2011;89(2):159-74.

Qin Y, Shuwei L, Guo Y, Liu Q, Zou J. Methane and nitrous oxide emissions from organic and conventional rice cropping systems in Southeast China. Biology and Fertility of Soils 2010;46:825-34.

Qiu H, Ge T, Liu J, Chen X, Hu Y, Wu J, et al. Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and structural modeling analysis. European Journal of Soil Biology 2018;84:27-34.

Rahmawati A, De Neve S, Purwanto BH. N_2O-N emissions from organic and conventional paddy fields from Central Java, Indonesia. Procedia Environmental Sciences 2015;28:606-12.

Robertson GP, Paul EA, Harwood RR. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science 2000;289 (5486):1922-5.

Said-Pullicino D, Cucu MA, Sodano M, Birk JJ, Glaser B, Celi L. Nitrogen immobilization in paddy soils as affected by redox conditions and rice straw incorporation. Geoderma 2014;228-229:44-53.

Setyorini D, Hartatik W. Nutrients balance under organic rice farming system in Central Java, Indonesia. IOP Conference Series: Earth and Environmental Science 2021;648:Article No. 012154.

Seufert V, Ramankutty N, Foley JA. Comparing the yields of organic and conventional agriculture. Nature 2012;485 (7397):229-32.

Seyfferth AL, Fendorf S. Silicate mineral impacts on the uptake and storage of arsenic and plant nutrients in rice (*Oryza sativa* L.). Environmental Science and Technology 2012; 46(24):13176-83.

Sparks DL, Page AL, Helmke PA, Loeppert RH. Methods of Soil Analysis, Part 3: Chemical Methods. Madison, Wisconsin, USA: Soil Science Society of America: American Society of Agronomy; 1996.

Sridevi S, Ramana MV. Nutrient budgeting in continuous rice-rice cropping system under long-term integrated nutrient management. Green Farming 2016;7:859-63.

Suwanmontri P, Kamoshita A, Fukai S. Recent changes in rice production in rainfed lowland and irrigated ecosystems in Thailand. Plant Production Science 2021;24(1):15-28.

Thailand Greenhouse Gas Management Organization. Carbon Credit Price [Internet]. 2021 [cited 2021 March 17]. Available from: http://carbonmarket.tgo.or.th/index.php?lang=EN&mod=Y2hhcnQ=&action=bGlzdA==.

Timsina J. Can organic sources of nutrients increase crop yields to meet global food demand? Agronomy 2018; 8(10):Article No. 214.

Vejchasarn P, Lynch JP, Brown KM. Genetic variability in phosphorus responses of rice root phenotypes. Rice 2016;9(1):Article No. 29.

Walkley A, Black IA. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 1934;37(1):29-38.

Witt C, Cassman KG, Olk DC, Biker U, Liboon SP, Samson MI, et al. Crop rotation and residue management effects on carbon sequestration, nitrogen cycling and productivity of irrigated rice systems. Plant and Soil 2000;225(1):263-78.

Xue Z, An S. Changes in soil organic carbon and total nitrogen at a small watershed scale as the result of land use conversion on the loess plateau. Sustainability 2018;10(12):Article No. 4757.

Yadav GS, Lal R, Meena RS, Babu S, Das A, Bhowmik SN, et al. Conservation tillage and nutrient management effects on productivity and soil carbon sequestration under double cropping of rice in north eastern region of India. Ecological Indicators 2019;105:303-15.

Yang J, Liang X, Li H, Chen Y, Tian G. Effects of no-till and rice varieties on nitrogen and phosphorus balance in rice fields. Environmental Sciences Europe 2020;32(1):Article No. 26.

Zhang W, Sheng R, Zhang M, Xiong G, Hou H, Li S, et al. Effects of continuous manure application on methanogenic and methanotrophic communities and methane production potentials in rice paddy soil. Agriculture, Ecosystems and Environment 2018;258:121-8.

Zhou G, Gao S, Lu Y, Liao Y, Nie J, Cao W. Co-incorporation of green manure and rice straw improves rice production, soil chemical, biochemical and microbiological properties in a typical paddy field in southern China. Soil and Tillage Research 2020;197:Article No. 104499.

Zhu Y-G, Williams PN, Meharg AA. Exposure to inorganic arsenic from rice: A global health issue? Environmental Pollution 2008;154(2):169-71.

Zomer RJ, Bossio DA, Sommer R, Verchot LV. Global sequestration potential of increased organic carbon in cropland soils. Scientific Reports 2017;7(1):Article No. 15554.

Low-Level Tritium Measurement in Tap Water in Bangkok Area and Annual Dose Estimation

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ABSTRACT

Monitoring of tritium concentration in tap water is an essential tool to determine the effective dose received from tap water. Liquid scintillation counting (LSC) is a widely used technique for determining tritium in water. Due to the very low activity of tritium in tap water, its detection requires a high-efficiency LSC with the lowest minimum detectable activity (MDA). Low-level tritium analysis in tap water were performed in two LSC models using conventional distillation techniques. The optimal conditions with the lowest MDAs were applied to determine the tritium concentration in the tap water distributed from two main sources located in the Bangkok metropolitan region: Bang Khen and Maha Sawat water treatment plants (WTPs). Twenty-six tap water samples were collected from petrol stations located around both water treatment plants. The results revealed that the amount of tritium in the tap water around the Bangkhen WTP was between 1.88-2.63 Bq/L with an average of 2.28±0.28 Bq/L, whereas those from the Maha Sawat WTP were between 2.01-2.69 Bq/L with an average of 2.44±0.26 Bq/L, which are far below the World Health Organization's (WHO) guideline limit (10,000 Bq/L) for drinking water. The annual effective dose (AED) for infants, children and adults obtained from tap water samples around the Bangkhen WTP were 0.010, 0.014, and 0.030 µSv/year, respectively, and those from the Maha Sawat WTP were 0.011, 0.015, and 0.032 µSv/year, respectively, which are far below the WHO's guideline limit (100 µSv/year).

1. INTRODUCTION

Tritium is a radioactive isotope of hydrogen that emits low-energy beta rays and has a half-life of 12.3 years (Nayak et al., 2019). Tritium can be formed naturally from the interaction of atmospheric nitrogen and oxygen atoms with high-energy cosmic rays (Popoaca et al., 2014), artificially via nuclear reactions (Duliu et al., 2018), and as a by-product of the operation of nuclear reactors (IAEA, 2010). From the mid-1950s to the early 1960s, artificially made tritium was widely dispersed during above-ground testing of nuclear weapons and has been decreasing ever since (USEPA, 2021). Today, tritium emission from nuclear power plants is a major occupational exposure pathway that impacts workers' health. A recent study reported high concentrations of tritium in the urine of nuclear reactor workers in China, resulting in higher internal radiation exposure caused by tritium compared to the general population (Chen et al., 2021). Tritium, both naturally occurring and artificially made, is usually in gaseous form for under controlled conditions, but when combined with oxygen is either in a liquid state, known as tritiated water (T_2O), or partially formed tritiated water (HTO) with high mobility in the environment (UNSCEAR, 2016; CNSC, 2010). Tritium may pose a health risk if ingested through drinking water, food, inhalation, or absorbed through the skin in large quantities (Matsumoto et al., 2021). The World Health Organization (WHO) has established guidelines for tritium in drinking water at 10,000 Bq/L. This represents the concentration of tritium that, if present

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in consumed drinking water, would result in an individual dose of 0.1 mSv (WHO, 2017). Therefore, measuring the amount of tritium in drinking water relative to established criteria is an important factor in determining the quality of water used for consumption.

Liquid scintillation counting is a conventional method for the measurement of low-level tritium in environmental samples (L'Annunziata et al., 2020; Theodorsson, 1999). The liquid scintillation counter (LSC) was first introduced in the 1950s and has continued to evolve for over 60 years. Hou (2018) recently reviewed the major developments of LSC's measurement techniques regarding instrumentation, methodology, and its applications in past decades. One major improvement regarding LSC instrumentation and methodology is the commercialization of triple-to-double coincidence ratio (TDCR) in liquid scintillation counting (Cassette and Bouchard, 2003), which has become a routine method for the measurement of beta-emitting nuclides in samples with varying quench levels. The first commercial TDCR-based LSC was introduced in 2008 by Hidex Oy, followed by two more types: the Hidex 300SL Super Low level and Hidex 600SL (Hou, 2018). For analyzing tritium in water samples, the detection limit can be improved by several techniques, such as reducing the quenching effect by using a commercially available tritium column filled with cation and anion exchange resins to purify water samples prior to tritium analysis. Another method with the lowest detection limit is the use of electrolysis to enrich tritium in water samples; however, the main disadvantage is the long analysis time (Hou, 2018). Other possible methods include using a new type of ultra-low background LSC system (e.g., AccuFLEX LSC-LB7, HITACHI ALOKA, Japan) with a 145 mL vial (Feng et al., 2020), or an ultra-low LSC (e.g., Quantulus 1220, PerkinElmer, USA) with commonly used 20 mL counting vials (Varlam et al., 2009).

Since the amount of tritium-in T_2O or HTO form-is present in the tap water samples at very low levels, it is, therefore, necessary to use a highly sensitive LSC with the lowest minimum detectable activity (MDA, Bq/L) for measuring tritium. Numerous recent studies have been especially focused on optimizing the counting performance for tritium analysis (Feng et al., 2020; Arun et al., 2019; Erchinger et al., 2017; Grahek et al., 2016). The results revealed that the main parameters affecting MDA were the volume ratios between the water samples and the scintillation cocktails, types of cocktails, types of LSC vials, and counting time. The optimal measurement conditions vary across laboratories, depending on available equipment and tools. A recent study determined tritium activity concentrations in tap, well, and spring water samples from Mersin province in Turkey using a Packard TriCarb 2900TR LSC system (Karataşli et al., 2017). The activity concentration of the tritium measured in water samples varied from <1.9 to 14.1±1.0 Bq/L with an average of 6.2±0.6 Bq/L. From literature review, there was only one study on the measurement of tritium activity concentration in tap water collected from different regions in Thailand using electrolytic enrichment and low background LSC (AccuFLEX LSC-LB7) with a sensitivity of less than 1 Bq/L (Rittirong et al., 2019). The tritium concentrations in tap water were in the range of 0.41-0.75 Bq/L. There are no research data on the concentration of tritium activity in tap water collected in the Bangkok metropolitan area. This research aimed to determine the concentration of tritium in tap water distributed from two main suppliers located in the Bangkok metropolitan region, namely, Bang Khen and Maha Sawat WTPs, to assess the radiological hazards arising from the consumption of tritium in tap water. Both WTPs receive raw water from different sources, the Chao Phraya River and the Mae Klong River, through the eastern and western water-supply canals, respectively. They supply tap water throughout Bangkok and surrounding areas. Therefore, the tap water produced by both WTPs represents the water supply that many people living in Bangkok and nearby areas use for their consumption. If tap water contains high concentrations of tritium, it will affect a large population. The important factors for low-level tritium analysis by two LSC instruments, Hidex 600SL and Quantulus 1220, were optimized using a conventional distillation technique and the optimal conditions were applied for measuring tritium in tap water samples. Determining the tritium levels in tap water is not only important for consumer safety but can also be used as an indicator for tritium leakage into the environment from nuclear activities.

2. METHODOLOGY

2.1 Sampling location

The Bang Khen (13.88165°, 100.55285°) and Maha Sawat (13.80973°, 100.41017°) WTPs are located 14.87 km north-east and 11.37 km north-west of Bangkok City, respectively. The sampling sites were 26 petrol stations located around the WTPs, which supplied water to those petrol stations. The water supply samples were collected in February 2020. The geographic coordinates and map of the sampling locations are illustrated in Table 1 and Figure 1, respectively.

2.2 Collection of water samples

Before sample collection, the tap was cleaned by washing the outside and inside of the faucet end with detergent and water and running it for at least 1 min. A 1 L Nalgene bottle and cap were rinsed twice by thoroughly shaking with tap water to flush and drain any contaminants that may have remained in the bottle. The tap water samples were then collected in the bottle to approximately 80% of the bottle volume.

2.3 Sample preparation

One hundred milliliters of the water sample were mixed with 0.5 g sodium hydroxide (Merck, Germany) and 0.1 g potassium permanganate (Merck, Germany) in a 500 mL round flask to oxidize the organic matter (ISO, 2019) and then distilled at 80-100°C. The first 20 mL of the distilled water was discarded, and the next 30 mL was collected (USEPA, 2019).

Table 1. Geographic coordinates of each sampling site

Sample code	Latitude (degree)	Longitude (degree)
BK1	13.85829	100.56803
BK2	13.89087	100.56803
BK3	13.93459	100.60911
BK4	13.93285	100.56958
BK5	13.86676	100.59108
BK6	13.85504	100.63265
BK7	13.84508	100.56572
BK8	13.80334	100.57800
BK9	13.83091	100.52550
BK10	13.82551	100.53104
BK11	13.91842	100.51613
BK12	13.89707	100.51545
BK13	13.84234	100.51856
MW1	13.81403	100.41250
MW2	13.82222	100.43685
MW3	13.80233	100.44860
MW4	13.83318	100.41459
MW5	13.87567	100.41240
MW6	13.86511	100.41029
MW7	13.78333	100.41557
MW8	13.78472	100.46134
MW9	13.76198	100.44424
MW10	13.78893	100.34289
MW11	13.77138	100.48790
MW12	13.78341	100.49165
MW13	13.79425	100.50666

BK: Bang Khen; MW: Maha Sawat

(a)

Figure 1. Maps of (a) sampling location in Bangkok metropolitan area and (b) 26 sampling sites generated using Google Maps; yellow pins represent 13 sites around Bang Khen WTP, and red pins represent 13 sites around Maha Sawat WTP.

(b)

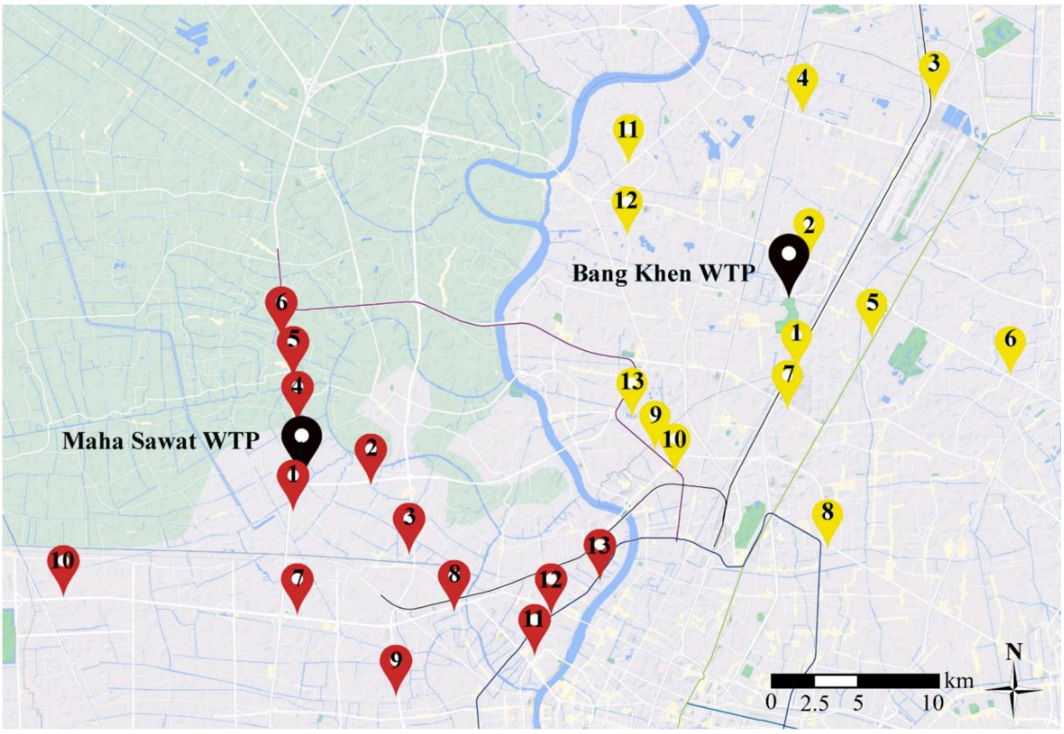

Figure 1. Maps of (a) sampling location in Bangkok metropolitan area and (b) 26 sampling sites generated using Google Maps; yellow pins represent 13 sites around Bang Khen WTP, and red pins represent 13 sites around Maha Sawat WTP (cont.).

2.4 Optimization of the counting performance

2.4.1 Quench curve generation

Two LSCs, the Hidex 600SL automatic (LabLogic Systems, UK) and Quantulus 1220 (PerkinElmer, USA) were used in this study. The efficiency of each tritium measurement was determined by generating a quench correction curve, which is the relationship between the counting efficiency and quench index parameters (QIPs) i.e., TDCR for Hidex and sample channel ratio (SCR) for Quantulus. The quenched tritium standard set (Eckert and Ziegler Analytics, USA) was measured by both LSCs for 60 min, with three measurements per vial. The results were used to generate quench curves, which were subsequently used for the counting efficiency and absolute activity or disintegrations per minute (DPM) calculations (Hou, 2018).

2.4.2 Sample-to-cocktail ratio

The effects of the sample-to-cocktail ratios on the MDA values were determined. The distilled water samples were mixed with the Ultima Gold LLT scintillation cocktail (Perkin Elmer, USA) in 20 mL LSC antistatic high-density polyethylene (HDPE) vials by varying the sample-to-cocktail volume ratio as follows:

- Hidex: 1:14, 2:13, 3:12, 4:11, 5:10, 6:9, and 7:8 mL
- Quantulus: 8:12, 9:11, and 11:9 mL

The vials were shaken well for 2 min and kept in the dark for 24 hours to reduce chemical quenching prior to LSC analysis. Tritium activity was counted for 180 min for Hidex and 480 min for Quantulus.

2.4.3 Vial type and counting time

The effects of vial type and counting time on MDA values were determined by using the optimal sample-to-cocktail ratio 6:9 for Hidex and 11:9 for Quantulus and comparing polyethylene vials and low potassium glass vials with the following counting times:

- Hidex: 60, 120, 180, 360, 540, 720, and 1,440 min
- Quantulus: 60, 120, 240, and 480 min

2.5 Tritium measurements

Measuring tritium in the tap water samples was performed using the optimum conditions obtained from the above experiments. The distilled tap water, tritium-free water and standard tritiated water were added to three LSC vials, followed by the scintillation cocktail. The vials were inverted several times to achieve adequate mixing. The samples were counted using the Hidex 600SL and Quantulus 1220 LSCs after allowing 24 h of adaptation in the dark. The TDCR outputs obtained from the Hidex were automatically calculated as the absolute sample activity (DPM), whereas the SCR outputs obtained from the Quantulus were manually converted to the counting efficiency. The tritium activity concentration, the annual effective dose and MDA values were then calculated using Equations (1), (2), and (3), respectively.

2.6 Calculations

Using the SCR values, the counting efficiency was determined from the quench curves. The tritium activity concentration, A (Bq/L), was then calculated using Equation (1) (Karataşli et al., 2017):

$$A\left(\frac{Bq}{L}\right) = \frac{R_s - R_b}{E \times V \times 60} \quad (1)$$

Where; R_s and R_b are the count rate of the sample and the background (cpm), respectively, E is the counting efficiency, and V is the sample volume (L).

The annual effective dose, AED (μSv/year) due to the ingestion of tritium in the drinking water samples was calculated using Equation (2) (Pintilie-Nicolov et al., 2021):

$$AED = A \times CF \times CR \times 10^6 \quad (2)$$

Where; A is the tritium activity concentration (Bq/L), CF is the dose coefficient (1.8×10^{-11} Sv/Bq for tritium), and CR is the consumption rate of drinking water (250, 350, and 730 L/year for infants, children, and adults, respectively) (WHO, 2017).

The MDA (Bq/L) was calculated based on the Currie equation (Currie, 1968) using Equation (3):

$$MDA\left(\frac{Bq}{L}\right) = \frac{\left(\frac{2.71}{T_s}\right)+3.29\left(\frac{R_b}{T_b}+\frac{R_b}{T_s}\right)^{1/2}}{E \times V \times 60} \quad (3)$$

Where; R_b is the count rate of the background (cpm), T_s is the counting time of the sample (min), T_b is the counting time of the background (min), E is the counting efficiency derived from the quench curve, and V is the sample volume (L).

3. RESULTS AND DISCUSSION

3.1 Quench curve generation

Since the counting efficiency is affected by the level of quenching in the sample, in order to determine the absolute sample activity, it is necessary to establish a quench correction curve to measure the quenching level in the samples. Theoretically, quench correction can be performed by one of the following methods: (1) internal standard method, (2) sample spectrum method, (3) external standard method, and (4) direct DPM method (L'Annunziata et al., 2020). These techniques allow for determining the detection efficiency of a particular sample and for converting the count rate (CPM) to the disintegration rate (DPM). In our study, the quench curves were obtained from a set of 10 quenched tritium standards containing constant tritium activity (222,540 Bq) with varying levels of quenching using the TDCR method for Hidex 600SL (Gudelis et al., 2017) and the SCR method (L'Annunziata et al., 2020) in the selected counting windows of 1-175 and 176-350 channels for Quantulus 1220. Figure 2(a) shows the quench curve for tritium, which shows a correlation between the TDCR value and the counting efficiency, which is a second-order polynomial curve given by the equation $y=-0.5856x^2+1.5468x-0.098$ ($R^2=0.9993$), where x is the TDCR value and y is the counting efficiency. Thereafter, the counting efficiency of an unknown sample can be determined from the measured TDCR value of the sample (Hou, 2018). The DPM value is then automatically calculated after the quench curve is generated and stored on a computer. Likewise, Figure 2(b) shows the quench curve obtained from Quantulus, showing a correlation between the SCR value and the counting efficiency, which is a logarithmic curve given by the equation $y=-0.114\ln(x)+0.4427$ ($R^2=0.9816$), where x is the SCR value and y is the counting efficiency. The tritium activity concentration is calculated from Equation (1) using the counting efficiency derived from the above equation.

3.2 Sample-to-cocktail ratio

The water sample to scintillation cocktail ratio is particularly important for measuring tritium using LSC (Lin et al., 2020). Therefore, the optimal sample-to-cocktail ratio needs to be determined. From the measurement results, it was found that the ratio between the volume of water and the Ultima gold LLT liquid scintillation cocktail influenced the MDA value, in which it was found that the MDA decreased when the sample-to-cocktail ratio increased (Figure 3). For Hidex, the lowest MDA was found at 6.9 Bq/L with a 7:8 sample-to-cocktail ratio, when samples were counted for 180 min (Figure 3(a)). However, the counting efficiency was found to be the highest at a 6:9 ratio. Therefore, a 6:9 sample-to-cocktail ratio was determined as the optimal ratio and was used for further experiments. For Quantulus, the lowest MDA was found at 1.02 Bq/L with an 11:9 sample-to-cocktail ratio when samples were counted for 480 minutes (Figure 3(b)).

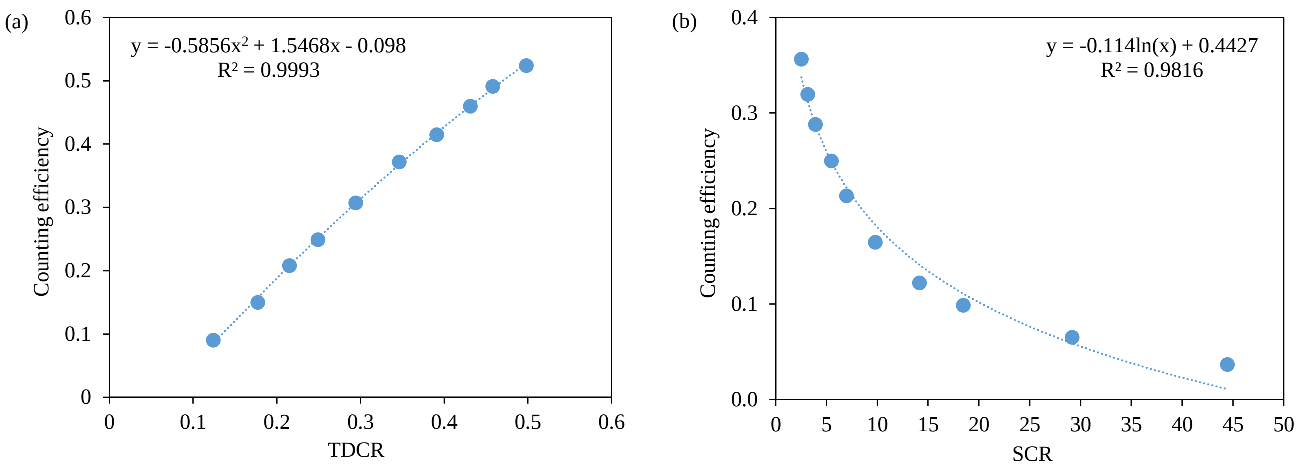

Figure 2. Quench curves for tritium counting using (a) TDCR and (b) SCR values as the quench index parameter

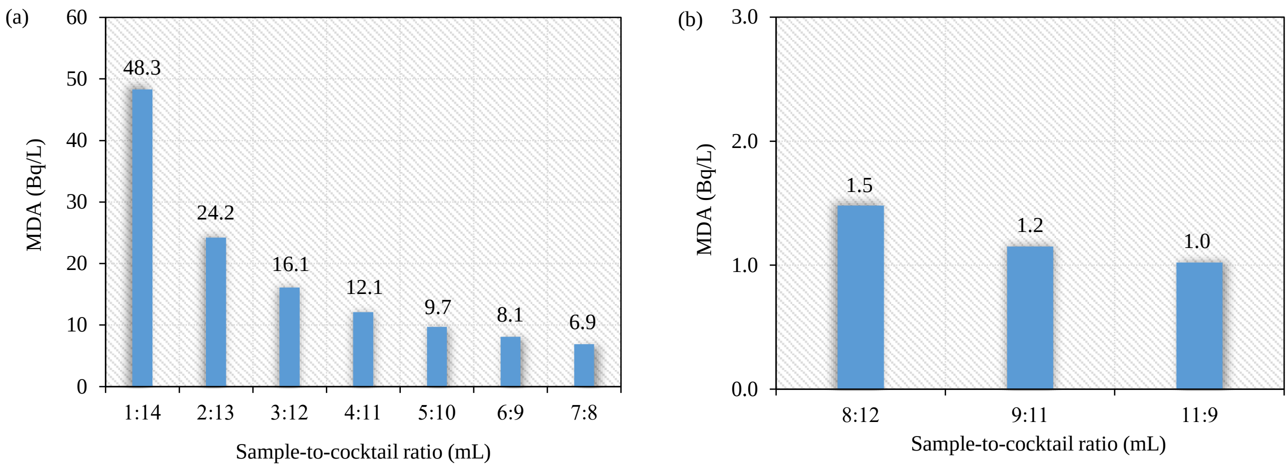

Figure 3. Change in MDA values in each sample-to-cocktail ratio: (a) Hidex and (b) Quantulus

3.3 Vial type and counting time

The effects of LSC vial type and counting time on MDA values were investigated. The results showed that the type of LSC vial and counting time influenced the MDA values, as shown in Figure 4. Plastic vials were found to have lower MDA values than glass vials for both LSCs. These results are consistent with a recent study (Arun et al., 2019) since plastic vials have lower background values than glass vials. The higher background value in the glass vial could be from K-40. Although LSC vials are often made of borosilicate glass, they are lower in potassium; however, their performance in the ^{3}H energy region was significantly affected by radioactive residues in the glass (Perkin Elmer, 2021). Therefore, plastic vials are appropriately used for measuring low-level tritium activity in environmental samples.

The counting time had an inverse relationship with the MDA value. The lowest MDA was found at the maximum counting time used in this study, i.e., 1,440 min for Hidex and 480 min for Quantulus. Therefore, counting times of 1,440 and 480 min were used for low-level tritium measurements in tap water samples using Hidex and Quantulus LSCs, respectively.

3.4 Optimum conditions

The optimum conditions for measuring tritium in tap water samples are summarized in Table 2. The lowest MDA of tritium measurements in tap water samples was found to be 2.7 and 1.0 Bq/L using Hidex and Quantulus LSCs, respectively. This result is consistent with previous studies by Hanslik et al. (2005), Palomo et al. (2007), Varlam et al. (2009), and Jakonić et al. (2014). The detection limits of standard or ultra-low background LSCs are around 1-3 Bq/L for non-enriched water samples. The Hidex 600SL LSC achieved a higher MDA of 2.7 Bq/L with the following optimum conditions: a sample-to-cocktail ratio of 6:9 counted for 1,440 min in a plastic vial. Although Hidex cannot determine the tritium content in tap water because it is below the MDA value, more importantly, the new MDA value, obtained by our laboratory, is significantly lower than the original 9.6 Bq/L using a 5:10 sample-to-cocktail ratio in a plastic vial. As tritium is a radioactive substance with limited health effects, due to its low energy emissions, measuring low levels of tritium in the environment is more useful for monitoring the impact of nuclear activities than public health concerns.

Table 2. The lowest MDA values obtained from the optimal conditions of the two LSCs

LSC	Sample-to-cocktail ratio	Vial Type	Counting time (min)	MDA (Bq/L)
Hidex 600SL	6:9	Plastic	1,440	2.7
Quanlutus 1220	11:9	Plastic	480	1.0

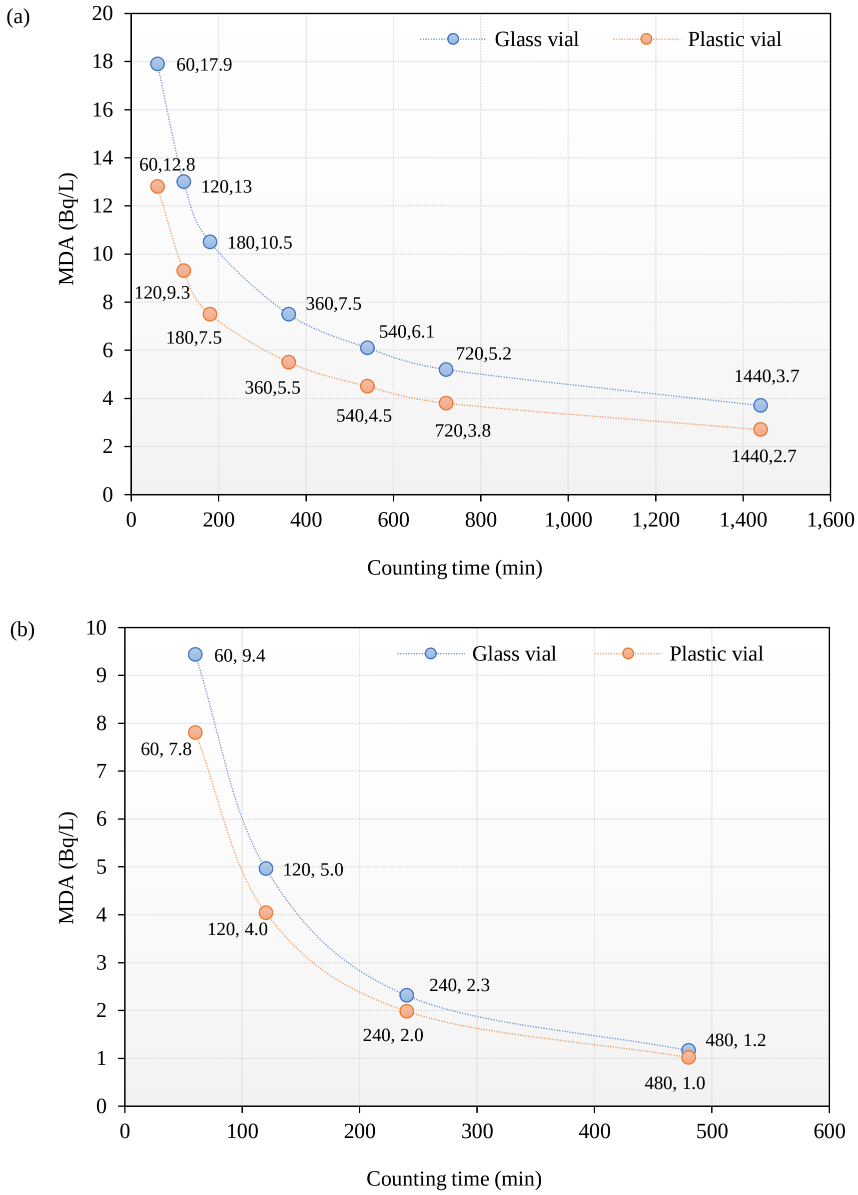

Figure 4. MDA values with different vial types and counting times: (a) Hidex and (b) Quantulus

3.5 Tritium concentration in tap water

Measuring tritium using the optimum parameters of both LSCs showed that Hidex was unable to detect tritium due to the tritium content being lower than its MDA value of 2.7 Bq/L, while the Quantulus, with a lower MDA of 1.0 Bq/L, was able to measure tritium. The results of the tritium concentrations with their respective MDAs from each sampling location are shown in Figure 5. The tritium concentration in tap water around the Bang Khen WTP location was between 1.88-2.63 Bq/L with an average of 2.28±0.28 Bq/L, whereas those from the Maha Sawat WTP were between 2.01-2.69 Bq/L with an average of 2.44±0.26 Bq/L. The average amount of tritium in tap water around the two WTPs was less than 10,000 Bq/L, which is a WHO guideline for drinking water quality (WHO, 2017). Our results are comparable with a recent study by Pédehontaa-Hiaa et al. (2020), which reported that the average tritium concentration in tap water in Lund, Sweden, measured in 2018-2019, was 1.5±0.6 Bq/L (MDA=1.2 Bq/L). It is noteworthy that the average tritium content in tap water supplied from the Bang Khen WTP was not significantly different from that supplied from the Maha Sawat WTP. Although the raw water source used in the production of the water supply of the two plants differs, the amount of tritium was not impacted in any way. The Bang Khen WTP uses raw water from the Chao Phraya River through the eastern water supply canal to the WTP, which is a total distance of about 18 km to produce tap water. The Maha Sawat WTP uses raw water from the Mae Klong River above the Mae Klong dam through the western water supply canal to the WTP, which is a total distance of 107 km (Metropolitan Waterworks Authority, 2020).

Based on our preliminary data, if tritium concentrations were assessed in water samples across the country, they could then be used as a basis for comparing tritium concentrations and assessing the future impact on nuclear power plants. However, if the tritium content is below 1.0 Bq/L, the electrolytic enrichment method is required since it provides an extremely low MDA value (Wallova et al., 2020; Lin et al., 2020), but is more laborious than the conventional distillation method (Hou, 2018).

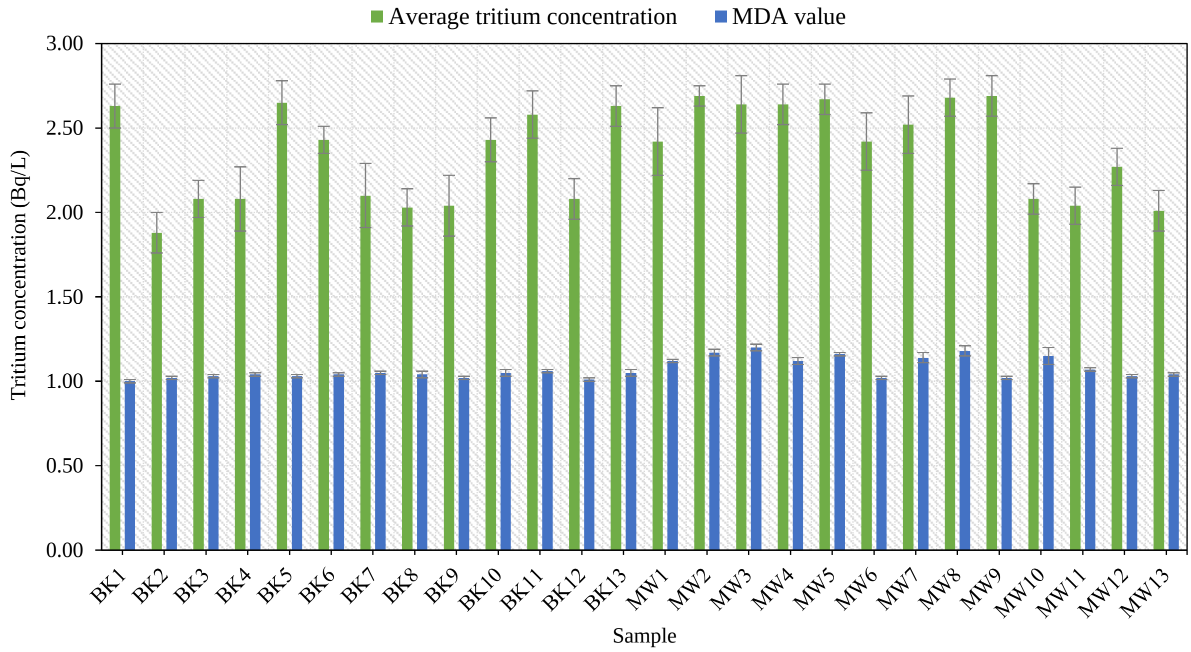

Figure 5. Average tritium concentrations in tap water collected from 26 stations around Bang Khen (BK) and Maha Sawat (MW) water treatment plants compared to their MDA values

3.6 The assessment of the annual effective dose

To assess the effective dose resulting from the ingestion of tritium in tap water, the AED per capita were calculated in infants, children and adults. The average AEDs from each sampling site are shown in Figure 6. The AED for infants, children and adults obtained from tap water samples around the Bangkhen WTP were 0.010, 0.014, and 0.030 µSv/year, respectively, and those from the Maha Sawat WTP were 0.011, 0.015, and 0.032 µSv/year, respectively, which are far below the WHO's guideline limit (100 μSv/year). Our results are comparable to a previous study by Karataşli et al. (2017), which reported that the AED values due to the intake of the tritium in natural water samples for infants, children, and adults in Turkey were averaged 0.024, 0.034, and 0.070 μSv/year, respectively.

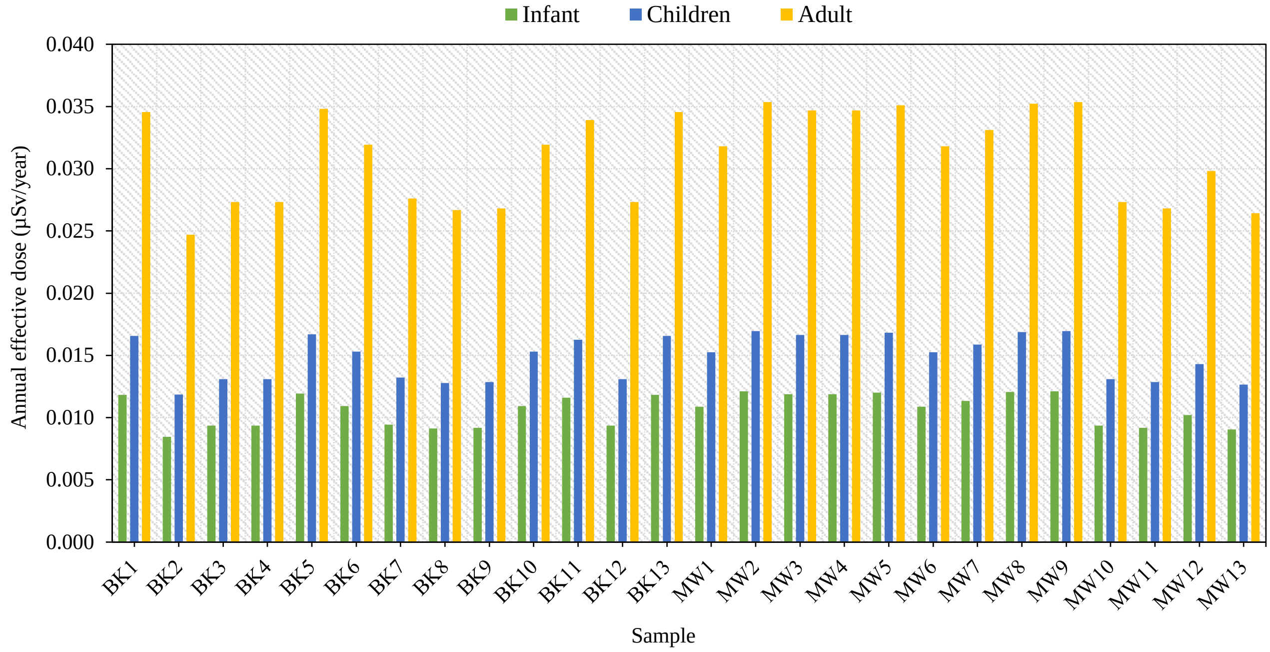

Figure 6. Annual effective dose in infants, children and adults derived from the average tritium concentration in tap water collected from 26 stations around Bang Khen (BK) and Maha Sawat (MW) water treatment plants

4. CONCLUSION

Due to the low level of tritium in tap water samples, the optimum conditions for Quantulus 1220 were successfully applied for directly measuring tritium without pre-enrichment which are simple and economical. The concentration of tritium in tap water around the Bangkhen WTP was between 1.88-2.63 Bq/L with an average of 2.28±0.28 Bq/L, whereas those from the Maha Sawat WTP were between 2.01-2.69 Bq/L with an average of 2.44±0.26 Bq/L, which is far below the WHO's guideline limit (10,000 Bq/L) in drinking water. The annual effective dose resulting from the ingestion of tritium in tap water from both WTPs are far below the WHO's guideline limit (100 μSv/year). As tritium is a radioactive substance with limited health effects due to its low energy emissions, measuring low levels of tritium in the environment is more useful in monitoring the impact of nuclear activities than public health concerns. This improvement could support environmental monitoring programs throughout the country.

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REFERENCES

Arun B, Vijayalakshmi I, Sivasubramanian K, Jose MT. Optimization of liquid scintillation counter for tritium estimation in water samples. Radiochemistry 2019;61:61-5.

Canadian Nuclear Safety Commission (CNSC). Health Effects, Dosimetry and Radiological Protection of Tritium INFO-0799. Canada: Minister of Public Works and Government Services; 2010.

Cassette P, Bouchard J. The design of a liquid scintillation counter based on the triple to double coincidence ratio method. Nuclear Instruments and Methods in Physics Research Section A 2003;505:72-5.

Chen F, Wang X, Li Y, Zhang Z. Analysis of tritium activity concentrations in urine and internal dose assessment for nuclear power plant workers. Radiation Medicine and Protection 2021;2(1):1-4.

Currie LA. Limits for qualitative detection and quantitative determination: Application to radiochemistry. Analytical Chemistry 1968;40:586-93.

Duliu OG, Varlam C, Shnawaw D. 18 years of continuous observation of tritium and atmospheric precipitations in *Ramnicu valcia* (Romania): A time series analysis. Journal of Environmental Radioactivity 2018;190-191:105-10.

Erchinger JL, Orrell JL, Aalseth CE, Bernacki BE, Douglas M, Fuller ES, et al. Background characterization of an ultra-low background liquid scintillation counter. Applied Radiation and Isotopes 2017;126:168-70.

Feng B, Chen B, Zhao C, He L, Tang F, Zhuo W. Application of a liquid scintillation system with 100-ml counting vials for environmental tritium determination: Procedure optimization, performance test, and uncertainty analysis. Journal of Environmental Radioactivity 2020;225:Article No. 106427.

Grahek Z, Breznik B, Stojković I, Coha I, Nikolov J, Todorović N. Measurement of tritium in the Sava and Danube Rivers. Journal of Environmental Radioactivity 2016;162-163:56-67.

Gudelis A, Gaigalaitė L, Gorina I, Butkus P. Use of the TDCR method for standardization of radionuclides in the FTMC. Proceedings part II of the 21st International Conference on Radionuclide Metrology and its Applications (ICRM 2017); 2017 May 15-19; Buenos Aires: Argentina; 2017.

Hanslik E, Jedinakova-Krızova V, Ivanovova D, Kalinova E, Sedlaova B, Simonek P. Observed half-lives of ^{3}H, ^{90}Sr and ^{137}Cs in hydrosphere in the Vltava River Basin (Bohemia). Journal of Environmental Radioactivity 2005;81:307-20.

Hou X. Liquid scintillation counting for determination of radionuclides in environmental and nuclear application. Journal of Radioanalytical and Nuclear Chemistry 2018; 318:1597-628.

International Atomic Energy Agency (IAEA). Good Practices in Heavy Water Reactor Operation: IAEA-TECDOC-1650. Vienna, Austria: International Atomic Energy Agency; 2010.

International Organization for Standardization (ISO). ISO 9698: Water Quality-Tritium-Test Method using Liquid Scintillation Counting. Geneva, Switzerland: Technical Committee ISO/TC 147; 2019.

Jakonić I, Todorović N, Nikolov J, Krajcar Bronić I, Tenjović B, Vesković M. Optimization of low-level LS counter Quantulus 1220 for tritium determination in water samples. Radiation Physics and Chemistry 2014;98:69-76.

Karataşli M, Turhan S, Kurnaz A, Varinlioğlu A, Gören E, Yeğingil Z. Determination of tritium activity concentration in natural water samples. Environmental Forensics 2017; 18(2):162-6.

L'Annunziata MF, Tarancón A, Bagán H, García JF. Chapter 6 - Liquid scintillation analysis: Principles and practice. In: L'Annunziata MF, editor. Handbook of Radioactivity Analysis. 4th ed. Amsterdam: Academic Press; 2020. p. 575-801.

Lin F, Yu T, Yu W, Ni J, Lin L. Electrolytic enrichment method for tritium determination in the Arctic Ocean using liquid scintillation counter. Acta Oceanologica Sinica 2020;39:73-7.

Matsumoto H, Shimada Y, Nakamura AJ, Usami N, Ojima M, Kakinuma S, et al. Health effects triggered by tritium: How do we get public understanding based on scientifically supported evidence? Journal of Radiation Research 2021;62(4):557-63.

Metropolitan Waterworks Authority. Important data of MWA. [Internet]. 2020 [cited 2020 Feb 3]. Available from: https://www.mwa.co.th/main.php?filename=stat&t=top. (in Thai).

Nayak SR, D'Souza RS, Kamath SS, Mohan MP, Bharath S, Shetty T, et al. Organically bound tritium: Optimization of measurements in environmental matrices by combustion method and liquid scintillation spectrometry. Journal of Radioanalytical and Nuclear Chemistry 2019;319(3):917-26.

Palomo M, Penalver A, Aguilar C, Borrull F. Tritium activity levels in environmental water samples from different origins. Applied Radiation and Isotopes 2007;65:1048-56.

Pédehontaa-Hiaa G, Holstein H, Mattsson S, Rääf CL, Stenström KE. Tritium in urine from members of the general public and occupationally exposed workers in Lund, Sweden, prior to operation of the European Spallation Source. Journal of Environmental Radioactivity 2020;213:Article No. 106141.

PerkinElmer. Liquid Scintillation Counting. [Internet]. 2021 [cited 2021 Sep 1]. Available from: https://www.perkinelmer.com/lab-products-and-services/application-support-knowledge base/radiometric/liquid-scintillation-counting.html.

Pintilie-Nicolov V, Georgescu PL, Iticescu C, Moraru DI, Pintilie AG. The assessment of the annual effective dose due to ingestion of radionuclides from drinking water consumption: Calculation methods. Journal of Radioanalytical and Nuclear Chemistry 2021;327:49-58.

Popoaca S, Bucur C, Simionov V. Determination of ^{3}H and ^{14}C in organic sample after separation through combustion method. Journal of Energy and Power Engineering 2014;8:1687-94.

Rittirong A, Noithong P, Hazama R, Sakuma Y, Saenboonruang K, Sudprasert W. Determination of tritium levels in tap waters collected from various regions in Thailand using liquid scintillation counting. Journal of Physics: Conference Series 2019;1285:Article No. 012021.

Theodorsson P. A review of low-level tritium systems and sensitivity requirements. Applied Radiation and Isotopes 1999;312:311-6.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Sources Effects and Risks of Ionizing Radiation, Report to the General Assembly, with Scientific Annexes: Annex C - Biological Effects of Selected Internal Emitters - Tritium. New York, USA: United Nations; 2016.

United States Environmental Protection Agency (USEPA). Radionuclide Basics: Tritium. [Internet]. 2021 [cited 2021 Aug 10]. Available from: https://www.epa.gov/radiation /radionuclide-basics-tritium.

United States Environmental Protection Agency (USEPA). EPA Method 906.0: Tritium in Drinking Water. [Internet]. 2019 [cited 2021 Jan 15]. Available from: https://www.epa.gov/sites/default/files/2015-06/documents/epa-906.0.pdf.

Varlam C, Stefanescu I, Duliu OG, Faurescu I, Popescu I, Dobrinescu D. Applying direct liquid scintillation counting to low level tritium measurement. Applied Radiation and Isotopes 2009;67(5):812-6.

Wallova G, Meresova J, Zvachova S, Petranova I, Sykora I. New electrolytic enrichment system for tritium determination in water research institute in Bratislava and it's first results of tritium activity in precipitation. Journal of Environmental Radioactivity 2020;216:Article No. 106177.

World Health Organization (WHO). Guidelines for Drinking-Water Quality. 4th ed. Geneva, Switzerland; 2017.

In Vitro and *Ex Situ* Biodegradation of Low-Density Polyethylene by a *Rhizopus* sp. Strain Isolated from a Local Dumpsite in North-East Algeria

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ABSTRACT

Low density polyethylene (LDPE) is the most abundant non-degradable plastic waste. Widely used in packaging material, it represents a serious threat to all ecosystems. In the present study, a *Rhizopus* sp. fungal strain was isolated from soil of a landfill located in north-east Algeria and cultured on potato dextrose agar. The *in vitro* biodegradability of pieces of the same plastic bag (0.2, 0.4, and 0.6 g) was estimated in minimal liquid medium and on minimal solid medium. Furthermore, biodegradation of plastic bag pieces was examined in seawater, tap water and soil. The isolated *Rhizopus* sp. strain could degrade the plastic bag waste. The highest *in vitro* rate occurred in the minimal liquid medium for both the 0.4-g and 0.6-g pieces (a 20% decrease in weight). In natural media, the highest weight decrease was different depending on the substrate: 5% in seawater for the 0.2-g piece, 10% in tap water for the 0.4-g piece and 8% in soil for the 0.4-g piece. This strain could also form a biofilm in Malt Extract Broth (MEB). These results revealed that the isolated *Rhizopus* sp. strain has considerable biodegradative ability based on different measures.

1. INTRODUCTION

Owing to their chemical stability, good mechanical properties, low production costs and simple processability, plastic materials are the main components in many manufacturing sectors such as packaging, clothing, construction, and automotive (Akhbarizadeh et al., 2020; Thiounn and Smith, 2020). Every year, over 320 million tons of plastic are produced, and this amount is predicted to double in 12 years (Ritchie and Roser, 2018).

Plastic is a polymer of long hydrocarbon chains with a high molecular weight, derived mainly from petrochemicals that are then synthetically arranged by certain chemical processes to produce the long polymer chains (Shimao, 2001). Polyethylene is a thermoplastic polymer produced by monomers of ethylene. There are numerous categories such as high-density polyethylene (HDPE), linear low-density polyethylene (LLDPE) and low-density polyethylene (LDPE), which is a thermoplastic made from petroleum. LDPE materials are generally used for manufacturing various containers, dispensing bottles, plastic bags, and various melded laboratory wares because of their light weight, strength, and durability (Pramila and Ramesh, 2011).

Plastics generate huge problems related to the environment, health, and the management of residual materials. In addition, some materials have generated considerable controversy regarding their toxicological risks due to the presence of additives such as phthalates or bisphenol A (Lewis, 2012). In fact, many countries have restricted the use of certain plastic products. Disposable plastic bags as well as polyethylene containers have been subjected to such measures (Lewis, 2012). Several processes are used to deal with this situation, namely landfills, incineration, and storage, without thinking about the consequences and possible negative effects of these techniques on the environment in the short or long term. On the other hand, recent studies have shown that the degradation

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of plastic bags by microorganisms, particularly fungi, is the main process of decontamination in sediments and deep soils (Bonanni et al., 2012). For example, Sowmya et al. (2014) affirmed the ability of *Penicillium simplicissimum* to use polyethylene as a carbon source and its ability to degrade it, while El Sayed et al. (2021) isolated and identified six fungal strains: *Aspergillus carbonarius*, *Aspergillus* sp., *Aspergillus flavus*, *Aspergillus ochraceus*, *Penicillium* sp., *Fusarium* sp. and proved their ability to use LDPE as a unique source of carbon and energy.

The aim of this work was to isolate and identify a fungal strain involved in plastic biodegradation from a landfill located in north-east Algeria and to evaluate its capacity to degrade plastic bag pieces and to form biofilm. The obtained fungal strain could be used as a tool to depollute contaminated soil.

2. METHODOLOGY

2.1 Study area and sample collection

A quantity of soil was collected from a public landfill area in north-east Algeria after elimination of the first 5 cm of the soil surface. The sample was transported in a sterile black plastic bag to the laboratory and kept at 4°C and used within 24 h (according to Boughachiche et al. (2005), with modification).

2.2. Isolation of fungal strains

Ten grams of soil was added to 90 mL of sterile physiological water. Then, 10^{-2}, 10^{-3} and 10^{-4} serial dilutions were prepared, and 0.1 mL of each dilution was inoculated on the surface of a Petri dish containing solid potato dextrose agar (PDA) supplemented with gentamicin. The plates were incubated at 27°C for one week (Davet and Rouxel, 1997). The isolated strains were further purified on solid PDA.

2.3. Identification and storage of fungi

Fungal strains were identified based on macroscopic and microscopic characteristics (Pardo-Rodríguez and Zorro-Mateus, 2021). Isolated strains were stored in slant PDA agar tubes (Botton et al., 1990).

2.4. Preparation of LDPE pieces

The LDPE films used in this study were plastic white bags of 50 µm thickness, flexible and solid. For the biodegradation studies, LDPE films were cut into small pieces (0.2, 0.4, and 0.6g) and were disinfected with 70% ethanol.

2.5. *In vitro* biodegradation test

2.5.1. Preparation of the minimal medium

Liquid minimal medium (MM) was prepared by adding 16 g K_2HPO_4, 2 g KH_2PO_4, 1 g $(NH_4)_2SO$, 0.475 g $MgSO_4$, 0.2 g NaCl, 0.01 g $CuSO_4$, 1 g $CaCO_3$ and 1 g $ZnSO_4$ in 1,000 mL of distilled water. Solid MM was obtained by adding 20 g agar to 1 L liquid MM (according to Nakajima-Kambe et al. (1999), with modifications). The initial pH of the medium was adjusted to 7.0.

2.5.2. Culture in liquid MM

The tested fungal strain was screened for its ability to degrade LDPE films. The biodegradation test was performed in liquid MM containing LDPE film as the sole carbon source. A piece of the same plastic bag (0.2, 0.4, and 0.6 g) was added to 250 mL Erlenmeyer flasks containing 150 mL of liquid MM. An agar disc of the fungal strain that had been cultured for seven days was inoculated in each Erlenmeyer flask (Bourzama et al., 2021). The Erlenmeyer flasks were incubated in a shaker incubator for one month at 27±2°C with rotation at 150 rpm. Every 10 days, the pieces were aseptically collected and weighed. The fungus *Aspergillus niger* was used as the control to confirm the degradation of LDPE films.

2.5.3. Culture on solid MM

A piece of plastic bag (0.2, 0.4, and 0.6 g) was added to Petri dishes containing solid MM. The fungal strain was added by touch. The plates were then incubated for 30 days at 27±2°C (Bourzama et al., 2021). A Petri dish containing solid MM without the piece of plastic bag served as the control.

2.6. *Ex situ* biodegradation test

The fungal strain was tested for its ability to degrade LDPE films in the presence of other carbon sources than LDPE. A piece of plastic bag piece was added to a flask containing 150 mL of sterile seawater, 150 mL of sterile tap water and 200 g of sterile soil. The fungal strain was added by the disc method. The weight of each piece was checked every 10 days during 1-month incubation. Flasks containing a plastic piece with 150 mL of sterile water, 150 mL of sterile tap water and 200 g of sterile soil, without addition of the fungal strain, served as the controls. All tests were carried out in triplicate.

2.7. Formation of biofilm by the fungal strain

Malt extract broth (MEB) was used to test the ability of the isolated fungal strain to form biofilm. MEB was prepared as follows: 5 g peptone, 10 g malt extract and 1,000 mL of distilled water. After autoclaving, MEB was poured into three Petri dishes. The tested fungal strain was inoculated by the touch technique and incubated for 3 days. Each day, one plate was examined using a binocular microscope (Siqueira and Lima (2013), with modifications).

2.8. Estimation of the biodegradation rate

The percentage weight loss of each plastic bag piece was calculated by the following equation (DSouza et al., 2021):

$$\%\text{ weight loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

The values obtained were then compared with the controls.

2.9. Carbon dioxide production

To estimate carbon dioxide (CO_2) production by the tested fungal strain during biodegradation, the cultures were observed by naked eye (according to Khruengsai et al. (2021), with modifications). Positive cultures were recognised by the presence of gas bubbles.

3. RESULTS

3.1 Screening and identification of LDPE-degrading fungal isolates

Growing the landfill soil samples on solid PDA for seven days yielded a single fungal strain. Based on its macroscopic and microscopic characteristics, the fungal isolate was identified as *Rhizopus* sp. (Figure 1).

(a) (b) (c)

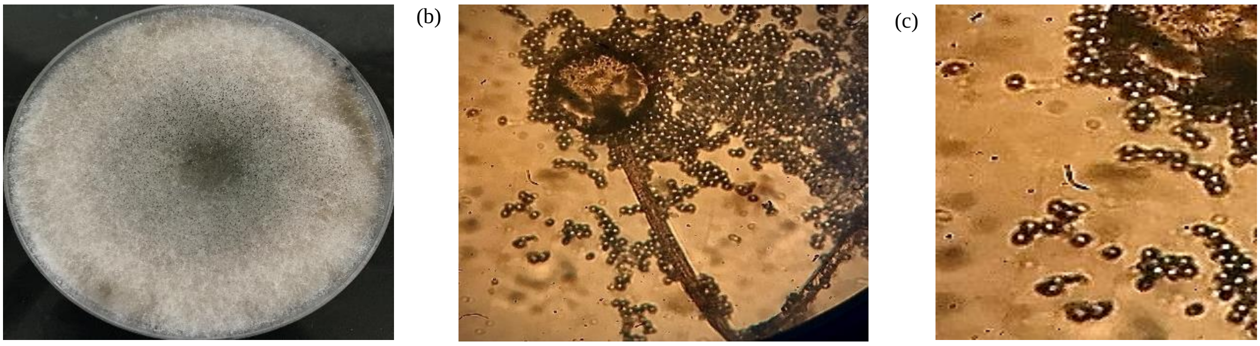

Figure 1. Morphology of the isolated *Rhizopus* sp. strain after 7 days of incubation: (a) macroscopic appearance, (b) microscopic appearance (40X magnification), and (c) spores of *Rhizopus* sp. under optical microscopy

3.2. *In vitro* biodegradation test

3.2.1. In liquid MM

The isolated *Rhizopus* sp. strain was tested for its ability to degrade plastic bag pieces in liquid MM. The cultures were incubated with continuous shaking (100 rpm) at 27±2°C at neutral initial pH, the weight change in plastic bag pieces was examined after 10, 20, and 30 days. Compared to the control *Aspergillus niger*, *Rhizopus* sp. showed better results.

The weight change of the 0.2-g plastic bag piece is presented in Figure 2(a). After the first 20 days, there had been no change in weight. After 30 days, the weight had decreased by 5%. Figure 2(b) shows the variation in the weight of the 0.4-g plastic piece. After 10 days, there had been a 16% decrease in weight. The weight continued to decrease, and at 30 days it had decreased by 20%. The 0.6-g plastic piece showed a weight loss similar to the 0.4-g plastic piece (Figure 2(c)).

3.2.2. On solid MM

On the plates with solid MM containing the plastic bag pieces, *Rhizopus* sp. appeared like a tangle of white filaments and small black and white dots. No growth was observed in the control plate (Table 1).

Table 1. Appearance of plates after incubation

Plate	Macroscopic observation
Plate with the 0.2-g piece	Growth of the tested fungal strain above the plastic bag piece
Plate with the 0.4-g piece	Growth of the tested fungal strain above the plastic bag piece
Plate with the 0.6-g piece	Growth of the tested fungal strain above the plastic bag piece
Control plate	Death of the tested fungal strain seeded on the plate

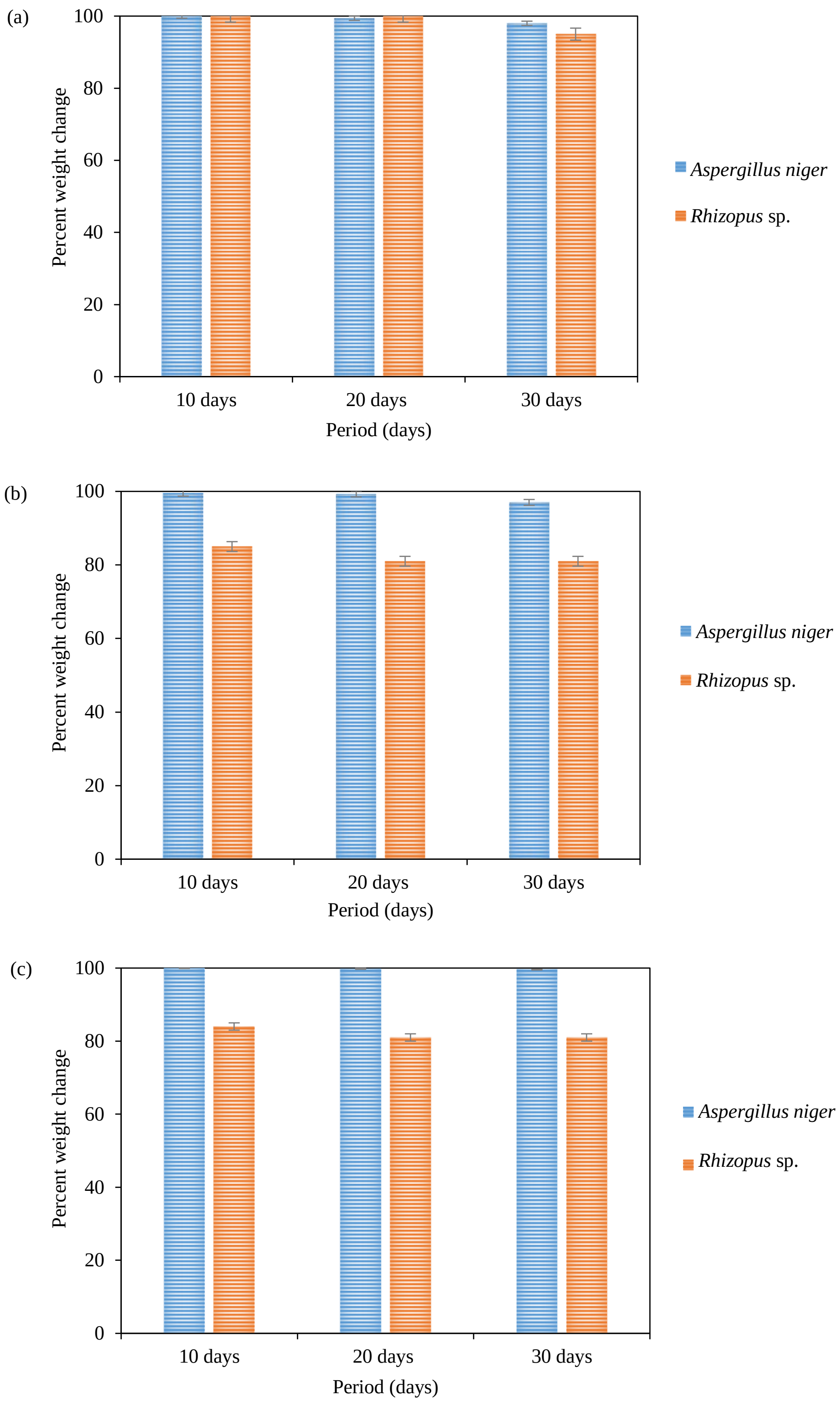

Figure 2. Percent weight change of (a) 0.2-g, (b) 0.4-g, and (c) 0.6-g plastic bag pieces incubated with the isolated *Rhizopus* sp. strain at 27±2°C for 30 days in liquid minimal medium compared with *Aspergillus niger*

3.3. *Ex situ* biodegradation test

3.3.1. In seawater

There was a slow weight loss of the 0.2-g and 0.4-g plastic bag pieces during the entire 30-days test period, 5% and 2.5%, respectively (Figure 3(a) and 3(b)). There was a slight variation in weight for the 0.6-g plastic bag piece (Figure 3(c)). After the first 10 days, there had been no change in weight. After 20 and 30 days, there had been a stable decrease in weight (1.5%).

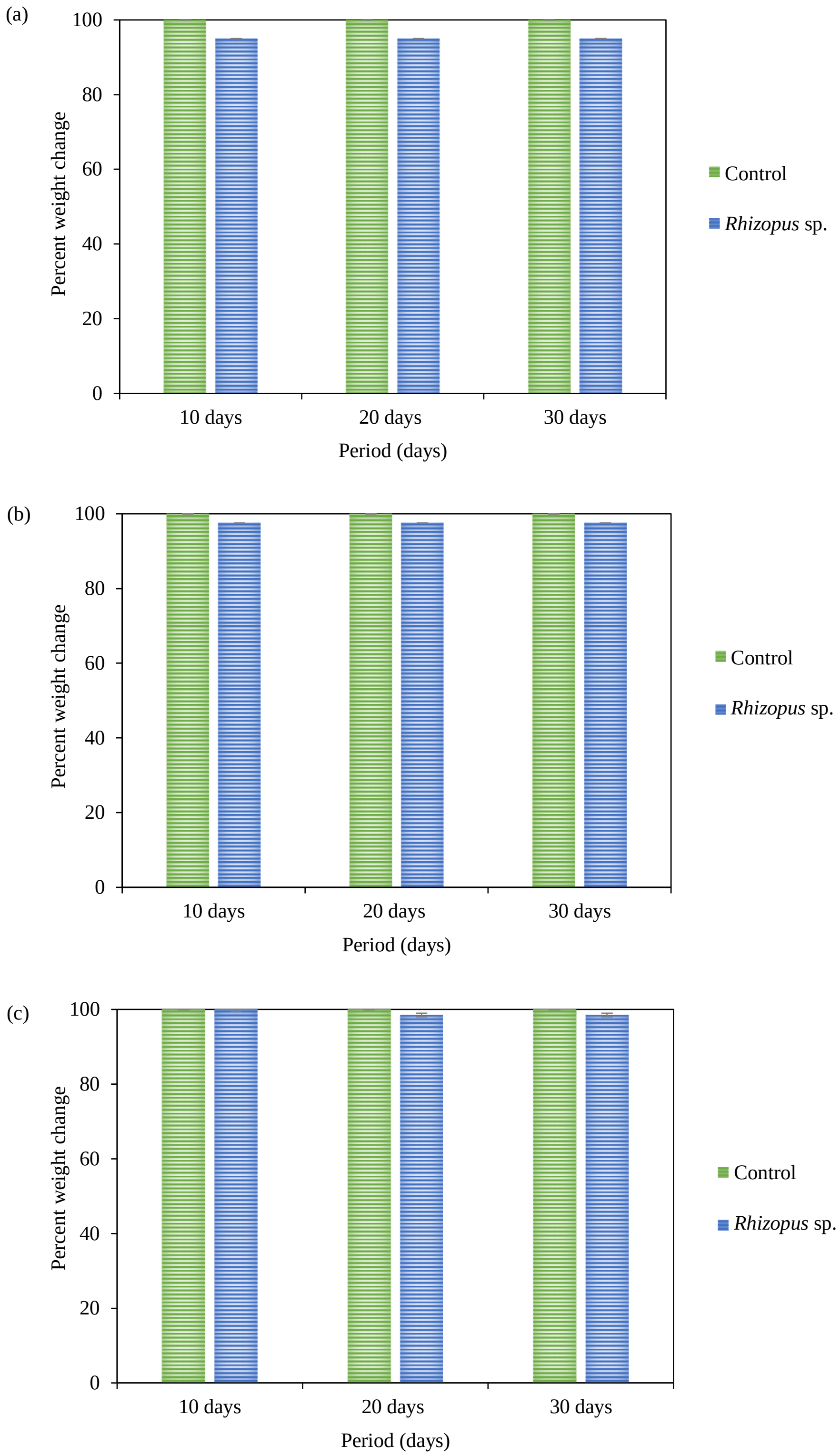

Figure 3. Percent weight change of (a) 0.2-g, (b) 0.4-g, and (c) 0.6-g plastic bag pieces incubated with the isolated *Rhizopus* sp. strain at 27±2°C for 30 days in seawater compared with the control

3.3.2. In tap water

The weight of the 0.2-g plastic bag piece had decreased by 5% after 30 days (Figure 4 (a)). The weight of the 0.4-g plastic bag piece also changed (Figure 4(b)). After 10 days, there had been no change. After 20 days, there had been a slight weight loss and it continued until day 30, when it had decreased by 10%. There had been no change in weight for the 0.6-g plastic bag piece after 20 days, but after 30 days the weight had decreased by 1.5% (Figure 4(c)).

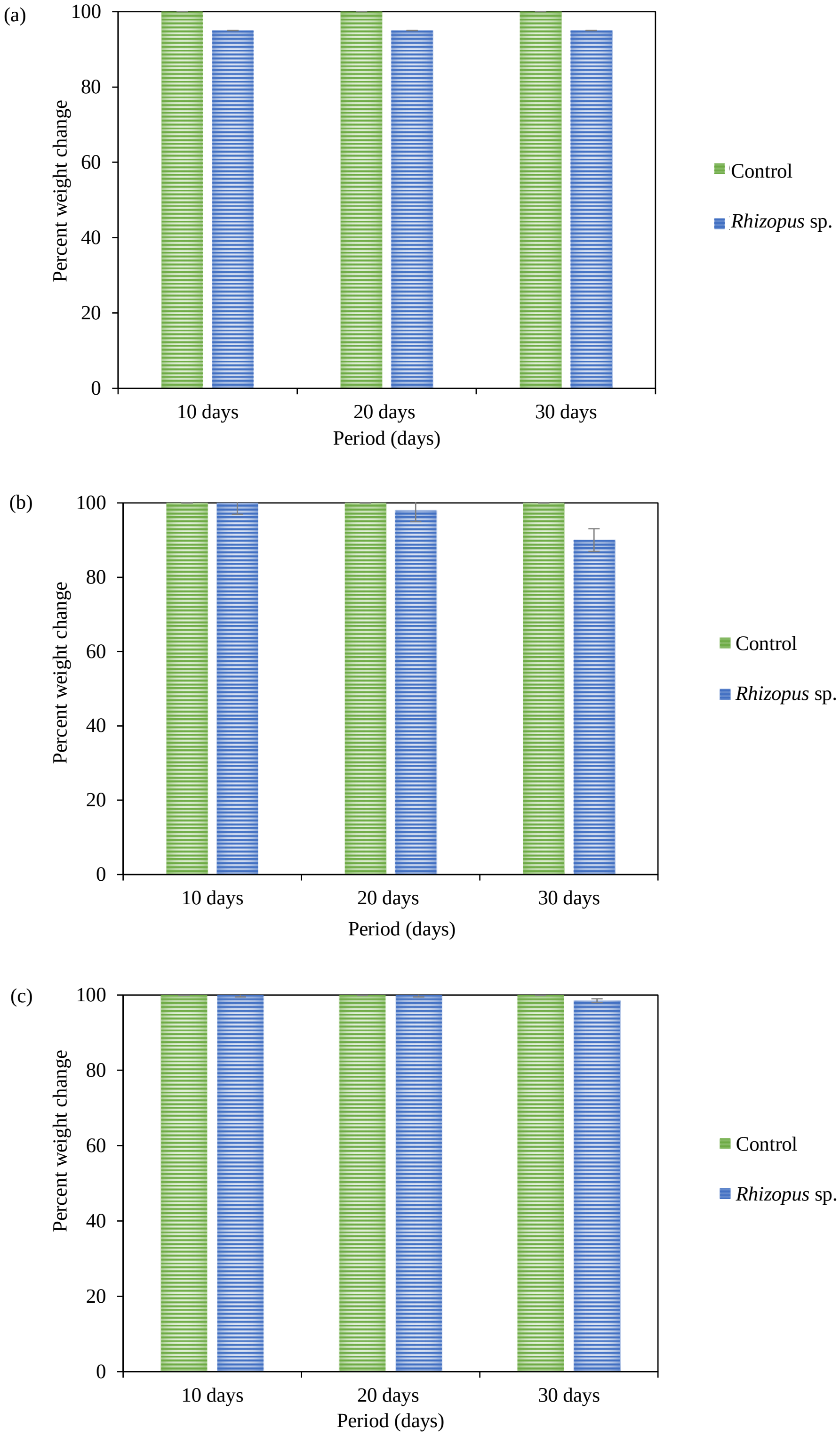

Figure 4. Percent weight change of (a) 0.2-g, (b) 0.4-g, and (c) 0.6-g plastic bag pieces incubated with the isolated *Rhizopus* sp. strain at 27±2°C for 30 days in tap water compared with the control

3.3.3. In soil

The weight of the 0.2-g plastic bag piece had not changed after 20 days of incubation. After 30 days, however, the weight had decreased by 5% (Figure 5(a)). The weight of the 0.4-g plastic bag piece varied during the experiment (Figure 5(b)). After 10 days, there had been no change in weight. After 20 days, there had been a slight weight loss that continued until 30 days, when it had reached 8%. Moreover, the 0.6-g plastic bag piece exhibited weight loss throughout the entire test period, reaching its maximum (6.5%) after 30 days (Figure 5(c)).

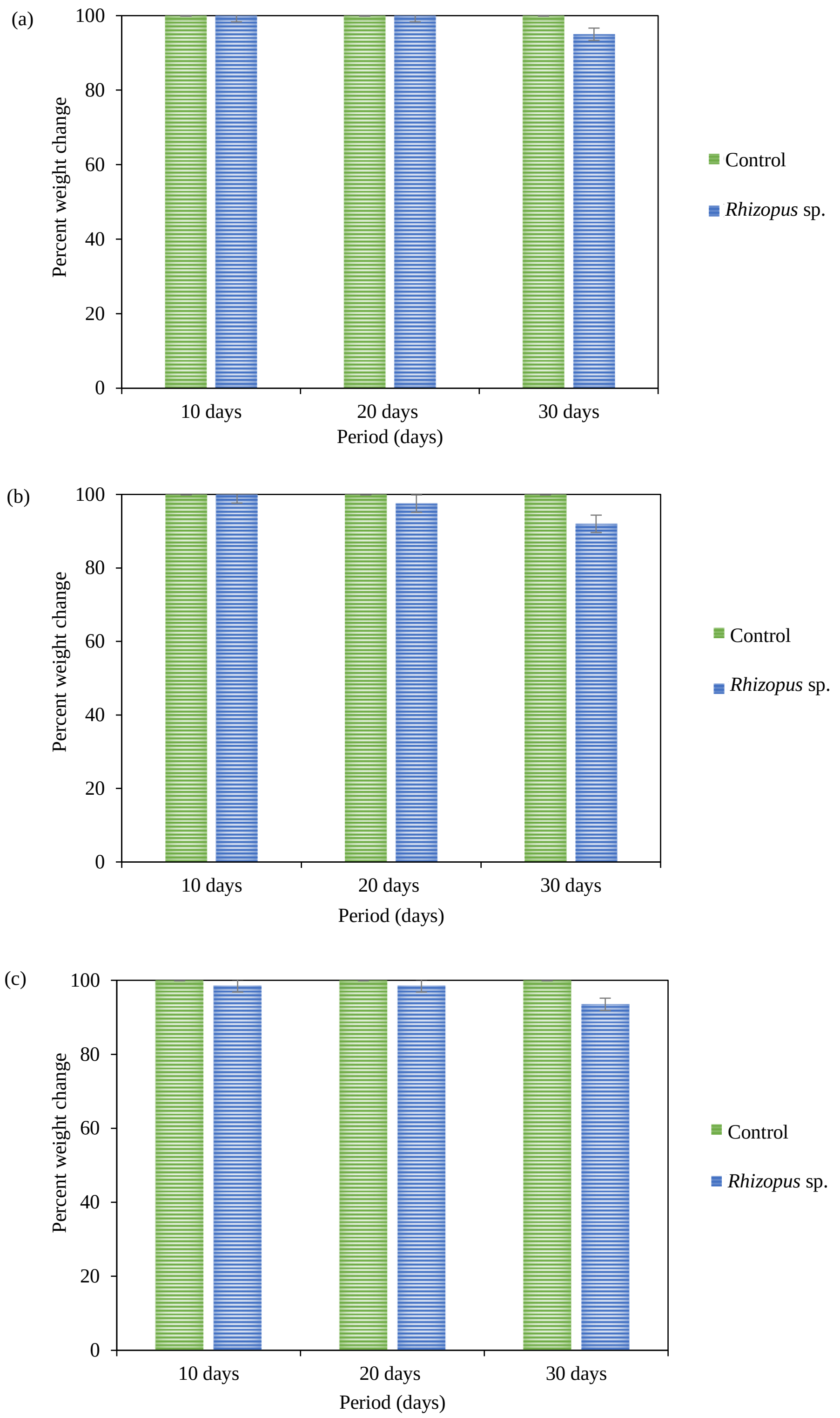

Figure 5. Percent weight change of (a) 0.2-g, (b) 0.4-g, and (c) 0.6-g plastic bag pieces incubated with the isolated *Rhizopus* sp. strain at 27±2°C for 30 days in soil compared with the control

3.4. CO_2 production

In vitro and *ex situ* biodegradation of polyethylene by the isolated fungal strain resulted in CO_2 production that was observed by naked eye as gas bubbles in all cultures except for the controls (Table 2).

3.5. Formation of biofilm

Rhizopus sp. was able to quickly form biofilm in MEB (Table 3).

Table 2. Production of carbon dioxide (CO_2) gas bubbles after 1 month of incubation of *Rhizopus* sp. *in vitro* and *ex situ* cultures

Test	Culture	Production of gas bubbles
In vitro	On liquid minimal medium	+
	On solid minimal medium	+
Ex situ	In seawater	++
	In tap water	+++

Note: +: low production of CO_2; ++: average production of CO_2; +++: good production of CO_2

Table 3. Microscopic observation of biofilm formation

Time	Biofilm formation
After 1 day	+
After 2 days	+
After 3 days	++

Note: +: beginning of biofilm formation; ++: actual formation of biofilm

4. DISCUSSION

There have been numerous studies evaluating the biodegradation of polyethylene by micro-organisms. Although many studies have used bacterial cultures in natural environments, only few studies have used fungal species in controlled environments (DSouza et al., 2021). The aim of this study was to isolate and identify a fungal strain from a landfill in north-east Algeria capable of degrading plastic bag pieces *in vitro* and *ex situ*. The isolated strain was identified as *Rhizopus* sp.

In liquid MM, the weight loss of LDPE pieces during the incubation with the tested fungus *Rhizopus* sp. resulted from the utilization of the polyethylene as the sole carbon source. The results indicated that this fungal strain was capable of degrading LDPE pieces compared to *A. niger*, which has been reported to have good degradation ability (Khruengsai et al., 2021). In fact, the degradation potential of the tested *Rhizopus* sp. strain is explained by the good adaptation of the fungus in liquid MM and its ability to produce the necessary biodegradation enzymes. In addition, the growth of the tested fungal strain on solid MM containing plastic bag pieces and the release of CO_2 gas bubbles affirms its capacity to degrade this polymer and to use it as a carbon source.

In *in vitro* and *ex situ* tests, the weight of the plastic bag pieces decreased. However, the degradation rate was higher in tests using MM compared with tests using natural media because the latter contain carbon sources other than polyethylene. Indeed, the greatest weight loss occurred in liquid MM (20%), while in natural media, the greatest weight loss occurred in tap water (10%). Moreover, environmental factors and the presence of another carbon sources have an important influence on the degradation of carbonyl polymers which leads to a good production of CO_2 in natural media compared to MM, in particular on the biological activity of fungal species (Lefaux, 2005).

The obtained results showed that the tested strain has a good biodegradation activity because different measures confirmed its potential to degrade plastic bag pieces. In fact, DSouza et al. (2021) obtained similar results with a maximum weight loss of LDPE estimated at 26.15% when using a consortium of *Aspergillus* species under controlled conditions.

Shah (2007) buried pieces of LDPE in soil mixed with wastewater sludge for a period of 10 months. Microscopic examination showed the presence of a fungal layer on the surface of the plastic material, indicating its use by microorganisms as a carbon source. On the other hand, DSouza et al. (2021) used a fungal consortium consisting of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus oryzae* for the biodegradation of LDPE in laboratory conditions. The weight loss of the samples was attributed to the degradation of the carbon polymers by fungal enzymes. The resulting monomers and oligomers were used directly by the fungal species as a unique carbon source. Furthermore, Torres et al. (1996) showed that some fungal strains can also use lactic acid and oligomers released from the abiotic degradation of polylactic acid polymer.

Polyethylene macromolecules are large and cannot be digested by intracellular enzymes because they cannot penetrate the microbial cell barrier (Ammala et al., 2011). Fungi produce extracellular enzymes such as laccases, peroxidases and esterases, which are directly involved in fixation on a polyethylene surface and subsequent biodegradation (Wei and Zimmermann, 2017). So, biodegradation occurs via secreted enzymes that digest polyethylene macromolecules until they are small enough for bioassimilation: the microbial cells metabolise it, assimilate it into their structure and then mineralise it (Wyart, 2007).

Enzyme-mediated degradation of poly-L-lactic acid was first reported by Williams (1981) using proteinase K from *Tritirachium album*. Oxidative degradation is an appropriate pathway for polymers lacking easily hydrolysable groups, such as polyethylene (Yuan et al., 2020). Indeed, microbial enzymes create functional groups that enhance the hydrophilicity of the polymer and improve its biodegradation (Shah et al., 2008). Moreover, in presence of *Aspergillus nomius* (Abraham et al., 2017) and *Rhizopus oryzae* (Awasthi et al., 2017), there was an increase in the amount of carbonyl groups in LDPE, a sign that was directly associated with peroxidase attack (Sudhakar et al., 2008).

Our results are comparable with the general model that describes the beginning of polymer biodegradation as a surface erosion process (Gajendiran et al., 2016). Microorganisms adhere to and colonise the plastic surface, a phenomenon involving the production and secretion of extracellular enzymes and other molecules that mediate biofilm formation (Koutny et al., 2006; Sen and Raut, 2015; Sheik et al., 2015), a fundamental step to initiate microbial biodegradation (Mathur et al., 2011). In fact, the large size of the enzymes can present an obstacle to penetrate deep into the material (Gajendiran et al., 2016). Besides, the undegraded macromolecules are too large to pass through the cell membranes (Koutny et al., 2006; Sen and Raut, 2015). Finally, biofilm formation can prevent the hydrophobicity of polyethylene and help microorganisms to survive in a nutrient-poor environment by using the solid substrate (Shah et al., 2008; Sen and Raut, 2015; Ojha et al., 2017; Yuan et al., 2020).

5. CONCLUSION

Compared to *Aspergillus niger*, the tested *Rhizopus* sp. strain has a good potential for plastic biodegradation as confirmed by several *in vitro* and *ex situ* tests: reducing the weight of plastic bag pieces, formation of visible biofilm on plastic bag pieces and CO_2 production. Based on the obtained results, it is evident that nature offers an alternative pathway to solve some environmental issues like plastic pollution. *Rhizopus* sp. could be used for the bioremediation of polluted environments to eliminate plastic waste that is harmful to the environment (flora and fauna) and to human health.

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REFERENCES

Abraham J, Ghosh E, Mukherjee P, Gajendiran A. Microbial degradation of low density polyethylene. Environmental Progress and Sustainable Energy 2017;36(1):147-54.

Akhbarizadeh R, Dobaradaran S, Schmidt TC, Nabipour I, Spitz J. Worldwide bottled water occurrence of emerging contaminants: A review of the recent scientific literature. Journal of Hazardous Materials 2020;392:Article No. 122271.

Ammala A, Bateman S, Dean K, Petinakis E, Sangwan P, Wong S, et al. An overview of degradable and biodegradable polyolefins. Progress in Polymer Science 2011;36:1015-49.

Awasthi S, Srivastava N, Singh T, Tiwary D, Mishra PK. Biodegradation of thermally treated low density polyethylene by fungus *Rhizopusoryzae*. 3 Biotech 2017;7:Article No. 73.

Bonanni PS, Schrott GD, Robuschi L, Busalmen JP. Charge accumulation and electron transfer kinetics in *Geobacter sulfurreducens* biofilms. Energy and Environnemental Science 2012;5(3):6188-95.

Botton B, Bretton A, Fevre M, Gautier S, Guy S, Guy PH, et al. Moisissures Utiles Et Nuisibles: Importance Industrielle. Paris, France: Masson; 1990. (in French).

Boughachiche F, Reguioua S, Zerizer H, Oulmi L, Boudemagh A, Kitouni M, et al. Production and preliminary characterization of antibiotics produced by an Actinomycetale Strain isolated from Ain Mlila Saltpans (Algerie). Antibiotiques 2005; 7(4):234-8.

Bourzama G, Iratni N, Ennaghra N, Ouled-Haddar H, Soumati B, Amour K, et al. *In vitro* removal of electronic and electrical wastes by fungi isolated from soil at Annaba Area in Northeast of Algeria. Environment and Natural Resources Journal 2021;19(4):302-9.

Davet P, Rouxel F. Détection Et Isolement Des Champignons Du Sol. Paris, France: INRA; 1997. (in French)

DSouza GC, Sheriff RS, Ullanat V, Shrikrishna A, Joshi AV, Hiremath L, et al. Fungal biodegradation of low-density polyethylene using consortium of *Aspergillus* species under controlled conditions. Heliyon 2021;7(5):e07008.

El Sayed MT, Rabie GH, Hamed EA. Biodegradation of low-density polyethylene (LDPE) using the mixed culture of *Aspergillus carbonarius* and *A. fumigates*. Environment, Development and Sustainability 2021;23:14556-84.

Gajendiran A, Krishnamoorthy S, Abraham J. Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. 3 Biotech 2016;6(52):1-6.

Khruengsai S, Sripahco T, Pripdeevech P. Low-density polyethylene film biodegradation potential by fungal species from Thailand. Journal of Fungi 2021;7(594):1-18.

Koutny M, Lemaire J, Delort AM. Biodegradation of polyethylene films with prooxidant additives. Chemosphere 2006;64(8): 1243-52.

Lefaux S. Biodégradation de films polymères à usage agricole: Caractérisation Physico-Chimique Des Résidus Et Identification Biomoléculaire Des Bactéries Actives [dissertation]. Le Mans, Université Du Maine; 2005. (in French)

Lewis H. Complying with Regulations. In: Verghese K, Lewis H, Fitzpatrick L, editors. Packaging for Sustainability. London: Springer; 2012. p. 155-70.

Mathur G, Mathur A, Prasad R. Colonization and degradation of thermally oxidized high-density polyethylene by *Aspergillus niger* (ITCC No. 6052) isolated from plastic waste dumpsite. Bioremediation Journal 2011;15(2):69-76.

Nakajima-Kambe T, Shigeno-Akutsu Y, Nomura N, Onuma F, Nakahara T. Microbial degradation of polyurethane, polyester polyurthanes and polyether polyurthanes. Applied Microbiology Biotechnology 1999;51:134-40.

Ojha N, Pradhan N, Singh S, Barla A, Shrivastava A, Khatua P, et al. Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. Scientific Reports 2017;7:Article No. 39515.

Pardo-Rodríguez ML, Zorro-Mateus PJP. Biodegradation of polyvinyl chloride by *Mucor* sp. and *Penicillium* sp. isolated from soil. Revista de Investigación Desarrollo e Innovación 2021;11(2):387-400. (in Spanish).

Pramila R, Ramesh VK. Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water: A SEM analysis. African Journal of Microbiology Research 2011;5(28):5013-18.

Ritchie H, Roser M. Plastic pollution [Internet]. 2018 [cited 2018 Sep]. Available from: https://ourworldindata.org/plastic-pollution.

Sen SK, Raut S. Microbial degradation of low density polyethylene (LDPE): A review. Journal of Environmental Chemical Engineering 2015;3:462-73.

Shah AA. Role of Microorganisms in Biodegradation of Plastics [dissertation]. Islamabad, Quaid-i-Azam University; 2007.

Shah AA, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: A comprehensive review. Biotechnology Advances 2008;26(3):246-65.

Sheik S, Chandrashekar KR, Swaroop K, Somashekarappa HM. Biodegradation of gamma irradiated low density polyethylene and polypropylene by endophytic fungi. International Biodeterioration and Biodegradation 2015;105:21-9.

Shimao M. Biodegradation of plastics. Current Opinion in Biotechnology 2001;12:242-7.

Siqueira VM, Lima N. Biofilm formation by filamentous fungi recovered from a water system. Journal of Mycology 2013; 2013:1-9.

Sowmya HV, Ramalingappa, Krishnappa M, Thippeswam B. Degradation of polyethylene by *Penicillium simplicissimum* isolated from local dumpsite of Shivamogga District. Environment, Development and Sustainability 2014;17:731-45.

Sudhakar M, Doble M, Murthy PS, Venkatesan R. Marine microbe-mediated biodegradation of low- and high-density polyethylenes. International Biodeterioration and Biodegradation 2008;61(3):203-13.

Thiounn T, Smith RC. Advances and approaches for chemical recycling of plastic waste. Journal of Polymer Science 2020;58(10):1347-64.

Torres A, Li SM, Roussos S, Vert M. Poly (lactic acid) degradation in soil or under controlled conditions. Journal of Applied Polymer Science 1996;62(13):2295-302.

Wei R, Zimmermann W. Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: How far are we? Microbial Biotechnology 2017;10(6):1308-22.

Williams DF. Enzymic hydrolysis of polylactic acid. Engineering in Medicine 1981;10(1):5-7.

Wyart D. Les polymères biodégradables [Internet]. 2007 [cited 2007 Oct 10]. Available from: https://www.techniques-ingenieur.fr/base-documentaire/archives-th12/archives-plastiques-et-composites-tiaam/archive-1/les-polymeres-biodegradables-am3579/?msclkid=3493e2cfb8d211ecb2bba1d72c6cb9e4. (in French).

Yuan J, Ma J, Sun Y, Zhou T, Zhao Y, Yu F. Microbial degradation and other environmental aspects of microplastics/plastics. Science of the Total Environment 2020;715:Article No. 136968.

Toxicological Effects of Tributyltin in Zebrafish (*Danio rerio*) Embryos

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ABSTRACT

Tributyltin (TBT) is known as an endocrine-disrupting chemical abundant in the aquatic environment. In the present study, zebrafish fish embryos were used to observe the chronic toxicity of TBT. Fish embryo toxicity analysis was carried out for different TBT concentrations (100, 50, 25, 12.5, 6.2, and 3.1 ng/L) and fertilized eggs were used to test each concentration effect. Fertilized eggs in 24-well plates (20 eggs in each well) were incubated at 26°C for four days and embryo coagulation, heartbeat of the embryo and mortality lethal endpoints (LC_{50} values) were recorded after 8, 24, 48, and 96 h. The results revealed that 100% coagulations of the embryos occurred at TBT doses of 50 and 100 ng/L. The coagulation significantly increased in a dose-dependent manner and TBT might induce coagulation of zebrafish embryos. Heartbeat changes were significantly decreased ($p<0.05$) in a dose-dependent manner at different TBT doses. LC_{50} values of TBT for zebrafish embryos were 19.9, 11.7, 7.3, and 5.2 ng/L at 8, 24, 48, and 96 h, respectively. The percentage of mortality was higher in embryos for the trace level of TBT, indicating that embryos are more sensitive to TBT toxicity. Hence, TBT is highly toxic and leads to a lethal effect on the zebrafish embryo, resulting in species extinction and declining biodiversity in the aquatic environment.

1. INTRODUCTION

Organotin compounds (OTs) are organometallic compounds that have been classified as Persistent Organic Pollutants (POPs) in the environment (Guruge et al., 2008; Ohura et al., 2015). Most OTs come from anthropogenic activities, with industrial production beginning in the 1960s (Sousa et al., 2014). Organotin chemicals are used as effective biocides, polyvinyl chloride stabilizers and industrial catalysts to manufacture silicone and polyurethane foams (Ain et al., 2021; Sousa et al., 2014). Organotins do not decompose thermally and have a low water solubility, allowing them to stay in the aquatic environment for a long time (Cui et al., 2011). Trisubstituted forms of organotin have the highest toxicological activity, and the triorganotins are the most toxic OTs; tributyltin (TBT) is the most toxic form and, considering their reproductive toxicity, OTs are also known as environmental hormones (Guruge et al., 2008; Sekizawa et al., 2003), which disrupt invertebrate and vertebrate reproductive cycles (Langston, 2020). A global ban on a number of OT substances and compounds was imposed in 2008 due to the harmful effects of OTs on living beings and aquatic habitats (IMO, 2001). Tributyltin is still utilized in agriculture and other industrial activities in developing countries with poor environmental monitoring due to its high effectiveness and low cost as antifouling biocides. With the busy maritime and heavy boating and shipping activities in coastal areas all around Sri Lanka and other industrial activities, high TBT usage and contamination in Sri Lanka have been recorded (Bandara et al., 2021). However, there are no records of the effect of TBT contamination to date.

Tributyltin has a high affinity for organic matter (Xiao et al., 2020) and has a half-life of 10-40 years in water and sediments (Guruge et al., 2008; Ohura et al., 2015). This could lead to an increase in their persistence in the environment, which is dependent on a large number of complex chemical structures and physicochemical characteristics (Corsi et al., 2020). As a result, TBT has hydrophobic, lipophilic, and ionic

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properties, which contribute to their toxicity to living organisms. These chemicals rapidly traverse cell membranes and accumulate in the adipose tissues of organisms due to their lipophilicity (Griffin et al., 2020). Their ionic characteristics improve their binding capacity to proteins like α-keratins and glutathione (Lv et al., 2021). Thus, TBT accumulates in living organisms following biomagnification along food chains (Bandara et al., 2021; Fent, 2004; Strand et al., 2003). Many harmful organic compounds accumulate in biota at different concentrations of magnitude and higher than in the ambient environment. These toxicants adversely affect the embryonic and larval stages of biota. This could result in the extinction and decline of affected species in the environment, reducing the diversity of the marine aquatic environment (He et al., 2020; Kim et al., 2018).

Recently, many toxicological studies have used fish and their different life stages as toxicological models due to many endocrine-disrupting chemicals (EDCs) being abundant in the aquatic environment. The results with early life stages of fish predict that about 70% of the long-term toxicity cases are from toxicants such as OTs (Lu et al., 2021). Therefore, the Fish Embryo Toxicity (FET) test would be an auspicious and less time-consuming tool for chemical testing (Santos et al., 2018). Since 2005, the fish embryo toxicity test has been required as part of routine whole effluent testing in Germany, and it has already been standardized at the international level (Lammer et al., 2009). With the majority of research relating to FET results focusing on Lethal Concentration (LC) values, coagulation and lack of heartbeat are the more commonly mentioned endpoints.

Most embryo toxicity tests have been conducted with zebrafish in the laboratories. There are numerous advantages of zebrafish embryo tests such as (1) a mature female zebrafish lays a large number of eggs (50-500) per time (Braunbeck et al., 2005). In the laboratory conditions, different life stages of embryos can easily be produced daily and parallel experimental treatments could be obtained (Scholz et al., 2008). Therefore, large numbers of analysis could be tested at the same time. (2) Due to the small size of embryos, the tests are conducted in 24-well plates. Hence, the tests require a small amount of substances which is particularly important when only limited test solutions are available. (3) The results of the embryo tests can be obtained rapidly (within two or three days) and less time-consuming (4) Sub-lethal endpoints are easy to identify. (5) Fish embryo toxicity tests are more sensitive than the fish cytotoxicity tests and the correlation between the acute fish embryo test and the fish test is perfect (Lungu-Mitea et al., 2020). In this study, zebrafish fish embryos were used to observe the chronic toxicity of TBT by covering the environmentally relevant concentrations of TBT (1, 10, and 100 ng/L) because no previous research in this area had been performed in Sri Lanka.

2. METHODOLOGY

2.1 Fish and rearing conditions

Wild-type zebrafish; *Danio rerio* were purchased from Angel aquarium, Sri Lanka. Fish were acclimatized for 14 days in glass tanks and the fish tanks were maintained in the laboratory at the Department of Zoology, University of Sri Jayewardenepura. A fourth-generation fish strain was derived from its own stock facilities and used to obtain the embryos.

2.2 Egg production of Zebrafish

The day before a test, male and female fish were placed in breeding chambers in 1:1 ratio immediately before the onset of darkness. Artificial plants were used as breeding stimulants and substrates. Mating, spawning and fertilization were observed after 30 min of the onset light in the morning. About 30-60 min after spawning, the spawning dishes were removed. Fertilized and non-fertilized eggs were separated and only fertilized eggs were transferred to a new tank with water. After determining of the overall egg number, viable (fertilized) eggs were kept for hatching. Fertilized eggs were distinguished by their transparency from non-fertilized eggs.

2.3 Fish embryo toxicity test (FET) with TBT

Standard TBT chloride and other chemicals (HPLC grade) were purchased from Sigma-Aldrich in Germany.

Fish embryo toxicity analysis was carried out for six different TBT concentrations (half diluted concentrations) of 100, 50, 25, 12.5, 6.2, and 3.1 ng/L (Log concentration 2, 1.7, 1.4, 1.1, 0.8, and 0.5 respectively). According to the method described by Lammer et al. (2009) fish embryo toxicity test was carried out with some modifications in the present study. In brief, embryo tests were initiated after 3 h. Twenty (20 eggs) fertilized eggs were selected for each test concentration and transferred to 24-well plates filled with 2 mL freshly prepared TBT concentrations (test solutions) and negative control (water). Fertilized eggs were placed in the 24-well-plates by using a sterilized pipette under the dissecting microscope. The 24-well plates were then covered with self-adhesive foil and incubated at 26±1°C for three days. Coagulation, the heartbeat of the embryo and mortality lethal endpoints were recorded using a dissecting microscope after 8, 24, 48, and 96 h. LC_{50} values were determined by graphing mortality percentage versus Log TBT concentrations. Triplicate experiments were performed for the FET test.

2.4 Statistical analysis

All assays were carried out in triplicates in three different experiments. All data are represented as the mean±standard deviation. Statistical comparison of the data was carried out using Pearson correlation and regression correlation of Minitab 17 for windows. The correlation value of p less than 0.05 was considered to be a statistically significant linear relationship.

The LC_{50} values were calculated using probit analysis. Statistical analyses were performed using SAS software and linear regression, version 2.17. A significant difference was considered as $p<0.001$ - highly significant.

3. RESULTS AND DISCUSSION

In the south Asian region, the lack of advanced detection methods for xenobiotics and their adverse effects are the major challenges for minimizing the trace level of pollutants and their massive impacts on the environment of Sri Lanka (Bandara et al., 2021). Therefore, in the present study, the embryonic effect of TBT was assessed for the first time in Sri Lanka.

3.1 Coagulation effect of zebrafish embryo

TBT exposure might cause the coagulation of the embryos. After 48 h, most of the embryos were coagulated and the embryos that did not coagulate showed normal development. A statistically significant increase in embryonic coagulation was observed at different TBT doses of 3.1, 6.2, 12.5, 25, 50, and 100 ng/L compared with the control group ($p<0.05$). The results revealed 100% coagulations of the embryos at TBT doses of 50 and 100 ng/L. Therefore, the coagulation is significantly increasing in a dose-dependent manner (Figure 1) and TBT might induce coagulation and infertility of the zebrafish embryos.

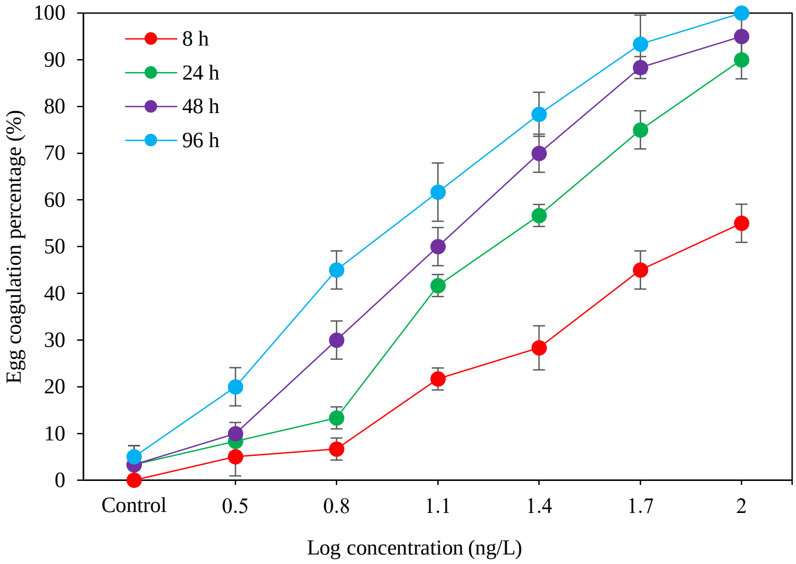

Figure 1. Effect of TBT exposure to the embryo coagulation of zebrafish; bpm; beats per minute

Mendis et al. (2018) revealed that coagulation of embryos is a common lethal effect with malathion compounds and it is consistent with the results of the present study. In the current test, the individual eggs were obtained from the same broodstocks and replicated to increase the reliability of the results. Low mortality rates observed in control indicate the experiment's reliability and it was essential to assume there was no inert material effect in the 24 well plates.

3.2 Heart toxicity of zebrafish embryo

As shown in Figure 2, heartbeat changes were found in a dose-dependent manner with, according to the results, a statistically significant decrease occurring at different TBT doses of 3.1, 6.2, 12.5, 25, 50, and 100 ng/L compared with the control group ($p<0.05$). This study demonstrated that TBT exposure caused a significant decrease in heart rate in a dose-dependent manner (Figure 2), and TBT might induce heart toxicity in the zebrafish embryos.

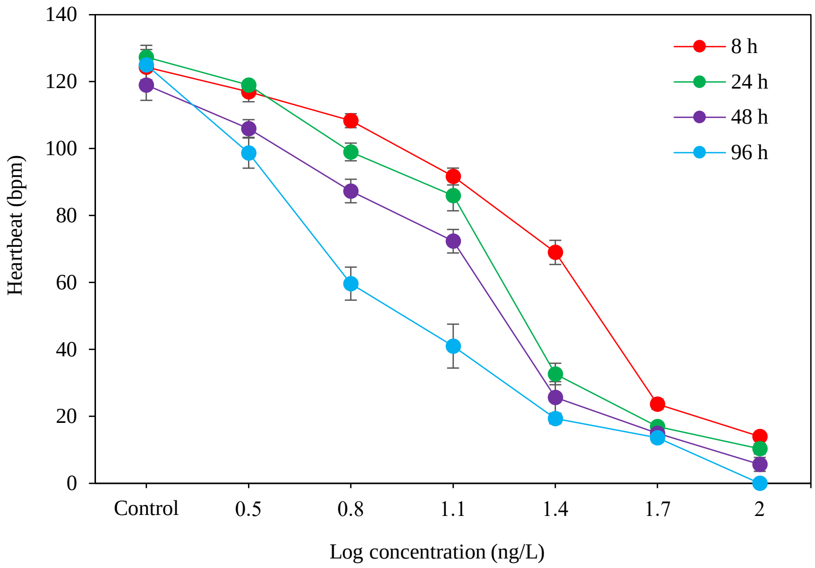

Figure 2. Effect of TBT exposure to the embryo heartbeat of Zebrafish; (n=20) bpm; beats per minute

The results revealed that tributyltin is highly toxic and lethal for the zebrafish embryo. After 48 h, most of the embryos were coagulated, and the heartbeat was drastically decreased. Both of these effects were statistically significant and in a dose-dependent manner. Treated embryos exposed to 100 ng/L and 50 ng/L showed total lethality while the control group had no effect. Similarly, Thitinarongwate et al. (2021) documented that the toxic effect of Zingiber essential oil appeared to occur in a concentration-dependent manner. The lowest dose of oil and control showed no mortality in the zebrafish embryos and a significantly increased mortality rate was in zebrafish embryos exposed to a high dose of oil. There was no viable zebrafish embryos in the groups treated with higher concentrations. The present study was aimed to observe the heart toxicity of zebrafish embryos against different TBT concentrations. The regular heartbeat rate of zebrafish embryos ranges from 120 to 180 per minute (Thitinarongwate et al., 2021). Heartbeat rate was not detected in the embryos at high TBT concentrations due to their early mortality.

3.3 Mortality rate and LC_{50} determination

The cumulative mortality of the embryos after TBT exposure is shown in Figure 3. Mortality was recorded at 8, 24, 48, and 96 h in order of increasing concentrations (Log concentration 0.5, 0.8, 1.1, 1.4, 1.7, and 2.0 ng/L). The median lethal concentration (LC_{50}) of TBT was calculated by sigmoidal regression using SAS software based on the zebrafish lethality curves (Figure 3). There was a significant ($p<0.0001$) increase in mortality of embryos in response to increasing TBT concentrations (Table 1).

Table 1. LC_{50} values of TBT at different time intervals

Exposure time (h)	LC_{50} (ng/L)	r^2 value	p value
8	19.9	0.9777	<0.0001
24	11.7	0.9343	<0.0001
48	7.3	0.9731	<0.0001
96	5.2	0.9816	<0.0001

The median lethal concentration (LC_{50}) values of TBT for zebrafish embryos were 19.9, 11.7, 7.3, and 5.2 ng/L at 8, 24, 48, and 96 h, respectively. The linear transformation of percentage mortality of embryo and TBT concentration are shown in Figures 3(a), 3(b), 3(c), and 3(d) for different exposure time. Remarkably, the percentage of mortality was higher in embryos for the trace level of TBT, indicating that embryos are more sensitive to TBT toxicity. Exposure to TBT for 8 h or more always caused mortality, increasing with longer exposure times. A continuous exposure until 96 h showed the highest toxicity (LC_{50}=5.2 ng/L). During the first 8 h of exposure, the compound showed less toxicity (LC_{50}=19.9 ng/L) compared to those exposed for 96 h post-fertilization ($p<0.0001$) when the embryos were already hatched. This result suggests that the continuous exposure of TBT causes the most toxicity with the lethal endpoint.

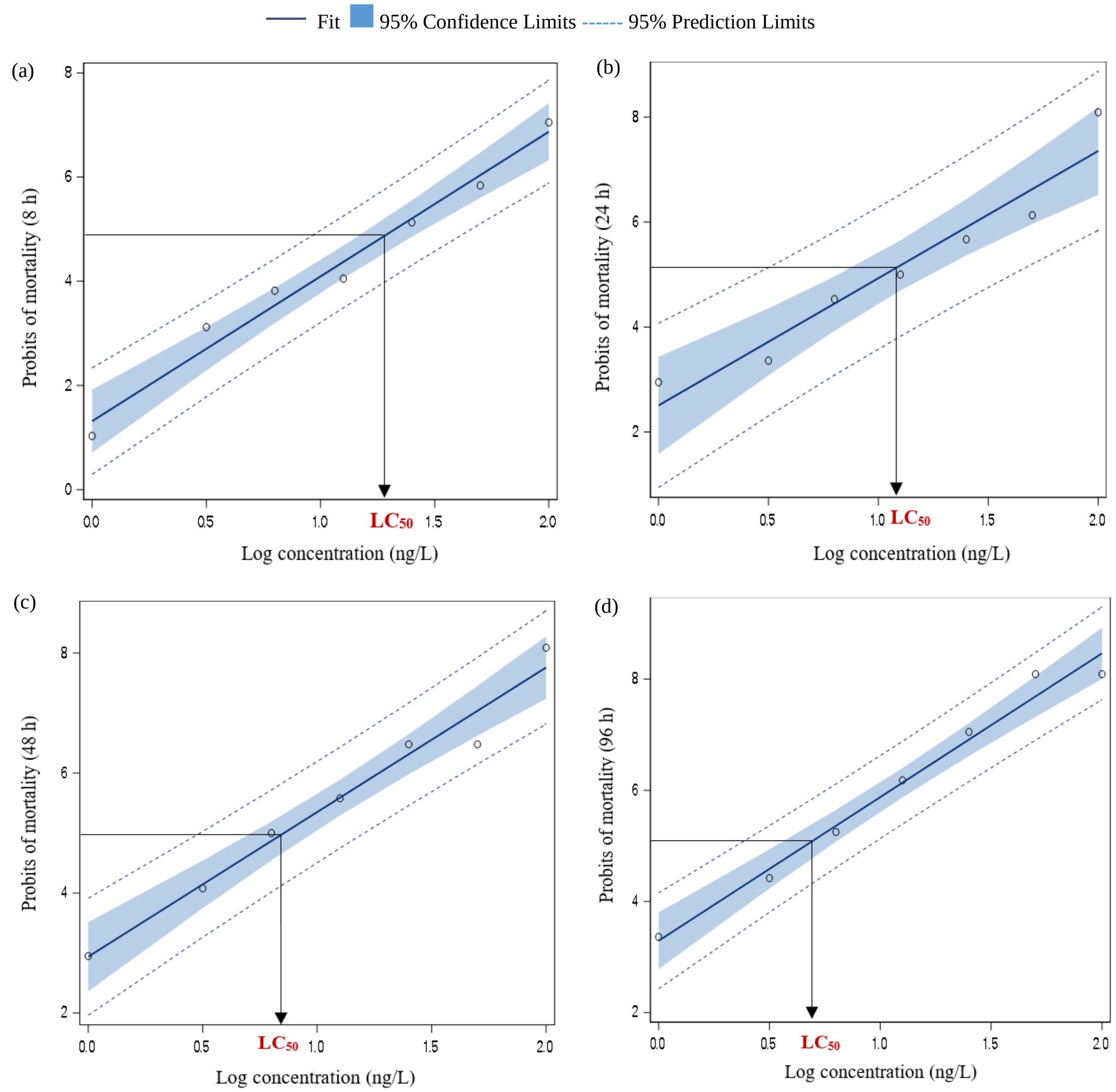

Figure 3. The linear transformation and relationship of probit at different concentrations of TBT were used to determine LC_{50} values for zebrafish embryo after (a) 8 h, (b) 24 h, (c) 48 h, and (d) 96 h of exposure to TBT.

The 50% lethal concentrations for each TBT concentration were calculated based on the egg embryos' mortality rate (0-100%). The concentrations were obtained in a geometric series in which each was 50% greater than the next lowest value as recommended by De Oliveira (2018). LC_{50} value was obtained based on cumulative mortality according to the three independent experiments using Regression Probit analysis. The results showed the most of the mortality in 100 and 50 ng/L after 8 h. LC_{50} values were high during the short exposure period and regularly reduced with increased exposure time. The LC_{50} values at 8, 24, 48, and 96 h were obtained as 19.9, 11.7, 7.3, and 5.2 ng/L, respectively. This significant reduction indicates the adverse chronic toxicity of TBT. These results are comparable to the findings of Dimitriou et al. (2003), which revealed that fish (Seabream fish) fertilized eggs were quite sensitive to both TBT and triphenyltin while LC_{50} for TBTCl and triphenyltin were 28.3 and 34.2 ng/L, respectively at 24 h. Moreover, much lower LC_{50} values at 96 h (<5 ng/L) have been reported for the embryo of two freshwater fish species, *Brevoortia tyrannus* and *Menidia beryllina* (Bushong et al., 1988). Therefore, TBT is responsible for the time-related toxicity of the embryos (Dimitriou et al., 2003). The concentrations of TBT compounds in studied water and sediments often exceed these tested levels. Consequently, fish embryos at the contaminated water bodies are at high risk since they float on the water surface or adhere to the benthic sediments. Even at low TBT concentrations, extremely low LC_{50} values could be estimated in these extreme cases of TBT pollution. It may cause direct mortality in aquatic animals during their early developmental stages. Bushong et al. (1988) observed that survival of euryhaline sheepshead minnow (*Cyprinodon variegatus*) was reduced to 59% compared to control when exposed to TBT from hatching to maturation and reproduction at 0.66 mg/L by day 145 and 100% at 5.4 mg/L by day 7, while survival and growth of the following generation were only unaffected at concentrations lower or equal to 1.3 mg/L. Furthermore, Pinkney et al. (1990) found significantly reduced larval survival in the first generation of euryhaline striped bass (*Morone saxatilis*) at TBT concentrations above 0.77 mg/L.

The present study identified that TBT might be a reason for reproductive impairments in zebrafish embryo. The findings may reveal that TBT pollution in water bodies in Sri Lanka might be an additional factor responsible for reducing natural populations in the environment by affecting the embryos and the larvae of animals. Human health implications from contaminated fish consumption should also be considered. Based on all this evidence, there is a need to develop safety and precautions regarding TBT usage. There is a necessity to be concerned that TBT might be entering into the human food chain through marine aquaculture of mollusks and fish despite the controls implemented in 1992. This study suggests that an extended monitoring program is required to check whether the 1992 legislation controlling the use of TBT is being enforced and whether it will be effective in the long term.

4. CONCLUSION

In this study, the chronic toxicity of TBT was assessed by using zebrafish embryos as a promising model to investigate the toxicity of hazardous chemicals. Coagulation, the heartbeat of the embryo and mortality lethal endpoints were recorded as the primary toxicological assays in the world. The results show that the coagulation and lack of heartbeat significantly increased with increasing TBT concentrations and the exposure time in a dose-dependent manner. LC_{50} values of TBT for zebrafish embryos were drastically decreased with increasing exposure time. The lethality was higher in embryos for the trace level of TBT, indicating the adverse chronic toxicity of TBT for the fish embryos. These adverse effects on fish embryos could easily threaten the ecosystem and biodiversity in Sri Lanka if tributyltin use is not strictly regulated.

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CONFLICTS OF INTEREST

The authors declare that they have no competing of interest as all were worked together and the manuscript has not been submitted simultaneously for publication elsewhere.

REFERENCES

Ain QU, Roy D, Ahsan A, Farooq MA, Aquib M, Hussain Z, et al. Endocrine-disrupting chemicals: Occurrence and exposure to the human being. In: Akash MSH, Rehman K, Hashmi MZ, editors. Endocrine Disrupting Chemicals-induced Metabolic Disorders and Treatment Strategies. Emerging Contaminants and Associated Treatment Technologies. Switzerland: Springe Cham; 2021. p. 113-23.

Bandara KRV, Chinthaka SDM, Yasawardene SG, Manage PM. Modified, optimized method of determination of Tributyltin (TBT) contamination in coastal water, sediment and biota in Sri Lanka. Marine Pollution Bulletin 2021;166:Article No. 112202.

Braunbeck T, Bottcher M, Hollert H, Kosmehl T, Lammer E, Leist E, et al. Towards an alternative for the acute fish LC_{50} test in chemical assessment: The fish embryo toxicity test goes multi-species-an update. ALTEX-Alternatives to Animal Experimentation 2005;22:87-102.

Bushong SJ, Hall JLW, Hall WS, Johnson WE, Herman RL. Acute toxicity of tributyltin to selected Chesapeake Bay fish and invertebrates. Water Research 1988;22:1027-32.

Corsi I, Bergami E, Grassi G. Behavior and bio-interactions of anthropogenic particles in marine environment for a more realistic ecological risk assessment. Frontiers in Environmental Science 2020;8:1-21.

Cui Z, Zhang K, Zhou Q, Liu J, Jiang G. Determination of methyltin compounds in urine of occupationally exposed and general population by in situ ethylation and headspace SPME coupled with GC-FPD. Talanta 2011;85:1028-33.

De Oliveira GAR, Leme DM, de Lapuente J, Brito LB, Porredon C, de Brito Rodrigues L, et al. A test battery for assessing the ecotoxic effects of textile dyes. Chemico-Biological Interactions 2018;291:171-9.

Dimitriou P, Castritsi-Catharios J, Miliou H. Acute toxicity effects of tributyltin chloride and triphenyltin chloride on gilthead seabream, *Sparus aurata* L., embryos. Ecotoxicology and Environmental Safety 2003;54:30-5.

Fent K. Ecotoxicological effects at contaminated sites. Toxicology 2004;205:223-40.

Griffin MD, Pereira SR, DeBari MK, Abbott RD. Mechanisms of action, chemical characteristics, and model systems of obesogens. BMC Biomedical Engineering 2020;2:1-13.

Guruge KS, Manage PM, Yamanaka N, Miyazaki S, Taniyasu S, Yamashita N. Species-specific concentrations of perfluoroalkyl contaminants in farm and pet animals in Japan. Chemosphere 2008;73:210-5.

He S, Li P, Li ZH. Review on endocrine disrupting toxicity of triphenyltin from the perspective of species evolution: Aquatic, amphibious and mammalian. Chemosphere 2020; 269:Article No. 128711.

International Marine Organisation (IMO). International convention on the control of harmful antifouling systems on ships [Internet]. 2001 [cited 2021 Mar 21]. Available from: https://www.imo.org/en/About/Conventions/Pages/International-Convention-on-the-Control-of-Harmful-Anti-fouling-Systems-on-Ships-(AFS).aspx.

Kim BM, Saravanan M, Lee DH, Kang JH, Kim M, Jung JH, et al. Exposure to sublethal concentrations of tributyltin reduced survival, growth, and 20-hydroxyecdysone levels in a marine mysid. Marine Environmental Research 2018;140:96-103.

Lammer E, Carr GJ, Wendler K, Rawlings JM, Belanger SE, Braunbeck T. Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 2009;149:196-209.

Langston WJ. Endocrine disruption and altered sexual development in aquatic organisms: An invertebrate perspective. Journal of the Marine Biological Association of the United Kingdom 2020;100:495-515.

Lu Y, Zhang Y, Zhong C, Martin JW, Alessi DS, Goss GG, et al. Suspended solids-associated toxicity of hydraulic fracturing flowback and produced water on early life stages of zebrafish (*Danio rerio*). Environmental Pollution 2021;287:Article No. 117614.

Lungu-Mitea S, Vogs C, Carlsson G, Montag M, Frieberg, K, Oskarsson A, et al. Modeling bioavailable concentrations in zebrafish cell lines and embryos increases the correlation of toxicity potencies across test systems. Environmental Science and Technology 2020;55:447-57.

Lv H, Wang J, Wang M, Shen L, Xiao L, Chen T, et al. Potent inhibition of tributyltin (TBT) and triphenyltin (TPT) against multiple UDP-glucuronosyltransferases (UGT): A new potential mechanism underlying endocrine disrupting actions. Food and Chemical Toxicology 2021;149:Article No. 112039.

Mendis JC, Tennakoon TK, Jayasinghe CD. Zebrafish embryo toxicity of a binary mixture of pyrethroid insecticides: d-tetramethrin and cyphenothrin. Journal of Toxicology 2018; 2018:1-8.

Ohura T, Sakakibara H, Watanabe I, Shim WJ, Manage PM, Guruge KS. Spatial and vertical distributions of sedimentary halogenated polycyclic aromatic hydrocarbons in moderately polluted areas of Asia. Environmental Pollution 2015; 196:331-40.

Pinkney AE, Matteson LL, Wright DA. Effects of tributyltin on survival, growth, morphometry, and RNA-DNA ratio of larval striped bass, *Morone saxatilis*. Archives of Environmental Contamination and Toxicology 1990;19:235-40.

Santos D, Vieira R, Luzio A, Felix L. Zebrafish early life stages for toxicological screening: Insights from molecular and biochemical markers. Advances in Molecular Toxicology 2018;12:151-79.

Scholz S, Fischer S, Gundel U, Küster E, Luckenbach T, Voelker D. The zebrafish embryo model in environmental risk assessment: Applications beyond acute toxicity testing. Environmental Science and Pollution Research 2008;15: 394-404.

Sekizawa J, Sutur G, Birnbaum L. Integrated human and ecological risk assessment: A case study of tributyltin and triphenyltin compounds. Human and Ecological Risk Assessment 2003;9:325-42.

Sousa AC, Pastorinho MR, Takahashi S, Tanabe S. History on organotin compounds, from snails to humans. Environmental Chemistry Letters 2014;12(1):117-37.

Strand J, Jacobsen JA, Pedersen B, Granmo A. Butyltin compounds in sediment and molluscs from the shipping strait between Denmark and Sweden. Environmental Pollution 2003;124:7-15.

Thitinarongwate W, Mektrirat R, Nimlamool W, Khonsung P, Pikulkaew S, Okonogi S, et al. Phytochemical and safety evaluations of *Zingiber ottensii* valeton essential oil in zebrafish embryos and rats. Toxics 2021;9:102-12.

Xiao X, Zhu S, Zou X, He G, Jiang J, Sheng GD. Relation of tributyltin and triphenyltin equilibrium sorption and kinetic accumulation in carp and *Ceratophyllum demersum*. Ecotoxicology and Environmental Safety 2020;192:Article No. 110289.

Climate Change Vulnerability Assessment for the Major Habitats and Species in Lung Ngoc Hoang Nature Reserve, Vietnamese Mekong Delta

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ABSTRACT

The study assessed the vulnerability of habitats and species to climate change in Lung Ngoc Hoang Nature Reserve (NR), Vietnam. The vulnerability assessment tools for habitat and species were developed by the International Union for Conservation of Nature (IUCN). Community members, NR managers and experts in the fields of environment, economic and rural development were involved in this study. The results showed that saltwater intrusion and inundation could cause serious threats to habitats (i.e., open water, Lung, agricultural and Melaleuca habitats) and freshwater species. The combined impacts of drought and high temperature potentially increase forest fires for the Melaleuca habitat and decrease the quantity and quality of open water habitats. The Melaleuca and Lung habitats have a high baseline conservation status, in which Melaleuca habitats are more vulnerable than Lung habitats. Conversely, open water and agricultural habitats are at low baseline conservation status, but open water habitats are more vulnerable. In addition, the proliferation of invasive alien species, encroachment on agricultural cultivation, and the degradation of water quality are also great threats to the NR. Key species, including *Melaleuca cajuputi*, *Elaeocarpus hygrophilus*, *Chitala ornate*, *Channa micropeltes*, were at low threat of climate change. However, *C. ornate* and *C. micropeltes* are seriously endangered by seawater intrusion, drought and poor water quality. The findings of this study can provide essential information for NR managers to formulate water management plans for the protection and management of the habitats and species in Lung Ngoc Hoang NR.

1. INTRODUCTION

The frequency of impacts caused by climate change has continuously increased from the late 19th century onwards (Khalid et al., 2021). It is even more serious since the distribution of these risks is uneven around the world, which depends on the characteristics of each region. For example, these can be geographic features, warming rates, development levels, vulnerability, adaptation, and mitigation strategies (Lee and Choi, 2018). Wetlands are internationally recognized for providing different services to humans and other living organisms (Tiner, 2014; Ricaurte et al., 2017). However, it is also deemed to be very vulnerable to climate change (Kelleway et al., 2017; Duncan et al., 2021). Lung Ngoc Hoang Nature Reserve (NR), also referred to as Lung Ngoc Hoang wetland, is located on low-lying land, with typical topography of the southern Hau River, Vietnam. Lung Ngoc Hoang NR, one of the green lungs in the Mekong Delta, has an important role in biodiversity conservation (Duong and Frederick, 2017). This wetland is well known for its untouched melaleuca forest ecosystem, which provides a favorable habitat for a wide range of indigenous fauna and flora. Consequently, this biodiversity also kindly supports local livelihoods.

However, the usage of canals to serve the fire prevention policy has directly affected the natural water circulation in the NR. Once the NR opens the sluice, the water level is low. At the end of the rainy season, the sluice will be closed to store water. This action has facilitated leaf decomposition in anaerobic conditions, which has caused black and polluted water canals. As a result, the deterioration of water quality

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will influence aquatic biodiversity. In addition, the NR is situated in the center of the Mekong Delta and the transition zone between the tidal currents of the East Sea and West Sea. Due to this special location and the effects of the Mekong River, the NR accumulates many pollutants and is also very sensitive to climate change (Hau Giang Department of Natural Resources and Environment) (Hau Giang DoNRE, 2021). Therefore, any development and plans of the province and neighboring provinces/cities (especially, Can Tho, Kien Giang and Soc Trang Provinces) can cause an array of consequences on the biodiversity and ecosystem of Lung Ngoc Hoang NR, which in turn affects local livelihoods. The sensitivity of the NR was even more enhanced by the establishment of large sluice gates on Cai Lon and Cai Be Rivers. The sluices were installed in 2017 to prevent increased salinity from the West Sea, which has changed the dynamic hydrological regime of Lung Ngoc Hoang NR to a static one. This change has caused water pollution due to an increase in agricultural and aquacultural runoff, industrial and urban waste, and an intensive invasion of alien species like water hyacinth.

Water is no longer circulated inside the NR in the end of the rainy season to dry season, leading to extremely low dissolved oxygen levels in the water bodies. It is necessary to develop a comprehensive management plan for Lung Ngoc Hoang NR that can alleviate the impacts of climate change and dam development on the environment and biodiversity of the area. The main objectives of this study were to assess the vulnerability of ecosystems, including major habitats and key species, to climate change in Lung Ngoc Hoang NR.

2. METHODOLOGY

2.1 Site description

Lung Ngoc Hoang NR, located in Phung Hiep District, Hau Giang Province, Vietnam, was established under the Prime Minister's Decision No. 13/2002/QD-TTg dated 14 January 2002 (The Prime Minister of Government, 2002). Lung Ngoc Hoang is situated in a network of national special-use forests. The NR's activities comply with the Government Decree No. 117/2010/ND-CP from 24 December 2010 on the organization and management of a system of national special-use forests (Vietnamese Government, 2010). The NR is located between latitudes 09°04′ to 09°45′ North and longitudes 105°39′ to 105°43′ East, with a total area of 2,799.97 ha (Forestry Research Institute, 2021), as presented in Figure 1. This area was formed during the sea recession and alluvial deposition. It mainly consists of coastal sediments and swamps, forming a low and fairly flat terrain, with an average elevation varying from 0.30 m to 1.50 m. The region has an open canal system through low-lying areas, such as Lai Hieu canal and Hau Giang 3 canal, connecting Lung Ngoc Hoang NR with adjacent areas. The system connects with Cai Lon and Cai Tau Rivers, which drain to the West Sea (Gulf of Thailand); and Sang Bo canal, Chu Ba and Xeo Xu canals emptying into Quan Lo-Phung Hiep Canal, connecting with the East Sea and the Ca Mau peninsula. Due to the interlaced canal system and geographic location, the hydrological regime is influenced by the intra-provincial rainfall regime, the flood zone of the Hau River, and the high tides of the East Sea and the West Sea. Sub-zones and plots in the NR are separated by a system of canals to facilitate commute and prevent forest fires. This area has freshwater all year round, although it has recently experienced slight saltwater intrusion. The average water level is 0.40 m, which increases to 1.1 m during the flood season and is less than 0.20 m during the dry season. The annual rainy season in Hau Giang begins in August and lasts for about five months, with the peak in October and November often coinciding with a period of locally heavy rain. Heavy rain and high tide occur simultaneously, increasing the water level and causing flooding in a large area over prolonged periods in the rainy season.

The reserve is located in the low-latitude belt of Vietnam, influenced by the tropical monsoon climate regime. The average annual temperature is 26.6°C, with an average relative humidity of 85%. There are two monsoon seasons in the year, including the northeast monsoon (from October to April) and the southwest monsoon (from May to November). The average rainfall in the NR is 1,700-1,800 mm/year, with the average number of rainy days per year varying from 120 to 135 days. These climate data were recorded at Vi Thanh hydro-meteorological station, Hau Giang Province, Vietnam (105°27′42″E, 9°49′04″E).

2.2 Vulnerability assessment

The Climate Change Vulnerability Assessment methodology employed in this study was developed by IUCN under the Mekong WET project (IUCN, 2017). The methodology focuses on participatory assessment and discussion with the community and local, regional and national authorities responsible for site management. The vulnerability assessment was carried out using two tools in an excel spreadsheet: a vulnerability assessment tool for habitat and a vulnerability assessment tool for species. To evaluate in the most general and objective way, a research team consisted of researchers with expertise in environment, economics and rural development, community members surrounding the NR (both male and female) and site managers. The study was conducted through field data collection and interviews in May 2021 in Lung Ngoc Hoang NR and the surrounding residential areas.

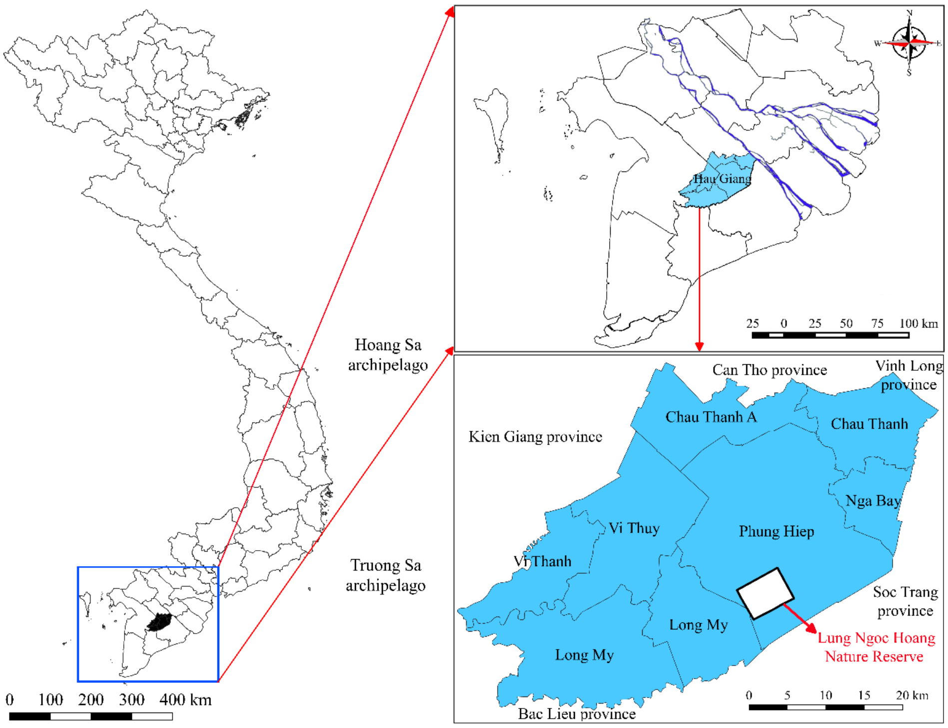

Figure 1. Location map of Lung Ngoc Hoang Nature Reserve, Hau Giang Province, Viet Nam

After consulting with managers at the NR and a group of experts (Table S1), the research team selected melaleuca, open water, agricultural land habitats (artificial habitat) and Lung habitats (natural habitats with the domination of grasslands). Besides that, four species were selected for climate vulnerability assessment, including two plant species (*Melaleuca cajuputi* and *Elaeocarpus hygrophilus*) and two fish species (bronze featherback (*Chitala ornate)* and the giant snakehead (*Channa micropeltes*). These are the main habitats and species representing a majority of the NR.

To evaluate the vulnerability of habitats and species, the research team and site managers assessed the baseline conservation status of the selected habitats and species. The next step was to project climate change impacts to 2050 based on NR or regional documents to address climate change threats to wetlands. These habitats and species were then appraised for their vulnerability to climate change (Table 1). Finally, potential threats to each habitat and species were identified by determining exposure, sensitivity, and adaptability to climate change. An average score of the factors (exposure, sensitivity and adaptability) represents the climate change vulnerability as a whole. In addition, the confidence score of the vulnerability assessment is also determined based on Table S2.

Table 1. Classification of climate change vulnerability

Climate change vulnerability	Category intervals
Very high	2.7-3.0
High	2.3-2.6
Moderate	1.9-2.2
Low	1.5-1.8
Very low	1.0-1.4

3. RESULTS AND DISCUSSION

3.1 Vulnerability of the habitats

The vulnerability assessment of each habitat is presented in Figure 2. In general, melaleuca habitats can be identified as the vulnerable habitats to climate change, followed by the open water habitats. However, the melaleuca habitats have a higher conservation status than the open water habitats. The vulnerability of Lung and agricultural habitats is observed to be lower. Lung habitats are given high conservation status because they host a number of important species like the giant snakehead.

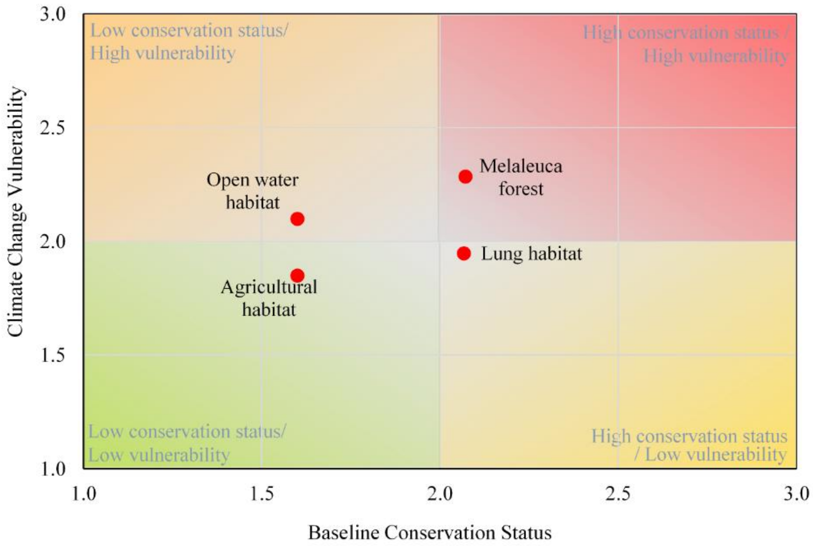

Figure 2. Current status of conservation and vulnerability to climate change in NR

3.1.1 Melaleuca forest habitat

In the NR, the melaleuca forest habitat is the most dominant (Table 2). It is protected and re-forested, covering more than 50% of the total wetland area. However, due to households encroaching on adjacent forest land for production, the forest area has decreased over the past 50 years. This is a threat in most protected areas in the Mekong Delta (Triet et al., 2019a).

The Melaleuca forest habitat does not host a diversity of plant species because of frequent flooding and high soil acidity (pH=4.53-5.65) (Hoa, 2015). Melaleuca trees (*Melaleuca cajuputi*) are the dominant species in the upper layer and other plant species (*Phragmites vallatoria* and *Stenochlaena palustris* with high densities of 4.56 and 2.11 tree/m^2, respectively) (Ni, 2018). According to Odum (2005), tropical plantations also have a low species composition. The melaleuca forest habitat reserve has a high economic value since locals can exploit wild forest honey. The melaleuca forest habitat can tolerate flooding to a moderate extent, but it is vulnerable to drought and fire. Therefore, the management board of Lung Ngoc Hoang NR always maintains a high water level to prevent fire. This control approach is likely to threaten other biodiversity and degrade water quality in the future due to the breakdown of organic matter in flooded environments and very little exchange of water with external bodies of water. Consequently, the Melaleuca forest habitat was determined to have a high conservation value and a baseline conversation rating of 2.1 (Figure 2). Besides the results of the baseline conservation status assessment, this was also reflected in the habitat's fire prevention plans in the dry season.

Table 2. The features of the habitats in Lung Ngoc Hoang Nature Reserve

Habitats	Area (ha)	Dominant species
Melaleuca forest	1482.70	*M. cajuputi*, *Phragmites vallatoria*, *Stenochlaena palustris*
Lung habitat	310.35	*Eupatorium odoratum*, *Cyclosorus parasiticus*, *Nypa fruticans*, and other vines.
Agricultural land	767.16	Rice, pineapple, lotus, fruit trees, and vegetables
Open water	182.44	*Eichhornia crassipes*, *Azolla pinnata*, *Pistia stratoides*, *Nymphaea rubra*, Lotus and several species of fish belong to families such as Channidae, Toxonidae, Loricariidae, Cobitidae, Hemiramphidae, Aplocheilidae
Others	57.32	The dominant species belong to families such as Asteraceae, Malvaceae, Fabaceae, Cactaceae commercially valuable species such as *Artocarpus heterophyllus*, *Durio zibethinus*, *Annona muricata*, *Musa paradisiaca*

Under climate change, survey results have shown that the Melaleuca forest habitat is most affected by drought and high temperatures (Table 3). In addition, prolonged flooding negatively impacts the existence and growth of species living in the habitat, which has been affected by rising water levels and heavy rainfall. The habitat is also vulnerable to extreme weather events, such as storms and high winds, which can topple and damage trees. Sea level rise and saltwater intrusion also threaten the Melaleuca forest habitats, with increased saltwater intrusion expected to impact the Mekong Delta, which the IPCC report predicts is one of the three most vulnerable coastal regions in the world (Nicholls et al., 2007). The Melaleuca habitat is moderately susceptible to drought, rising temperature and sea level. However, the ability of this habitat to adapt to new conditions in the context of climate change is assessed at a relatively high level (Table 2). The Melaleuca forest habitat was assessed to be highly vulnerable to the impacts of climate change, with a score of 2.3 (Figure 2). The results of vulnerability for Melaleuca forest habitat were similar to the other wetlands in the Mekong Delta area, including Phu My conservation area (score 2.4), U Minh Thuong National Park (score 2.0) (Triet et al., 2019a; Triet et al., 2019b).

3.1.2 Lung habitat

Lung habitat is the local term used to define areas of lowland topography where wetland grasslands are dominant all year round. This habitat accounted for approximately 11.08% of the total reserve area, as presented in (Table 2). The area has remained mostly unchanged thus far, except for the expanding grassland area. This expansion has led to dredging around the border edge to create a path for boats around the Lung habitat to support the tourism development. The Lung habitat plays an important role in maintaining fish stocks by providing habitat and spawning grounds for various fish and other species. The layers of thick vegetation make an ideal shelter and breeding ground for many reptiles, amphibians, and insects and provide habitats for birds. The area has highly diverse vegetation with supporting species (Table 2). The area is strictly managed and protected in the core zone of the NR where the anthropogenic impacts are limited. Nevertheless, this cannot prevent the invasion of many exotic species that can diminish habitat diversity and be a major threat to fish. As a freshwater wetland, the habitat is significantly affected by the saline intrusion. Lung habitat recovers relatively quickly from extreme weather events but requires frequent flooding to regenerate; therefore, this habitat cannot withstand drought. Overall, the initial conservation status is just above average (score 2.1) (Figure 2), reflecting both positive and negative impacts on habitat features.

Under climate change, the Lung habitat is vulnerable to drought, high temperatures, hydrological changes, saline intrusion, and high flooding (Table 3), which can lead to a loss of habitats for plants and animals and breeding grounds for fish species. Lung habitat areas are the most susceptible to drought and hydrological changes due to changes in flood regimes caused by anthropogenic factors combined with climate change. In addition, because this is a wetland area, floods or prolonged rain often lead to inundation and mass deaths of plants and animals. Lung habitats can adapt and recover from changes in rainfall and flooding. However, there is not ample area for the habitat to expand due to grey infrastructures such as dikes, canals, and embankments. Thus, extreme weather can cause temporary disturbances but is not expected to cause permanent damage to the habitat. The vulnerability of this habitat to the impacts of climate change (score 1.9) was lower than the Melaleuca habitat (score 2.3) (Figure 2).

3.1.3 Open water habitat

Open water habitat covers all water bodies and canals in Lung Ngoc Hoang NR. Its area is approximately 182.44 ha, accounting for about 6.42% of the total NR area. The area has recently increased because these canals have become wider and deeper by NR activities and natural landslides on the banks. In the baseline vulnerability analysis, the habitat is characterized by a high diversity of aquatic plants, especially a high proportion of exotic species. The habitat integrity is threatened by invasive alien plants, although the management manually removes these species each year. In addition, water pollution from the melaleuca habitat and agricultural production threatens open water habitats. The vulnerability analysis showed a low baseline conservation status of 1.6 (Figure 2). Although this habitat has a low conservation level and is not currently in danger, it is considered a critically important habitat in the NR because it is the main water source for local people in domestic and agricultural activities and other creatures in this study area.

An assessment of habitat exposure, sensitivity and adaptability revealed that the open water habitat is highly vulnerable to climate change (Table 3).

Table 3. Summary of key climate issues in the habitats of Lung Ngoc Hoang Nature Reserve

Habitats	Climate issues	Exposure level	Sensitivity level	Adaptivity
Lung	Drought, high temperature, saltwater intrusion, hydrological change, flood	Average	High	High
Melaleuca forest	Drought, high temperature, sea-level rise, flood, hydrological change, storms and high wind	Average	Average	High
Open water	Drought, high temperature, saltwater intrusion, hydrological change	High	High	Average
Agriculture	Floods, droughts, other extreme weather events (early rain, storms), saltwater intrusion	Low	Average	High

Hydrological changes and saltwater intrusion are the two most pressing threats. Moreover, the habitat is also at risk from heavy rainfall and high temperatures. The impacts of saline intrusion were visible in 2017-2018 when the salinity levels at the site reached 0.8‰, leading to some trees shriveling and losing their leaves. However, the system was otherwise able to withstand this level of salinity. The site also saw changes in hydrological conditions through extreme weather events at the beginning of the rainy season in 2021. Locals reported prolonged and heavy rainfall and a complete lack of a dry period have led to waterlogged conditions. Increased rainfall also led to impacts on water quality through increased erosion and sediment accumulation. Higher temperature is expected to reduce dissolved oxygen concentration, leading to decreased respiration capacity for freshwater species, especially white fish (*Chitala ornate*, *Barbonymus gonionotus*, *Parachela siamensis*, *Corica laciniata*, etc.). An increase in water temperature will also affect the survival of aquatic organisms if it exceeds their heat tolerance. The open water and Lung habitats mainly have aquatic plants and vines, which are able to regenerate after weather events much more quickly than Melaleuca forest habitats, dominated by thick trees that need a recovery period. Open water habitats are vulnerable to dry season impacts, with increased evaporation due to higher temperatures and severe droughts that can lower water levels and reduce habitat for aquatic animals. These consequences can lead to changes in the flora and fauna composition in the habitat. Overall, open water habitats were identified as having a high vulnerability (score of 2.1) (Figure 2) to the combined effects of climate change, especially in the dry season. The previous study by Scott et al. (2018), Triet et al. (2019a) and Ly et al. (2019) also evaluated high vulnerability for open water habitats, ranging from 2.1-2.4.

3.1.4 Agricultural habitat

Agricultural habitats, including the areas for agricultural production, such as rice, perennial crops, and other annual crops, are significantly impacted by human activities. These habitats are assessed as having a low baseline conservation level (score around 1.6) (Figure 2), which is lower than Boeung Prek Lapov protected landscape (score 1.7) (Ly et al., 2019). This disparity is explained by the degree of contribution to livelihoods and the influence of objective factors such as externalities. During the last five years, the agricultural habitat has increased, with an area of about 767.16 ha, accounting for 27.40% of the total area. Agricultural habitats are quite common in the Mekong Delta, and the agricultural land area tends to increase near protected areas (Triet et al., 2019a). This may partly explain the baseline conservation value of the habitat. The agricultural habitat contains the most economic species. According to a report by Ni (2018), there are about 156 species of higher plants in agricultural habitats at the site. The main exploited species are rice, pineapple, lotus, fruit trees, and vegetables. In addition, some households take advantage of the vacant space to raise livestock and poultry and use canals to raise fish and grow vegetables. This habitat is often disturbed, affecting plant species through chemical spraying and land reclamation activities. This can add nutrients to the soil and limit the development of pests and diseases to save costs and bring high efficiency in production. Agricultural habitats are also vulnerable to prolonged rains and floods. Alien species are also present in agricultural habitats, though occurring in low densities, and therefore presenting a relatively lower threat than in Lung and open water habitats.

The survey results indicated that the vulnerability of the agricultural habitat to the impacts of climate change is low, with major threats including floods, droughts, and other extreme weather events such as early rains and storms. Although flooding can cause damage to the crops, people can take advantage of the floods to diversify their livelihoods and increase their incomes by catching fish, farming fish and growing snails. Floods also help them improve the land quality by washing away pests and diseases from rice cultivation and providing nutrients to the soil. High temperature and heavy rain affect crop productivity and lead to economic losses in the area (Van et al., 2021). Agricultural habitats are most affected by climate change impacts from April to May due to the highest temperature of the year with saltwater intrusion (Hau Giang DoNRE, 2021). According to Trinh et al. (2021) and Van et al. (2021), climate change, such as the high temperature, earlier rainfall, and sea-level rise, is forecast to significantly impact agriculture in Vietnam (MoNRE, 2016). Overall, agricultural habitat is adaptable to the predicted extreme weather and climate conditions because it is expected that farmed plant and animal species can be adjusted to suit new climatic conditions. Therefore, the agricultural habitat is assessed as having a low vulnerability to climate change (score of about 1.6) (Figure 2). It is supported by Ly et al. (2019) that suggested that rice fields have low climate change vulnerability (score 1.9).

The main threats to these habitats arise from large-scale hydrological changes and pollution caused by the accumulation, extraction, and diversion of river flows for agriculture, industry and hydroelectricity. Furthermore, agricultural encroachment and invasive alien species considerably increase pressure on all four habitats. In the future, the habitats in Lung Ngoc Hoang NR will be susceptible to saltwater intrusion and flooding due to sea-level rise, as predicted by 2100. Since this is a seasonal freshwater wetland, saltwater and permanent flooding will far exceed the capacity of the habitats.

3.2. Vulnerability of species

The reasons for choosing these species are presented in Table 4. Lung Ngoc Hoang NR is known as the cradle of freshwater fishes where they gather to lay eggs during the breeding season in the west of Hau River. This area acts as a buffer zone that connects Long Xuyen Quadrangle with Ca Mau peninsula. According to previous studies conducted by the Management Board of the NR, the number of *C. ornate* and *C.micropeltes* have recently increased. *C. ornate* and *C. micropeltes* are also on the List of Rare and Precious Species in Vietnam that need to be protected and restored (MoARD, 2019).

Table 4. Rationale for choosing the evaluation species

Species	Reason for evaluation
Melaleuca *(M.cajuputi)*	Typical species, abundant in the reserve
Elaeocarpus (*E. hygrophilus*)	Investment in additional planting, large area, listed in the Red Book of Vietnam
Bronze featherback (*C. ornate*)	Large numbers, representative of fish populations in the area, on the List of Rare and Precious Species
Giant snakehead (*C. micropeltes*)	Large numbers but rare elsewhere in the Mekong Delta, representing fish populations in the region, on the List of Rare and Precious Species

3.2.1 Melaleuca cajuputi

M. cajuputi, the most dominant species of Melaleuca in the world, is also a typical and dominant forest tree species in the acid sulfate soil wetlands of the Mekong Delta. All *M. cajuputi* in the NR are planted without the existence of the natural one, which is a unique feature of this NR from other protected areas and national parks. The *M. cajuputi* in the study area are less than 10 years old and have a trunk height between 6.78-12.40 m and a diameter between 8.75-10.67 cm. Those over 10 years old have a height of 11.12-14.68 m and a diameter of 11.61-16.63 cm. The total wood reserve of Lung Ngoc Hoang NR is about 187,351.5 m^3.

M. cajuputi can reproduce quickly and have a large dispersal range because of their small seeds and large annual seed production. In terms of the baseline conservation status, *M. cajuputi* can tolerate prolonged waterlogging but still require drying time to ensure optimal growth. Permanent waterlogging leads to poor growth and inhibits natural regeneration. Although they are freshwater species, they can tolerate low salinity for short periods. While some trees were planted before the establishment of the NR, such as in 1984 and 1994-2002, others were planted later in 2006 and 2015. The trees have been grown in favorable conditions with rarely negative impacts, thus having a high density of 4,000-5,100 trees/ha. The annual water storage to prevent bushfires in the dry season has negatively affected the growth of *M. cajuputi*. In addition to the threat of prolonged flooding, some high-grade melaleuca trees in strictly protected areas cannot be assisted by silviculture like thinning and cleaning forest. Analysis of *M. cajuputi* in the NR yielded a baseline conservation score of 1.7, with a confidence level of 2.7 (Figure 3).

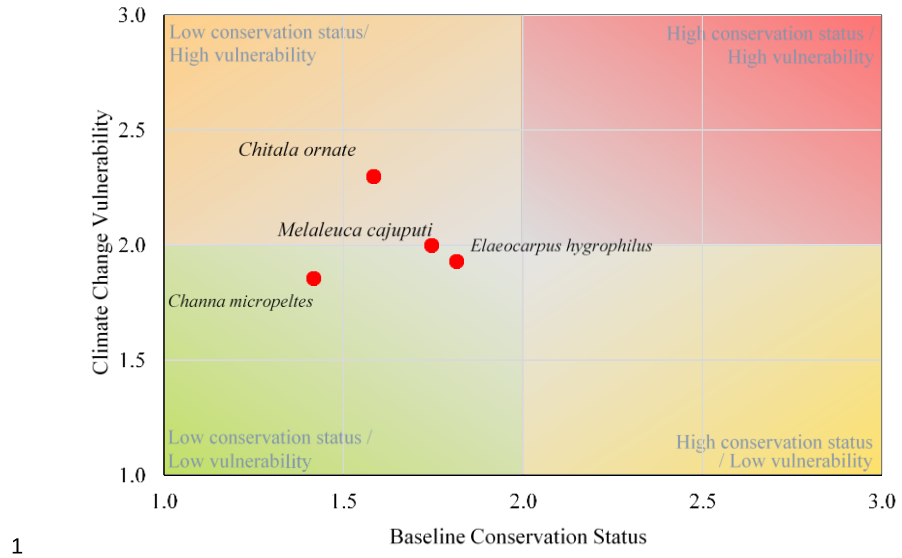

Figure 3. Basic conservation status and vulnerability of species

In terms of the vulnerability assessment to climate change, the results showed that saline intrusion would threaten the survival and development of mature *M. cajuputi*. The impacts of climate change are expected to influence seeds and seedlings of the tree disproportionately. Thanks to a wide canopy of mature melaleuca trees, the plants growing under have suffered fewer impacts from climate change. Many studies have reported that melaleuca trees can tolerate various extreme conditions such as floods, droughts, and mild saline conditions (Cuong et al., 2004; Tanaka et al., 2011; Tran et al., 2013). Besides, their distribution in various environments is also testament to their great adaptability to different climates. Mature *M. cajuputi* and seedlings can develop suckers from their roots, which helps them resist flooding. Wetlands are one of the most affected by climate change (IPCC, 2007), but melaleuca species are well adapted to inundation conditions (Cuong et al., 2004; Tran et al., 2013). According to Tran et al. (2013), *M. cajuputi* can also survive a shift to a colder and drier climate. Due to these aforementioned features, Melaleuca species possesses a remarkable potential to regenerate. Besides seeds, *M. cajuputi* can regenerate by shoot or clone-regeneration. *M. cajuputi* has an average exposure to climate change with high tolerance and adaptability. The vulnerability assessment to climate change ranked melaleuca (*M. cajuputi*) in the group of moderate vulnerability, with a score of 2.0 at a confidence level of 2.5 (Figure 3). According Triet et al. (2019a), Triet et al. (2019b) and Triet et al. (2019c) showed that *M. cajuputi* are highly vulnerable in U Minh Thuong national park, Phu My species-habitats conservation area, and Lang Sen wetland reserve, respectively. One of the reasons for this difference is due to the characteristics of the soil environment, the conservation level of the area, and the geographical location.

3.2.2 Elaeocarpus hygrophilus

Elaeocarpus (*E. hygrophilus*) is a species in the Elaeocarpaceae family. It is a medium-sized evergreen tree, about 10-15 m height, with white wood and a thornless trunk and branches. The tree bears olive-colored, smooth, oval-shaped fruits with two pointed ends, measuring 3-3.5 cm long and 1.5-2 cm wide, with one seed per fruit (Figure 4). The Elaeocarpus trees are located along the canals and on the degraded acid sulfate soil. Due to their low economic value, they only grow in a limited potential area with favorable ecological conditions. This species is on the Viet Nam Red List. In 2007, the management board of the NR planted more than 10,000 Elaeocarpus trees. These planted trees were propagated from other native trees and planted from Elaeocarpus seeds. Thus, the quantity and the area of trees have been greatly increased thanks to these effective conservation measures.

Figure 4. Elaeocarpus (*E. hygrophilus*)

Elaeocarpus, a canopy tree, requires a spacious place to grow well. Thus, once planted in a dense forest area, the tree often grows slowly and reaches high but is unable to create a canopy. The trees in the study area often suffer from pests and diseases, which has resulted in a decrease in *E. hygrophilus* biomass. Although the percentage of seedlings from seeds is very high, they have to compete for light with other species; thus, seedlings frequently fail. The dispersal rate depends on the water flow and the space availability for the tree to drop seeds. In the current conditions, the seed dispersal capacity of the tree is about average. Mature trees can withstand flooding and are moderately resilient to drought. The seeds are covered with hard shells, which has facilitated their recovery from droughts and floods. The main threats to *E. hygrophilus* in the NR include pests and diseases and a lack of suitable habitat. The analysis recorded a baseline conservation score of about 1.8 that was higher than melaleuca (Figure 3). This species is likely to be affected by floods, droughts, and salinity intrusion in the future, based on the forecast until 2100 from the MoNRE (2016). The species habitat is mostly in canals and ditches; thus, they can survive in similar habitats in other areas without being inhibited by changes in climatic factors. *E. hygrophilus* was determined to have a moderate level of vulnerability to climate change in the NR (score 1.8, confidence level 3.0) (Figure 3). When subjected to the direct pressures of climate change, this species can remain relatively ecologically connected.

3.2.3 Bronze featherback

Lung Ngoc Hoang NR is known as the "fish navel" of the Mekong Delta. The bronze featherback (*C. ornate*) (Figure 5) is a typical species in this area with a well-developed population. The assessments have observed an increasing yield with a high commercial value. The fish usually live alone and then gather to spawn once a year. They are highly fertile and often spawn in tree holes, where there is the shade of trees on the open water. Invasive weeds in the Lung habitat interfere with this reproduction. Persistent rain and subsequent black water from the melaleuca forest habitat have caused slow growth or even death of the fish. According to the managers, there are other reasons for the death of fish, such as climate change (high temperature and saline intrusion) and the residues of pesticides. However, these topics need further investigation. Habitat degradation and local fishing are the main threats to bronze featherback. They are also preyed upon by birds and other fishes within the NR, such as *Anhinga melanogaster* and *Channa striata*, and their juveniles are especially vulnerable because they live near the surface of the water.

Figure 5. Bronze featherback (*C. ornate*)

Climate change can cause certain impacts on the species survival. Droughts are considered moderate threats, but the consequences of long-term hydrological changes are assessed to be more significant to their habitat. The presence of the Cai Lon-Cai Be dam will lead to a change in the hydrological regime, namely less seasonal variation in water flow and level. In addition, the water management regime in the NR may affect water quality, which exceeds the tolerance of the species. Inundation due to sea-level rise can also reduce the reproduction ratio by narrowing suitable habitats. As a freshwater fish, the *C. ornate* may also be threatened by the saline intrusion, as predicted by the MoNRE (2016) and Triet et al. (2019b). If the sea level rises according to the forecast, by 2100, the habitat of the bronze featherback is likely to be completely lost in the Lung Ngoc Hoang NR. The vulnerability of *C. ornate* is classified as highly vulnerable (score 2.3) with a confidence level of 3.1 (Figure 3); this was similar to the study of Triet et al. (2019a) at U Minh Thuong National Park (score 2.2). This can be explained by the similarity of geographical conditions (wetland areas) and large populations but not as abundant elsewhere in the Mekong Delta.

3.2.4 Giant snakehead

The giant snakehead (*C. micropeltes*) is a highly important economic fish and is listed as a protected species in the list of inland freshwater protected areas of Viet Nam (Figure 6) (The Prime Minister of Government, 2008). Thus, fishing the *C. micropeltes* within the NR is prohibited. The fish is found in several protected areas and is currently assessed as a species of least concern (LC) (Allen and Ng, 2020). According to the officials at the NR, the *C. micropeltes* have high reproduction rates, but the number of individuals is lower than that of the bronze featherback. This may be because, during the period of low water levels, the *C. micropeltes* move out of the forest. The giant snakehead fish is a large fish, preying on other fish and feeding on each other if there is a lack of food. Therefore, they require a large habitat, and areas with high densities of *C. micropeltes* are less likely to have other fish species. The distribution characteristics revealed that most live in inland slow-flowing or still freshwater bodies such as rivers, lakes, canals, ponds, and lagoons (Rainboth, 1996; ICEM, 2013). They breed and raise their juveniles in stagnant water 30-100 cm deep and around 27°C (ICEM, 2013). *C. micropeltes* are resilient because the spawning cycle is short, from April to June per year. Due to their high economic value, giant snakehead fish are facing with threats from fishing, especially people from outside the NR. The basic conservation status of *C. micropeltes* is 1.4, a low level (Figure 3).

(a) (b)

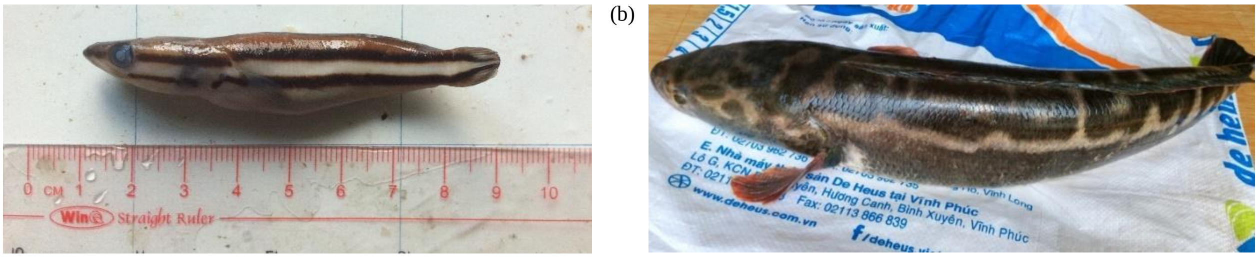

Figure 6. (a) A juvenile giant snakehead, (b) An adult giant snakehead (*C. micropeltes*)

Climate change threatens to have moderate impacts on the development and survival of the *C. micropeltes*. The species spawns in stagnant areas where flooding can reduce the availability of suitable habitats for spawning. However, *C. micropeltes* are tolerant of high water pollution and low pH and can withstand the effects of drought, prolonged rain, and changes in hydrology. Furthermore, the species has a low vulnerability to rising temperatures because the expected temperature rise in the area is within the tolerance range (7-35°C). They can survive in freshwater and brackish water; therefore, seawater intrusion may have only a moderate impact. In addition, the young *C. micropeltes* is known to migrate over short distances on land to find other water bodies, using its ability to breathe air (Pijper, 2021), which allows it to relocate to new habitats to avoid the effects of climate change. Adult giant snakehead fish completely lose this ability and are thus more vulnerable. The climate change vulnerability of *C. micropeltes* is relatively low at 1.9 (Figure 3). Given the quality of the species data, our overall confidence level for this assessment is very high.

4. CONCLUSION

Saltwater intrusion, droughts, high temperature, and permanent inundation can seriously threaten all future habitats and species. The proliferation of invasive alien species is also the main risk to the open water and Lung habitats. The Melaleuca and Lung habitats have a high baseline conservation status. The Melaleuca habitat is found to be more vulnerable than the Lung habitat. Open water and agricultural habitats are assessed as having low baseline conservation status, and open water habitats are identified as more vulnerable than agricultural habitats. The Melaleuca habitat is influenced by non-climatic factors such as encroachment for agricultural cultivation, the deterioration of water quality, and thick tree density. Most of the species selected for vulnerability assessment have a low baseline conservation level. The adaptability to climate change of *C. ornate* is much lower than that of the other species. *M. cajuputi* and *E. hygrophilus* have high adaptability under the impacts of climate change. Increasing salinity, doughts, and hydrological changes can moderately impact on the living environment of *C. micropeltes* and *C. ornate*. The findings in this study could assist the managers in formulating water management plans to ensure the development and limit the risk of the habitats and species in Lung Ngoc Hoang NR.

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REFERENCES

Allen DJ, Ng HH. *Channa micropeltes*: The IUCN red list of threatened species [Internet]. 2020 [cited 2021 June 23]. Available from: https://www.iucnredlist.org/species/172432/89799044.

Cuong NV, Quat HX, Chuong H. Some comments on indigenous Melaleuca of Vietnam. Journal of Agriculture and Rural Development 2004;11:1600-2.

Duncan R, William G, Lisa G, Jamie R, Katrina W, Danial K. Intertidal wetland vegetation dynamics under rising sea levels. Science of the Total Environment 2021;766;Article No. 144237.

Duong MT, Fredecrick FP. Survey the composition and distribution of fungi species in the natural reserve wetland Lung Ngoc Hoang, Vietnam. Journal of Advances in Technology and Engineering Studies 2017;3:19-26.

Forestry Research Institute. Sustainable Forest Management Plan of Lung Ngoc Hoang Nature Reserve, Hau Giang Province, Period 2021-2030. Lung Ngoc Hoang, NR: Hau Giang, People's Committee of Hau Giang Province; 2021.

Hau Giang Department of Natural Resources and Environment (Hau Giang DoNRE). Develop and Update an Action Plan to Respond to Climate Change in Hau Giang Province for the period of 2021-2030, with a Vision to 2050. Hau Giang: Hau Giang DoNRE; 2021.

Hoa NTH. Current Status of Soil and Water Quality in Lung Ngoc Hoang Nature Reserve [dissertation]. Can Tho, Can Tho University; 2015.

Intergovernmental Panel on Climate Change (IPCC). Summary for policymakers. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE, editors. Climate Change 2007: Impacts, Adaptation and Vulnerability. Cambridge, UK: Cambridge University Press; 2007. p. 7-22.

International Centre for Environmental Management (ICEM). USAID Mekong ARCC Climate Change Impact and Adaptation on Fisheries. Bangkok, Thailand: ICEM; 2013.

International Union for Conservation of Nature and Natural Resources (IUCN). Guidance Notes for Climate Change Vulnerability and Disaster Risk Assessment for the CAWA and Mekong WET Projects. Gland, Switzerland: IUCN; 2017.

Kelleway JJ, Cavanaugh K, Rogers K, Feller IC, Ens E, Doughty C, et al. Review of the ecosystem service implications of mangrove encroachment into salt marshes. Global Change Biology 2017;23:3967-83.

Khalid Z, Meng X, Rana IA, Rehman M, Su X. Holistic multidimensional vulnerability assessment: An empirical investigation on rural communities of the Hindu Kush Himalayan region, Northern Pakistan. International Journal of Disaster Risk Reduction 2021;62:Article No. 102413.

Lee JS, Choi HI. Comparison of flood vulnerability assessments to climate change by construction frameworks for a composite indicator. Sustainability 2018;10:Article No. 768.

Ly S, Hour P, Tomos A. Climate change vulnerability assessment for Boueng Prek Lapouv protected landscape, Cambodia. Bangkok, Thailand: IUCN ARO; 2019.

Ministry of Agriculture and Rural Development (MoARD). Decision 06/2015/QD-BNNPTNT Dated April 27, 2019 on the Announcement of the List of Rare and Endangered Aquatic Species in Vietnam that Need to be Protected, Restored and Developed. Hanoi, Vietnam: MoARD; 2019.

Ministry of Natural Resources and Environment (MoNRE). Climate Change and Sea Level Rise Scenarios for Vietnam. Hanoi, Vietnam: MoNRE; 2016.

Ni DV. Current Status of Biodiversity at Lung Ngoc Hoang Nature Reserve. Hau Giang, Vietnam: Department of Science and Technology of Hau Giang province; 2018.

Nicholls RJ, Wong PP, Burkett VR, Codignotto JO, Hay JE, Mclean RF, et al. Coastal systems and low-lying areas. In: Parry ML, Canziani OF, Palutikof JP, Linden PJVD, Hanson CE, editors Climate Change 2007: Impacts, Adaptation and Vulnerability. Cambridge, UK: Cambridge University Press; 2007.

Odum EP, Barrett GW. Fundamentals of Ecology. 5th ed. London, UK: Saunders Comp.; 2005.

Pijper. *Channa micropeltes*: Giant Snakehead [Internet]. 2021 [cited 2021 June 25]. Available from: https://aquainfo.org/channa-micropeltes-giant-snakehead/.

Rainboth WJ. Fishes of the Cambodian Mekong. Rome, Italy: Food and Agriculture Organization; 1996.

Ricaurte LF, Olaya-Rodríguez MH, Cepeda-Valencia J, Lara D, Arroyave-Suárez J, Max Finlayson C, et al. Future impacts of drivers of change on wetland ecosystem services in Colombia. Global Environmental Change 2017;44:158-69.

Scott A, Cranmer C, Thongsavath O, Xeuasing K. Climate Change Vulnerability Assessment, Xe Champhone Ramsar Site, Lao PDR. Vientiane, Lao PDR: IUCN; 2018.

Tanaka K, Masumori M, Yamanoshita T, Tange T. Morphological and anatomical changes of *Melaleuca cajuputi* under submergence. Trees 2011;25:695-704.

The Prime Minister of Government. Decision 1479/QD-TTg Dated October 13, 2008 on Decision Approving the Planning of the System of Inland Water Conservation Zones up to 2020. Hanoi, Vietnam: Vietnamese Government; 2008.

The Prime Minister of Government. Decision No. 13/2002/QD-TTg Dated January 14, 2002 on the Establishment of Lung Ngoc Hoang Wetland Nature Reserve, Can Tho Province (Now Hau Giang Province). Hanoi, Vietnam: Vietnamese Government; 2002.

Tiner R. Wetlands. In: Wang Y, August PV, Chen JM, Congalton RG, Giles F, Fox, JM et al, editors. Encyclopedia of Natural Resources. UK: Taylor and Francis; 2014.

Tran DB, Dargusch P, Moss P, Hoang TV. An assessment of potential responses of *Melaleuca* genus to global climate change. Mitigation and Adaptation Strategies for Global Change 2013;18:851-67.

Triet T, Dung NTK, Thuyen LX, Dao TTA. Climate Change Vulnerability Assessment U Minh Thuong National Park, Vietnam. Vietnam: IUCN; 2019a.

Triet T, Dung NTK, Thuyen LX, Dao TTA. Climate Change Vulnerability Assessment Phu My Species and Habitat Conservation Area, Vietnam. Vietnam: IUCN; 2019b.

Triet T, Dung NTK, Thuyen LX, Dao TTA. Climate Change Vulnerability Assessment Lang Sen Wetland Reserve, Vietnam. Vietnam: IUCN; 2019c.

Trinh TA, Feeny S, Posso A. Chapter 17: The impact of natural disasters and climate change on agriculture: Findings from Vietnam. In: Chaiechi T, editor. Economic Effects of Natural Disasters: Theoretical Foundations, Methods, and Tools. USA: Academic Press; 2021. p. 261-80.

Van CT, Duong PTT, Nga DT, Ninh LV. Study on assessing the impact of climate change (temperature and rainfall) on rice yield in the Long Xuyen Quadrangle region (LXQR)-Vietnam. Vietnam Journal of Hydrometeorology 2021;7:65-73.

Vietnamese Government. Decree No. 117/2010/ND-CP Dated December 24, 2010 on Organization of Management of Special-Use Forest System. Hanoi, Vietnam: Vietnamese Government; 2010.

Comparative Study of Carbon Stock and Tree Diversity between Scientifically and Conventionally Managed Community Forests of Kanchanpur District, Nepal

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ABSTRACT

The present study was accomplished to assess and compare tree diversity, carbon stock, and to find the relationship between carbon stock and tree diversity in scientifically and conventionally managed community forests (CFs) of Kanchanpur District, Nepal. A total of 94 sample plots were overlaid with a systematic random sampling method (51 plots in scientifically managed Singhapur CF and 43 plots were established in conventionally managed Kalika CF). The height and DBH of each tree were measured to calculate biomass and carbon stock. Shannon-Wiener and Simpson's indexes were calculated for tree diversity. The data were pooled and analyzed using MS Excel and SPSS software. The values were statistically compared using a t-test. The total carbon stock and tree diversity were higher in scientifically managed CF (207.58 tons/ha and H= 0.97) than conventionally managed CF (183.72 tons/ha and H=0.85). *Shorea robusta* has a major contribution on total carbon stock in both CFs (Kalika: 66.34% and Singhapur: 70.43%) followed by *Terminalia tomentosa* (Kalika: 24.65% and Singhapur: 13.36%). The t-test did not show any significant difference for the mean values of carbon stocks and tree diversity between the CFs at a 5% level of significance. However, carbon stock showed a weak but positive relationship with species richness and negative with evenness. The result of the study recommends managing forests scientifically for increased tree diversity leading to enhanced carbon deposition.

1. INTRODUCTION

The sum of carbon that is sequestered from the atmosphere, and deposited within the forest ecosystems (in the form of living biomass, soil carbon, deadwood, and litter) is known as forest carbon stock (FAO, 2011a). During photosynthesis, carbon is typically stored as biomass in plants (Suryawanshi et al., 2014). Of the total dry biomass of the tree, 43-50% is carbon (Malhi et al., 2002). It is re-discharged to the atmosphere if biomass is destroyed (Vashum and Jayakumar, 2012). Forests play a crucial role in the global carbon cycle and carbon balance; storing a large amount of terrestrial carbon (~80% of above ground and ~40% of belowground carbon) (Canadell and Raupach, 2008; Pan et al., 2013); a serving as a natural buffer against climate change and related challenges (Fahey et al., 2010). The accumulation of carbon in woods vegetation differs by topographical area, plant species, structure and composition, canopy cover, and management practices (Ruiz-Benito et al., 2014; Pandey and Bhusal, 2016; Dieler et al., 2017). The estimation of carbon storage provides valuable information for greenhouse gas (GHG) reduction (Johnston and Radeloff, 2019; Adame et al., 2020). The information can be useful to formulate and implement programs and strategies related to climate change (Saatchi et al., 2011; Avitabile et al., 2016).

About 5-30 million plant species are expected to be found in the world (only 5-10% of them are identified so far) (García et al., 2008; Kunzig, 2008; Mora et al., 2011). Tropical and subtropical regions host the maximum floral diversity (WCMC, 1992).

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Tropical forests alone store about 25% of the terrestrial carbon stock (Bonan, 2008). Nepal hosts 3.2% of the world's known flora; ranks in 31st and 10th position in terms of flowering plant diversity in the world and asia respectively (GoN/MoFSC, 2014). The floral diversity is on the blink of extinction due to anthropogenic factors (such as deforestation, forest conversion, and overgrazing) and natural factors (such as climate change, invasion of alien species, and disasters) (Ribeiro et al., 2014; Thapa and Maharjan, 2014; Chaudhary et al., 2016). Due to the fragmentation of forests, causing a threat to flora, there is an increased worldwide concern on floral species richness, composition, and forest structure (Myers et al., 2000). The assessment of the floral diversity is essential to conserve, protect, and manage the floral species in the particular area (Georgieva et al., 2013; Akinyemi and Oke, 2014). The species diversity has a positive and independent relationship with carbon stock (Poorter et al., 2015; Banik et al., 2018).

About 18% of atmospheric carbon dioxide (CO_2) emissions can be reduced through pausing deforestation (IPCC, 2007). The proper forest management focused on Reducing Emissions from Deforestation and Forest Degradation (REDD+) programs contributes to achieving emission reduction (Skutsch and Laake, 2008). Community forests (CF) plays a pivotal role in carbon storage through forest management and conservation which ultimately contribute to the REDD and REDD+ mechanisms (Maraseni et al., 2005; Pandey et al., 2014). About 7% of the forests in the world are managed under community forestry programs (FAO, 2011b). Scientific managed forest (SFM) employs the silvicultural system which aims to improve forest growth, productivity, species diversity, regeneration, and stand dynamics (Nguyen and Baker, 2016; Awasthi et al., 2020); whereas, conventional forest management lacks this system. In Nepal, SFM has been employed mainly in *S. robusta* forests that are among the major tropical forests having enough carbon-storing potential (Shrestha, 2008).

Several pieces of research have been conducted to assess carbon stock in different regions of Nepal (Bohara et al., 2021; Charmakar et al., 2021; Måren and Sharma, 2021; Regmi et al., 2021). However, a comparative study of carbon stock and tree diversity between scientifically managed CF that employs the silvicultural system and conventionally managed CF that lacks the system was not conducted yet in Nepal. Hence, this study was performed to assess and compare carbon stock, tree diversity and to find out the relationship between tree diversity and carbon stock in scientifically and conventionally managed community forests (CF) of Kanchanpur District, Nepal. The results of the study will be useful to managers for the conservation of forest biomass and diversity. The study will help to provide baseline information regarding the present carbon pools of the proposed study areas for REDD+ as well as help users, officials, and managers to assess the forest carbon stock and relationship with tree diversity in other parts of the tropical regions.

2. METHODOLOGY

2.1 Study area

The study was conducted in two CFs: Kalika CF and Singhapur CF of Kanchanpur District (Figure 1). Kanchanpur (Latitude: 28º38′ to 29º28′ N and longitude: 80º03′ to 80º33′ E) is located in Sudur Pashchim Province of Nepal; covers an area of 1,610 km^2 with a population of 171,304 (CBS, 2011). It is bordered by India in the south and west while Kailali and Dadledhura Districts border in the east and north respectively. The elevation and climate of the district vary from 176 m.s.l. of the lower tropical region to 1,528 m.s.l. of the sub-tropical region. The dominant tree species consists of *Shorea robusta* (Sal), *Terminalia tomentosa* (Saj), *Dalbergia sissoo* (Sisso), and *Acacia catechu* (Khayer) species. Kalika CF (area: 1.665 km^2) is conventionally managed while Singhapur CF (area: 2.05 km^2) is scientifically managed (since 2017 A.D.). Kalika CF lies in ward No. 11 of the Suklaphanta municipality consisting of a total household of 271 with a population of 1,122. Singhapur CF lies in ward No. 6 of Krishnapur municipality consisting of a total household of 314 with a population of 1,834. Both the CFs lie in the tropical climatic zone with *Shorea robusta* as a dominant species.

2.2 Data collection

2.2.1 Sampling design and data collection

The inventory in both CFs was taken using a systematic random sample procedure with a sampling intensity of 0.5%. GPS was used to collect and locate the coordinates of the sample plots. Arc GIS was used to assign the sample plots to the map. Altogether 94 concentric circular plots (43 plots in Kalika CF and 51 plots in Singhapur CF) of size 200 m^2 for a tree (>10 cm), 25 m^2 for a sapling (<10 cm), and 10 m^2 for seedling were overlaid (Figure 2). The reasons behind selecting the circular plots are easier to layout and less perimeter coverage with a greater area which reduces the probable bias due to border trees (Subedi et al., 2010). DBH of tree and sapling were measured by using Diameter tape, height of the tree and sapling was measured with Abney's level, and seedlings were counted and height was measured by measuring tape.

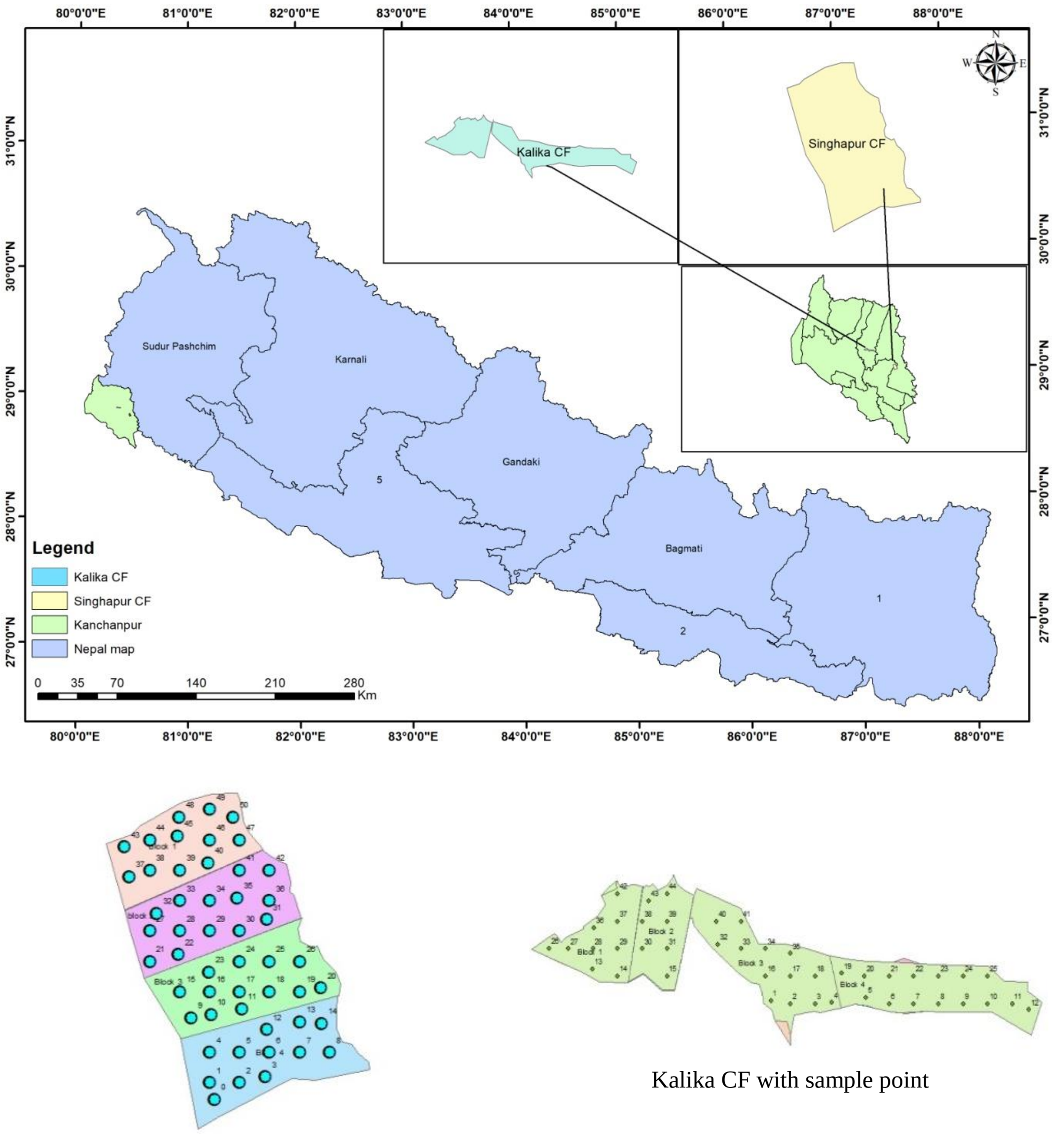


Figure 1. Map of the study area showing Kalika CF and Singhapur CF

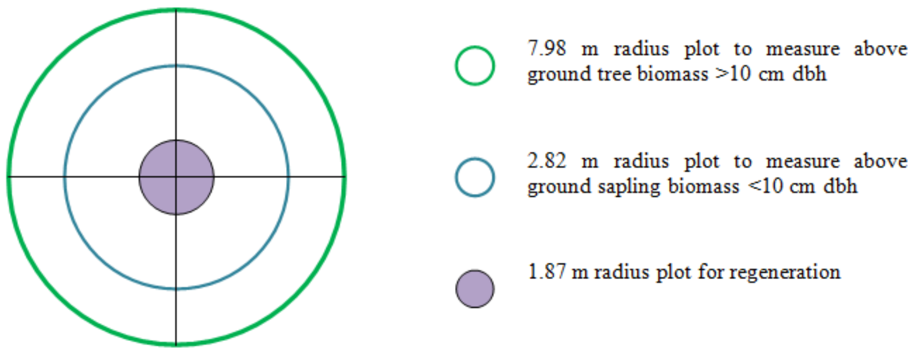


Figure 2. Concentric sample plots used for the inventory

2.3 Data analysis

2.3.1 Carbon stock estimation

The carbon stock measuring guideline in CFs (Subedi et al., 2010) was followed for the inventory. The allometric equation was used to calculate the above-ground biomass of a tree (DBH>10 cm) (Chave et al., 2005). A national allometric biomass table was used to determine the biomass of saplings (DBH<10cm) (Tamrakar, 2000). Below ground biomass (root biomass) was estimated from a root shoot ratio of 0.125 (MacDicken, 1997). Then summation of total biomass was done and multiplied with a default carbon fraction value (0.47) which resulted in the total carbon stock (IPCC, 2006). Similarly, to estimate the carbon stock of a single tree species, density values of the entire forest for that species were summed. The percentage contribution on carbon stock of each tree species was estimated by dividing the amount of carbon stock of a specific species in a forest by the sum of carbon stock/ha of all species in the same forest.

Above ground tree biomass (AGTB) = 0.0509 × δD2H; where, δ=wood density (g/cc), D=DBH (cm), H=height of the tree (m).

Above ground sapling biomass [Ln (AGSP)] = a + b ln (dbh); where AGSP is in Kg, Ln=natural log, a and b are constants, and D=DBH (cm).

2.3.2 Tree diversity estimation

Shannon-Wiener index was calculated for the species diversity. Species dominance was calculated using the Simpson index. The degree of the relative dominance of each species in that area was estimated using Pielou evenness (e). Species richness was determined by Margalefs' richness index.

$$\text{Shannon-Wiener Index (H)} = \sum \frac{ni}{n} \times \ln \frac{ni}{n}$$

$$\text{Simpson Index (D)} = \sum \frac{ni}{n}$$

$$\text{Pielou Evenness (e)} = \frac{H}{\log S}$$

$$\text{Margalefs' Richness Index (d)} = \frac{S}{\log N}$$

Where; n=total number of individuals of all species in that vegetation type, ni=importance value of species, S=number of species, and ni/n=importance probability of each species in a population.

Ms Excel and SPSS software (version 20) were used to analyze the data. Before applying a hypothesis test, a non-parametric normality test was performed. The distribution of variation in total carbon stock, H, and D was tested using a one-sample K-S normality test. The t-test was used to examine the variation in H, D, d, and e in two community forests. The relationship between total carbon stock and plant diversity was investigated using the correlation coefficient test. The significance level was α=0.05.

3. RESULTS

3.1 Density and diameter relationship

The density of individual trees at different DBH classes was found to be different in two CFs. The distribution curve for tree species (>10 cm diameter) showed a subsequent decrease in individual numbers from lower DBH class to higher DBH class in both CFs (Figure 3(a) and 3(b)). However, there was variation in the DBH of trees between the CFs. In Kalika CF, there was old stock of trees with a maximum diameter of 108 cm (Figure 3(a)) while in Singhapur CF trees were comparatively younger with a maximum diameter of 92 cm (Figure 3(b)).

3.2 Species distribution at different growth phases

The number of seedlings and saplings was highest in comparison to other successive development phases in both CFs. In the case of Kalika CF, seedlings and saplings contributed 46.17%, followed by mature regeneration (24.59%), tree (20.56%), and pole stage (8.65%) (Figure 4(a)). While, in Singhapur CF seedlings and saplings contributed 52.61%, followed by mature regeneration (23.60%), tree (12.53%), and pole stage (11.31%) (Figure 4(b)). The total number of seedlings, saplings, and trees was higher in Singhapur CF than Kalika CF (Figures 4(a) and 4(b)).

3.3 Density, basal area, and species wise carbon stock contribution

The density of *Shorea robusta* was highest in both CFs (Kalika: 190.48 per ha and Singhapur: 271.43 per ha), followed by *Terminalia tomentosa* (50 per ha and Singhapur: 40.48 per ha) and so on (Table 1). The basal area of the species in both CFs was maximum for *Shorea robusta* (Kalika: 57.61 m^2/ha and Singhapur: 48.63 m^2/ha), followed by *Terminalia tomentosa* (Kalika: 13.93 m^2/ha and Singhapur: 7.54 m^2/ha) and so on (Figure 5). The contribution of *Shorea robusta* was maximum (Kalika: 66.34% and Singhapur: 70.43%) followed by *Terminalia tomentosa* (Kalika: 24.65% and Singhapur: 13.36%) to the total carbon stock (Table 2).

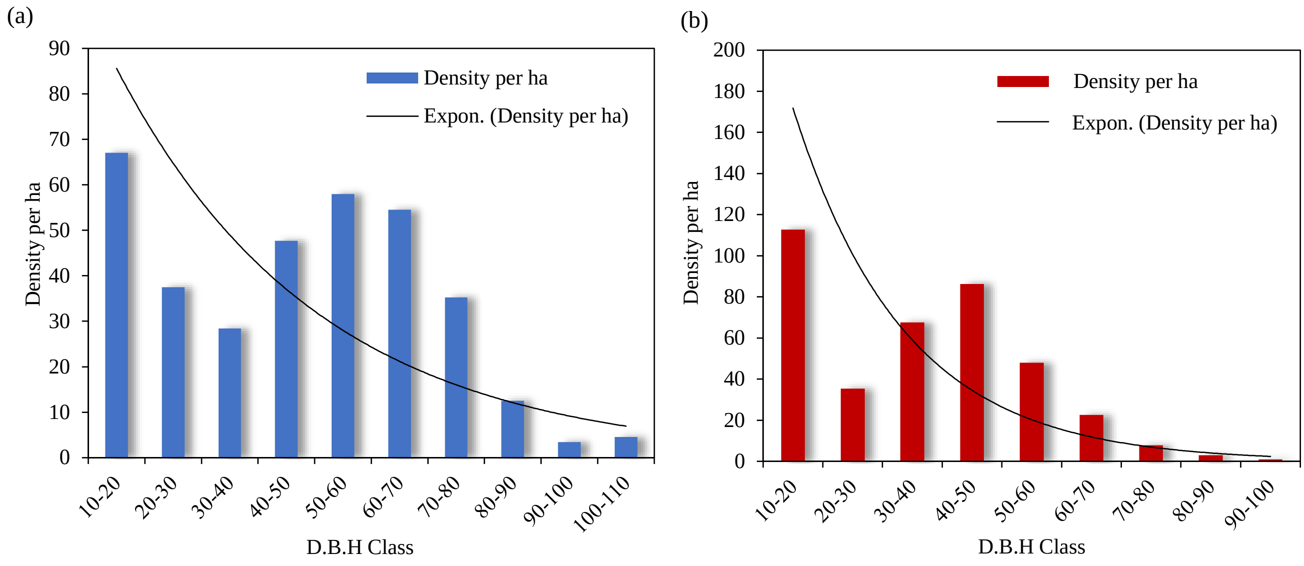


Figure 3. Density diameter curve of trees >10 cm and taller than 137 cm in (a) Kalika CF and (b) Singhapur CF

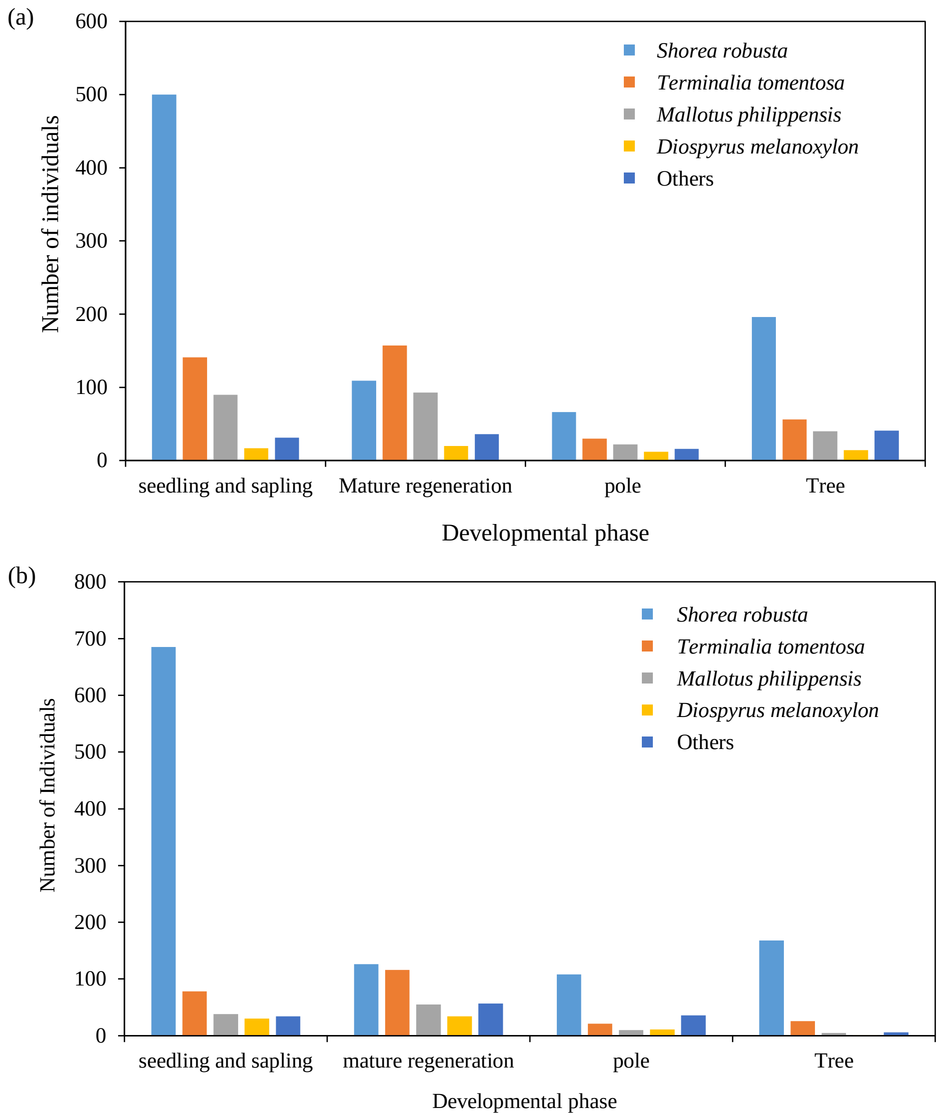


Figure 4. Number of individuals of species at different developmental phases in (a) Kalika CF and (b) Singhapur CF

Table 1. Tree species and their density per ha in the CFs

Kalika CF		Singhapur CF	
Species name	Density (per ha)	Species name	Density (per ha)
Shorea robusta	190.48	*Shorea robusta*	271.43
Terminalia tomentosa	50.00	*Terminalia tomentosa*	40.48
Anogeissus latifolia	7.15	*Anogeissus latifolia*	16.35
Lagerstroemia parviflora	29.77	*Lagerstroemia parviflora*	36.27
Mallotus philippensis	30.96	*Mallotus philippensis*	30.72
Diospyrus melanoxylon	29.77	*Diospyrus melanoxylon*	21.91
Syzygium cumini	10.27	*Syzygium cumini*	1.93
Acacia catechu	5.95	*Rhus wallichii*	1.93
Rhus wallichii	1.20	*Ficus* sp.	13.47
Diploknema butyracea	1.20	*Terminalia chebula*	0.97
Terminalia belirica	2.39	*Adina cardifolia*	0.97
Dalbergia sissoo	4.77	*Powlenia tomentosa*	1.93
		Eucalyptus camaldulensis	1.93
		Madhuca indica	0.97

Table 2. Species contribution on the carbon stock

Kalika CF		Singhapur CF	
Species name	Carbon stock (%)	Species name	Carbon stock (%)
Shorea robusta	66.34	*Shorea robusta*	70.43
Terminalia tomentosa	24.65	*Terminalia tomentosa*	13.36
Anogeissus latifolia	0.36	*Anogeissus latifolia*	2.18
Lagerstroemia parviflora	2.12	*Lagerstroemia parviflora*	3.93
Mallotus philippensis	1.38	*Mallotus philippensis*	6.33
Diospyrus melanoxylon	2.45	*Diospyrus melanoxylon*	1.71
Syzygium cumini	2.06	*Syzygium cumini*	0.99
Acacia catechu	0.46	*Rhus wallichii*	0.04
Rhus wallichii	0.11	*Ficus* sp.	0.40
Madhuca indica	0.13	*Terminalia chebula*	0.16
		Terminalia belirica	0.01
		Adina cardifolia	0.14
		Powlenia tomentosa	0.16
		Eucalyptus camaldulensi	0.22

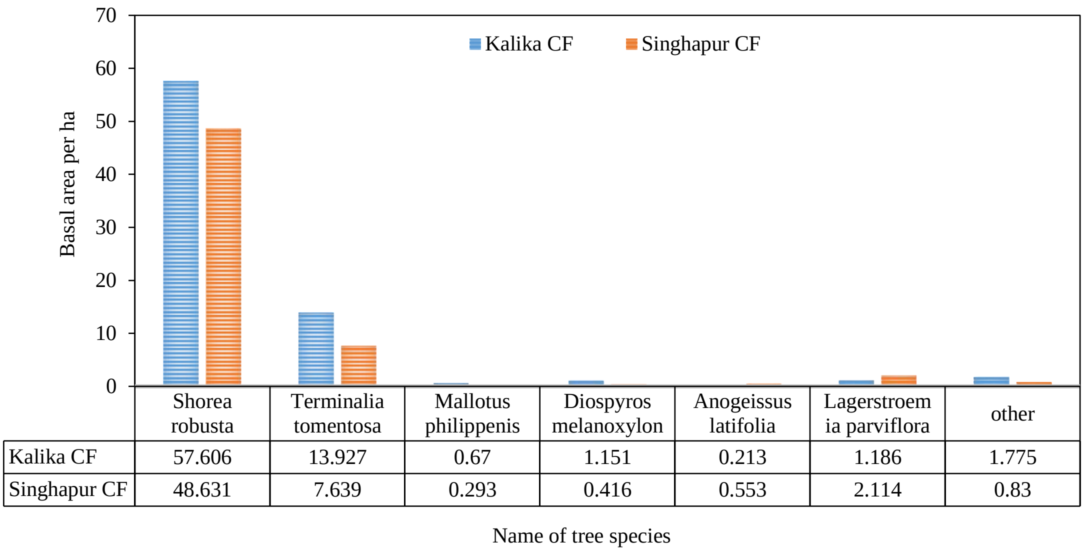


Figure 5. Tree species wise basal area in Kalika CF and Singhapur CF

3.4 Total carbon stock

The total carbon stock was estimated to be 183.72 tons/ha in Kalika CF and 207.579 tons/ha in Singhapur CF (Figure 6). The mean value of carbon stock (of tree layers) was 183.722±14.13 tons/ha and 207.58±11.50 tons/ha, respectively in Kalika CF and Singhapur CF. However, the t-test between Kalika CF and Singhapur CF did not show any significant difference in mean values of carbon stock.

3.5 Tree diversity in the CFs

The Shannon-Wiener diversity was greater in Singhapur CF (H=0.97) than Kalika CF (0.85). This indicates the higher tree diversity in Singhpur CF than Kalika CF. The Simpson's index, species richness, and evenness also support this statement. Statistically, the t-test showed that there were no significant differences in the values of Shannon-Wiener indices, Simpson's index, species richness, and evenness (0.83<0.5) between the CFs at 5% level of significance (Table 3).

3.6 Relationship between carbon stock with species richness, and evenness in the CFs

The r^2 values in both CFs ranged from 0.0657 to 0.149, indicating a positive but weak relationship of carbon stock with species richness (Figures 7(a) and 7(b)). The values of R^2 ranged from 0.0342 to 0.061 which showed a weak and negative relationship of carbon stock with species evenness in the CFs (Figures 8(a) and 8(b)).

Table 3. Diversity indices for tree species

Biodiversity indices	Kalika CF	Singhapur CF
Shannon-Wiener biodiversity index	0.845	0.9745
Simpson's index	0.4907	0.479
Average species richness	3.078	3.023
Simpson's evenness (mean value)	0.715	0.73058

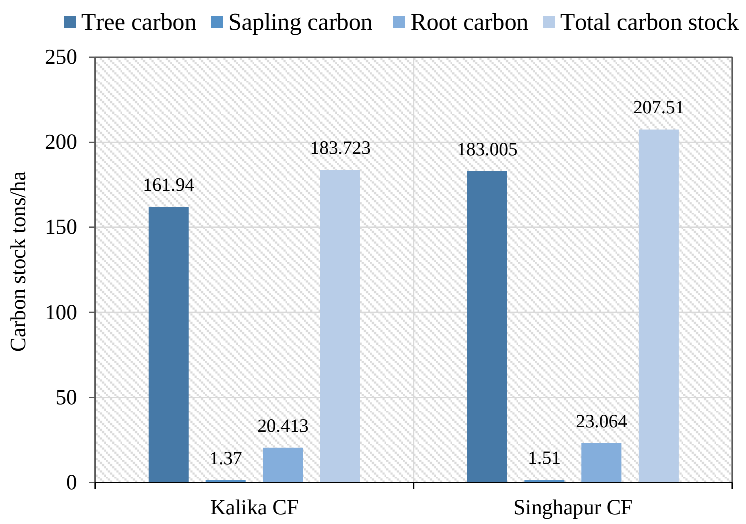


Figure 6. Total carbon stock in the CFs

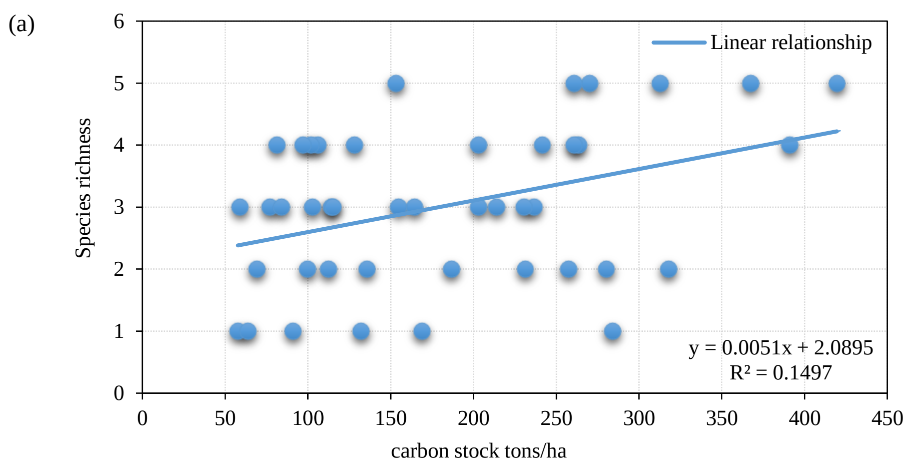


Figure 7. Relationship between carbon stock and species richness in (a) Kalika CF and (b) Singhapur CF

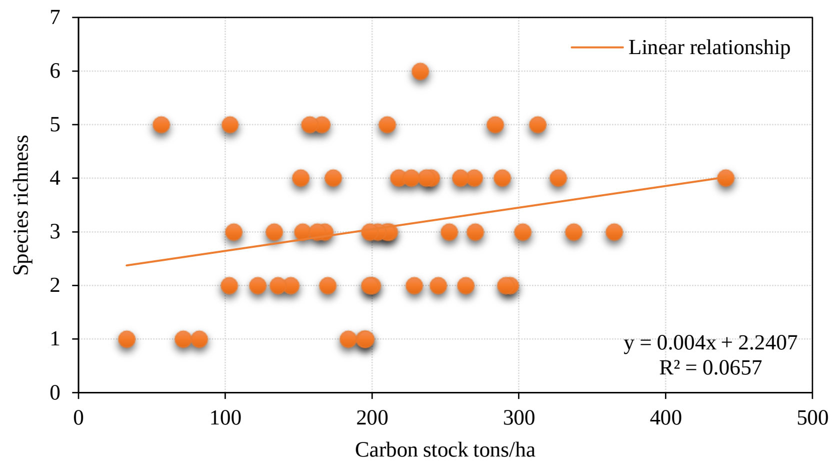
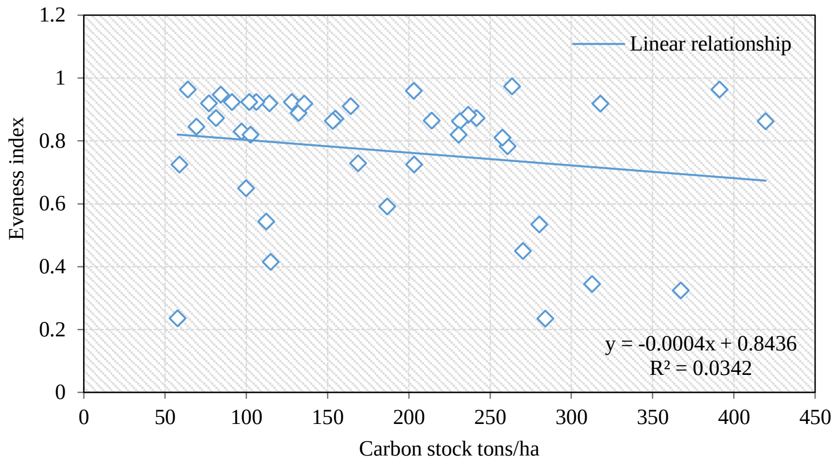


Figure 7. Relationship between carbon stock and species richness in (a) Kalika CF and (b) Singhapur CF (cont.)



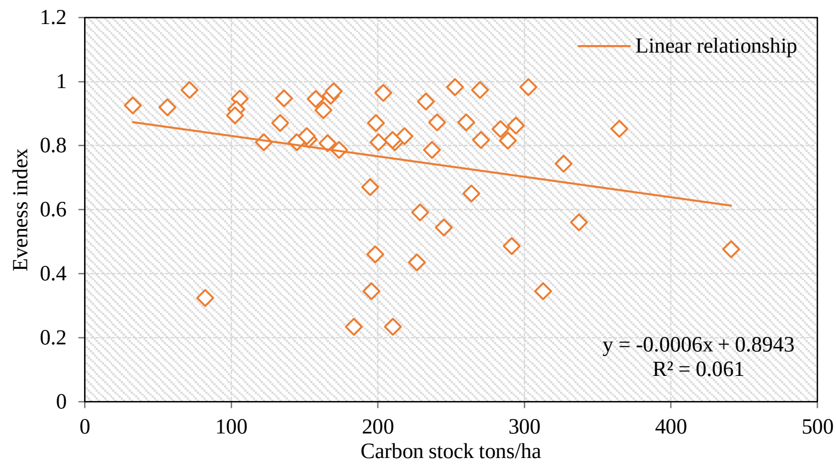


Figure 8. Relationship between carbon stock and species evenness in (a) Kalika CF and (b) Singhapur CF

4. DISCUSSION

The overall number of individuals of species was seen to be decreasing from the early regeneration phase to subsequent development phases in both CFs. The number of regeneration and pole phases was higher in Singhapur CF than Kalika CF, but large trees were higher in Kalika CF. This result indicates that the number of individuals at different phases was affected by the disturbance and two different management practices. The total number of seedlings, saplings, and trees was higher in Singhapur CF than Kalika CF. *S. robusta* was the dominant species in both the CFs in all successive developmental phases (seedlings, saplings, pole, and tree). The higher density of *S.*

robusta might be due to the presence of low canopy cover allowing abundant sunlight to reach the understory which favors the abundant growth of seedlings as well as saplings of the species (Sapkota et al., 2009; Joshi et al., 2020). The high abundance of *S. robusta* in Singhapur CF than Kalika CF might be due to the artificial regeneration and management strategies i.e., the annual regeneration is done each year in the different compartment, fire line construction and fencing has set free the Singhapur CF from livestock and anthropogenic disturbance. The great density and number of seedlings and saplings show that the CF has a good regeneration capacity (Pallardy, 2010). Variations in regeneration, sapling, and mature tree size may be attributable to variations in any of the study site's location characteristics, such as topography, climate, soil nutrients, stand, disturbances (type and intensity) (Gautam and Devoe, 2006; Sapkota et al., 2009). The study area has two different management practices which might be reason for the variation in regeneration, sapling, and mature tree size.

The total carbon stock was estimated to be greater in Singhapur CF than Kalika CF. The variation in carbon stock between two CFs may be due to drivers and management factors influencing them (Mandal et al., 2013). As Klika CF was stressed by the high anthropogenic disturbances and stand structure was poor as comparison to the Singhapur CF. The higher carbon stock might also be due to the greater density of matured trees. The study conducted by (Neupane and Sharma, 2014) in two *S. robusta*-dominated CFs of Gorkha District Laxmi Mahila CF and Jalbire Mahila CF) found higher carbon stock in Jalbire Mahila CF which contained a higher proportion of mature trees. Sejuwal (1994) has reported a carbon stock of 468 tons/ha in *S. robusta* forest (tree layer only) of Chitwan National Park which is higher than the present study. This might be due to the maturity of the forest (old aged stand store more carbon) (Singh et al., 2006). The standing carbon stock of trees depends upon the succession stage of the forest and the carbon sequestration potential depends on the age, forest type, stand condition, density, and size of trees (Brown et al., 1989; Dixon et al., 1994; Dieler et al., 2017). The FRA report of 2014 showed that the total carbon stock from the forests (*S. robusta* dominated) of the Terai region of Nepal be 89.18 tons/ha. In contrast to this, the present study reported higher carbon stock where *S. robusta* was the highest contributor of carbon stock in both the CFs.

In the present study, the tree diversity (Shannon-Wiener diversity) was higher in Singhapur CF which is regenerated than the Kalika CF which is old. The Simpon's index was higher in Kalika CF with dominant species: *S. robusta*. However, the t-test showed no significant difference in values of Shannon-Wiener and Simpson's diversity index at a 5% significant level. Mandal et al. (2013) has also reported no significant difference in Shannon-Wiener and Simpson's diversity index in three collaborative forests of the Terai region. The species richness and evenness were higher in Singhapur CF. The diversity of both CFs was found to be lower than the *S. robusta*-dominated CF of hilly Nepal (H=2.42) (Sapkota et al., 2009), Namjung CF (H=1.09), and Khari CF (H=1.30) of Gorkha district (Shrestha, 2005). In the present CFs, carbon stock and species richness showed a weak but positive relationship. Mandal et al. (2013) have also found weak but positive relation (hump-shaped relationship) between carbon stock and species richness in collaborative forests of Terai. Nakakaawa et al. (2010) have found a positive relationship between tree carbon stock and species diversity in pilot carbon offset projects in southwestern, Uganda. Wang et al. (2011) have also reported a positive correlation of carbon stock with diversity in the Spruce-dominated forest of Uganda. The relationship of carbon stock with species evenness was weak and negative. The present finding is similar to a study (Vance-Chalcraft et al., 2010) that found a negative relationship of aboveground biomass with species evenness in Puerto Rico's subtropical forest. Mandal et al. (2013) have also reported a weak but negative relationship (opposite hump-shaped relationship) of carbon stock with species evenness.

5. CONCLUSION

The study showed that the carbon stock and tree diversity were higher in scientifically managed CF than the conventionally managed CF. Scientifically managed CF has good regeneration status with higher seedlings and saplings. *S. robusta* was the most dominant species with the highest contribution to the carbon stock in both the CFs. Carbon stocks of both CFs have a positive relationship with the species richness of the trees that indicates species diversity has a positive impact on carbon sequestration potentiality. As a result, this study strongly encourages the use of sustainable forest management practices or silvicultural systems in community-managed forests. These community-managed forests should also be included in the REDD+ system so that they may profit from carbon credits, which will assist to improve forest conditions and provide a source of cash for the local community.

REFERENCES

Adame P, Cañellas I, Moreno-Fernández D, Packalen T, Hernández L, Alberdi I. Analyzing the joint effect of forest management and wildfires on living biomass and carbon stocks in Spanish forests. Forests 2020;11(11):Article No. 1219.

Akinyemi DS, Oke SO. Floristic composition and structural diversity of Shasha forest reserve in Ile-Ife, Southwestern Nigeria. Notulae Scientia Biologicae 2014;6(4):433-40.

Avitabile V, Herold M, Heuvelink GB, Lewis SL, Phillips OL, Asner GP, et al. An integrated pan-tropical biomass map using multiple reference datasets. Global Change Biology 2016; 22(4):1406-20.

Awasthi N, Aryal K, Chhetri BB, Bhandari SK, Khanal Y, Gotame P, et al. Reflecting on species diversity and regeneration dynamics of scientific forest management practices in Nepal. Forest Ecology and Management 2020;474:Article No. 118378.

Banik B, Deb D, Deb S, Datta BK. Assessment of biomass and carbon stock in sal (*Shorea robusta* Gaertn.) forests under two management regimes in Tripura, Northeast India. Journal of Forest and Environmental Science 2018;34(3):209-23.

Bohara B, Miya MS, Timilsina S, Gautam D, Regmi S. Biomass and carbon stock variation along slopes in tropical forest of Nepal: A case of depard community forest, Makwanpur, Nepal. Journal of Multidisciplinary Applied Natural Science 2021;1(2):89-99.

Bonan GB. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 2008;320(5882):1444-9.

Brown S, Gillespie AJ, Lugo AE. Biomass estimation methods for tropical forests with applications to forest inventory data. Forest Science 1989;35(4):881-902.

Canadell JG, Raupach MR. Managing forests for climate change mitigation. Science 2008;320(5882):1456-7.

Central Bureau of Statistics (CBS). National Population and Housing Census 2011, National Report. Kathmandu: National Planning Commission; 2011.

Charmakar S, Oli BN, Joshi NR, Maraseni TN, Atreya K. Forest carbon storage and species richness in FSC certified and non-certified community forests in Nepal. Small-Scale Forestry 2021;20(2):199-219.

Chaudhary RP, Uprety Y, Rimal SK. Deforestation in Nepal: Causes, consequences and responses. In: Shroder JF, Sivanpillai R, editors. Biological and Environmental Hazards, Risks, and Disasters. Netherlands: Elsevier; 2016. p. 335-72.

Chave J, Andalo C, Brown S, Cairns MA, Chambers JQ, Eamus D, et al. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. Oecologia 2005;145(1):87-99.

Dieler J, Uhl E, Biber P, Müller J, Rötzer T, Pretzsch H. Effect of forest stand management on species composition, structural diversity, and productivity in the temperate zone of Europe. European Journal of Forest Research 2017;136(4):739-66.

Dixon RK, Solomon AM, Brown S, Houghton RA, Trexier MC, Wisniewski J. Carbon pools and flux of global forest ecosystems. Science 1994;263(5144):185-90.

Fahey TJ, Woodbury PB, Battles JJ, Goodale CL, Hamburg SP, Ollinger SV, et al. Forest carbon storage: Ecology, management, and policy. Frontiers in Ecology and the Environment 2010;8(5):245-52.

Food and Agriculture Organization of the United Nations (FAO). FAO Glossary of Terms. FAO, Rome, Italy [Internet]. 2011a [cited 2022 Jan 2]. Available from: http://www.fao.org/faoterm.

Food and Agriculture Organization of the United Nations (FAO). State of the World's Forests 2011. Rome, Italy: FAO; 2011b.

Gautam KH, Devoe NN. Ecological and anthropogenic niches of sal (*Shorea robusta* Gaertn. f.) forest and prospects for multiple-product forest management: A review. Forestry 2006;79(1):81-101.

Georgieva N, Pachedjieva K, Lyubenova M. SCI "Zapadna Stara Planina and Predbalkan": Floristic studies on xerothermic oak forests. Bulgarian Journal of Agricultural Science 2013; 19(2):218-21.

Governement of Nepal/Ministry of Forest and Soil Conservation (GoN/MoFSC). Nepal National Biodiversity Strategy and Action Plan 2014-2020. Kathmandu, Nepal: GoN/MoFSC; 2014.

García JMG, Torre FE, Gómez JCG. Trends in taxonomy today: An overview about the main topics in taxonomy. Zoológica Baetica 2008;19:15-49.

Intergovernmental Panel on Climate Change (IPCC). IPCC Guidelines for National Greenhouse Gas Inventories: Volume 1-5. Hayama, Japan: Institute for Global Environmental Strategies; 2006.

Intergovernmental Panel on Climate Change (IPCC). Summary for policymakers. In: Solomon S, Qin D, Manning M, Chen Z, Marquis, M, Averyt, KB, et al, editors. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Cambridge, UK: Cambridge University Press; 2007.

Johnston CM, Radeloff VC. Global mitigation potential of carbon stored in harvested wood products. Proceedings of the National Academy of Sciences 2019;116(29):14526-31.

Joshi R, Singh H, Chhetri R, Yadav K. Assessment of carbon sequestration potential in degraded and non-degraded community forests in Terai Region of Nepal. Journal of Forest and Environmental Science 2020;36(2):113-21.

Kunzig R. Are hotspots the key to conservation. Scientific American 2008;18(4):42-9.

MacDicken KG. A Guide to Monitoring Carbon Storage in Forestry and Agroforestry Projects. USA: Winrock Internationl Institute for Agricultural Development; 1997.

Malhi Y, Meir P, Brown S. Forests, carbon and global climate. Philosophical Transactions A: Mathematical, Physical and Engineering Sciences 2002;360(1797):1567-91.

Mandal RA, Dutta IC, Jha PK, Karmacharya S. Relationship between carbon stock and plant biodiversity in collaborative forests in Terai, Nepal. International Scholarly Research Notices 2013;2013:Article No. 625767.

Maraseni TN, Cockfield G, Apan A. Community based forest management systems in developing countries and eligibility for clean development mechanism. Journal of Forest and Livelihood 2005;4(2):31-42.

Måren IE, Sharma LN. Seeing the wood for the trees: Carbon storage and conservation in temperate forests of the Himalayas. Forest Ecology and Management 2021;487:Article No. 119010.

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B. How many species are there on Earth and in the ocean? PLoS Biology 2011;9(8):e1001127.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000;403(6772):853-8.

Nakakaawa C, Aune J, Vedeld P. Changes in carbon stocks and tree diversity in agro-ecosystems in south western Uganda: What role for carbon sequestration payments? New Forests 2010;40(1):19-44.

Neupane B, Sharma RP. An assessment of the effect of vegetation size and type, and altitude on above ground plant biomass and carbon. Journal of Agricultural and Crop research 2014; 2(3):44-50.

Nguyen TT, Baker PJ. Structure and composition of deciduous dipterocarp forest in Central Vietnam: Patterns of species dominance and regeneration failure. Plant Ecology and Diversity 2016;9(5-6):589-601.

Pallardy SG. Physiology of Woody Plants. USA: Academic Press; 2010.

Pan Y, Birdsey RA, Phillips OL, Jackson RB. The structure, distribution, and biomass of the world's forests. Annual Review of Ecology, Evolution, and Systematics 2013;44:593-622.

Pandey HP, Bhusal M. A comparative study on carbon stock in Sal (*Shorea robusta*) forest in two different ecological regions of Nepal. Banko Janakari 2016;26(1):24-31.

Pandey SS, Maraseni TN, Cockfield G. Carbon stock dynamics in different vegetation dominated community forests under REDD+: A case from Nepal. Forest Ecology and Management 2014;327:40-7.

Poorter L, van der Sande MT, Thompson J, Arets EJ, Alarcón A, Álvarez-Sánchez J, et al. Diversity enhances carbon storage in tropical forests. Global Ecology and Biogeography 2015; 24(11):1314-28.

Regmi S, Dahal KP, Sharma G, Regmi S, Miya MS. Biomass and carbon stock in the Sal (*Shorea robusta*) forest of Dang District Nepal. Indonesian Journal of Social and Environmental Issues 2021;2(3):204-12.

Ribeiro S, Fernandes JP, Espírito-Santo MD. Diversity and floristic patterns of mediterranean grasslands: The relative influence of environmental and land management factors. Biodiversity and Conservation 2014;23(12):2903-21.

Ruiz-Benito P, Gómez-Aparicio L, Paquette A, Messier C, Kattge J, Zavala MA. Diversity increases carbon storage and tree productivity in Spanish forests. Global Ecology and Biogeography 2014;23(3):311-22.

Saatchi SS, Harris NL, Brown S, Lefsky M, Mitchard ET, Salas W, et al. Benchmark map of forest carbon stocks in tropical regions across three continents. Proceedings of the National Academy of Sciences 2011;108(24):9899-904.

Sapkota IP, Tigabu M, Oden PC. Tree diversity and regeneration of community-managed Bhabar lowland and Hill Sal forests in central region of Nepal. Bois Et Forêts Des Tropiques 2009;300(2):57-68.

Sejuwal M. Aboveground Biomass Estimation in Tropical Forest of Royal Chitwan National Park [dissertation]. Central Department of Botany, Tribhuvan University; 1994.

Shrestha BB. Fuelwood harvest, management and regeneration of two community forests in Central Nepal. Himalayan Journal of Sciences 2005;3(5):75-80.

Shrestha BP. An Analytical Study of Carbon Sequestration in Three Different Forest Types of Mid Hills of Nepal [dissertation]. Institute of Forestry, Tribhuvan University; 2008.

Singh JS, Gupta SR, Singh SP. Ecology Environment and Resource Conservation. New Dehli: Anamaya Publishers; 2006.

Skutsch M, Van Laake PE. Redd as multi-level governance in-the-making. Energy and Environment 2008;19(6):831-44.

Subedi BP, Pandey SS, Pandey A, Rana EB, Bhattarai S, Banskota TR, et al. Forest Carbon Stock Measurement: Guidelines for Measuring Carbon Stocks in Community-Managed Forests. Kathmandu, Nepal: Asia Network for Sustainable Agriculture and Bioresources (ANSAB); 2010.

Suryawanshi MN, Patel AR, Kale TS, Patil PR. Carbon sequestration potential of tree species in the environment of North Maharashtra University Campus, Jalgaon (MS) India. Bioscience Discovery 2014;5(2):175-9.

Tamrakar PR. Biomass and Volume Tables with Species Description for Community Forest Management. Kathmandu, Nepal: Ministry of Forest and Soil Conservation, and Natural Resource Management Sector Assistance Program; 2000.

Thapa N, Maharjan M. Invasive alien species: Threats and challenges for biodiversity conservation: A case study of Annapurna Conservation Area, Nepal. Proceedings of the International Conference on Invasive Alien Species Management; 2014 Mar 15-27; Chitwan, Nepal: 2014.

Vance-Chalcraft HD, Willig MR, Cox SB, Lugo AE, Scatena FN. Relationship between aboveground biomass and multiple measures of biodiversity in subtropical forest of Puerto Rico. Biotropica 2010;42(3):290-9.

Vashum KT, Jayakumar S. Methods to estimate above-ground biomass and carbon stock in natural forests: A review. Journal of Ecosystem and Ecography 2012;2(4):1-7.

Wang W, Lei X, Ma Z, Kneeshaw DD, Peng C. Positive relationship between aboveground carbon stocks and structural diversity in spruce-dominated forest stands in New Brunswick, Canada. Forest Science 2011;57(6):506-15.

World Conservation Monitoring Centre (WCMC). Global Biodiversity: Status of the Earths Living Resources. London: Chapman and Hall; 1992.

Estimation of Effects of Air Pollution on the Corrosion of Historical Buildings in Bangkok

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ABSTRACT

Historical buildings are recognized as the valuable cultural heritage of a nation. They may suffer material deterioration unavoidably because of exposure to air pollution. We used geographic information systems with dose-response functions (DRFs) to estimate the corrosion of copper and Portland limestone, and their risk of corrosion with regard to historical buildings in Bangkok, Thailand. The first step was to find a suitable spatial interpolation method considering the air pollution and meteorological measurement data for 2010-2019 from 26 monitoring stations in Bangkok and its neighborhoods. Applying multiple performance measures, the inverse distance weighting (IDW) method was found to be the most suitable. Predictions of the pollutant concentration in the spatial atmosphere showed that the concentration of all pollutants (SO_2, NO_2, O_3, and PM_{10}) tends to increase in 2028. Air pollution exposure time duration tends to be a key factor affecting the corrosion of material. The results of spatial corrosion estimations indicated that in 2010, the corrosion of copper and Portland limestone were at acceptable levels; however, the estimated corrosion levels for 2019 and 2028 are higher and beyond the acceptable levels. Moreover, both materials in the Rattanakosin historical area exceed their tolerable corrosion rates with considerably serious risks in 2028. The results can be further used to establish active measures to reduce the rate of corrosion of historical buildings in Bangkok.

1. INTRODUCTION

Although historical buildings indicate the historical origin of a nation, they also are a part of international cultural heritage and should be conserved. Many historical buildings suffer damage due to air pollution from various sources such as traffic activities, road and building construction, and industries. Pollutants are deposited and accumulated on the building surface. Under suitable atmospheric conditions of temperature, rainfall, and humidity, these pollutants can corrode the building materials; ancient materials are also affected by exposure time and material type (Barnoos et al., 2020; Richards et al., 2020; El-Gohary and Moneim, 2021). The impact of atmospheric pollution on material corrosion has been investigated for decades. The International Co-operative Program on Effects on Materials including Historic and Cultural Monuments (ICP Materials) and the MULTI-ASSESS project of the United Nations Economic Commission for Europe (UNECE) (Tidblad et al., 2001; Reiss et al., 2004) initially conducted field experiments to observe building material corrosion and measure air pollutants and meteorological parameters. The collected data were analyzed using multiple regression methods to develop dose-response functions (DRFs). DRFs were used to estimate the effect of atmospheric pollution on historical buildings (Christodoulakis et al., 2016; Onchang and Hawker, 2019; Broomandi et al., 2021). Building material deterioration under defined temporal and spatial conditions were estimated using a geographic information system (GIS) with DRFs.

Interpolation methods in GIS (e.g., inverse distance weighting (IDW), spline, and kriging) were used to estimate the spatial contribution of atmospheric pollutants and corrosion of materials in areas of interest (Castillo-Miranda et al., 2017; Eslami and Ghasemi, 2018; Castillo-Miranda et al., 2021). IDW determines

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values at nearby locations using a linear-weighted combination set of sample points. It provides an option to adjust smoothness of the results by setting power k, which controls the degree of local influence (Chang, 2018). Spline estimates values by minimizing overall surface curvature, resulting in smoothly varying surfaces of the parameters (Childs, 2004). Kriging is based on a geostatistical method for spatial interpolation. Unlike other interpolation methods, it can generate prediction errors to assess the quality of prediction. Kriging relies on the semivariogram, which quantifies spatial autocorrelation in the dataset. The semivariogram fitted with a mathematical function will be used for estimating the semivariance at any given distance. There are several types of kriging; ordinary and universal kriging are the commonly used methods (Chang, 2018).

However, to the best of our knowledge, a limited evaluation process to validate these interpolation methods prior to their application was reported in the previous studies. For example, Spain (De la Fuente et al., 2013) and Turkey (Karaca, 2013) selected the kriging method to interpolate spatial distribution of air pollution with no evaluation of the method, while Castillo-Miranda et al. (2021) used root means square as a single indicator to select the two methods-IDW and kriging-for mapping of the contribution of nitric acid to atmospheric corrosion on zinc material. Thus, this possibly raises questions regarding the validity of the results.

Bangkok was ranked as the world's number one tourist destination in 2018 by the Mastercard Global Destination Cities Index, with the number of tourists reaching 22.78 million in 2018 (Mastercard, 2019). The ancient religious monuments located in the historical Rattanakosin area in Bangkok are popular tourist destinations. However, according to the United Nations Environment Program (UNEP), Bangkok is an urban area plagued by air pollution over the years (UNEP, 2019). The high degree of air pollution, causing corrosion of the monuments in Bangkok, seems unavoidable. This, in turn, affects tourism in the long run.

This study aimed to assess material corrosion throughout Bangkok and its historical area (Rattanakosin) using GIS with DRFs. Statistical analysis was applied for selecting interpolation methods. The spatiotemporal impacts of atmospheric pollution on the monument materials were identified. The results can be used to formulate suitable conservation and restoration plans for the historical buildings.

2. METHODOLOGY

2.1 Data collection

2.1.1 Spatial data

Digital topographic maps of Bangkok, collected from Bangkok Metropolitan Administration GIS Center, were based on the Universal Transverse Mercator (UTM) with World Geodetic System (WGS) 1984 Zone 47. Geographic coordinates of the Buddhist monuments in the Rattanakosin historical area, registered as ancient monuments in the Royal Thai Government Gazettes for 1949-2009 (Ministry of Culture, 2017), were collected from the Fine Arts Department, Ministry of Culture. These monuments are located in 12 Buddhist temples. Coordinates of air quality monitoring stations in Bangkok and its neighborhood (total: 26 stations) were received from the Pollution Control Department of Thailand (PCD) (Figure 1).

2.1.2 Attribute data

Data of monument names and construction materials were obtained from the Fine Arts Department. Annual air quality and meteorological data (2010-2019), comprising air pollutant (SO_2, NO_2, O_3, and PM_{10}) concentrations, temperature, relative humidity, and precipitation amount from the 26 stations, were collected from the PCD. H^+ concentrations in precipitation were received from the database of Acid Deposition Monitoring Network in East Asia (EANET).

2.2 Dose-response functions

Multipollutant DRFs obtained from ICP Materials and the MULTI-ASSESS project of UNECE (European Union, 2005; Kucera, 2014) were used to estimate the deterioration rates of copper and Portland limestone (Table 1), which are commonly used in the monuments (Karaca, 2013; Onchang and Hawker, 2019). DRFs were previously used to examine the building material deterioration world-wide (Broomandi et al., 2021). Since DRFs are provided on an annual basis, all air pollutant and meteorological data were gathered on an annual basis. Deterioration rates can be expressed as either material loss (ML) (g/m^2) or surface recession (R) (μm). R can be obtained by dividing ML by material density (g/cm^3).

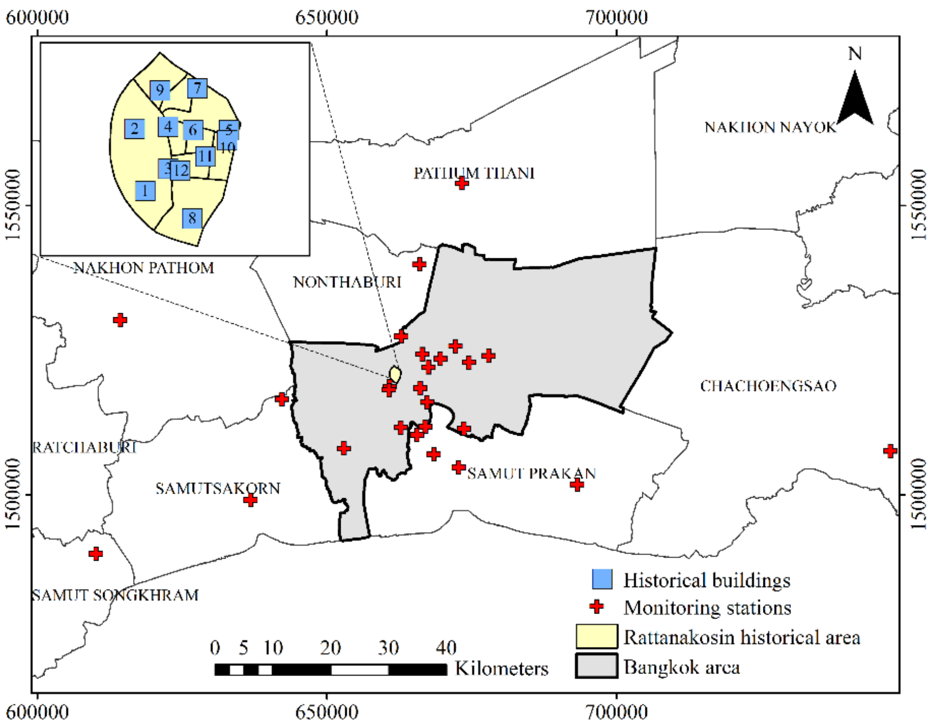

Figure 1. Study area: Bangkok and its surrounding provinces

Note: Numbers in the Rattanakosin historical area indicate the Buddhist historical buildings in the following temples: (1) Wat Phra Chettuphon Wimon Mangkhalaram; (2) Wat Mahathat Yuwarajarangsarit Rajaworamahavihara; (3) Wat Ratchapradit Sathitmahasimaram; (4) Wat Buranasiri Matayaram; (5) Wat Ratchanatdaram; (6) Wat Mahannapharam; (7) Wat Bowonniwet Wihan Ratchaworawihan; (8) Wat Rat Burana Ratchaworawihan; (9) Wat Chana Songkhram Ratchawora Maha Wihan; (10) Wat Thep Thidaram Woraviharn; (11) Wat Suthat Thepwararam Ratchaworamahawihan; and (12) Wat Ratchabophit Sathitmahasimaram Ratchaworawihan

Table 1. Dose-response functions (DRFs) for multipollutant situations and temperature functions of unsheltered copper and Portland limestone

Material	Dose-response function (DRF)	Temperature function
Copper[a]	$ML = 3.12 + \{1.09 + 0.00201[SO_2]^{0.4}[O_3]Rh_{60}e^{f(T)} + 0.0878Rain[H^+]\}t$	$f(T) = -0.032(T-10)$ (when $T \geq 10°C$)
Portland limestone[b]	$R = 4.0 + 0.0059[SO_2]RH_{60} + 0.054Rain[H^+] + 0.078[HNO_3]Rh_{60} + 0.0258PM_{10}\}\ t$	

Note: ML is material loss (g/m^2); R is surface recession (μm); $[SO_2]$, $[NO_2]$, $[O_3]$, and $[PM_{10}]$ are the concentrations of these air pollutants in $\mu g/m^3$; Rh is relative humidity (%) (Rh_{60}, which is equal to (Rh-60) when Rh>60 and 0 otherwise); Rain is the cumulative amount of precipitation (mm) over the exposure period (t) (years); (H^+) is the concentration of H^+ in the precipitation (mg/L); t is duration of exposure in years; T is the temperature in °C. The annual HNO_3 concentrations ($\mu g/m^3$) were calculated as $HNO_3=516\ e^{-3400/(T+273)} \times ([NO_2][O_3]Rh)^{0.5}$. [a]European Union (2005); [b]Kucera (2014).

2.3 Tolerable corrosion rate

The tolerable corrosion rate (Table 2) is the value representing the technical and economic considerations for a particular material and application for its annual restoration work (Kucera, 2014). It was introduced in the MULTI-ASSESS project by considering two main factors: (1) tolerable corrosion before action based on the stage of deterioration when the restoration starts; and (2) the tolerable time between maintenance based on how often it is acceptable to restore the object. The tolerable corrosion rate (K_{tol}) can be calculated as a multiple (n) of the background corrosion rate:

$$K_{tol} = n \times K_b \tag{1}$$

Where; n is a factor, and K_b is the background corrosion rate.

The corrosion rates of the two materials calculated from DRFs in this current study were compared with their tolerable corrosion rates. The selection of n (Table 2) to analyze the acceptable corrosion rates is important. If n is too high or low, the calculated tolerable corrosion rate value may be higher or lower than the actual value. The tolerable corrosion rate chosen in this study was 2.5 (n=2.5), as it corresponds to the target for protecting the basic structural materials and monuments for 2020 of the ICP Materials Project (UN ECE, 2009) and was also used in previous studies (De la Fuente et al., 2013; Karaca, 2013). Note that the tolerable corrosion rates used in this study are based on the European context. Thus, using this approach for other areas raises the concern of uncertainty in the results.

Table 2. Tolerable corrosion rates (µm/year) for n=1.0 (background corrosion rate), 1.5, 2.0, and 2.5

Material	n=1.0	n=1.5	n=2.0	n=2.5
Copper	0.32	0.50	0.64	0.80
Portland limestone	3.2	5.0	6.4	8.0

Source: Kucera (2014)

2.4 Mapping

2.4.1 Evaluation of spatial interpolation methods

We used ArcMap version 10.1 software to generate a GIS-based spatial distribution of air pollution and material corrosion in Bangkok. Initially, we validated the estimation of the spatial distribution of air pollutants by four spatial interpolation methods-IDW, spline, ordinary kriging, and universal kriging. The estimated pollutant concentrations were compared with the measured ambient air quality data averaged over all the monitoring stations. For comparison, the following statistical indicators were used: (1) fractional bias (FB)-a measure of the mean relative bias, indicating only systematic errors, and the arithmetic difference between the estimated and observed values; (2) geometric mean bias (MG)-an index for determining the overestimation or underestimation or scatter; (3) normalized mean square error (NMSE)-an estimator of the overall deviation between the estimated and measured values; (4) geometric variance (VG)-factor representing the scatter of the estimated values; (5) correlation coefficient (R)-a measure of how well the estimated values follow trends in measured values; (6) fraction of predictions within a factor of two of estimations (FAC2)-representing the estimation scatter of the spatial estimation method (Dixon and Venkatesh, 2016). The interpolation methods are validated if MG, VG, R, and FAC2 are close to 1, and FB and NMSE are close to 0. These statistical performance measures can be expressed as follows:

$$FB = \frac{\overline{C_o} - \overline{C_p}}{0.5\,(\overline{C_o} + \overline{C_p})} \qquad (2)$$

$$MG = Exp\,(\overline{\ln C_o} - \overline{\ln C_p}) \qquad (3)$$

$$NMSE = \frac{\overline{(C_o - C_p)^2}}{\overline{C_o}\,\overline{C_p}} \qquad (4)$$

$$VG = Exp\,[\overline{(\ln C_o - C_p)^2}] \qquad (5)$$

$$R = \frac{\overline{(C_o - \overline{C_o})(C_p - \overline{C_p})}}{\sigma_{C_p}\sigma_{C_o}} \qquad (6)$$

$$\text{FAC2=the fraction of data that satisfies } 0.5 \leq \frac{C_p}{C_o} \leq 2.0 \qquad (7)$$

Where; C_o is the measured value; C_p, the estimated valued; $\overline{C}_o$, the average of the measured values; $\overline{C}_p$, the average of the estimated valued; σ_{C_o}, the standard deviation of the measured value; and σ_{C_p}, the standard deviation of the estimated value.

2.4.2 Spatial corrosion analysis of historical buildings

The estimated material corrosions were quantified using DRFs (in Table 1) with air pollutants and meteorological input parameters obtained from the monitoring stations in 2010 and 2019. Future corrosion in 2028 was predicted. We projected the values of future pollution and meteorological parameters from the observed data for 2010-2019 using exponential triple smoothing in Microsoft Excel (version 2016) (Microsoft, 2021). Then, we interpolated the obtained corrosion values using the suitable spatial interpolation method selected based on the results of statistical analyses.

3. RESULTS AND DISCUSSION

3.1 Appropriate spatial estimation methods

The validation assessment results of spatial air pollution interpolation methods are listed in Table 3. As each indicator has advantages and disadvantages, multiple performance indicators should be considered in the validation exercise. The overall results in Table 3 suggest that IDW is the appropriate method. FB and MG indicate IDW has lower relative bias and systematic error. NMSE and VG show that IDW has lower systematic and unsystematic (random) errors. FAC2 is probably the most robust because it is not overly influenced by outliers (Chang and Hanna, 2004); it also indicates that IDW performs better than other methods. Our finding agrees with Vorapracha et al. (2015) who evaluated three spatial interpolation methods-IDW, ordinary kriging, and universal kriging-with observed PM_{10} (particulate matter: ≤10 µm) concentrations using root mean square error as the statistical measure. The IDW method was commonly used for spatial air pollution estimation (Jumaah et al., 2019; Pharasit and Chaiyakam, 2019). Therefore, we selected the IDW method to spatially estimate the corrosion of historical building materials unlike previous studies such as those of De la Fuente et al. (2013) and Karaca (2013), who used ordinary kriging without considering statistical indications.

Table 3. Results of the statistical analyses for evaluating interpolation methods

Air pollutants	Interpolation method	FB	MG	NMSE	VG	R	FAC2
SO_2	IDW	-0.05*	0.96*	0.01*	1.01*	0.14	1.05*
	Spline	-0.11	1.39	0.49	5.31	0.00	1.11
	Kriging (Ordinary)	-0.16	0.87	0.06	1.04	0.15	1.17
	Kriging (Universal)	-0.38	0.80	0.27	1.10	0.16*	1.30
NO_2	IDW	0.05	1.05	0.00*	1.00*	0.07	0.95
	Spline	0.30	-	1.06	-	-0.03	0.74
	Kriging (Ordinary)	-0.02*	0.99*	0.01	1.01	0.20*	1.02*
	Kriging (Universal)	-0.03	0.98	0.02	1.02	0.07	1.03
O_3	IDW	-0.05*	0.95*	0.01*	1.01*	0.06	1.05*
	Spline	-0.20	-	0.63	-	0.03	1.22
	Kriging (Ordinary)	-0.11	0.90	0.02	1.02	0.07	1.11
	Kriging (Universal)	-0.34	0.73	0.17	1.17	0.09*	1.41
PM_{10}	IDW	0.10*	1.21	0.08*	1.43	0.08*	0.91*
	Spline	-0.23	0.93*	0.36	1.50	0.02	1.26
	Kriging (Ordinary)	0.15	1.25	0.09	1.32*	0.00	0.86
	Kriging (Universal)	0.18	1.37	0.13	1.73	0.03	0.83

Note: (*) indicates the best method and (-) represents missing statistical values.

3.2 Spatial estimation of air pollutant concentrations

The interpolation of air pollutant concentrations derived from the IDW method in Bangkok in 2010 and 2019 are shown in Figures 2-5. A comparison of the air pollutant concentrations in these two years showed that the concentrations of all pollutants, except PM_{10}, decreased. The reductions in NO_2 and SO_2 may be attributed to the improvements in vehicle technologies and fuel quality (Holnicki et al., 2021). Because NO_2 is one of the primary air pollutants forming O_3, a decrease in NO_2 is likely related to the reduction in O_3 (Lee et al., 2021; Phonphinyo and Sakunkoo, 2021). The reductions in SO_2, NO_2, and O_3 concentrations during these two years agrees with the reported air quality data for Bangkok (PCD, 2020). The increment in PM_{10} may be related to the rise in construction activities, e.g., the construction of condominiums and sky train lines in Bangkok and its neighborhood. Sanecharoen et al. (2019) reported that the Landsat satellite images for 2008-2014 indicate land-use changes in Bangkok. They discovered that natural areas were being replaced by artificial objects, as indicated by increasing number and density of high-rise buildings in the city. Interestingly, the higher air pollution zone found in the western part of Bangkok indicates the possible influence of fuel combustion in industries in Samut Sakhon province (Department of Industrial Works, 2021).

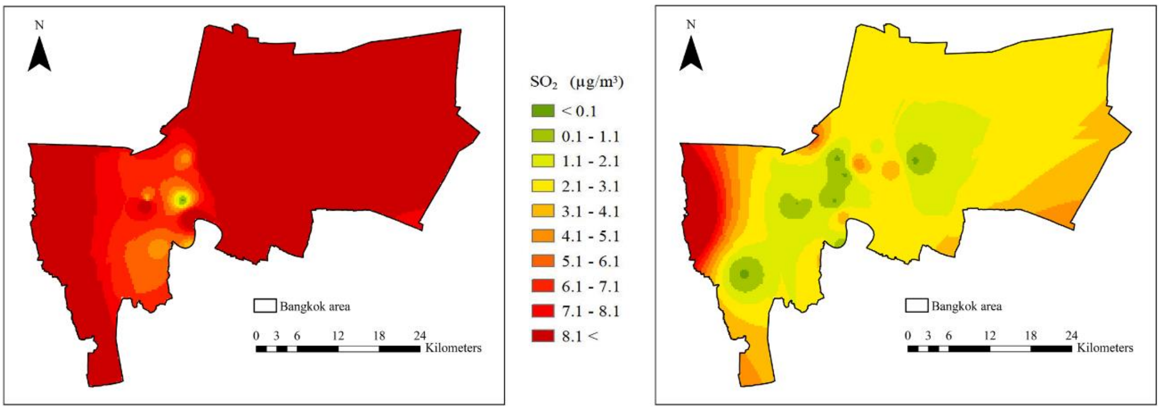

Figure 2. SO_2 concentrations in the study area in 2010 (left) and 2019 (right)

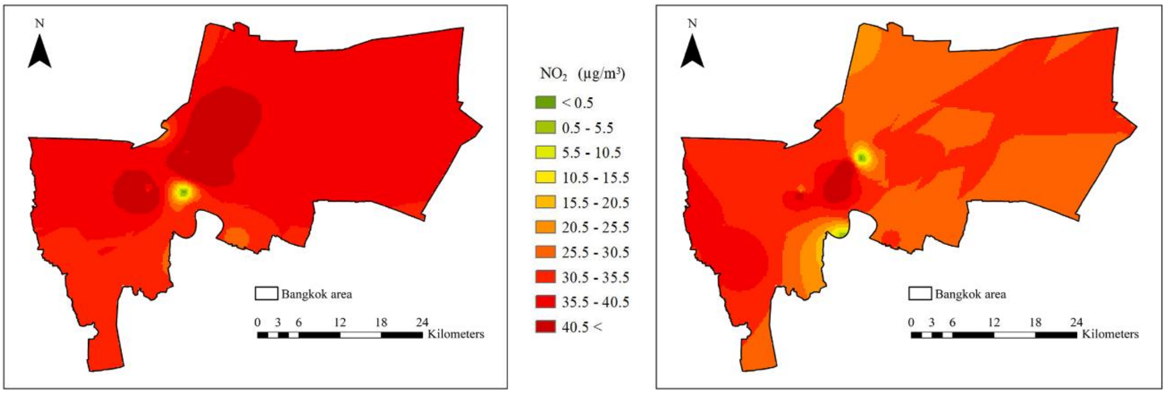

Figure 3. NO_2 concentrations in the study area in 2010 (left) and 2019 (right)

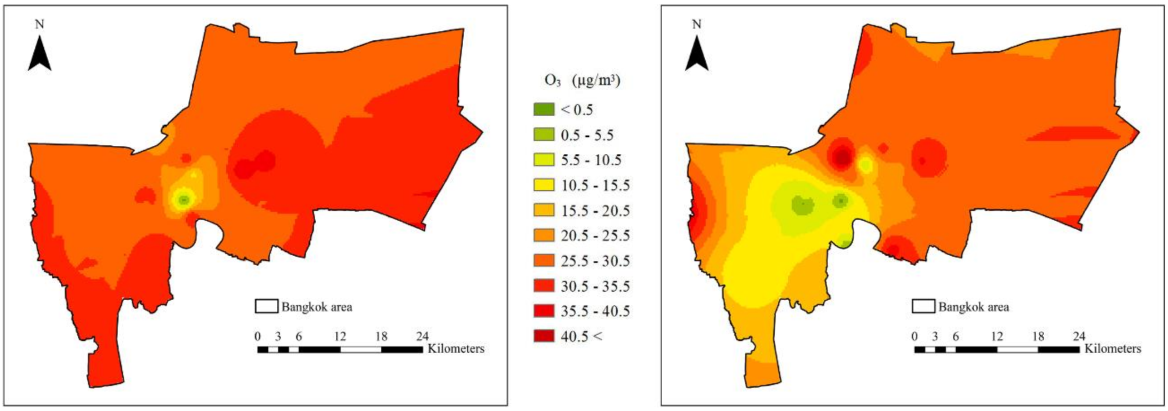

Figure 4. O_3 concentrations in the study area in 2010 (left) and 2019 (right)

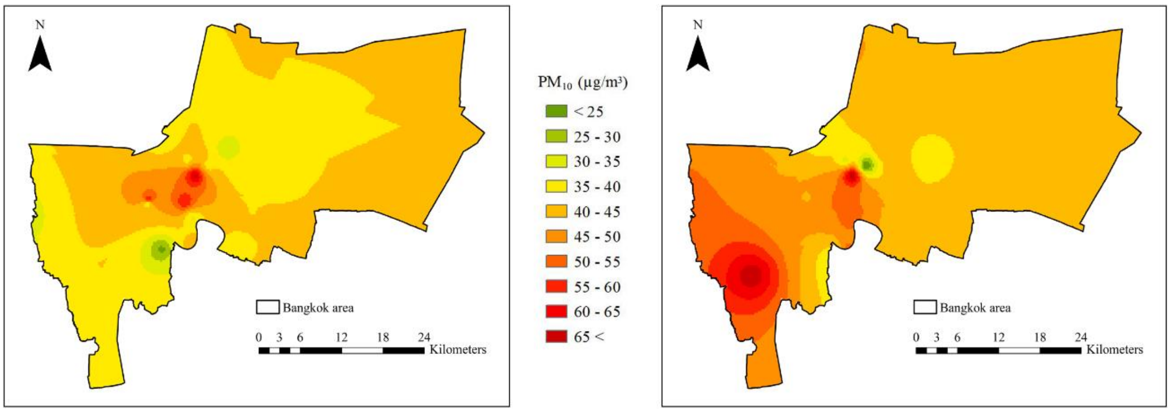

Figure 5. Particulate matter (size ≤10 µm; PM_{10}) concentrations in the study area in 2010 (left) and 2019 (right)

3.3 Corrosion of building materials

The estimated corrosions of copper and Portland limestone in 2010 and 2019 are shown in Figures 6 and 7, respectively. Both materials underwent lower corrosion in 2010 than in 2019. Interestingly, the decrease in the concentrations of air pollutants, namely, SO_2, NO_2, and O_3, were seen with the increase in corrosion rates. This is due to the duration of exposure time, which plays an important role affecting the corrosion as seen in the DRFs (Table 1). Other factors including precipitation, relative humidity and temperature were also responsible for the corrosion. However, this study found their changes over the years were minor. The density of buildings and industries in the area indirectly affect the corrosion of materials. Recently, a study reported the influence of building and industrial densities on land surface temperature in Bangkok (Sanecharoen et al., 2019). As seen from the DRFs (Table 1), temperature plays an important role in material recession and is used as a variable to explain material corrosion.

The prediction results of air pollution and meteorological parameters for corrosion forecast in 2028 are as follows: air pollution (SO_2, 8 µg/m^3; NO_2, 29 µg/m^3; O_3, 29 µg/m^3; PM_{10}, 44 µg/m^3; and HNO_3, 1.5 µg/m^3); meteorology (temperature, 27.2°C; relative humidity, 70.9%; precipitation, 658.1 mm; and H^+ in the precipitation, 3.04 mg/L). The corrosion forecasts for 2028 (Figure 8) for both materials are much higher than the corrosion estimates for 2010 and 2019 (Figures 6 and 7).

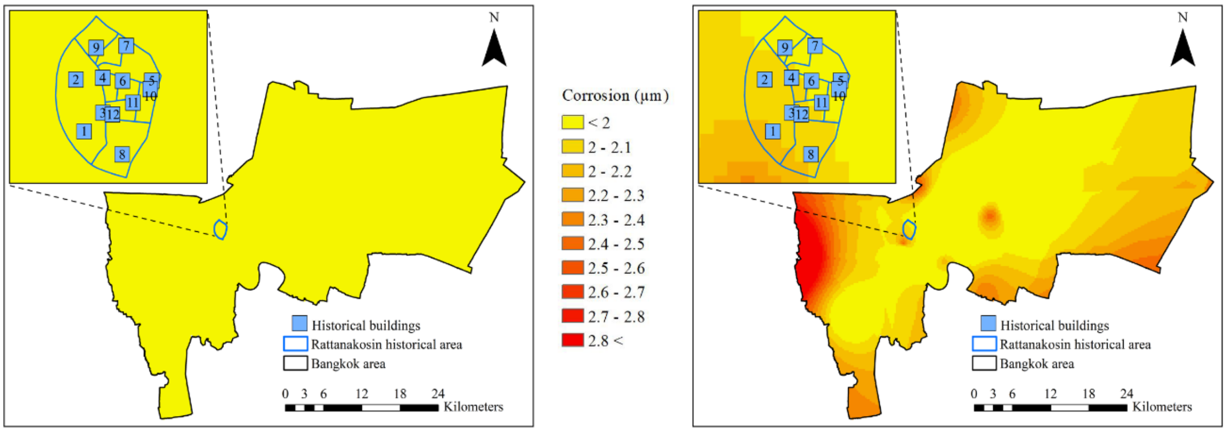

Figure 6. Corrosion of copper in the study area in 2010 (left) and 2019 (right)

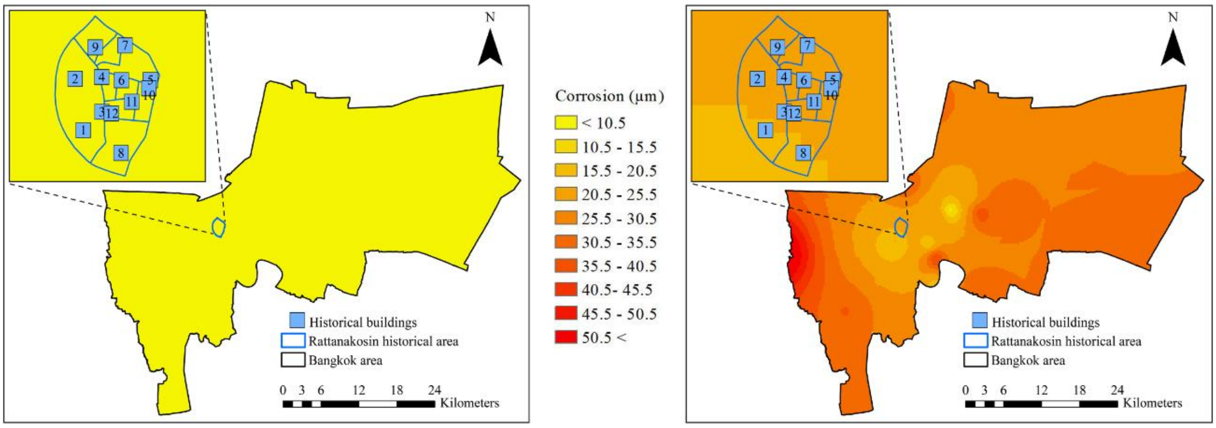

Figure 7. Corrosion of Portland limestone in the study area in 2010 (left) and 2019 (right)

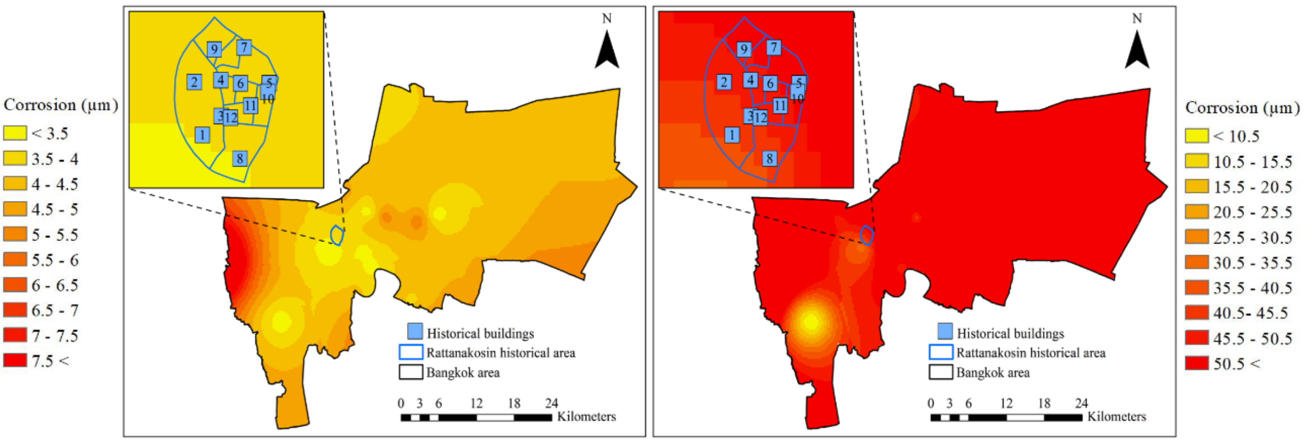

Figure 8. Corrosion of copper (left) and Portland limestone (right) in 2028

The corrosion of the two materials in 2010, 2019, and 2028 with their corresponding tolerable corrosion rates are listed in Table 4. The average corrosion rates of the two materials exceeded their tolerable corrosion rates in 2019 and 2028. The difference between the estimated and tolerable corrosion rates is much larger for Portland limestone than for copper. Overall, the results suggest an urgent need for air pollution mitigation together with measures to prevent building material corrosion both in the current and future situations. Table 4 presents a comparison of the average corrosion rates for copper and Portland limestone in this study for Bangkok with those reported for other cities. For 2010, Bangkok exhibited a lower average corrosion rate for copper and higher average corrosion rate for Portland limestone compared to Istanbul, Turkey. The concentrations of all pollutants (except O_3) and relative humidity in Istanbul were higher those in Bangkok, while the temperature and H^+ concentration in precipitation were lower in Istanbul (Karaca, 2013).

Table 4. Comparison of case studies on monument corrosion

Case study	Year	Material	Corrosion (µm)			
			Minimum	Maximum	Average	Standard deviation
Current study	2010	Copper	0.5	1.0	0.6	<0.1
		Portland limestone	5.8	9.7	7.4	0.3
Current study	2019	Copper	1.6	3.9	2.1	0.3
		Portland limestone	10.4	58.4	29.8	4.7
Current study	2028	Copper	3.3	10.7	4.4	0.8
		Portland limestone	6.8	220.9	67.3	20.1
Madrid[a]	2010	Copper	-	-	-	-
		Portland limestone	4.4	5.2	-	-
Istanbul[b]	2010	Copper	0.8	2.1	1.1	0.3
		Portland limestone	4.0	7.6	5.2	0.7
			Tolerable corrosion rates (µm)[c]			
		Copper	0.8			
		Portland limestone	8.0			

Note: [a]De la Fuente et al. (2013), [b]Karaca (2013), and [c]referred to the values in Table 2

Considering the DRF of copper (Table 1), the air pollutants and relative humidity in Istanbul may exhibit a stronger effect on the corrosion of copper than the conditions in Bangkok. The corrosion risk for Portland limestone is higher in Bangkok than in Istanbul. Similar to the above interpretation, the DRF variables, including temperature, H^+ concentration in precipitation, and calculated HNO_3 concentrations are higher for Bangkok than for Istanbul. Hence, these parameters may demonstrate a greater effect on the corrosion of Portland limestone in Bangkok. Note that in the Istanbul case study (Karaca, 2013), the cumulative amount of precipitation was not considered; hence, this variable was excluded in this comparison.

3.4 Corrosion of monuments in the Rattanakosin historical area

Estimates of the material corrosion in the Rattanakosin historical area are listed in Table 5. A comparison of the estimates for 2019 and 2028 shows that the corrosion rates of all monuments increased and exceeded the tolerable corrosion rates of the materials.

For copper, the highest risk in 2019 corresponded to the monument in temple No. 1 (2.1-2.2 µm), and more serious risks in 2028 correspond to more monuments (temples No. 2-7 and 9-12), with corrosion rates of 3.5-4.0 µm. For Portland limestone, the corrosion rates in 2019 and 2028 far exceeded its tolerable corrosion rate (8.0 µm). The highest risks were in the range of 20.5-25.5 µm in 2019 and exceeded 50.5 µm in 2028.

Note that the estimation accuracy of corrosion risk depends on many factors, such as building damage directionality considering the current exposure conditions, material composition, structural features of different ancient sites, and validity of DRFs under different atmospheric conditions. More research is required to consider these factors.

Table 5. Analysis of the corrosion risk of the historical monuments in the study area

No.	Temple	Material	Corrosion (µm)		
			2010*	2019**	2028**
1	Wat Phra Chettuphon Wimon Mangkhalaram	Copper	0.6-0.7	2.1-2.2	<3.5
		Portland limestone	7.0-7.4	15.5-20.5	35.5-45.5
2	Wat Mahathat Yuwarajarangsarit Rajaworamahavihara	Copper	0.6-0.7	2.0-2.1	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
3	Wat Ratchapradit Sathitmahasimaram	Copper	0.6-0.7	2.0-2.1	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	40.5-45.5
4	Wat Buranasiri Matayaram	Copper	0.6-0.7	2.0-2.1	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
5	Wat Ratchanatdaram	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	>50.5
6	Wat Mahannapharam	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
7	Wat Bowonniwet Wihan Ratchaworawihan	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	>50.5
8	Wat Rat Burana Ratchaworawihan	Copper	0.6-0.7	2.0-2.1	<3.5
		Portland limestone	7.0-7.4	15.5-20.5	40.5-45.5
9	Wat Chana Songkhram Ratchawora Maha Wihan	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
10	Wat Thep Thidaram Woraviharn	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	>50.5
11	Wat Suthat Thepwararam Ratchawora Mahawihan	Copper	0.6-0.7	<2.0	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
12	Wat Ratchabophit Sathitmahasimaram Ratchaworawihan	Copper	0.6-0.7	2.0-2.1	3.5-4.0
		Portland limestone	7.0-7.4	20.5-25.5	45.5-50.5
			Tolerable corrosion rates (µm)[a]		
		Copper	0.8		
		Portland limestone	8.0		

Note: *Since the estimation results did not show the differences among the monuments in the year 2010 (Figure 6 left and Figure 7 left), the annual corrosion of copper and Portland limestone were in the range of 0.6-0.7 and 7.0-7.4 µm, respectively. **is the corrosion rate higher than the tolerable corrosion rate of copper material and Portland limestone (0.8 and 8.0 µm, respectively, in Table 2). Underlines are the highest corrosion rates found in the individual year. [a]refers to the values in Table 2

4. CONCLUSION

The appropriate GIS-based interpolation method to reproduce the spatial distribution of ambient air pollution was identified as IDW from the results of statistical analyses. In Bangkok, the concentrations of all pollutants, except PM_{10}, decreased from 2010 to 2019. However, the prediction shows that all pollutants return to increase in 2028. We utilized DRFs with IDW to calculate the spatial corrosion rates of copper and Portland limestone in 2010, 2019, and 2028. The material corrosion rates increased and exceeded their tolerable corrosion rates in 2019 and 2028. Air pollution concentrations together with the duration of exposure time affects the material corrosions. The corrosion rates of monument materials increased beyond their tolerable limit in the Rattanakosin historical area in Bangkok. The results of the current study can serve as concrete information to initiate strategic actions to conserve historical buildings.

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REFERENCES

Barnoos V, Oudbashi O, Shekofteh A. The deterioration process of limestone in the Anahita Temple of Kangavar (West Iran). Heritage Science 2020;8(66):1-19.

Broomandi P, Tleuken A, Zhaxylykov S, Nikfal A, Kim JR, Karaca F. Assessment of potential benefits of traffic and urban mobility reductions during COVID-19 lockdowns: Dose-response calculations for material corrosions on built cultural heritage. Environmental Science and Pollution Research 2021;29(5):6491-510.

Castillo-Miranda JO, Torres-Jardon R, Garcia-Reynoso JA, Mar-Morales BE, Rodriguez-Gomez FJ, Ruiz-Ruarez LG. Mapping recession risk for cultural heritage stone in Mexico City due to dry and wet deposition of urban air pollutants. Atmósfera 2017;30(3):189-207.

Castillo-Miranda JO, Rodriguez-Gomez FJ, Genesca-Llongueras J, Ruiz-Suarez LG, Garcia-Reynoso JA. Estimation and mapping of the contribution of nitric acid to atmospheric corrosion of zinc. Global Journal of Environmental Science and Management 2021;7(4):523-42.

Chang JC, Hanna SR. Air quality model performance evaluation. Meteorology and Atmospheric Physics 2004;87:167-96.

Chang KT. Introduction to Geographic Information Systems. 9th ed. New York: McGraw-Hill Education; 2018.

Christodoulakis J, Tzanis CG, Varotsos CA, Ferm M, Tidblad J. Impacts of air pollution and climate on materials in Athens, Greece. Atmospheric Chemistry Physics Discussions 2016; 17(1):1-27.

Childs C. Interpolating surfaces in ArcGis spatial [Internet]. 2004 [cited 2022 May 14]. Available from: https://www.esri.com/news/arcuser/0704/files/interpolating.pdf.

De la Fuente D, Vega JM, Viejo F, Diaz I, Morcillo M. Mapping air pollution effects on atmospheric degradation of cultural heritage. Journal of Cultural Heritage 2013;14(2):138-45.

Department of Industrial Works. Factory information for officials [Internet]. 2021 [cited 2022 Jan 20]. Available from: http://reg.diw.go.th/executive/Prov3.asp?prov=74.

Dixon B, Venkatesh U. GIS and Geocomputation for Water Resource Science and Engineering. 1st ed. UK: John Wily and Sons Ltd; 2016.

El-Gohary MA, Moneim AA. The environmental factors affecting the archaeological buildings in Egypt, "II Deterioration by severe human activities". Periodico Di Mineralogia 2021; 90(2):41-55.

Eslami A, Ghasemi SM. Determination of the best interpolation method in estimating the concentration of environmental air pollutants in Tehran City in 2015. Journal of Air Pollution and Health 2018;3(4):187-98.

Holnicki P, Nahorski Z, Kaluszko A. Impact of vehicle fleet modernization on the traffic-originated air pollution in an urban area: A case study. Atmosphere 2021;12(12):Article No. 1581.

Jumaah HJ, Ameen MH, Kalantar B, Rizeei HM, Jumaah SJ. Air quality index prediction using IDW geostatistical technique and OLS-based GIS technique in Kuala Lumpur, Malaysia. Geomatics, Natural Hazards and Risk 2019;10(1):2185-99.

Karaca F. Mapping the corrosion impact of air pollution on the historical peninsula of Istanbul. Journal of Cultural Heritage 2013;14(2):129-37.

Kucera V. Chapter VI: Mapping of effects on materials. In: Kucera V, editor. Manual on Methodologies and Criteria for Modeling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends. Berlin: Federal Environmental Agency (Umweltbundesamt); 2014. p. 1-16.

Lee HJ, Chang LS, Jaffe DA, Bak J, Liu X, Abad GG, et al. Ozone continues to increase in East Asia despite decreasing NO_2: Causes and Abatements. Remote Sensing 2021;13(11):1-17.

Mastercard. Global destination cities index [Internet]. 2019 [cited 2021 Aug 31]. Available from: https://newsroom.mastercard.com/wp-content/uploads/2019/09/GDCI-Global-Report-FINAL-1.pdf.

Microsoft. Forecast.ets function [Internet]. 2021 [cited 2021 Oct 31]. Available from: https://support.microsoft.com/en-us/office/forecast-ets-function-15389b8b-677e-4fbd-bd95-21d464333f41.

Ministry of Culture. Attachment of Act on Ancient Monuments, Antiques, Objects of Art and National Museums (No.2), B.E. 2535: Volume 109. Bangkok: Office of Archaeology, Fine Arts Department; 2017. (in Thai).

European Union. Model for Multi-Pollutant Impact and Assessment of Threshold Levels for Cultural Heritage: EU 5FP RTD Project. Stockholm, Sweden: MULTI- ASSESS Publishable Final; 2005.

Onchang R, Hawker DW. A computational program for estimating atmospheric corrosion of monuments. Environment and Natural Resources Journal 2019;17(3):19-28.

Pharasit M, Chaiyakarm T. Geoinformatics application on air quality assessment: A case study in Bangkok. Thai Science and Technology Journal 2019;28(5):744-58.

Phonphinyo S, Sakunkoo P. The amount of ambient ozone and nitrogen dioxide that relate with traffic around Bueng Srithan in Khon Kaen University area. Research and Development Health System Journal 2021;14(2):135-43.

Pollution Control Department (PCD). Thailand State of Pollution Report 2019. Bangkok, Thailand: PCD; 2020.

Reiss D, Rihm B, Thoni C, Faller M. Mapping stock at risk and release of zinc and copper in Switzerland: Dose response functions for runoff rates derived from corrosion rate data. Water, Air, and Soil Pollution 2004;159:101-13.

Richards J, Bailey R, Mayaud J, Viles H, Guo Q, Wang X. Deterioration risk of dryland earthen heritage sites facing future climatic uncertainty. Scientific Reports 2020;10(1):1-9.

Sanecharoen W, Nakhapakorn K, Mutchimwong A, Jirakajohnkool S, Onchang R. Assessment of urban heat island patterns in Bangkok metropolitan area using time: Series of LANDSAT thermal infrared data. Environment and Natural Resources Journal 2019;17(4):87-102.

Tidblad J, Kucera V, Mikhailov AA, Henriksen J, Kreislova K, Yates T, et al. UN ECE ICP materials: Dose-response functions on dry and wet acid deposition effects after 8 years of exposure. Water, Air, and Soil Pollution 2001;130,1457-62.

United Nations Economic Commission for Europe (UN ECE). Executive body for the convention on long-range transboundary air pollution [Internet]. 2009 [cited 2020 Jan 12]. Available from: https://www.unece.org/fileadmin/DAM/env/documents/2009/EB/wge/ece.eb.air.wg.1.2009.16.e.pdf.

United Nations Environment Programme (UNEP). Air pollution is choking Bangkok, but a solution is in reach [Internet]. 2019 [cited 2020 Jan 12]. Available from: https://www.unep.org/news-and-stories/story/air-pollution-choking-bangkok-solution-reach.

Vorapracha P, Phonprasert P, Khanaruksombat S, Pijarn N. A comparison of spatial interpolation methods for predicting concentrations of particle pollution (PM_{10}). International Journal of Chemical, Environmental and Biological Sciences 2015;3(4):302-6.

Wastewater Treatment Efficiency by a Freshwater Phylactolaemate Bryozoan and Experimental Feeding with Protozoa

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ABSTRACT

The wastewater treatment ponds of the King's Royally Initiated Laem Phak Bia Environmental Research and Development (LERD) Project in west-central Thailand provide habitats for freshwater bryozoans, which are colonial invertebrate animals. Bryozoans sieve food particles out of the water using a retractable lophophore and can play an important ecological role in wastewater treatment. In this unique environment, we: (1) investigated the efficiency of a phylactolaemate bryozoan (*Plumatella casmiana*) in wastewater treatment, measured by BOD_5, chlorophyll a and turbidity; and (2) determined the role of protozoans in the diet of the bryozoan *P. casmiana*. Comparison of growth rate and fecal pellet characteristics between protozoan-fed bryozoans and phytoplankton-fed bryozoans was investigated. At the end of our wastewater treatment experiment, water quality parameters were markedly improved in the treatment with bryozoans compared to the control (without bryozoans). The treatment efficiency levels for BOD_5, turbidity, and chlorophyll a were 24.04%, 59.21%, and 55.13%, respectively. The growth rates of bryozoans in the experimental treatment increased over time. Our study also revealed that this bryozoan can feed on a diet of protozoans under experimental conditions. However, the average daily growth rate of protozoan-fed bryozoans -20 zooids per day was lower than that of phytoplankton-fed bryozoans 19 zooids per day. This may have been due to incomplete digestion of protozoans or insufficient nutrition in the bryozoans. The results from this study provide better understanding of bryozoan ecology and their role in wastewater treatment systems.

1. INTRODUCTION

Bryozoans are moss-like invertebrates that coexist in colonies, where one colony consists of many zooids joined together (Schwaha et al., 2016). They are hermaphroditic and can reproduce both sexually and asexually (Schwaha et al., 2020). Most freshwater bryozoans can be propagated from encapsulated seed-like structures called statoblasts. These statoblasts are found in most bryozoans and are important in phylactolaemate systematics (Hirose et al., 2011). One species, *Plumatella casmiana* Oka, 1907 is capable of producing statoblasts that are dormant as well as statoblasts ("leptoblasts") that germinate immediately upon release from the colony (Wood, 2015). Bryozoans feed by filtering suspended solids such as microorganisms, algae, plankton and other debris from the surrounding water (Morris et al., 2002; Rutkauskaitė-Sucilienė and Šatkauskienė, 2016; Nimtim et al., 2020). They can also consume protozoans and rotifers (Wood, 2015). The food items are captured by an array of ciliated tentacles and then digested in a simple gut (Ryland, 2020). Undigested particles are packaged together and expelled in the form of fecal pellets (Wood, 2015). Wright (2014) and Yepes-Narváez (2020) reported that a single bryozoan zooid can filter up to 8.8 mL of water per day; thus, these animals have the potential to remove suspended particles and algae from water and contribute to nutrient cycling and regulation of the metabolism of freshwater ecosystems (Todini et al., 2018). Bryozoans also represent a food source for many aquatic species and provide microhabitats and refuge for smaller invertebrates (Gorgoglione et al., 2016).

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Most bryozoans (Phylum Bryozoa) are marine. However, some species can be found in freshwater habitats; these are members of Class Phylactolaemata and are referred to as phylactolaemate bryozoans (Massard and Geimer, 2007). Bryozoans grow on submerged objects such as glass, plastic and wood (Wood, 2009) in shallow water, and they can live in a wide variety of environments. They usually thrive in warm waters between 15ºC and 28ºC. However, some species can survive in cold (below 10ºC) and very warm waters (above 32ºC or more). A study by Shrivastava and Rao (1985) showed that *Plumatella emarginata* could survive in Bhopal Lake in India during the summer, when water temperatures rose to 34ºC.

Bryozoans can survive in both clean water and water that has been contaminated with pollutants such as wastewater treatment systems. Bryozoans are also tolerant to a wide range of pH, but thrive in slightly alkaline water bodies (Wood, 2015). Because bryozoans are sedentary organisms and have a certain degree of tolerance to water contamination, they are one of the most suitable organisms for use as bioindicators; they are capable of assessing water quality in a fixed location continuously and accurately (Seansupha, 2009). Bryozoans can also be problematic in the environment. They cause clogging of various pipes and drainage systems (Wood, 2005) via floatoblasts, which are characterized by their buoyancy and mass production. In marine environments, bryozoans spread easily by currents or by attaching to transport ships as well as in ballast water. Therefore, bryozoans are among the most invasive alien species and can greatly affect native species (Kocak et al., 2019).

In Thailand, a total of 18 species of freshwater bryozoans have been identified from 26 provinces (Wood et al., 2006). A member of the family Plumatellidae, *Plumatella casmiana* has a worldwide distribution (Wood et al., 2010). This paper deals primarily with *P. casmiana*, which is a phylactolaemate bryozoan. *P. casmiana* is the predominant bryozoan found in wastewater treatment systems of the King's Royally Initiated Laem Phak Bia Environmental Research and Development (LERD) Project in Thailand. In fact, the study by Nimtim (2020) identified three species of freshwater bryozoans living in these wastewater treatment ponds: *P. casmiana*, *P. vorstmani* Toriumi, 1952 and *P. vaihiriae* Hastings, 1929. Wastewater in the LERD Project is treated by a series of oxidation ponds. Notably, bryozoans can grow well in some of these wastewater treatment ponds; they tend to settle on the outfall of these ponds (Nimtim et al., 2020), where oxygen and food are plentiful and the colonies are beyond the reach of predatory fish. Thriving bryozoan colonies in this environment could be due to the combination of rich organic food materials coming from domestic wastewater as well as dense populations of highly nutritional phytoplankton. In particular, *Spirulina platensis* (Gomont) Geitler 1925 is dominant in oxidation ponds (Chaichana and Dumpin, 2016).

Bryozoans can consume a variety of foods such as plankton and organic particles suspended in the wastewater ponds of the LERD Project. Protozoans are also diverse, abundant and one of the main biological components in these man-made ecosystems (Madoni, 2011). Protozoans may be an important alternative food source for bryozoan nutrition. There is strong evidence that some species of freshwater bryozoans can feed not only on algae but also on protozoans (Wood, 2019). Nimtim et al. (2020) suggested that the role of ciliates in bryozoan nutrition at the LERD Project site should be further investigated. Nutrients derived from protozoa (as a food) may increase the growth rate of bryozoans and help maintain healthy communities of bryozoans in wastewater treatment ponds.

Research on the role of bryozoans in wastewater treatment is limited, especially in Thailand. Much of the research has focused on bryozoans in natural habitats. To gain more insight into the biology and ecology of freshwater bryozoans in the LERD Project (wastewater treatment ponds), this study aimed to explore the role of *P. casmiana* in wastewater treatment, particularly in terms of reduction of organic content (BOD_5), chlorophyll a, and turbidity. Since oxidation ponds are a rich source of food to bryozoans, knowledge of protozoa as a food source of bryozoans is important, but currently this topic is poorly understood. This study thus further examined the role of protozoans as a food source for bryozoans and characterized the fecal pellets after protozoan digestion. The results should add to our understanding of the efficiency of the *P. casmiana* digestive system and create wider interest in wastewater treatment and research in Thailand and the international research community.

2. METHODOLOGY

2.1 Study site

The wastewater treatment system at the LERD Project was established in 1991 with the aim of treating domestic wastewater from Phetchaburi municipality, Thailand. The municipal wastewater here is composed of organic matter, inorganic substances, oil and various flotation agents, solids, detergents, and nutrients. The LERD Project handles approximately 3,600 to 6,000 m^3/d. There is a total of five ponds, comprising one sedimentation pond, three oxidation ponds and one stabilization pond (Figure 1). The ponds range in surface area from 10,134-42,878 m^2, with a combined storage capacity of approximately 20,000 m^3/d. Wastewater flows by gravity from pond 1 to pond 5, and then is discharged to a nearby mangrove forest and then to the Gulf of Thailand. When the wastewater enters the system, the BOD_5 ranges from 1,000-1,600 mg/L. After treatment in all five ponds, the BOD_5 is reduced to only 10-15 mg/L.

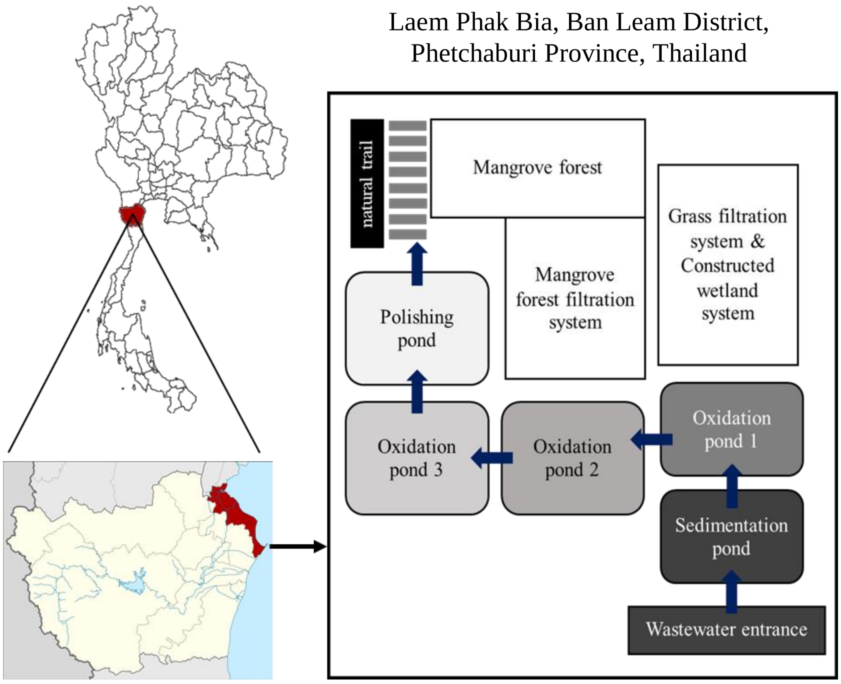


Figure 1. Map and flow diagram of the LERD Project's wastewater treatment system (13.0471°N, 100.0844° E)

2.2 Sampling of bryozoans and bryozoan culture

Portions of bryozoan colonies (*P. casmiana*) were collected by hand from the edge of the outflow weir of a pond where bryozoans were abundant (Figure 2). The color of living colonies of these bryozoans is light to dark brown and colonies are soft. Colonies are seen as tubular branches and look like tree roots. Portions of bryozoan colonies were then placed in a polyethylene bottle containing a small amount of water from the sampling point. The bryozoan samples were cultured and used for further experiments in the laboratory of the LERD Project.

Colonies of bryozoans were torn into small fragments about 0.5 cm in diameter, and then viewed under a stereo microscope (Nikon SMZ 445) to confirm that the bryozoans were alive. Then, 4-5 pieces of bryozoan colony were placed in plastic Petri dishes inside a plastic container filled with water from oxidation pond 2. We used water from oxidation pond 2 because bryozoans were most abundant there, and the environmental conditions were suitable for bryozoans. The Petri dishes were attached to the wall of the container using a pair of small magnets. The dishes were left at room temperature (30°C) for a few days until each bryozoan colony had attached to the Petri dish. Then, the bryozoans were nurtured for another 3-5 days and supplied with constant aeration (Figure 3), with the water being renewed daily (3-4 L) to provide food for the bryozoan colonies.

Figure 2. Location of bryozoan colonies collected from oxidation pond 2 at the LERD Project indicated by red arrow (above); and living bryozoan colonies on hand (below left) and under microscope (below right).

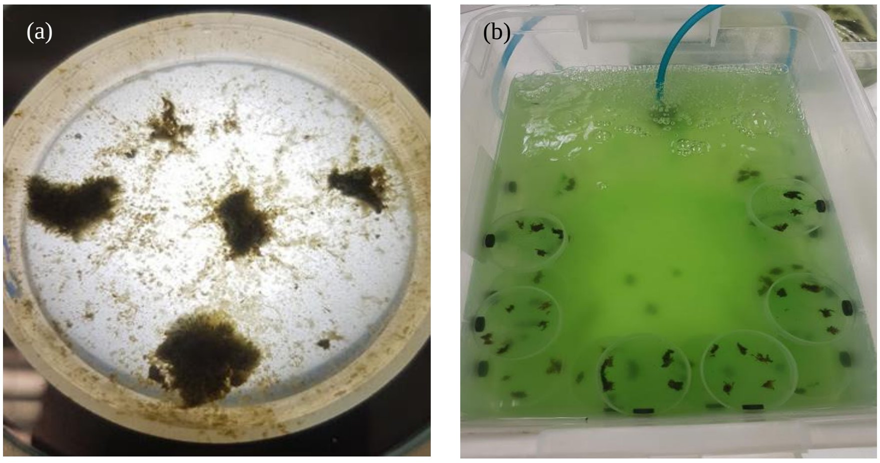


Figure 3. (a) Colonies of *P. casmiana* (Oka, 1907) fully established in a Petri dish; and (b) Petri dishes attached with paired magnets to the walls of a container

Water samples (n=3) from oxidation pond 2 were examined for several parameters. We measured temperature (ºC), pH, dissolved oxygen (mg/L) and conductivity (µs/cm) using a multimeter analyzer (WTW ProfiLine Cond 3310). Plant nutrients (mg/L) were also measured: soluble reactive phosphorus (SRP) by ascorbic acid method, ammonium nitrogen by phenol hypochlorite method, total nitrogen by the Kjeldahl method and total phosphorus by the vanadomolybdate method. Phytoplankton was collected by filtration of 10 L of water through a plankton net (20-micron mesh). Phytoplankton species were identified and counted for density (unit/L) using Sedgewick Rafter counting chamber. Analysis of water and biological samples was determined at Department of Environmental Technology and Management, Faculty of Environment, and Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok.

2.3 Efficiency of wastewater treatment by bryozoans

In this experiment, we explored the role of the freshwater bryozoan *P. casmiana* in wastewater treatment based on the method of Nimtim et al. (2020). The experiment included a control (without bryozoans) and an experimental treatment with bryozoans, containing around 100-150 zooids. Both the experimental and control treatments were done in triplicate. The water used in the experiment was taken from oxidation pond 2. Water was poured into 18 beakers of 1,000 mL (Figure 4). Each beaker was aerated at a constant rate to circulate food particles throughout water column and to maintain oxygen level, and was kept at room temperature (30-35ºC) with light: dark (L:D) conditions of 12:12 h. We measured turbidity using a turbidity meter (WTW Turb® 430 IR) and measured chlorophyll a by an acetone extraction method, with wavelengths determined by spectrophotometer. Turbidity and chlorophyll a were measured at 0, 24, 48, and 72 h. BOD_5 measurement was done by Azide modification method at 0 and 72 h to compare the values between the beginning and end of the experiment. Wastewater treatment efficiency was calculated using the formula [(In-Out)/In] × 100, where In is the water quality before the experiment and Out is the water quality after the experiment. The growth of bryozoans was estimated by counting the number of zooids at 0, 24, 48, and 72 h, and then the average daily growth rate of bryozoans (ADG) was estimated using the formula (W2-W1)/time, where W1 is the number of zooids before the experiment and W2 is the number of zooids after the experiment. Water chemistry analysis was conducted in the laboratories of the LERD Project and of Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok.

2.4 Feeding experiment of bryozoans using protozoans as diet

The bryozoans used in this study were cultured using the same procedure as described in section 2.2. For protozoan culture, water collected from oxidation pond 2 was poured into 50 mL Petri dishes and then three boiled mung bean seeds were added to each dish as a food source for the protozoans (Figure 5). The protozoans were grown for 3-5 days before they were used to feed the bryozoans. After 3-5 days, the water in the Petri dishes became milky white, indicating a dense population of protozoans (Figure 5). The density of cultured protozoans per 1 mL was calculated to obtain an estimate of the number of protozoa.

Figure 4. Experimental setup to investigate wastewater treatment efficiency by bryozoans

We divided the experiment into a control and experimental treatment, both performed in triplicate. The bryozoan colonies were placed in Petri dishes. In the control, three Petri dishes with colonies of bryozoans were attached to the wall of a 600 mL beaker and the bryozoans were fed daily by phytoplankton in water from oxidation pond 2 (600 mL of pond water).

In the experimental treatment, three Petri dishes with colonies of bryozoans were used and protozoans were provided as bryozoan diet. Prior to the experiment, pond water (500 mL) was taken from oxidation pond 2 and left for five days (we waited until phytoplankton in the water died and settled to the bottom of beaker). The resulting clarified water without phytoplankton was then mixed with protozoans obtained from our protozoan culture. On day 0, the bryozoans were fed with protozoans (500 mL of clarified pond water + 100 mL of protozoans). During days 1-5, 50 mL of protozoans was added to the beaker containing Petri dishes. The experiment was conducted for six days. The feeding of bryozoans on the protozoans was observed and photographed under a light microscope (Leica DM500).

We measured the growth rate of bryozoans by counting the number of zooids on days 0 to 5 under a stereo microscope, and then estimated the average daily growth rate. We also examined the fecal pellets produced by the bryozoans for both the control and experimental treatment. A pointed dropper was used to collect fecal pellets from the Petri dishes. The shape, appearance, color, and detail of fecal pellets were examined under a microscope at 400X magnification (Wood, 2021) and fecal pellets were photographed and compared.

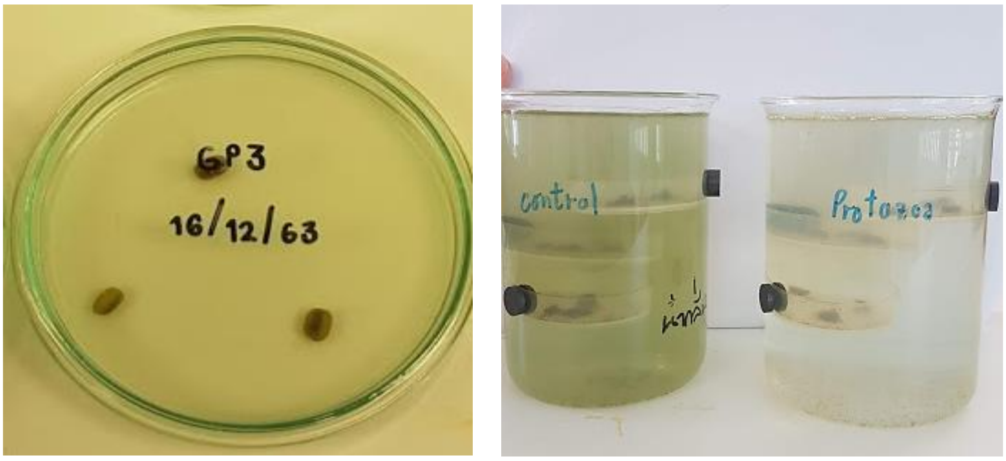


Figure 5. Culture of protozoans in Petri dish (left); and bryozoan feeding experiment using protozoans as main diet (right)

Data were analyzed using descriptive statistics (mean and standard deviation). Error bars are also presented in bar and line graphs. A statistical test was applied for comparing the control and the experimental treatment using the Student's t-test. Means with a significance value of less than 0.05 were considered significantly different. The statistical analysis was performed using the IBM SPSS Statistics software, authorized user version 22.

3. RESULTS

3.1 Efficiency of wastewater treatment by bryozoans

We gathered general information on water chemistry and phytoplankton communities in oxidation pond 2 since water from this pond was used in our experiments. Water chemistry results are presented in Table 1. Temperature, pH, conductivity, and dissolved oxygen were in suitable ranges for bryozoan survival and growth. Nutrient concentrations were high, especially nitrogen. Oxidation pond 2 was classified as hypereutrophic (TP>0.1 mg/L and TN>1.5 mg/L).

Phytoplankton were classified into two divisions (Table 2). Cyanobacteria represented the largest group in oxidation pond 2, with *Spirulina platensis* as the most abundant species followed by the genera *Oscillatoria* and *Microcystis*. High nutrient concentrations corresponded well with high densities of phytoplankton.

Table 1. Water quality measurements (mean±SD) in oxidation pond 2

Parameter	Value
Temperature (°C)	30.69±0.71
pH	8.44±0.49
Dissolved oxygen (mg/L)	5.28±1.98
Conductivity (μs/cm)	496.63±16.49
NH_4^+-N (mg/L)	0.51±0.03
SRP (mg/L)	0.64±0.03
Total nitrogen (mg/L)	2.92±0.00
Total phosphorus (mg/L)	0.67±0.00

Table 2. Species and densities of phytoplankton in oxidation pond 2

Phytoplankton	Density (unit/L)
Division Cyanophyta	
Class Cyanophyceae (Blue-green algae)	
Merismopedia sp.	200
Microcystis sp.	15,050
Spirulina platensis (Nordstedt) Geitler	1,062,500
Oscillatoria sp.	85,500
Anabaena sp.	6,025
Division Chlorophyta	
Class Chlorophyceae (Green algae)	
Pandorina morum (Müller) Bory	150
Tetraedron gracile (Reinsch) Hansgirg	50
Tetraedron hastatum (Reinsch) Hansgirg	75
Tetraedron trigonum (Naegeli) Hansgirg	175
Radiococcus sp.	425
Actinastrum hantzschii Lagerheim	75
Coelastrum astroideum De Notaris	200
Coelastrum microporum Nägeli	75
Crucigenia sp.	800
Scenedesmus acuminatus (Lagerheim) Chodat	100

Table 2. Species and densities of phytoplankton in oxidation pond 2 (cont.)

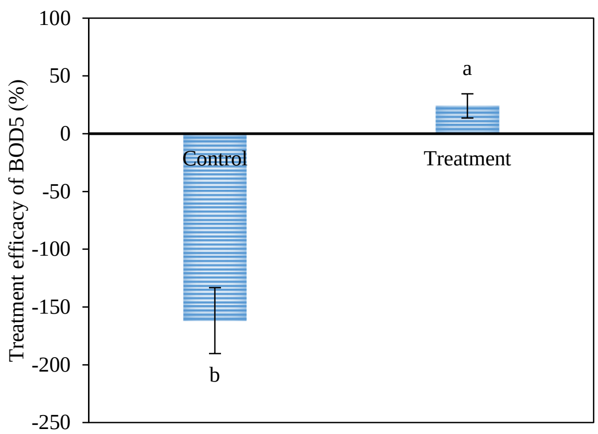
Phytoplankton	Density (unit/L)
Scenedesmus quadricauda (Turpin) Brébisson	225
Scenedesmus sp.	125
Pediastrum duplex Meyen	100
Pediastrum simplex (Meyen) Lemmermann	50
Class Euglenophyceae (Euglenoids)	
Euglena sp.	1,275
Lepocinclis ovum (Ehrenberg) Lemmermann	25
Phacus longicauda (Ehrenberg) Dujardin	25
Trachelomonas hispida (Perty) Stein	25

The wastewater treatment efficiency of *P. casmiana* was determined by measuring the change in BOD_5, chlorophyll a concentration, and turbidity before and after the experiment. The results are shown in Table 3. In the experimental treatment (with bryozoans), after 72 h, the levels of BOD_5, chlorophyll a and turbidity were reduced. In the control (without bryozoans), chlorophyll a and turbidity values were reduced, while BOD_5 was elevated.

Statistical analysis revealed that after 72 h, the BOD_5 and turbidity levels between the control and treatment were significantly different at the 95% confidence level. In the control, treatment efficiency levels for BOD_5 and turbidity were -161.77±49.39% and 24.09±8.74%, respectively. In the experimental treatment, treatment efficiency levels for BOD_5 and turbidity were 24.04±18.13% and 59.21±15.40%, respectively. The efficiency of chlorophyll a removal in the control and experimental treatment was 36.89±5.30% and 55.13±14.34% (Figure 6).

Table 3. Water quality between control without bryozoans and experimental treatment with bryozoans

Variable	Prior to experiment	After experiment	
		Control	Treatment
BOD_5 (mg/L)	7.30±1.05		
- After 72 h		18.8±1.97	5.2±1.03
Chlorophyll a (mg/L)	0.27±0.01		
- After 24 h		0.37±0.01	0.37±0.02
- After 48 h		0.36±0.01	0.30±0.02
- After 72 h		0.17±0.01	0.11±0.04
Turbidity (NTU)	33.7±1.16		
- After 24 h		34.90±0.30	31.20±0.30
- After 48 h		31.63±0.49	22.50±3.06
- After 72 h		25.27±2.11	12.36±4.24



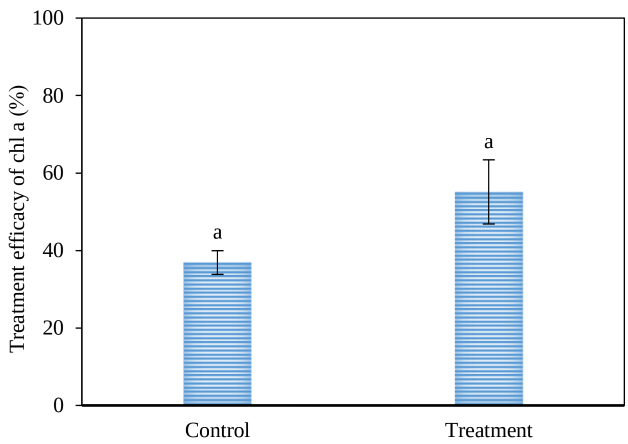


Figure 6. Comparison of wastewater treatment efficiency (BOD_5, turbidity and chlorophyll a) between control and experimental treatment (mean values with error bars). Different letters (a and b) above columns indicate significant differences between control and experimental treatment.

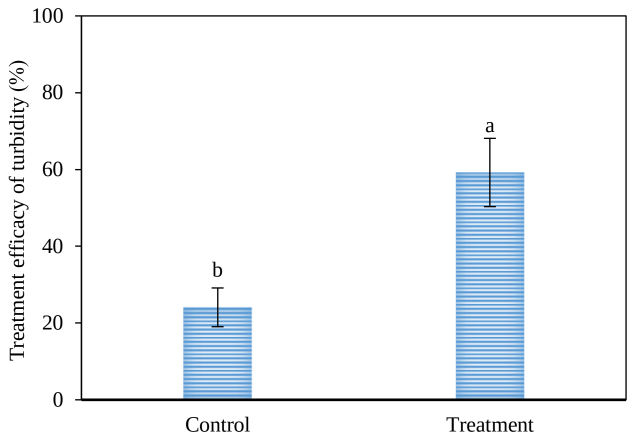


Figure 6. Comparison of wastewater treatment efficiency (BOD_5, turbidity and chlorophyll a) between control and experimental treatment (mean values with error bars). Different letters (a and b) above columns indicate significant differences between control and experimental treatment (cont.).

The results showed that during the experiment, the bryozoan growth rate increased over time (Figure 7). The average daily growth rate of bryozoans was 54±22 zooids per day.

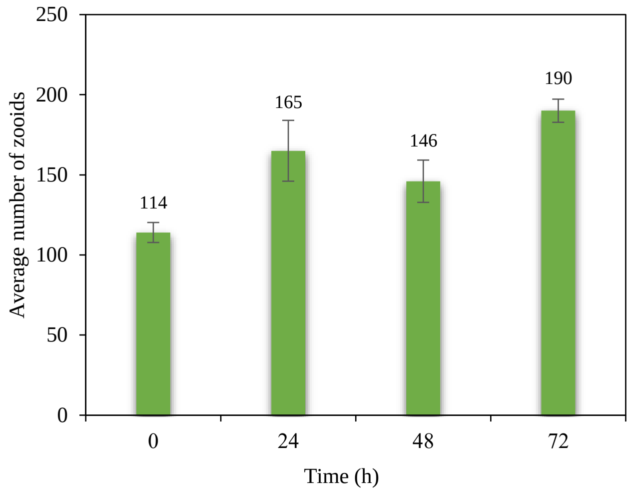


Figure 7. Growth rate of bryozoans (based on number of zooids) during 72 h of feeding experiment (mean values with error bars)

3.2 Feeding studies of bryozoans using protozoa

In this experiment, protozoans were cultured as diet for bryozoans. The protozoans were identified as members of the Phylum Ciliophora (*Colpidium, Paramecium, Tetrahymena*). The density of protozoans was about 1,600 individuals/mL. The bryozoans were able to feed successfully on the protozoans. The lophophore of a bryozoan aids in waving protozoans into the mouth and further into the digestive system (Figure 8). The feeding rate of bryozoans was approximately 2 individual protozoans per minute during the experiment.

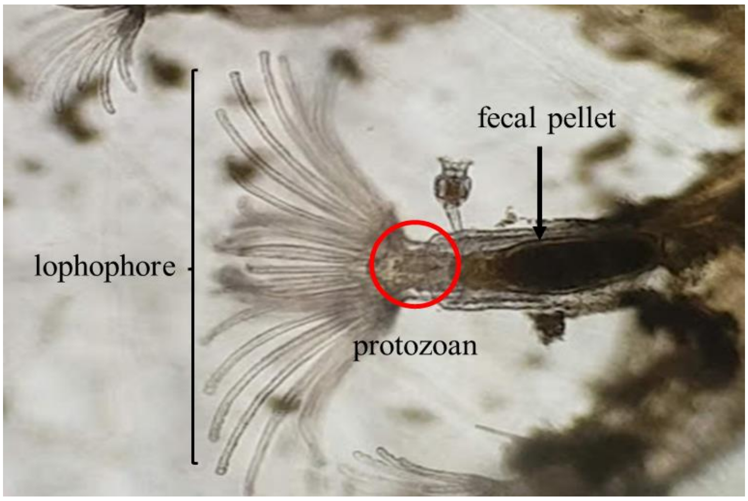


Figure 8. Protozoan (in red circle) entering the gastrointestinal tract of a bryozoan and presence of a fecal pellet (arrow) inside the bryozoan. Length of bryozoan is approximately 0.5 mm (Wood, 2021).

We examined the characteristics of fecal pellets produced by bryozoans feeding on protozoans and compared them with fecal pellets of phytoplankton-fed bryozoans in the control. The fecal pellets of protozoan-fed bryozoans were translucent and consisted of large fragments (Figure 9(a)), indicating incomplete digestion of the protozoans. In contrast, the fecal pellets of bryozoans in the control contained greenish particles from ingested phytoplankton. This was seen as a large mass of unidentified microscopic cells (Figure 9(b)).

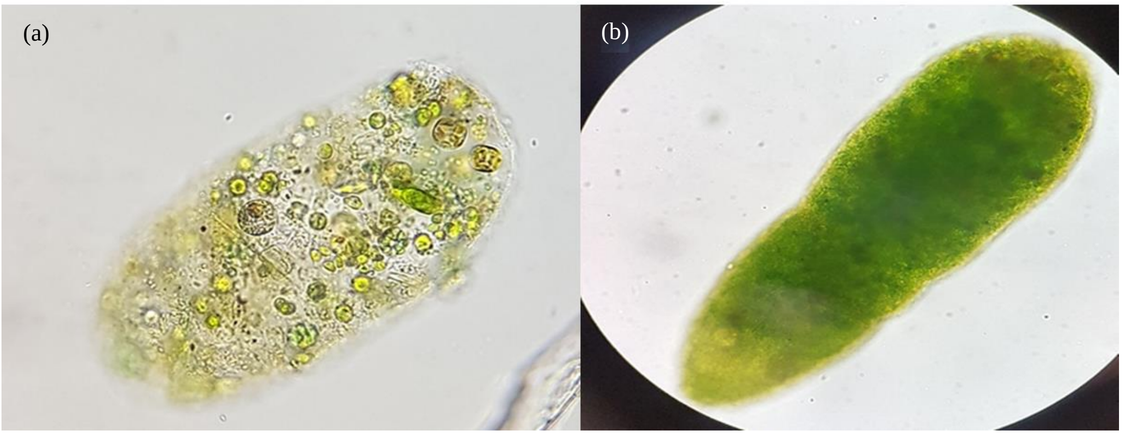


Figure 9. Fecal pellet of protozoan-fed bryozoan (a) and fecal pellet of phytoplankton-fed bryozoan in the control (b). Length of fecal pellet is approximately 100 µm (Wood, 2021).

Figure 10 shows the growth rates of protozona-fed bryozoans compared to bryozoans in the control that were fed with phytoplankton from oxidation pond 2. Based on the number of zooids, bryozoans in the control had relatively higher growth than protozoan-fed bryozoans, for which growth tended to decrease gradually throughout the experiment. The average daily growth rates of protozoan-fed bryozoans and phytoplankton-fed bryozoans were -20±6 and 19±34 zooids per day.

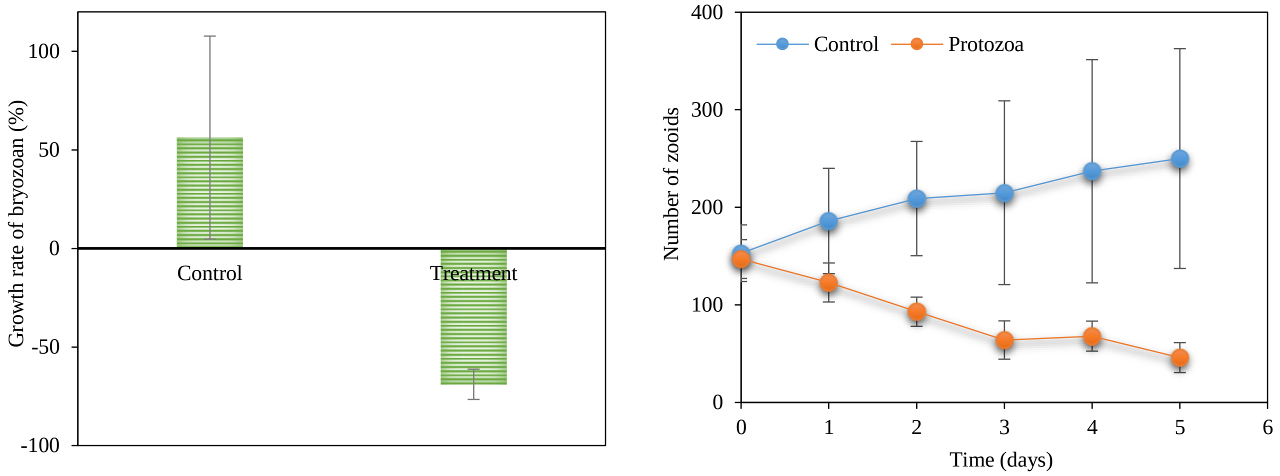


Figure 10. Comparison of growth rates of bryozoans between control (bryozoans fed with phytoplankton) and treatment (bryozoans fed with protozoans) (mean values with error bars)

4. DISCUSSION

Bryozoans are invertebrate animals that have the potential to be biological filters (Rutkauskaitė-Sucilienė and Šatkauskienė, 2016) and are part of the cycling of organic matter (Todini et al., 2018) in wastewater treatment ponds. The present study showed that in the laboratory, water quality (BOD_5 and turbidity in particular) in the LERD Project tended to improve in the presence of bryozoans. The increase of BOD_5 in the control may be linked to an increase in organic components from different sources such as phytoplankton, zooplankton and other microscopic organisms (Minnesota Pollution Control Agency, 2008). Further research should investigate the population dynamics and composition of microbial communities in wastewater ponds. The improvement in water quality in the experimental treatment could have resulted from the filter-feeding activity of the bryozoans, reducing the number of organic particles, small organisms and perhaps dissolved organic materials (Wood, 2021). The lophophores of bryozoans are used to carry these organic food materials into the mouth (Riisgård and Manríquez, 1997), with the ingested suspensions used for bryozoan growth and reproduction (Tamberg and Shunatova, 2016). Increasing growth rates of bryozoans toward the end of the experiment corresponded well with the continuous improvement in water quality. This was consistent with a previous study at the LERD Project, in which the filtration rates of suspended particles by bryozoans (*P. casmiana* and *P. vorstmani*) were as high as 74% (Nimtim et al., 2020). When food is digested, bryozoans excrete small oval fecal pellets that themselves are an important food source for aquatic life in natural systems, such as planktonic and benthic organisms (Orellana et al., 2019; Wood, 2019). Therefore, it is suggested that bryozoan communities in wastewater treatment ponds should be maintained for the benefit of water quality improvement. However, there are many factors that should be considered to support the colonization of bryozoans, such as control of water level and environmental conditions. In addition, bryozoan colonies in wastewater treatment ponds should be protected from fish predation, such as by putting fine screening over the colonies.

The food sources of bryozoans are diverse, such as plankton and organic matter suspended in water. Protozoans are also an alternative important diet for bryozoans (Wood, 2015), especially those that are smaller in size, such as *Paramecium*. In the present study, protozoans were offered as diet for the bryozoans, and we confirmed that *P. casmiana* can consume protozoans. This was consistent with a recent experimental feeding study by Wood (2021), revealing that several bryozoan species (*Fredericella indica* Annandale, 1909, *L. carteri* Hyatt, 1866) and *Plumatella emarginata* Allman, 1844) ingested protozoans. After digestion, the bryozoans released fecal pellets that were composed of digested and undigested protozoans. In contrast, bryozoans that were fed with phytoplankton released fecal pellets that had different characteristics in terms of color and internal composition. We found that the fecal pellets of phytoplankton-fed bryozoans contained densely packed, tiny phytoplankton cells. These tiny green particles in fecal pellets could be *Spirulina platensis* (Gomont) Geitler 1925, which is a cyanobacterium found predominantly in wastewater treatment ponds at the LERD Project (Nimtim, 2020). The fecal pellets of phytoplankton-fed bryozoans in the present study were similar to those in the study by Wood (2021), who described green fecal pellets of *Fredericella indica* after feeding on cultures of *Chlamydomonas* sp. Thus, the composition and characteristics of fecal pellets depend on the food types consumed by the bryozoans, and these can be distinguished under a microscope.

The growth rates of the protozoan-fed bryozoans were relatively slow, perhaps because the bryozoans could not break apart larger protozoans in the gut. Furthermore, the presence of some undigested particles in the fecal pellet may be the result of those items spending little time in the stomach. Therefore, the full complement of nutritional substances could not be absorbed and used for growth. The ability of bryozoans to capture food in water is another important factor in their growth. Because protozoans move quickly, bryozoans may have difficulty capturing them. Previous research also showed factors that influence the capture of particles by bryozoans, including characteristics of the food (such as type, size, and concentration) (Pratt, 2008; Todini et al., 2018). Okamura and Doolan (1993) suggested that large colonies of bryozoans ingested greater numbers of particles than small colonies, and that feeding of both large and small colonies depended on flow rate (feeding increased with flow). In contrast, the bryozoans that fed on phytoplankton tended to grow faster because the water from the pond offered a greater variety of food sources, especially phytoplankton (together with zooplankton, protozoans and other microscopic organisms) that are the staple foods of bryozoans (Amui-Vedel et al., 2007). In particular, dominant *S. platensis* can provide high nutritional value and may have produced the fast growth of bryozoans in this experiment, although the food eaten passes through the gut in less than an hour and looks apparently intact in fecal pellets (Cancino et al., 2019). However, the results from the present study differed from Wood (2021), who reported that phylactolaemate bryozoans (different species from this study) are carnivorous and feed on protozoans and other small zooplankton, such as rotifers, from which they get most of their nutrition and results in faster growth than those with any other diet. The results of this research on the diet of bryozoans have demonstrated that bryozoans are omnivorous and can be a link to the transfer of energy in ecosystems. In addition, bryozoans can regulate population dynamics not only of phytoplankton but also of protozoans and possibly other microscopic animals in the wastewater treatment system.

5. CONCLUSION

This study has highlighted the role of the freshwater bryozoan *P. casmiana* in wastewater treatment at the LERD Project. It was clearly demonstrated that bryozoans aid in improving the water quality, especially in terms of BOD_5 and turbidity. This is because suspended organic food particles in water are removed through filtration by bryozoans. Therefore, maintaining communities of bryozoans in wastewater treatment ponds can be beneficial to overall wastewater treatment efficiency. This study also has provided further insights into the biology and ecology of bryozoans in terms of their diet. It was demonstrated that protozoans can be a food source for bryozoans. However, protozoan-fed bryozoans had slower growth rates than those feeding on phytoplankton. This may be due to incomplete digestion of protozoans by bryozoans, as fecal pellets of protozoan-fed bryozoans contained some undigested particles. More research is still needed on the effects of wastewater contaminants (such as heavy metals, pharmaceuticals, and personal care products) on the growth, reproduction and survival of bryozoans in wastewater treatment systems.

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REFERENCES

Amui-Vedel A-M, Hayward PJ, Porter JS. Zooid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. Journal of Experimental Marine Biology and Ecology 2007;353:1-12.

Cancino JM, Orellana MC, Wood TS, Chaichana R. An energy budget for *Plumatella casmiana* Oka, 1907 at Laem Phak Bia, Thailand. Proceedings of the 18th International Bryozoology Association Conference; 2019 June 16-22; Technical University of Liberec, Liberec: Czech Republic; 2019.

Chaichana R, Dampin N. Unialgal blooms of cyanobacteria in oxidation ponds of the King’s Royally Initiated Laem Phak Bia Environmental Research and Development Project, Thailand. EnvironmentAsia 2016;9:150-7.

Gorgoglione B, Kotob MH, El-Matbouli M. Migrating zooids allow the dispersal of *Fredericella sultana* (Bryozoa) to escape from unfavourable conditions and further spreading of *Tetracapsuloides bryosalmonae*. Journal of Invertebrate Pathology 2016;140:97-102.

Hirose M, Dick MH, Mawatari SF. Are *Plumatellid statoblasts* in freshwater bryozoans phylogenetically informative? Zoological Science 2011;(5):318-26.

Kocak F, Kucuksezgin F, Bakal I. Impact of environmental variables on fouling bryozoan species in the Eastern Aegean Sea. Marine Pollution Bulletin 2019;141:46-51.

Madoni P. Protozoa in wastewater treatment processes: A mini review. Italian Journal of Zoology 2011;78(1):3-11.

Massard JA, Geimer G. Global diversity of bryozoans (Bryozoa or Ectoprocta) in freshwater. Hydrobiologia 2007;595:93-9.

Minnesota Pollution Control Agency. Turbidity: Description, Impact on Water Quality [Internet]. 2008 [cited 2022 May 15]. Available from: https://www.pca.state.mn.us/sites/default/files/wq-iw3-21.pdf.

Morris DJ, Morris DC, Adams A. Development and release of a malacosporean (Myxozoa) from *Plumatella repens* (Bryozoa: Phylactolaemata). Folia Parasitologica 2002;49(1):25-34.

Nimtim M, Chaichana R, Wood TS. Role of freshwater bryozoans in wastewater treatment ponds at Laem Phak Bia Environmental Research and Development Project site, Phetchaburi Province, Thailand. Agriculture and Natural Resources 2020;54:649-56.

Nimtim M. Ecology and Roles of Bryozoans in Wastewater Treatment System at Laem Phak Bia Environmental Study and Development Project Phetchaburi Province [dissertation]. Thailand: Kasetsart University; 2020.

Okamura B, Doolan L. Patterns of suspension feeding in the freshwater bryozoan *Plumatella repens*. Biological Bulletin 1993;184:52-6.

Orellana MC, Cancino JM, Wood TS, Chaichana R. Feeding and faecal pellet production in *Plumatella casmiana* Oka, at Laem Phak Bia, Thailand. Proceedings of the 18th International Bryozoology Association Conference; 2019 June 16-22; Technical University of Liberec, Liberec: Czech Republic; 2019.

Pratt MC. Living where the flow is right: How flow affects feeding in bryozoans? Integrative and Comparative Biology 2008; 48(6):808-22.

Riisgård HU, Manríquez P. Filter-feeding in fifteen marine ectoprocts (Bryozoa): Particle capture and water pumping. Marine Ecology Progress Series 1997;154:223-39.

Rutkauskaitė-Sucilienė J, Šatkauskienė I. Species Composition of freshwater bryozoan (Bryozoa) in some regions of Lithuania. Proceedings of the 10th International Scientific Conference; 2016 May 19-20; Vilnius, Lithuania; 2016.

Ryland JS. Moss animal: Encyclopaedia Britannica, [Internet]. 2020 [cited 2022 Jan 11]. Available from: https://www. britannica. com/animal/moss-animal.

Seansupha S. Effect of Water Quality Change to Growth of Freshwater Bryozoans [dissertation]. Thailand: Kasetsart University; 2009.

Schwaha T, Hirose M, Wanninger A. The life of the freshwater bryozoan *Stephanella hina* (Bryozoa, Phylactolaemata): A crucial key to elucidating bryozoan evolution. Zoological Letters 2016;2:2-25.

Schwaha T, Ostrovsky AN, Wanninger A. Key novelties in the evolution of the aquatic colonial phylum Bryozoa: Evidence from soft body morphology. Biological Reviews 2020;95: 696-729.

Shrivastava P, Rao KS. Ecology of *Plumatella emarginata* (Ectoprocta: Phylactolaemata) in the surface waters of Madhya Pradesh with a note on its occurrence in the protected waterworks of Bhopal (India). Environmental Pollution Series A, Ecological and Biological 1985;39(2):123-30.

Tamberg Y, Shunatova N. Feeding behavior in freshwater bryozoans: Function, form, and flow. Invertebrate Biology 2016;135:138-49.

Todini C, Elia A, Selvaggi R, Scoparo M, Taticchi M. Food selection by *Plumatella geimermassardi* Wood and Okamura, 2004 (Phylactolaemata, Bryozoa). Knowledge and Management of Aquatic Ecosystems 2018;419(26):1-11.

Wood TS, Anurakpongsatorn P, Mahujchariyawong J. Freshwater bryozoans of Thailand (Ectoprocta and Entoprocta). The Natural History Journal of Chulalongkorn University 2006; 6(2):83-119.

Wood TS, Anurakpongsatorn P, Mahujchariyawong J. An Introduction to the Freshwater Bryozoans of Thailand. Bangkok, Thailand: Kasetsart University Press; 2010.

Wood TS. Bryozoa. In: Gene EL, editor. Encyclopedia of Inland Waters. Amsterdam, Netherlands: Elsevier; 2009. p. 250-9.

Wood TS. Chapter 16: Phyla ectoprocta and entoprocta (bryozoans). In: Thorp JH, Rogers DC, editors. Thorp and Covich's Freshwater Invertebrates. 4th ed. Massachusetts, USA: Academic Press; 2015. p. 327-45.

Wood TS. Digestion and nutrition in freshwater bryozoans (Phylactolaemata). Invertebrate Biology 2021;140(2):e12314.

Wood TS. Phylactolaemate bryozoans feed the zoobenthos. Australasian Palaeontological Memoirs 2019;52:163-7.

Wood TS. The pipeline menace of freshwater bryozoans. Denisia 2005;16:203-8.

Wright J. Bryozoa moss animals [Internet]. 2014 [cited 2022 Jan 11]. Available from: https://animaldiversity.org/accounts/Bryozoa.

Yepes-Narváez V. Environmental Ecology of Marine Bryozoans (Phylum Bryozoa) and Ascidians (Tunicata: Ascidiacea) Under Multi-stressor Scenarios [dissertation]. UK: Manchester Metropolitan University; 2020.

Indoor Air Quality in Public Health Centers: A Case Study of Public Health Centers Located on Main and Secondary Roadsides, Bangkok

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ABSTRACT

This cross-sectional study investigated the indoor air quality (IAQ) of public health centers (PHCs) and primary health care units in Bangkok to determine the impact of the different locations and ventilation systems concerning IAQ. Three indoor air pollutants: $PM_{2.5}$, CO_2, and CO were measured in three areas (medical record departments, outpatient departments and examination rooms) of six PHCs located in two different locations (main and secondary roadsides). The results showed that the average levels of $PM_{2.5}$, CO_2, and CO in the PHCs located on main roadsides were higher than those located on secondary roadsides. Among these parameters, only CO was found to significantly differ between those two locations indicating the result of vehicles and traffic sources regarding indoor CO level. Furthermore, all parameters were compared among the sampling areas with different ventilation systems; natural ventilation and air conditioner with and without ventilation fan. The amounts of all three pollutants significantly differed in each area with different ventilation systems. The average levels of $PM_{2.5}$ and CO_2 were the highest in areas with air conditioner without ventilation fan, while the level of CO was the highest in areas with natural ventilation. The ventilation was proved to be a key measure to improve IAQ. PHCs should consider ventilation efficacy to improve the IAQ by using ventilation fans in rooms using air conditioners. Finally, the average levels of all parameters were found below the recommended values in related standards, indicating safe IAQ for people working and receiving services in PHCs.

1. INTRODUCTION

Indoor air quality (IAQ) is one of the environmental risks concerning people's health, affecting their comfort and well-being when occupying a building (Luksamijarulkul et al., 2019). Currently, most people spend more than 90% of their time in various activities in indoor environments such as in an office, school, and their residence (Giulio et al., 2010). Thus, IAQ has become an important environment issue, especially in workplaces. Poor IAQ could lead to discomfort and sickness, and result in decreased work performance decrease and absenteeism in the workplace (Indoor Air Quality Management Group, 2019). Some equipment in buildings such as heaters, ventilation systems and air conditioners could cause high accumulation of air pollutants resulting in low IAQ (Brickus et al., 1998).

Public health centers (PHC) have roles and responsibilities to provide health services covering primary health care needs of people in Bangkok. Currently, 69 PHCs are distributed in 50 district areas in Bangkok to support convenient health services and reduce patient crowding in hospitals. In 2019, the monthly average number of patients totaled 2,423 (~80 people daily) depending on the service area of each center (Office of Public Health System Development, 2020). Moreover, PHCs are workplaces having strict requirements about IAQ because these places serve patients with communicable diseases who could spread pathogens through air transmission, so

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these places always use disinfectants inside the building (Hellgren et al., 2008). Thus, controlling IAQ remains an important role to prevent the spread of infectious diseases and protect hospital personnel and sensitive patients (Leung and Chan, 2006).

The location of the PHC is an important factor for patients to receive services, especially, PHCs located midtown on main roadsides that are easy to approach, and always receive many people requesting services. However, location has been found to be related to the potential sources of pollutants, such as busy roads, that affect the IAQ (Karner et al., 2010). A related study reported that people residing, working or attending school near major roads appeared to be at greater risk of a variety of short and long term health effects (Mullen et al., 2011). In Bangkok, major sources of air pollution include large numbers of vehicles, construction of buildings and sky train facilities, and open burning, especially grilling food using charcoal. Vehicles were indicated to be the main cause of air pollution problems in 2018; they generate high concentrations of pollutants including black smoke, nitrogen dioxide (NO_2), ozone gas (O_3), particulate matter with a diameter of 2.5 micrometers or less ($PM_{2.5}$), and particulate matter with a diameter of 10 micrometers or less (PM_{10}) causing problems in areas near roadsides (PCD, 2019).

Related research of IAQ in nine advanced level hospitals from 11 provinces in 2014 revealed that a high percentage of examination rooms from all hospitals exceeded the maximum permissible levels of air quality parameters, including CO_2 (15.59%), relative humidity (43.48%), temperature (87.68%), $PM_{2.5}$ (1.70%), formaldehyde (19.51%), bacteria (50.00%), and mold (25.75%) (Chokwinyou et al., 2014). More recently, a study measuring IAQ in Thailand concluded that the most problematic parameters included temperature, relative humidity and dust (PM_{10}, $PM_{2.5}$) due to activities of building users and insufficient ventilation (BOED, 2018). In addition, the Environmental Sanitation Office, Bangkok Metropolitan Administration conducted a survey to measure air quality in 27 buildings of PHCs in 2019 and found IAQ parameters in waiting rooms, OPDs and nursing laboratories of several PHCs were above the maximum permissible levels, including $PM_{2.5}$ (18.52% of total survey), PM_{10} (16.05% of total survey), and CO_2 (12.35% of total survey) exceeding the Singapore recommended values of less than 35 $\mu g/m^3$, 50 $\mu g/m^3$, and less than 1,000 ppm, respectively (Office of Public Health System Development, 2020).

This study aimed to reveal the IAQ of PHCs (involving three main parameters: $PM_{2.5}$, CO_2, and CO) located in different locations, main and secondary roadsides, which were compared to determine the effect of both locations on the IAQ. Results were also compared among different indoor areas to reveal the effect of ventilation on IAQ. The results can provide information to establish guidelines or suggestions to improve IAQ minimizing the health risk of the patient and staff in PHCs.

2. METHODOLOGY

2.1 Study area

This cross-sectional study was conducted in six of a total 69 PHCs in Bangkok from May to July, 2021 during the COVID-19 outbreak. These six sampling sites were selected using purposive sampling method and dividedg in two groups; three PHCs (PHC_A, PHC_B, and PHC_C) located on main roadsides, and another three PHCs (PHC_D, PHC_E, and PHC_F) located on secondary roadsides (small streets with two lanes) (Figure 1).

2.2 Data collection

In each PHC, samples were collected at three different areas including the medical records department (MRD), outpatient department (OPD), and examination room (EXR) hosting different activities, numbers of people and ventilation systems. Each sampling point was collected at two different service time intervals: 8.00 to 11.30 and 12.30 to 16.00. Triplicate samples were collected from three different weekdays; Monday, Thursday and Friday. For each sampling time, the three main parameters measured were $PM_{2.5}$, CO_2, and CO, and ventilation in the area was recorded while sampling. The sample sizes totaled 108 samples per parameter.

2.3 Indoor air quality measurement

The level of $PM_{2.5}$ was determined using a particle measurement instrument (DustTrak™ II Aerosol Monitor 8530). The instrument showed real-time aerosol mass readings with gravimetric sampling and zero calibration performed with a zero (HEPA) filter, before starting the sampling. The measurement ranged from 0.001 to 400 $mg/m^3$and particle size range was approximately 0.1 to 10 µm. CO and CO_2 were measured using an indoor air quality meter (HD37AB1347). The measurement range was 0 to 5,000 ppm for CO_2 and 0 to 500 ppm for CO. Both instruments were calibrated before initiating this study. For each measurement, the instruments were placed in the breathing zone at a height of 90 to 100 cm above the floor and in the middle of the room.

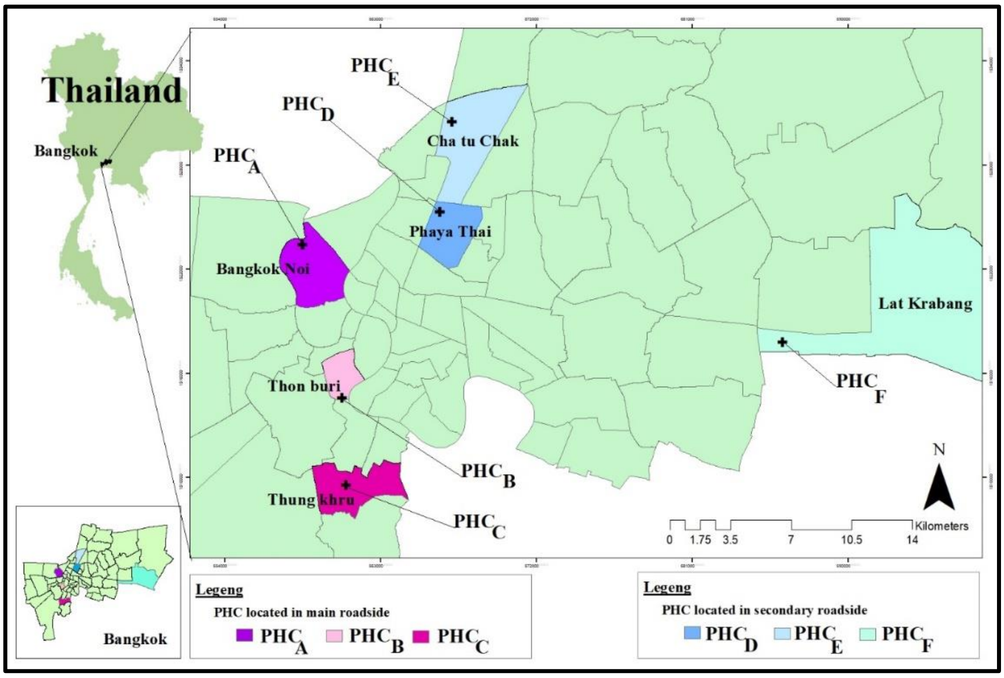

Figure 1. Map of sampling sites, Public Health Centers in Bangkok, Thailand

2.4 Statistical analysis

Descriptive statistics were used to illustrate PHC characteristics. The normal distribution of the results was checked using the Kolmogorov-Smirnov test, and each sample was assumed to be drawn from a non-normal distribution. The results obtained for IAQ parameters ($PM_{2.5}$, CO_2, and CO) were compared using the Mann-Whitney U test for different locations (main and secondary roadsides). The Kruskal-Wallis Test was used to compare the results of all measurements between different ventilations (natural ventilation, air conditioners without ventilation fan and air conditioners with ventilation fans). The critical level for statistical significance was used as $p<0.05$ and all data were analyzed using SPSS, Version, 18.0.

3. RESULTS AND DISCUSSION

3.1 Ventilation of the sampling areas in PHCs

In the selected PHCs, all the buildings were up to 30 years old. The sampling areas used different ventilation systems during sampling although they were located in the same type of sampling area: MRD, OPD or EXR. Most had air conditioners (83.3%) but some areas in some PHCs had natural ventilation provided by windows and doors (16.7%). Ventilation systems of the sampling areas could be divided in three types: natural ventilation, air conditioner without ventilation fan and air conditioner with ventilation fan as shown in Table 1.

Table 1. Ventilation systems of the areas during sampling (N=108)

Type of ventilation	Sampling area			Total (%)
	MRD	OPD	EXR	
Natural ventilation	0	7	11	18 (16.7%)
Air conditioner without ventilation fan	31	14	22	67 (62.0%)
Air conditioner with ventilation fan	5	15	3	23 (21.3%)
Total	36	36	36	108 (100%)

3.2 Comparison of IAQ between groups of PHCs with different locations

The IAQ in two groups of PHCs, located on main and secondary roadsides were compared to investigate the effect between those locations on IAQ in PHCs and the result are shown in Table 2. The average levels of $PM_{2.5}$, CO_2, and CO in the PHCs located on main roadside were higher than those located on secondary roadside which proved that these different locations had an effect on IAQ in PHCs. However, only the CO was found to differ significantly between these two groups ($p<0.05$); the CO in PHCs on the main roadside was higher due to the higher traffic volume which was the main source of CO from the road. Babayiğit et al. (2014) reported in a case study of IAQ at primary schools in Turkey that the higher level of CO in schools located on a main street and close to heavy traffic significantly differed from the level in schools far from heavy traffic. However, Chamseddine et al. (2019) studied the indoor CO level from three hospitals in Beirut, Lebanon and found that the CO level at the lower floor was slightly higher than those at the upper floor primarily from the result of outdoor vehicle induced emission.

3.3 Comparison of IAQ in the sampling areas with different ventilation systems

To investigate the effect of ventilation on IAQ, the results of $PM_{2.5}$, CO_2, and CO measurement in each sampling area among three different ventilation systems were compared as shown in Table 3. The results of all $PM_{2.5}$, CO_2, and CO significantly differed in each area with different ventilation systems ($p<0.05$) indicating effect of ventilation systems on these indoor pollutants.

Table 2. Comparison of IAQ parameters in PHCs between the groups located on the main and secondary roadside

IAQ Parameter	Location of PHC		p-value
	Main roadside, (n=54)	Secondary roadside, (n=54)	
$PM_{2.5}$ ($\mu g/m^3$)	10.4±5.1	9.0±3.0	0.12
CO_2 (ppm)	777.0±290.1	668.4±119.4	0.13
CO (ppm)	2.5±1.4	1.6±0.8	<0.001*

*: Mann-Whitney U test

Table 3. Comparison of IAQ parameters among sampling areas in PHCs with different ventilation systems

IAQ parameter	Type of ventilation			p-value
	Natural ventilation, (n=18)	Air conditioner without ventilation fan, (n=67)	Air conditioner with ventilation fan, (n=23)	
$PM_{2.5}$ ($\mu g/m^3$)	9.4±3.3	10.5±4.7	7.7±2.6	0.034*
CO_2 (ppm)	538.3±62.0	793.1±253.6	662.0±94.8	<0.001*
CO (ppm)	2.5±0.9	2.2±1.4	1.2±0.6	0.001*

*: Kruskal- Wallis Test

For $PM_{2.5}$, the highest average level was found in area with air conditioners without ventilation fan (10.5±4.7 $\mu g/m^3$), following by natural ventilation (9.4±3.3 $\mu g/m^3$) and air conditioner with ventilation fan (7.7±2.6 $\mu g/m^3$), respectively. The highest average level in the area with air conditioner without ventilation fan was due to the accumulation of fine particles from outdoor sources such as diesel vehicles with no or less ventilation to the outside. However, the ventilation fan proved to reduce $PM_{2.5}$ level in the area with air conditioner. Lomboy et al. (2015) studied the characterization of $PM_{2.5}$ in an urban tertiary care hospital in the Philippines and found that the level of $PM_{2.5}$ in the mechanically ventilated wards were predominantly affected by the air conditioning system, human activities and the infiltration of contaminated outdoor air. Furthermore, a case study in Bangkok regarding the relationship between indoor and outdoor $PM_{2.5}$ concentrations in different conditions (open and closed ventilation systems) reported the $PM_{2.5}$ concentrations in the closed ventilation area in each place in terms of Indoor/Ambient ratio (I/A ratio) ranged from 0.37 to 3.57, indicating that outdoor $PM_{2.5}$ can enter the buildings through the ventilation system and infiltration (Sompornrattanaphan et al., 2018). Similarly, indoor $PM_{2.5}$ concentrations in noncentral air-conditioned hospitals were reported to be associated with outdoor concentrations indicating the impact of ambient air quality in urban centers on IAQ (Nimra et al., 2021). Lee et al. (2020) studied the intervention effect on IAQ in hospitals and found that only $PM_{2.5}$ concentrations decreased in rooms employing an air-cleaner. However, the highest average level of $PM_{2.5}$ at 10.5±4.7 μg/m^3 in this study was still below 15 μg/m^3 (24-hours) following the new WHO air quality guidelines (WHO, 2021) indicating low risk from $PM_{2.5}$ exposure inside the PHC building.

For CO_2, the highest average level (793.1±253.6 ppm) was found in the area with air conditioner without ventilation fan followed by air conditioner with ventilation fan (662.0±94.8 ppm) and natural ventilation (538.3±62.0 ppm). High CO_2 levels were detected in some areas, the maximum level at 1,708 ppm, which was higher than 1,000 ppm, indicating inadequate ventilation in the building (NIOSH, 1987). The source of indoor CO_2 was from people inside the building, both patients and staff under inadequate ventilation of the area resulting in indoor CO_2 accumulation. The natural ventilation via door and window openings proved to be the best choice to reduce indoor CO_2 effectively. Furthermore, appropriate outpatient appointment systems limiting and distributing the patient number during service times can be applied to reduce occupant density and time patients spent in the room. An onsite measurement of indoor CO_2 in the outpatient waiting rooms at a Chinese hospital revealed that CO_2 level varied with people density and measured CO_2 concentration exceeded the threshold of 1,000 ppm in hospital departments that were too crowed and during rush hour period (Tang et al., 2020). However, a related study of indoor CO_2 in another hospital in China, found that the cause of indoor CO_2 level related to patient habits to maintain thermal comfort including closing and opening windows or doors and turning the air-conditioner on and off. Increasing indoor CO_2 level resulted from the closed rooms where indoor air could not be diluted by fresh air from outside (Zhou et al., 2015). Similarly, Argunhan and Avci (2018) evaluated IAQ of the university classrooms in Turkey and found that increased CO_2 levels were associated with the number of people in the area together with closing doors and windows. However, Zuraimi et al. (2007) studied the effect of ventilation strategies of child care centers on IAQ in Singapore and found that the ventilation rates of the air conditioners were significantly lower than those of natural ventilation resulting in higher levels of occupant-related pollutants such as CO_2. In addition, the highest average level of CO_2 at 793.1±253.6 ppm was still below the threshold limit value of 5,000 ppm as the American Conference of Governmental Industrial Hygienists standard (ACGIH, 2013) indicating low risk from CO_2 exposure inside the PHC buildings.

For CO, the highest average level in the area with natural ventilation was 2.5±0.9 ppm, followed by air conditioner without ventilation fan (2.2±1.4 ppm) and air conditioner with ventilation fan (1.2±0.6 ppm). The highest average level in areas with natural ventilation was due to the outdoor source affecting IAQ. Ventilation fans were proven to reduce CO in the area with air conditioners. Mendes et al. (2013) studied the IAQ in elderly care centers in Portugal and found that maximum CO level was in the room with an open window next to a heavily trafficked road. Similarly, Synnefa et al. (2003) investigated the IAQ in 15 school buildings in Athens, Greece and found that CO level in several classrooms was higher than the recommended limits due to insufficient natural ventilation and lack of mechanical ventilation. However, the highest average level of CO at 2.5±0.9 ppm was still below the threshold limit value of 25 ppm (8 h) as the ACGIH standard (ACGIH, 2013) indicating low risk from CO exposure inside the PHC building.

Finally, an overview of the results in Table 3 revealed that better ventilation could provide better IAQ by reducing indoor air pollutants. Areas using air conditioner with ventilation fan had lower levels of $PM_{2.5}$, CO_2, and CO comparing with those areas without ventilation fan. Thus, ventilation fan openings in room using air conditioners should be employed to reduce health risks from indoor air pollutants. For the areas using natural ventilation, all three parameters were also determined in safe levels for people's health. However, the results in this study were from a survey in a short period which did not represent conditions all the year round, especially during the peak period of $PM_{2.5}$ in winter. Thus, the long term study covering the peak period is of interest for the further study.

4. CONCLUSION

Safety and health of staff and patients from $PM_{2.5}$, CO_2, and CO when working and receiving services in PHCs was ensured by the results in this study. Although all average levels of $PM_{2.5}$, CO_2, and CO were higher in PHCs located on the main roadside, only CO significantly differed between both locations

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which could indicate the sources from vehicle and traffic outside the buildings have an effect on the IAQ. However, comparing IAQ among three different ventilation systems, the average levels of all measured parameters in the area with different ventilation systems significantly differed indicating the effect of ventilation on indoor air pollutants. Ventilation was proved to be a key measure to improve IAQ. For this reason, PHCs should consider ventilation efficacy to improve the IAQ by using ventilation fans in rooms using air conditioners during service times.

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REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH). Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, Ohio: ACGIH; 2013.

Argunhan Z, Avci AS. Statistical evaluation of indoor air quality parameters in classrooms of a university. Advances in Meteorology 2018;2018;Article No. 4391579.

Babayiğit MA, Bakir B, TEKBAŞ ÖF, Oğur R, Kilic A, Ulus S. Indoor air quality in primary schools in Keçiören, Ankara. Turkish Journal of Medical Sciences 2014;44(1):137-44.

Brickus LS, Cardoso JN, Aquino Neto FR. Distributions of indoor and outdoor air pollutants in Rio de Janeiro, Brazil: Implications to indoor air quality in bayside offices. Environmental Science and Technology 1998;32(22):3485-90.

Bureau of Occupational and Environmental Diseases (BOED). Guide to measuring indoor air quality for Entrance and exit channels. 1st ed. Bangkok, Thailand: Aksorn Graphic and Design; 2018.

Chamseddine A, Alameddine I, Hatzopoulou M, El-Fadel M. Seasonal variation of air quality in hospitals with indoor-outdoor correlations. Building and Environment 2019; 148:689-700.

Chokwinyou P, Pensuk C, Paweenkitiporn W. Indoor air quality in hospitals and hotels, Thailand. Environmental Health Journal 2014;16:51-66.

Indoor Air Quality Management Group. Guidance Notes for the Management of Indoor Air Quality in Offices and Public Places. Hong Kong: Government of the Hong Kong Special Administrative Region; 2003.

Giulio MD, Grande R, Campli ED, Bartolomeo SD, Cellini L. Indoor air quality in university environments. Environmental Monitoring and Assessment 2010;170(1):509-17.

Hellgren UM, Palomaki E, Lahtinen M, Riuttala H, Reijula K. Complaints and symptoms among hospital staff in relation to indoor air and the condition and need for repairs in hospital buildings. Scandinavian Journal of Work Environment and Health 2008;34(4):58-63.

Karner A, Eisinger DS, Niemeier D. Near-roadway air quality: Synthesizing the findings from real-world data. Environmental Science and Technology 2010;44(14):5334-44.

Lee HJ, Lee KH, Kim DK. Evaluation and comparison of the indoor air quality in different areas of the hospital. Medicine (Baltimore) 2020;99(52):e23942.

Leung M, Chan AH. Control and management of hospital indoor air quality. Medical Science Monitor 2006;12(3):17-23.

Lomboy MFTC, Quirit LL, Molina VB, Dalmacion GV, Schwartz JD, Suh HH, et al. Characterization of particulate matter 2.5 in an urban tertiary care hospital in the Philippines. Building and Environment 2015;92:432-9.

Luksamijarulkul P, Somjai N, Nankongnap N, Pataitiemthong A, Kongtip P, Woskie S. Indoor air quality at different sites of a governmental hospital, Thailand. Nursing and Palliative Care 2019;4:1-5.

Mendes A, Pereira C, Mendes D, Aguiar L, Neves P, Silva S, et al. Indoor air quality and thermal comfort: Results of a pilot study in elderly care centers in Portugal. Journal of Toxicology and Environmental Health 2013;76(4-5):333-44.

Mullen NA, Bhangar S, Hering SV, Kreisberg NM, Nazaroff WW. Ultrafine particle concentrations and exposures in six elementary school classrooms in northern California. Indoor Air 2011;21(1):77-87.

National Institute for Occupational Safety and Health (NIOSH). Guidance for Indoor Air Quality Investigations. Cincinnati, Ohio: NIOSH; 1987.

Nimra A, Ali Z, Nasir ZA, Tyrrel S, Safdar S. Characterization of indoor air quality in relation to ventilation practices in hospitals of Lahore, Pakistan. Sains Malaysiana 2021; 50(6):1609-20.

Office of Public Health System Development, Health Department, Bangkok Metropolitan Administration. Annual reports and key statistics 2019 Bangkok [Internet]. 2020 [cited 2021 Sep 11]. Available from: https://webportal.bangkok.go.th /health/Annual-reports-and-key-statistics.

Pollution Control Department (PCD), Ministry of Natural Resources and Environment. Booklet on Thailand State of Pollution 2018. 1st ed. Bangkok, Thailand: S. Mongkon Press Limited Partnership; 2019.

Sompornrattanaphan M, Thongngarm T, Tantilipikorn P, Kreetapirom P, Foo J. The contribution of outdoor fine particulate matter to indoor air quality in Bangkok Metropolitan Region, Thailand: Are indoor dwellers safe? Siriraj Medical Journal 2018;70(4):265-71.

Synnefa A, Polichronaki E, Papagiannopoulou E, Santamouris M, Mihalakakou G, Doukas P, et al. An experimental investigation of the indoor air quality in fifteen school buildings in Athens, Greece. International Journal of Ventilation 2003;2(3):185-201.

Tang H, Ding J, Lin Z. On-site measurement of indoor environment quality in a Chinese healthcare facility with a semi-closed hospital street. Building and Environment 2020;173:Article No. 106637.

World Health Organization (WHO). WHO Global Air Quality Guidelines: Particulate Matter ($PM_{2.5}$ and PM_{10}), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva, Switzerland: WHO; 2021.

Zhou Q, Lyu Z, Qian H, Song J, Möbs VC. Field-measurement of CO_2 level in general hospital wards in Nanjing. Procedia Engineering 2015;121:52-8.

Zuraimi MS, Tham KW, Chew FT, Ooi PL. The effect of ventilation strategies of child care centers on indoor air quality and respiratory health of children in Singapore. Indoor Air 2007;17(4):317-27.

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Electronic Waste (E-Waste) Management of Higher Education Institutions in South Central Mindanao, Philippines

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ABSTRACT

E-waste management is undoubtedly one of the important environmental concerns at present because the dependence on electronic devices has increased. There have been policies, legal provisions, and advocacy undertakings educating and introducing ways to manage and properly dispose of e-waste in the region yet there is no clear understanding of these practices, particularly in Higher Education Institutions (HEIs). The present study evaluates the e-waste management implementation of HEIs in South Central Mindanao, Philippines using survey questionnaires and in-depth interviews with 13 HEI representatives. The surveys showed that 39% of the HEIs have an annual budget of less than PhP100,000.00 (USD 1,950). Moreover, 23% of HEIs annually purchase 10-60 units of Information and Communication Technology (ICT) equipment. E-waste management among HEIs shows that 53.8% of ICT and electronic devices end up in landfills and 23.1% in informal sectors like junk shops. It can be noted that the yearly generation of e-waste among HEIs highly impacts the rise in the usage of IT equipment and electronic devices. Lack of awareness, e-waste disposal facilities, priorities, audit resolution and procedure and no definite legislation or laws among HEIs are the main challenges for e-waste management in the region. This study prompts mainstream e-waste management in HEIs and enlightens the public about appropriate strategies to address this issue. Furthermore, the findings of this study can be useful in formulating national and regional e-waste management plans and programs.

1. INTRODUCTION

The rapid advancement in technology, the constant introduction of innovative designs and smart functions, appealing marketing, and compatibility issues have caused the reduction in lifespan and accelerated obsolescence of electronic products, resulting in escalated quantities of e-waste (Kiddee et al., 2013; Agamuthu et al., 2015; Cucchiella et al., 2015; Kitila and Woldemikael, 2019). The Philippines is also facing these kinds of challenges due to the increasing usage of Information and Communication Technology (ICT) equipment and electronic devices both in basic education and higher learning institutions and with the onset of COVID-19 where online classes were implemented. Poor enforcement of environmental laws, lack of e-waste legislation or frameworks and shortage of e-waste management facilities have led to critical human and environmental problems, particularly in developing countries where 80% of discarded electronics from developed nations are transported (Lundgren, 2012; Kiddee et al., 2013; Kitila and Woldemikael, 2019). Several studies have reported that toxic metals and harmful chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) can be emitted from e-waste, adversely affecting human health through food chain contamination and direct occupational exposure on workers who engage in primitive recycling (Williams et al., 2008; Robinson, 2009; Kiddee et al., 2013).

The dilemma of e-waste generation in the country has become even more intricate to handle due to the importation of used and scrap electronics from developed nations; thus, the Philippines is beset with e-waste problems from two sources-the locally generated ones and the imported e-waste that contains discarded

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products from other countries (Gutierrez and Agarrado, 2011; Sasaki, 2021). In the case of government offices and institutional users of electronics (e.g., computers and laptops), obsolete products are cast-off through a lengthy and tiresome procedure of bidding by recyclers to procure the electronics for discarding (Alam, 2016). According to Celestial et al. (2018), there were 119 registered Treatment, Storage and Disposal (TSD) facilities in the Philippines as of 2011, but only 23 facilities are handling e-waste and only one in South Central Mindanao. There is no doubt that there will be a paucity of proper e-waste disposal schemes and facilities in the Philippines, especially in the region. Most obsolete electronics are simply stored, discarded with municipal wastes, or crudely recycled (Alam, 2016; Rosete and Valdez, 2018). Despite the existence of Philippine Republic Act No. 6969 or the Toxic Substances and Hazardous and Nuclear Wastes Control Act of 1990 which controls substances with toxic components, there is no clear-cut provision for e-wastes, absence of appropriate recycling facilities and a lack of skilled manpower (Peralta and Fontanos, 2006). Hence, e-waste management in the country, including among HEIs, remains implausible. E-waste management entails the process of collecting e-waste, recovering, and recycling material in a safe manner and disposal of e-waste through appropriate techniques to reduce its adverse effects on the environment.

HEIs in the Philippines are currently intensifying the use of ICT, thereby increasing their procurement and consumption of electronic equipment such as computers, laptops, and printers for efficient delivery of learning. Considering their reliance on electronic goods, along with frequent upgrading or replacement of such equipment, higher learning institutions markedly contribute to the generation of e-waste (Agamuthu et al., 2015).

Given the gaps, this study seeks to evaluate the e-waste management implementation of HEIs in South Central Mindanao, Philippines. As emerging academic institutions in the region often institute higher standards for e-waste management for the protection of people, the environment, and other organizations, the results of this study not only contribute to the higher learning institutions but can also be used to support the policymakers, especially with mainstreaming the e-waste management system through revisiting audit procedures and establishment of appropriate recycling facilities. Furthermore, the findings could provide baseline information on e-waste management in the Philippines considering only a few to none have explored e-waste management among HEIs in the country.

2. METHODOLOGY

The study was conducted in South Central Mindanao, Philippines. It covers four provinces and one highly urbanized city. The study focuses on the province of Cotabato which covers 17 municipalities and one component city, with a total of 30 public and private (colleges and universities) (Figure 1). Letters were sent to 30 higher learning institutions as survey respondents. Of these, only 13 (4 public and 9 private) universities and colleges agreed to provide data for the study. This number is ample enough to represent the current condition of the institution toward e-waste management in the region as they are major institutions in the region. Primary information was gathered from the key respondents designated by each HEI, such as the ICT Laboratory Aid, Property Custodian and Electronics/Electrical Laboratory In-charge. These individuals were chosen by their ICT personnel because of their proficiency and profound link with the subject under investigation.

2.1 Data collection and analysis

Each key respondent was sent a survey questionnaire consisting of 19 items relating to the annual purchase of electronic devices by the HEIs, options available for management concerning the functional and/or damaged electronic items, solid waste disposal practices and e-waste management activities organized by the HEIs. Prior to the survey, the developed questionnaires were tested and verified to draw recommendations for improvement and assess their efficiency in capturing data. Since the study was carried out from January through June 2020, during the onset and height of the COVID-19 pandemic, the survey was delivered online through Google forms. A link was sent to each respondent through email.

To verify and seek to get more details about the survey results, the key respondents were visited by the research team for a key informant interview (KII). During the interview, the survey respondent was asked the same questions developed in the online survey from Google forms. By doing so, the survey responses were verified, validated, and elucidated. Descriptive statistics such as frequency, percentage and mean were used to analyse the collected data.

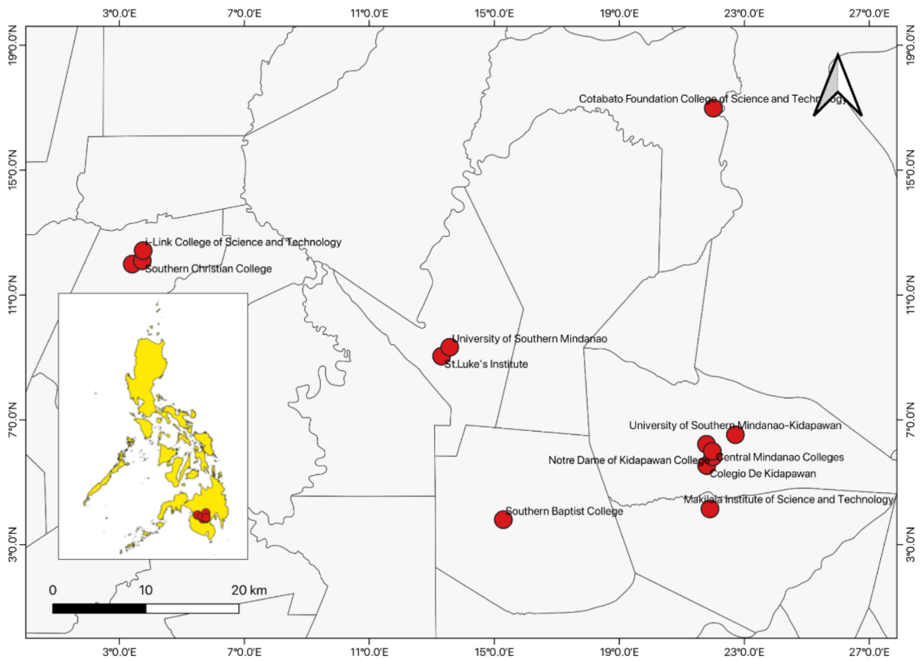


Figure 1. Map of South-Central Mindanao (13 colleges and universities)

3. RESULTS AND DISCUSSION

3.1 Annual purchase of electronic devices among HEIs

Colleges and universities need various types of electrical and electronic equipment in carrying out their daily undertakings, particularly ICT devices (e.g., desktop computers, laptops, computer mice, Wi-Fi routers, printers, copiers, televisions, projectors, and sound systems). Most of the interviewed learning institutions procure 1-9 electronic units yearly and allocate an annual budget of less than PhP 100,000.00 or USD 1,953.24 (38.5%) (Figure 2(a)). More than twenty-three percent (23%) have an annual purchase of 10-69 units of electronics and a minor proportion (15.4%) acquire over 70 units in a year. By and large, HEIs with more personnel and students procure more than 100 units of electronics and spend between PhP 100,000.00 and PhP 1,000,000.00 (USD 195,324) annually. The annual purchases typically consist of photocopiers, laptops, desktops, television, computer mouse, Wi-Fi router, printer, LCD projectors, mobile phones, electric fans, water dispensers, air conditioners, computer wires, and an LED wall. These electronic devices are important tools for HEIs, aiding them in intensifying their ICT efforts and realising their education and research objectives. HEIs relentless demand for electronic items, due to their need for more advanced technology or design, additional appliance, and features lacking in older and worn-out equipment, make HEIs one of the leading generators of e-waste.

The perceived life span of acquired items among HEIs such as laptops, desktop computers, television, and Wi-Fi router last for more than five years (Figure 2(b)), which agrees with the study of Islam et al. (2021), while mouse, LCD projectors, and printers have a relatively shorter life span of two years. Because of the frequent usage and multiple users of printers and LCD projectors, it is estimated to have a shorter life span.

HEIs have various considerations regarding whether to repair an impaired electronic device or not, such as (1) price, (2) warranty, (3) knowledge and skills in repairing, and (4) availability of spare parts as shown in Figure 2(c). Over seventy-six percent (76.9%) of the respondents indicated that the cost for repair relative to purchasing a new product is the factor most considered, closely followed by the validity of warranty service (69.2%). When the cost for repair is comparably higher than the price for a new item, HEIs would rather replace the impaired product. Public HEIs opted to replace impaired products especially when there was no allotment for repair indicated in the procurement plan. When the product is under warranty and it's already impaired, only the supplier can repair otherwise warranty will be void. The product that required higher knowledge and skills in repairing that are hard to find, thus HEIs opted to replace with a new one.

The number of HEI-owned ICT and electronics equipment being repaired yearly is between 10-39 and a marginal proportion (7.7%) renders 70-99 items for repair as presented in Figure 2(d). Regarding the results of KII, HEIs opt not to repair their equipment because of warranty service for some recently acquired electronic devices and the limited availability of needed spare parts.

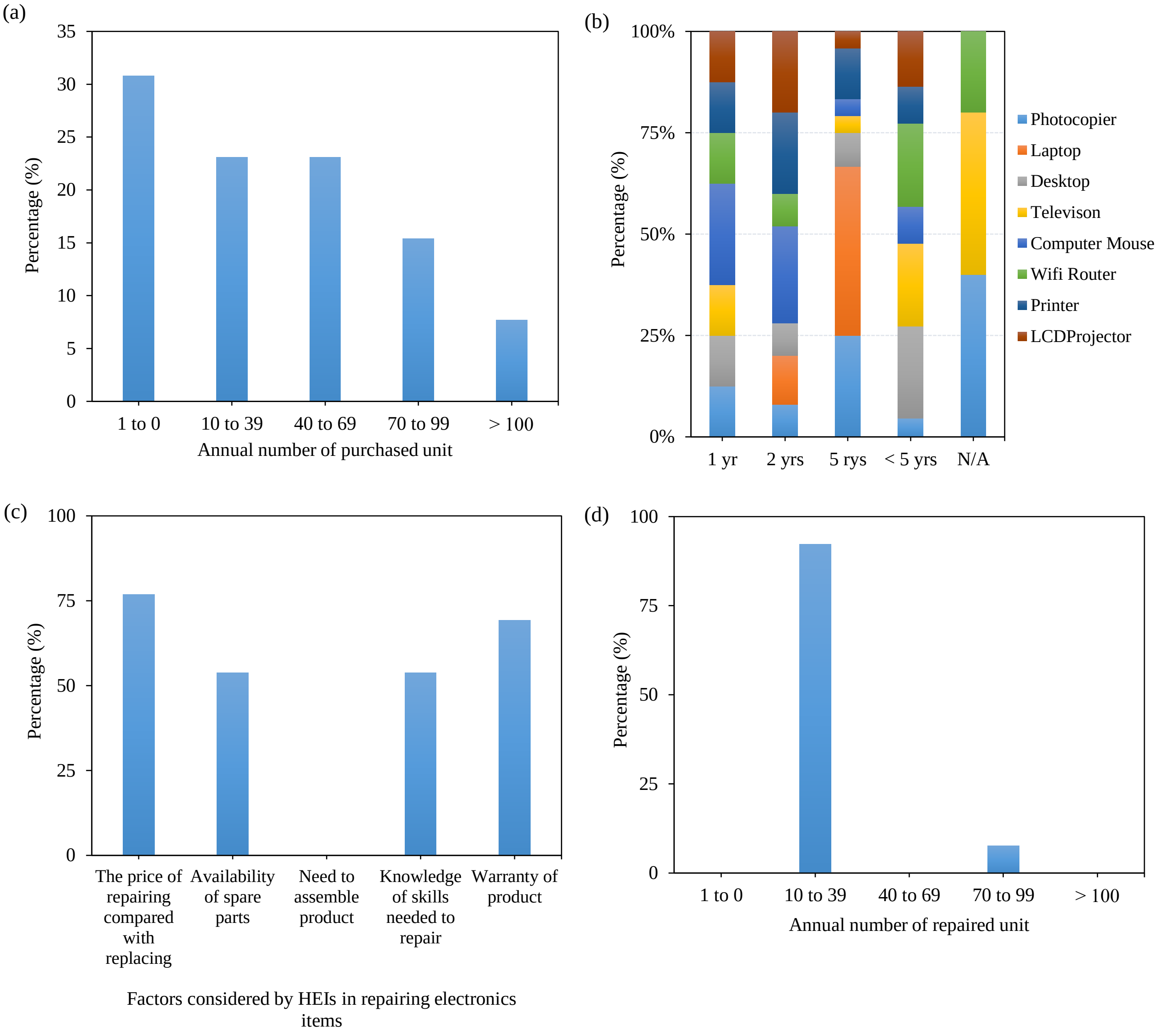


Figure 2. (a) HEIs number of units purchased yearly, (b) perceived life span of electronic equipment, (c) factors considered by HEIs in repairing electronics items, and (d) number of electronic and electrical equipment repaired yearly

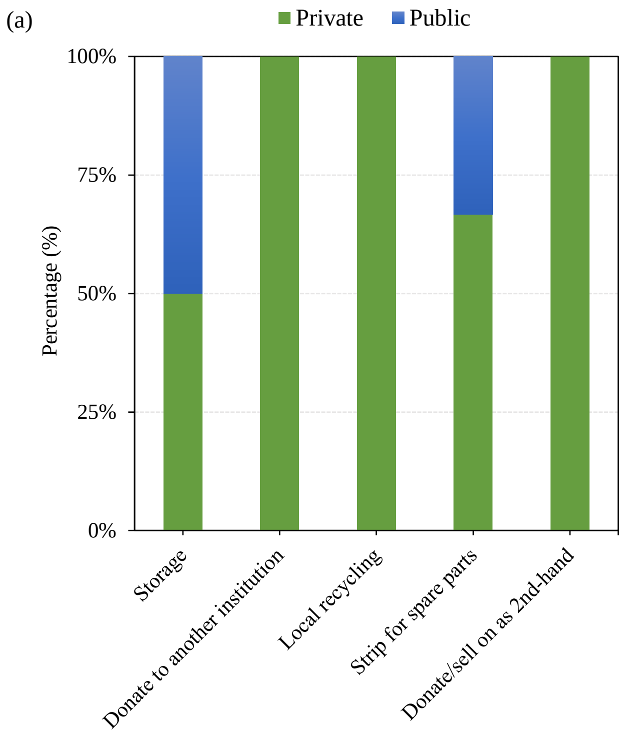
3.2 Management practices for both functional and damaged electronic items

With the number of electronic and electrical equipment repaired annually and having reached their useful life, they become part of e-waste because they are working but are outdated, or damaged/unusable. It was found out that most of the workingelectronic and electrical devices that are no longer used by the HEIs for obsolete electronic items end up in any of these routes: storage room, other agencies/offices/ schools through donation, or sold as a second-hand product which is brought to a local recycling centre or stripped for spare parts (Figure 3(a)). The majority of HEIs, both private and public colleges and universities end up putting their electronic and electrical devices into storage followed by stripping for spare parts or materials. This is consistent with extant studies suggesting that placing in storage is one of the practised modes of disposal for e-wastes (Agamuthu et al., 2015; Alam, 2016; Kitila and Woldemikael, 2019). In the absence of precise directives for its management, e-wastes are typically stored for a certain period due to a perceived value but eventually run through other phases such as reuse, recycling, and the landfill (Peralta and Fontanos, 2006; Oteng-Ababio, 2012).

Dismantling of out-modelled or obsolete electronic devices ranked as the second most adapted management option for e-wastes. Electronic items usually consist of spare parts and assemblies that have economic value, and these functional components are often recovered by recyclers. Through recycling, natural resource depletion and reducing greenhouse gases can be addressed (Ko et al., 2021). Through a process known as cannibalization, functional components of discarded computers and laptops are stripped and are used to create a new unit, subsequently sold at second-hand markets (Carisma, 2009) and the recovery of waste materials (reuse and recycle) is the technique to reach the goal of using everything and nothing left (Ko et al., 2021).

The results reveal that few of HEIs donate working electronic equipment because of an existing protocol among government learning institutions: obsolete electronic devices must be turned over to General Services Offices (GSO) or property officers. Accordingly, only private tertiary schools can perform other disposal modes-the doling out or selling off obsolete electronics as second-hand products, and taking them to local recycling centres- due to the prevailing audit procedures and regulations for public HEIs.

While Figure 3(b) presents non-working and damaged/unusable electronic and electrical devices as practised by surveyed HEIs, disposal methods for unusable electronics are largely like those working but obsolete devices, with storing and stripping for spare parts as the topmost responses. Damaged electronics are temporarily kept for a short time until they are picked up by garbage collectors or scavengers. Other discarded devices are also dismantled to recover useful subassemblies and components that can be utilized for other purposes. Despite its relative advantages, recycling and/or selling discarded devices are placed only by private institutions. Recycling is deemed as an effective yet underutilized e-waste management alternative (Kitila and Woldemikael, 2019), rendering several positive implications including pollution prevention; avoiding the need to extract limited resources; reducing energy used in manufacturing new products. As elicited from the KII, most of the selected tertiary schools do not practice recycling, stemming from the absence of definite e-waste disposal policies and regulations, facilities and the established audit procedure and resolution for government-administered HEIs.



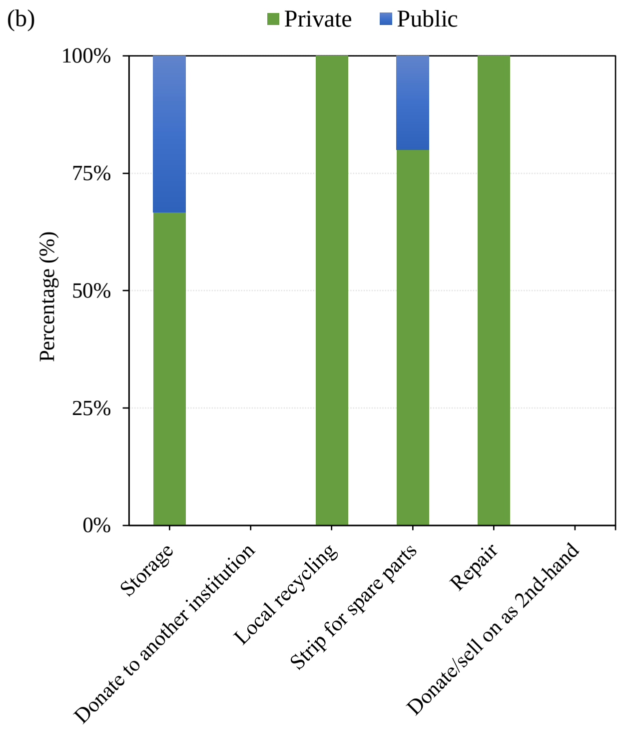


Figure 3. (a) Management of working electrical/electronic devices and (b) management of non-working and damaged electronic devices

The management practice differs according to the devices, the most common management alternatives for both functional but out-outdated devices and non-working items are recycling and dismantling/stripping of usable assemblies or spare parts are the topmost response (Figure 4(a)). Since repairing is also an integral management option for HEIs, cost, warranty validity and availability of spare parts as prime considerations. Disposing of trash is common for computer mice and computer wires while laptop and desktop computers are for donating to civic organizations or schools. Only a few engaged in selling and sorting electronic and electrical devices.

Among the salient findings of the present study is solid waste including e-wastes disposal practices of colleges and universities in South Central Mindanao (Figure 4(b)). Solid wastes generated by HEIs primarily ended-up in either of these routes: landfills (53.8%), informal e-waste sectors such as junk shops (23.1%) and returned to GSO for audit purposes (7.7%). Remarkably, a sizeable proportion (15.4%) also expressed that they completely lack solid waste disposal procedures in their institution. In the absence of e-waste regulations and clear policies, discarding e-wastes through local garbage collection offers the simplest option for HEIs.

This observation is congruent with other studies indicating that landfilling remains the most practised means of disposal for various types of wastes, including e-waste (Lundgren, 2012; Agamuthu et al., 2015; Rosete and Valdez, 2018). Despite its practicality, discarding of e-waste in the general waste stream has critical setbacks: possible leaching of highly toxic elements such as Ba, Cd, Ar, Se, Cu, Pb, Hg and Li, and contamination of soil and water bodies (Tiwari and Dhawan, 2014; Ponghiran et al., 2021), and shortage of landfill space (Rosete and Valdez, 2018).

Public awareness has an imperative role in addressing issues and challenges associated with e-waste; hence this aspect was also explored in the present study. Only 46.2% (Figure 4(c)) of the respondents expressed awareness of the notion of e-waste management, suggesting that adoption of this concept among HEIs in South-Central Mindanao is flimsy. Lundgren (2012) remarked that public awareness about e-waste management is generally low, with cognizance of the hazardous nature of e-waste and the crude waste management methods in developing nations as the weakest facets. Rautela et al. (2021) noted that setbacks in e-waste management are attributed to various factors including lack of technical skills, weak financial support, shortage of infrastructure and facilities, and poor community engagement.

HEIs' low level of awareness about e-waste management is further reflected by their low engagement in activities relevant to this concept (Figure 4(d)). Most of the surveyed learning institutions do not spearhead endeavours related to the disposal and processing of e-waste. As deduced from the survey, volunteer activities, recycling events and e-waste collection drives are mostly conducted by private tertiary schools, hence, HEIs in South Central Mindanao have generally low participation in e-waste management initiatives.

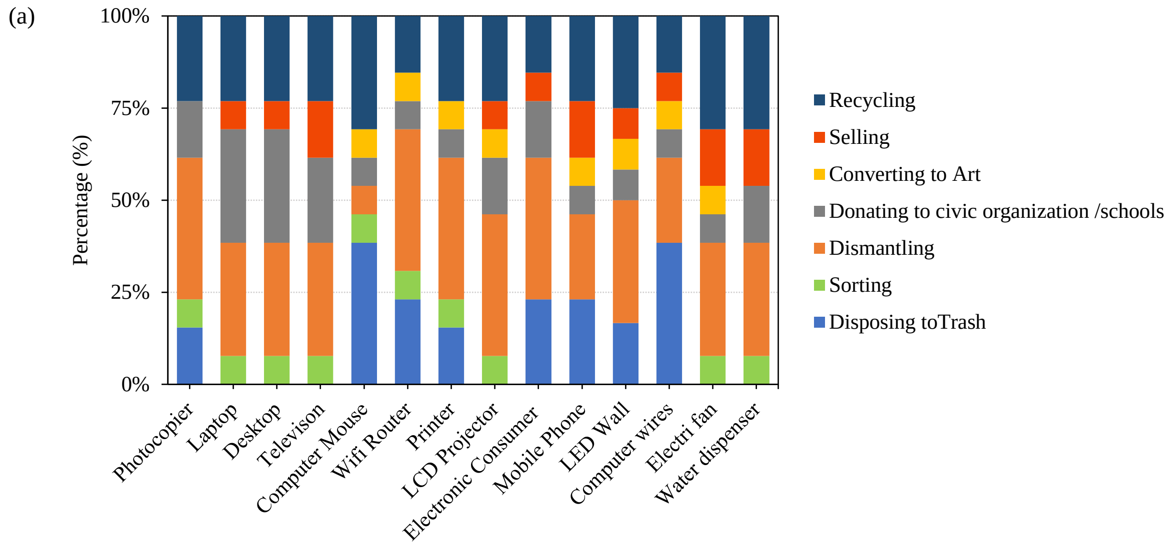


Figure 4. (a) Management practices for functional and nonworking items, (b) solid waste disposal practices of HEIs, (c) HEIs sponsored activities relating to e-waste management, and (d) awareness about e-waste management among HEIs

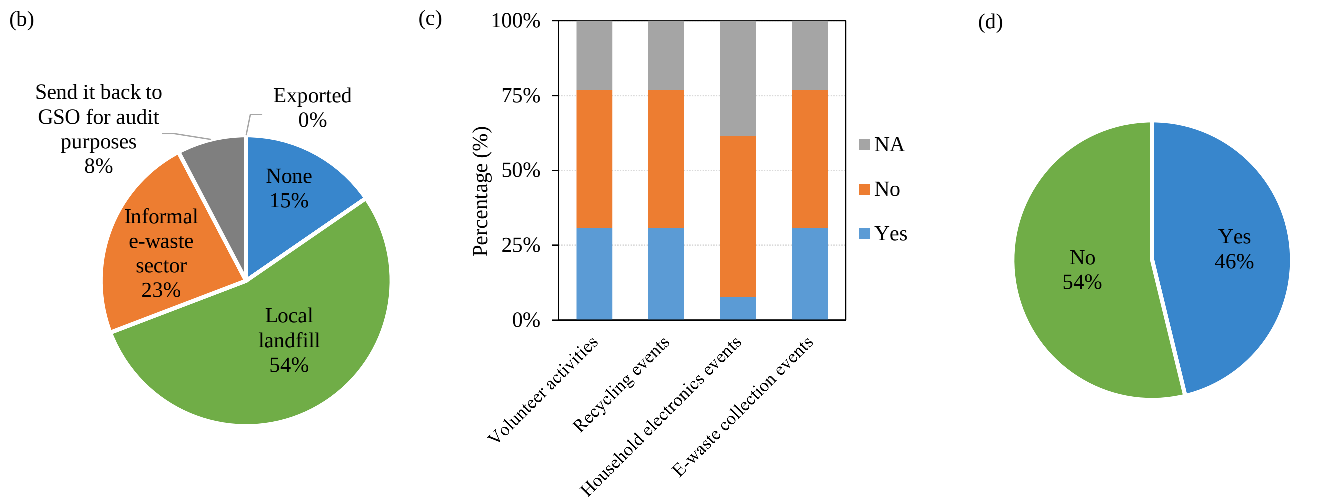


Figure 4. (a) Management practices for functional and nonworking items, (b) solid waste disposal practices of HEIs, (c) HEIs sponsored activities relating to e-waste management, and (d) awareness about e-waste management among HEIs (cont.)

HEI representatives were also asked about their perception of e-waste as a problem. Whilst the perceived intensity of the e-waste problem varied for each respondent, e-waste is largely viewed as a problem by HEIs in South Central Mindanao, with 38.5% considering it as an extremely serious dilemma (Figure 5). Despite their high level of cognizance of the challenges associated with e-waste, all surveyed HEIs do not have comprehensive e-waste management rules and regulations. Moreover, discussion about e-waste management is not integrated into their regular operations. Collaboration and partnership with other stakeholders/sectors do not exist. As deduced from the KII, only 7.7% of the HEIs indicated that they work in partnership with their respective Local Government Unit- Municipal Environmental and Natural Resources Office (LGU-MENRO). E-waste has been considered an escalating concern due to its environmental and human health problems (Raghupathy et al., 2010; Rautela et al., 2021). Considering its impacts, proper administration of generated e-waste must be incorporated into the management plan of HEIs and other concerned agencies.

3.3 E-waste management framework of HEIs in South Central Mindanao

Following our findings, a framework showing the e-waste management of learning institutions in South Central Mindanao was developed (Figure 6). It also shows that HEIs' sources of electronic devices are brand new with the annual number of purchases made and the budget allotted for ICT equipment and electronic devices. Because of the life span of the acquired items and management processes, it compounded the number of discarded electronic and electrical devices, thus HEIs necessitate in managing e-waste. The management alternatives for both functional but outdated devices and non-working items are namely: storing, donating, selling, recycling, dismantling, and disposing of landfills. The most common practices for e-waste disposal in HEIs are storing and dismantling/stripping usable assemblies or components. HEIs have no other options but to store discarded electronic and electrical devices due to audit procedures, including lack of awareness, facilities, guidelines, priorities, and non-existent e-waste management. Also, recycling, donating, and selling were influenced by audit procedures and warranty, thus discarded items were stored for some time.

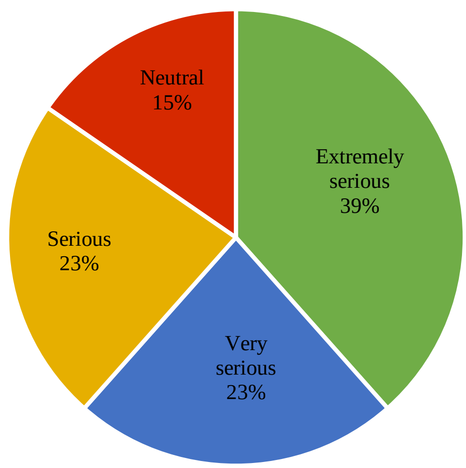


Figure 5. HEIs' perception of e-waste as a problem

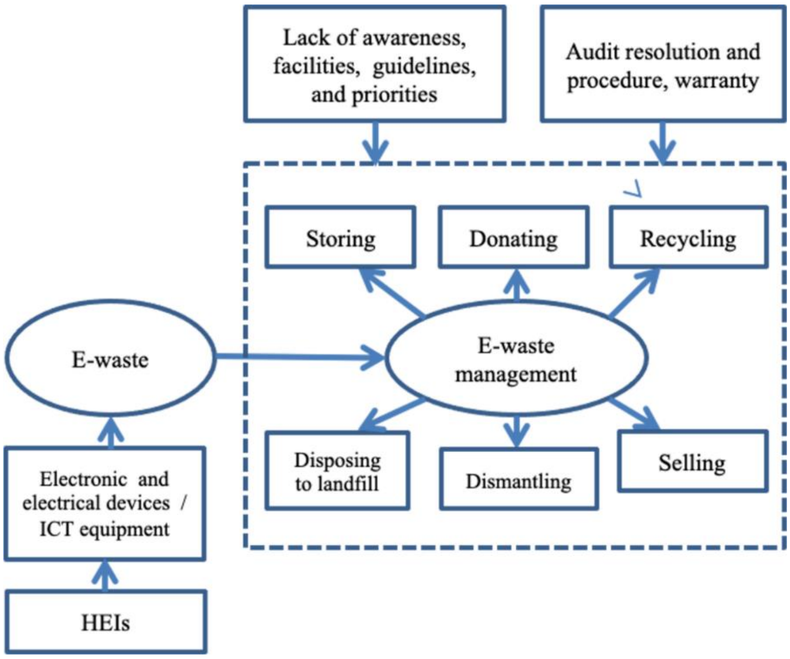


Figure 6. E-waste management framework of HEIs in South Central Mindanao

3.4 Recommendations

The Department of Environmental and Natural Resources (DENR) categorized e-wastes as a new class of miscellaneous waste through Administrative Order No. 2013-22, yet a comprehensive legal framework and mechanism for e-waste are non-existent in universities and colleges. The government should revisit the audit procedure and resolutions to address the compounded e-waste product in the storage area of HEIs. Formulating appropriate legislation and laws that need to be implemented by the government HEIs based on scientific evidence. It is important to provide recycling facilities for e-waste and technical support and capacity-building programs to the recyclers. There must be personnel assigned to this area to closely monitor the volume of e-waste generated in the schools. Annual inventory of equipment issued among faculty and personnel must be incorporated into the e-waste management plan.

Because of the low level of awareness among HEIs regarding e-waste and the adverse effect on human health and the environment, dissemination and information should be done. Also, include in the awareness program the disposal and accredited e-waste TSD facilities. Part of the awareness activities is to conduct recycling events and e-waste collection drives. The HEIs should provide repair services to salvage some damaged electronics devices to reduce stored e-waste products, ensure after-sale services of the supplier and encourage extended producer responsibility (EPR). When the life span of equipment has reached end-of-life and becomes non-functional, the supplier will collect and be responsible for disposing of their product. The HEIs should formulate policy and guidelines on e-waste management because the majority of HEIs are highly reliant on ICT equipment and electronic devices. Furthermore, every HEI should give utmost priority to e-waste disposal.

4. CONCLUSION

The present study has evaluated the e-waste management in higher learning institutions of South-Central Mindanao, Philippines and substantial findings were obtained from the current work. Although only 13 out of 30 HEIs responded, the sample consisted of a mix of private and public universities/colleges, small and large populations, and proximity to major municipalities. Also, the large number of repeated responses across the sampled universities/colleges suggests that a similar situation holds in other universities. The sizeable contribution of educational institutions in generating e-waste has been documented and attributed to various reasons including increasing demand for more advances, innovative designs and features, impairment of devices and rapid obsolescence rate of electronic products. Remarkably, the adoption of recycling, deemed as the most efficient management alternative, is ineffective. The major setback affecting e-waste management in HEIs may be associated with the administrative aspect-public schools that are not able to implement other disposal options such as donating, selling, and recycling due to audit procedures and regulations. The solid waste disposal of HEIs is also flimsy, with most of the generated wastes ending up in the landfill. Being the foundation of education and development, institutional policy on handling e-waste should be integrated into the management plan of all universities, colleges, and other concerned agencies. Furthermore, policymakers can also make a better strategy on how to mainstream the increasing e-waste management problems based on the baseline information provided in this study.

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REFERENCES

Agamuthu P, Kasapo P, Nordin NA. E-waste flow among selected institutions of higher learning using material flow analysis model. Resources, Conservation and Recycling 2015;105: 177-85.

Alam ZF. The assessment of the e-waste management generated from cellular phones, laptops, and personal computers in the Philippines. Manila Journal of Science 2016;9:27-42.

Carisma B. Drivers of and Barriers to E-Waste Management in the Philippines [dissertation]. Sweden: Lund University; 2009.

Celestial RG, Mauricio ED, Tan LB, Gumaru JC, Mondragon RO. E-waste management in the Philippines. Agro-Industrial Waste Management 2018;5(1):1-2.

Cucchiella F, D'Adamo I, Koh SL, Rosa P. Recycling of WEEEs: An economic assessment of present and future e-waste streams. Renewable and Sustainable Energy Reviews 2015; 51:263-72.

Gutierrez R, Agarrado G. New age trade agreements and their possible contribution to toxic trade. Environmental Law Network International 2011;2:Article No. 46.

Islam MT, Dias P, Huda N. Young consumers'e-waste awareness, consumption, disposal, and recycling behavior: A case study of university students in Sydney, Australia. Journal of Cleaner Production 2021;282:1-18.

Kiddee P, Naidu R, Wong MH. Electronic waste management approaches: An overview. Waste Management 2013;33(5): 1237-50.

Kitila AW, Woldemikael SM. Waste electrical and electronic equipment management in the educational institutions and governmental sector offices of Addis Ababa, Ethiopia. Waste Management 2019;85:30-41.

Ko CO, Tun Y, Lwin NH, Moe T, Eaindray J. Estimation of the recyclable waste amount collected by informal recycling shops: Case study in Nay Pyi Taw, Myanmar. Environment and Natural Resources Journal 2021;19(2):103-11.

Lundgren K. The Global Impact of E-Waste: Addressing the Challenge. Geneva, Switzerland: International Labour Organization; 2012.

Oteng-Ababio M. Electronic waste management in Ghana-issues and practices. In: Curkovic S, editor. Sustainable Development-Authoritative and Leading-Edge Content for Environmental Management. IntechOpen; 2012. p. 149-66.

Peralta GL, Fontanos PM. E-waste issues and measures in the Philippines. Journal of Material Cycles and Waste Management 2006;8(1):34-9.

Ponghiran W, Charoensaeng A, Khaodhiar S. The environmental impact assessment of gold extraction processes for discarded computer RAM: A comparative study of two leaching chemicals. Journal of Material Cycles and Waste Management 2021;23(4):1412-22.

Raghupathy L, Krüger C, Chaturvedi A, Arora R, Henzler MP. E-waste recycling in India: Bridging the gap between the informal and formal sector. Hamburg, Germany: International Solid Waste Association, World Congress; 2010.

Rautela R, Arya S, Vishwakarma S, Lee J, Kim KH, Kumar S. E-waste management and its effects on the environment and human health. Science of the Total Environment 2021; 773:Article No. 145623.

Robinson BH. E-waste: An assessment of global production and environmental impacts. Science of the Total Environment 2009;408(2):183-91.

Rosete MA, Valdez KG. Assessing the level of awareness of electronic waste among the business economics majors of the University of Santo Tomas College of Commerce and Business Administration. Review of Integrative Business and Economics Research 2018;7(4):216-37.

Sasaki S. The effects on Thailand of China's import restrictions on waste: Measures and challenges related to the international recycling of waste plastic and e-waste. Journal of Material Cycles and Waste Management 2021;23(1):77-83.

Tiwari D, Dhawan NG. E-waste management: An emerging challenge to manage and recover valuable resources. International Journal of Environmental Research and Development 2014;4(3):253-60.

Williams E, Kahhat R, Allenby B, Kavazanjian E, Kim J, Xu M. Environmental, social, and economic implications of global reuse and recycling of personal computers. Environmental Science and Technology 2008;42(17):6446-54.

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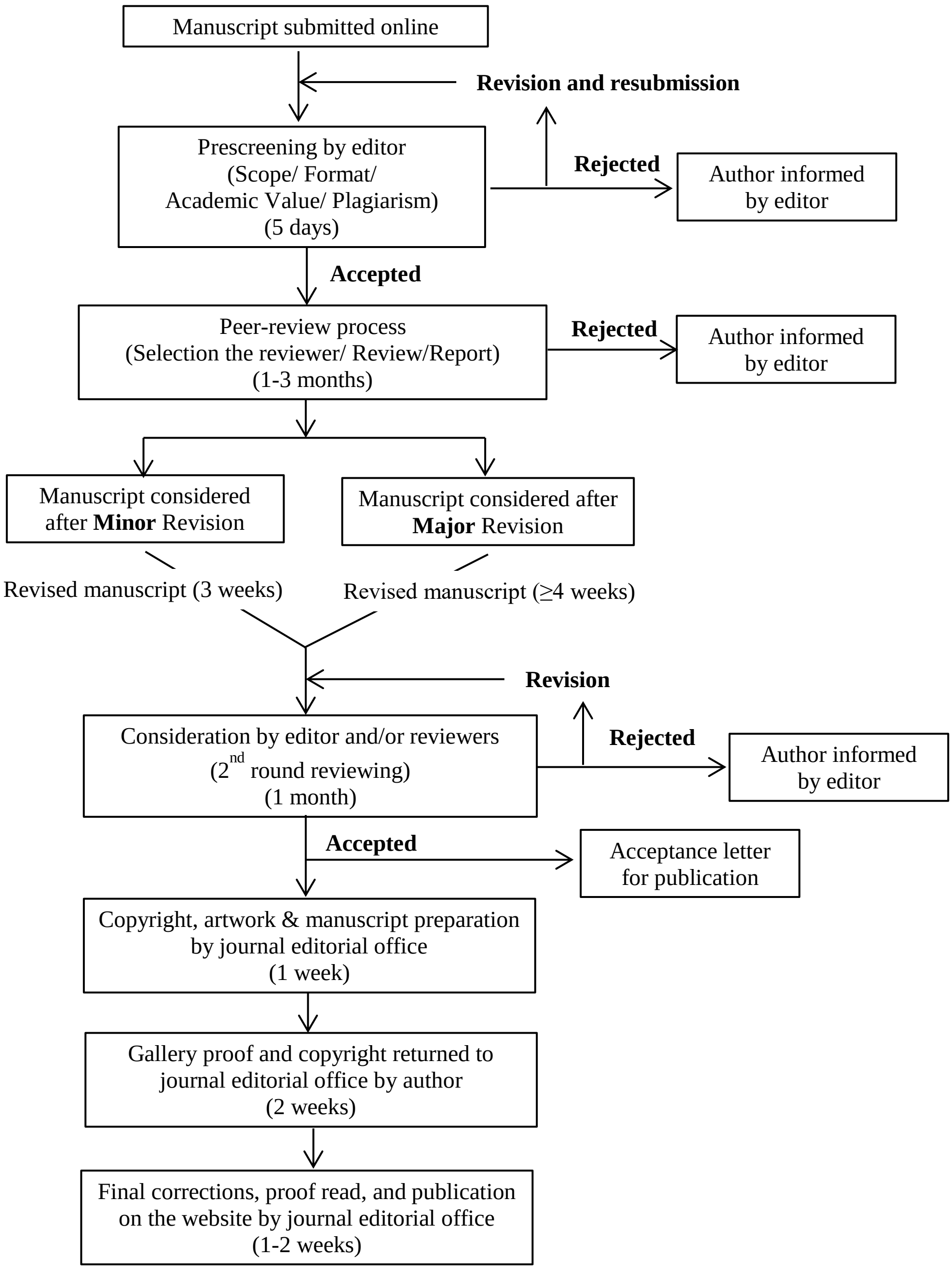

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Journal article
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Published in conference proceedings
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Ph.D./Master thesis

Shrestha MK. Relative Ungulate Abundance in a Fragmented Landscape: Implications for Tiger Conservation [dissertation]. Saint Paul, University of Minnesota; 2004.

Website

Orzel C. Wind and temperature: why doesn't windy equal hot? [Internet]. 2010 [cited 2016 Jun 20]. Available from: http://scienceblogs.com/principles/2010/08/17/wind-and-temperature-why-doesn/.

Report organization:

Intergovernmental Panel on Climate Change (IPCC). IPCC Guidelines for National Greenhouse Gas Inventories: Volume 1-5. Hayama, Japan: Institute for Global Environmental Strategies; 2006.

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