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Recycling of Piggery Effluent for *Azolla microphylla* Production and Application in Vermiculture for Enhancing Nutrients Quality and Driving BCG Economy Model

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Abstract

Piggery wastewater is a major source of environmental problems, while *Azolla microphylla (AM)* is a good phytoremediator and an essential source of nutrients and protein. The effect of surface areas and time on biomass production and nutrients of *AM* was investigated. Meanwhile, efficiencies of *AM* in removing contaminants were also identified. A completely randomized design (CRD) with three treatments and three replicates, nine plastic containers with three different sizes of surface areas including large (L, 0.47 m²), medium (M, 0.22 m²), and small (S, 0.16 m²) were selected for recycling piggery wastewater and *AM* production. *AM* and cow dung (CD) were mixed 50:50 in a plastic bucket 30 cm in diameter and 18 cm high by following CRD for application biomass of *AM* in vermiculture. It revealed that the large area was the most convenient size for increasing biomass (399%) and doubling time around (3-6 days) within 14 days. Chemical oxygen demand (COD) and biological oxygen demand (BOD) were reduced well in the M for 2 weeks. Nitrate nitrogen (NO_3^--N) and phosphate (PO_4^{3-}) were removed effectively in the M and L within 3 weeks, respectively. Interestingly, initial total Kjeldahl nitrogen (TKN, 1.65%), crude protein (10.3%), and total phosphorous (TP, 0.37%) were increased in biomass of *AM* by 2.41%, 15.04%, and 0.43%, respectively. Copper (Cu) and Zinc (Zn) was up taken 54% and 11% from piggery effluent, meanwhile, these were increased in *AM* in respective 22%, and 111%. The C/N ratio was significantly reduced in vermicompost of cow dung with *AM* (CDA) approximately (13.5), while the vermicompost and compost of cow dung (CD) were more than (15 and 17.8), respectively. The pH, organic matter (OM), organic carbon (OC), Cu, and Zn showed better in vermicompost of CDA than in the compost. The L size was effective in producing biomass of *AM* within 14 days, and the *AM* has effectively removed pollutants from piggery effluent in M and S. Hence, *AM* not only improved water quality but also produced its biomass for sustainable agriculture and environment friendly. Whereas, the vermicompost of CDA was a good one for improving nutrient quality and earthworm production.

Keywords: *Azolla microphylla*; Wastewater; Vermicompost; Nutrient

1. Introduction

Insufficient freshwater resources occur around the world, especially in arid nations when demography and development are being increased, while freshwater sources are limited. Besides, the agricultural sector has been accounting for 70% of global water consumption or 95% for some developing countries, and it is also a significant player in environmental pollution (FAO, 2017). In this sector, piggery farms are a major source of environmental influence. Those farms caused a great deal of pollution, especially water pollution. For instance, piggery farm in Brazil has been consumed freshwater roughly 15 liters for each animal per day, occurring unsustainable freshwater that enables to generate a vast of wastewater, loading contaminants such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorous (TP), copper (Cu) and zinc (Zn) (Velho *et al.,* 2012). Basing Tokhun *et al.* (2010) reported that the number of pigs around 9,645 could produce wastewater 103,3050 L/day, and consists of BOD and COD in respective around 3,555 mg/L and 4,889 mg/L. Furthermore, piggery waste and wastewater contained abundances of TP and TN contents approximately 2,765 ppm and 4,546 ppm, respectively, investigated from 41 industrial pig farms in South Korea (Zhang *et al.,* 2017), which induced eutrophication, causing depletion of oxygen for organisms' breath (Aczel, 2019). These components could result in soil and crop contamination (Mora-Orozco *et al.,* 2018), public water bodies lower quality, and harm animals and people's health (Olanrewaju and Olowoyeye, 2018). Besides, small-scale industrial pig farms (29 sites) in China reported that slurry yields were produced annually on average 216, 333, and 773 tons per year per pig farm from manure, urine, and washing water, respectively. A large amount of this excess was a significant effect on the quality of surface and groundwater, and agricultural land due to the high amount of available phosphorous, and heavy metals, Cu and Zn contents (Zhang *et al.,* 2017). However, some elements such as TN, TP, Cu, and Zn are necessary for the crop to thrive well. The concentration of Cu and Zn in water less than 0.2 ppm and 2 ppm are essential micronutrients, yet more than 1 ppm and 2 ppm, respectively are toxins (USEPA, 2012).

Azolla, meanwhile, is a floating-aquatic fern consisting of *Anabaena azollae* that could fix nitrogen from the atmosphere around 1.1 tons per hectare that were significantly higher than legume only 0.4 ton per hectare (Hall *et al.,* 1995; Muradov *et al.,* 2014) and store it in the cavity of *Azolla*, inducing *Azolla* to raise its biomass, doubling time around 2 to 6 days (Arora and Singh, 2003; Box, 2018; Golzary *et al.,* 2020). Moreover, it could produce biomass of approximately 15 tons per hectare spending time around 3 months by initial inoculation of 1.3 kg/m^2 (Bocchi and Malgioglio, 2010). As well as, it enables to thrive in the effluent (Forni *et al.,* 2001), and remove heavy metals (Talebi *et al.,* 2019). Some previous studies showed that this fern provides numerous advantages which have been widely used in uptaking nutrients and pollutants from different sources such as the distillery, textile, domestic, sewage, municipal, industry, and aquaculture (Taylor *et al.,* 2016; Akinbile *et al.,* 2018; Amare *et al.,* 2018; Box, 2018; Naghipour *et al.,* 2018; Talebi *et al.,* 2019). Moreover, it has been used as feed for the animal (fish, duck, cattle, and poultry) (Shiomi and Kitoh, 2001; Datta, 2011; Raja *et al.,* 2011; Brouwer *et al.,* 2018), green manure or biofertilizer for crops and soil fertilization (Bocchi and Malgioglio, 2010; Zhao *et al.,* 2014; Yao *et al.,* 2018; Razavipour *et al.,* 2018; Kumar *et al.,* 2019, 2020), saline soil amendment (Sharifi *et al.,* 2019), source of protein for many purposes (Brouwer *et al.,* 2017, 2018, 2019), and the cover of water evapotranspiration (Kimani *et al.,* 2020).

Besides, vermiculture is useful biotechnology for improving nutrient quality and environmental management, using earthworms to decompose waste or organic materials. Earthworms have a special muscular gizzard (muscle stomach) that can crush organic waste, small living creatures, dead larva, protozoa, and other microorganisms (nematodes, arthropods) into tiny pieces of 2 - 4 micrometers. On the other side, by lowering chemical fertilizer and pesticide use, it may be possible to boost nutrients and crop yield, and environment friendly (Sinha *et al.,* 2002; Edwards *et al.,* 2011; Visuvasam Motcha, 2017). Whereas, *AM* can be easily cultivated, contains high nutrients, and decays quickly. However, there is no previous research on applying biomass of *AM* to enhance vermicompost quality (earthworm cast). Therefore, this research was carried out to maximize the benefits of piggery effluent and *AM* biomass which could drive the Bio-Circular-Green economy (BCG) economy model meaning that we try to use renewable biological resources and convert them into additional products to keep a balance of the economy, society and environment. The first objective was to identify the effective surface areas and the period for producing high biomass of *AM*, and PW5 quality. The second objective was to compare the nutrient quality between earthworm cast and compost by using *AM*. We hypothesized that *AM* performs better growth in the large surface area within 14 days and PW could be recycled. Whereas, the vermicompost provided better quality than traditional compost.

2. Materials and methods

2.1 Experimental site

Piggery wastewater was collected from a storage pond of a small-scale farm located in latitude 16031'21.2''N and longitude 102055'45.5''E, while *AM* deriving from Department of Soil Science and Environment located in latitude 16031'21.2"N and longitude 102055'45.5"E, Khon Kaen University, which has been being cultured for experiment purposes.

2.2 Experimental design

2.2.1 Biomass production of AM and recycling PW5

AM is a high potential fern in biomass productivity and tolerance salinity in comparison to the other species (Arora and Singh, 2003), and it could be cultured in all seasons (Arora and Saxena, 2005). It has been cultured in big plastic containers at the DSSE before inoculation to the experiment. A completely randomized design (CRD) consisted of three replicates with three treatments of different-surface areas of plastic containers including large (L, 0.47 m^2), medium (M, 0.22 m^2), and small (S, 0.16 m^2) was carried out in a polyethylene greenhouse condition at 30.5 ± 3 °C within 21 days. Untreated piggery wastewater gathering from the location mentioned above was diluted with tap water at a ratio (5:95) to get a concentration of piggery wastewater at 5% (PW5). Each treatment was inoculated 74 g of the fresh biomass of *AM*, adapted from (Amare *et al.,* 2018), with a total amount volume of 60 liters of PW5 without compensation. All of the containers were covered with a white net to prevent the insect affecting (Figure. 1).

2.2.2 Application biomass of AM in Vermiculture

The *AM* was grabbed after recycling PW5 to apply in vermiculture (using earthworm to decompose). The earthworm species *Eudrillus ugeniae* was selected. *AM* and cow dung (CD) were mixed 50:50 in a plastic bucket 30 cm in diameter and 18 cm high under the nursery (Bhat *et al.,* 2015; Ananthavalli *et al.,* 2019). The completely randomized design (CRD) was applied by inoculation of 5 *Eudrillus ugeniae* to each treatment (Natarajan and Devi, 2014) within 30 days (Petmuenwai *et al.,* 2013).

2.3 Data collection

2.3.1 Biomass production of AM and recycling PW5

Samples of PW5 were collected simultaneously from each container every week, and immediately transferred to the laboratory for physicochemical analysis, and stored in a refrigerator at a temperature of 4 ± 2 °C for further examination by following the standard procedures below. Whereas, fresh biomass of the *AM* was grabbed every week by using stainless steel mesh and keeping in shade about one hour before weighting. A digital balance (model Ohaus PA 2102) was used to weigh the biomass. The constant dry weight of *AM* was dried in an oven at 70 °C for 24 h (Kimani, 2020). Then, the dried biomass was finely ground by a portable blender passed through a 0.45 mm mesh sieve.

2.3.2 Physiochemical analysis in AM

The relative growth rate (RGR) of the *AM* was calculated by using the formula (1):

$$RGR\ (g/g/d) = \frac{\ln(DWf) - \ln(DWi)}{Tf - Ti} \quad (1)$$

where DWf and DWi were the final and initial dried weight (g) in respective, while Tf and Ti were the duration (days) of the final and initial inoculation. Eq. 2 was applied in previous studies (Sudiarto *et al.,* 2019; Kimani, 2020). Whereas doubling time (DT) calculation was calculated by following the equation (2) below:

$$DT\ (days) = \frac{\ln(2)}{RGR} \qquad (2)$$

where ln (2) is equal to about 0.693 (Kimani, 2020), while RGR is the value derived from the equation (1).

TKN was examined by following the Kjeldahl method (ICARDA, 2013). Whereas crude protein was multiplied with a factor of 6.25, Crude protein = %TKN * 6.25 was adapted from AOAC 954.01 (AOAC, 1990). TP was measured by Spectro UV-2550, following the spectrophotometric vanadium phosphomolybdate method (FAO, 2008), while Cu and Zn were examined by the AAS method (APHA, 2017).

The bioconcentration factor (BCF) of heavy metal (Cu and Zn) in *AM* was calculated by the formula (3) which was applied in the previous studies (Adel *et al.,* 1998; Akhtar *et al.,* 2019).

$$BCF = \frac{Cf\ in\ plant}{Ci\ in\ PW5} \qquad (3)$$

where Cf in the plant was the ion concentration of heavy metal (mg/kg) containing in *AM* (basis dry) after the completed experiment, while Ci in PW5 was the initial concentration of heavy metal (mg/L) in piggery wastewater.

2.3.3 Physiochemical analysis in PW5

BOD was analyzed suddenly by preparing two sets; one was for DO_0 (analysis in an initial day) and another for DO_5 (storing in incubation at temperature 20 ± 2 ^oC for 5 days) following APHA 4500 - O C, azide modification method, while COD was analyzed following APHA 5220 C, close reflux, titrimetric method (APHA, 2017). Whereas temperature, DO, pH, and EC was measured by a calibrated multiple-parameter meter, Oakton ® Waterproof PCD 650, USA. Whereas NO_3^--N was examined by the brucine method (USEPA, 1971), and $PO4_3^-$ followed by APHA 4500 P. F, the automated ascorbic acid reduction method (APHA, 2017). TSS was followed by APHA 2540 B, the glass fiber filter disc, and dried at a temperature of 103 - 105 ^oC, while TDS was examined by APHA 2540 C. dried at 180 ± 2 ^oC (APHA, 2017). The last ones, Cu and Zn were analyzed by following the atomic absorption spectrometry method (AAS) APHA 3030 E, (APHA, 2017).

Efficiencies of removal contaminants were calculated by the following formula (4):

$$R\ (\%) = \left(1 - \frac{final\ concentration}{Initial\ concentration}\right) \times 100 \qquad (4)$$

which was applied to ascertain the efficiency rate of *Azolla* species (Taylor *et al.,* 2016; Akinbile *et al.,* 2018; Amare *et al.,* 2018; Naghipour *et al.,* 2018), and vermi-filtration in piggery wastewater treatment (Mungruaiklang and Iwai, 2021).

2.3.4 Application biomass of AM in Vermiculture

These parameters: pH, EC, OC, OM, Cu, Zn, and C/N ratio were estimated in cow dung (CD) and the *AM* before the experiment. The EC (1:5 H_2O) was measured using a soil bridge method; pH (1:5 H2O) was measured by glass electrode (ICARDA, 2013); OC (%) was analyzed using a Walkley & Black method; and the OM (%) was calculated following the OC values by multiplying with factor 1.724 (FAO, 2019). This formula was followed by the previous study (Vodounnou *et al.,* 2016).

2.4. Statistical analysis

The data was shown by the mean values of triplicate ± standard deviation (SD). The Statistix program, version10 (USA), was used for statistical analysis in this study. Moreover, all data in this experiment were analyzed by following analysis of variance (ANOVA) and mean comparisons tool to identify significant differences with a confidential interval of 95%, based on Fisher's least significant difference (LSD) procedure (p-value ≤ 0.05).

3. Results

3.1 Biomass production of AM, RGR, and DT

Fresh biomass of *AM* was harvested and weighted every week (Table 1). The results revealed that different sizes of surface areas including large (L, 0.47 m^2), medium (M, 0.22 m^2), and small (S, 0.16 m^2) and time were significant differences ($P \leq 0.05$). The L size was the most convenient for increasing biomass in accumulation 243%, 399%, and 406% in periods 7 days, 14 days, and 21 days, respectively. The following was the M which increased 181%, while the small was 100% for 14 days, and decreased within 21 days (Figure 2). Doubling time (DT) and relative growth rate (RGR) were also significant differences in various surface areas and time ($P \leq 0.05$). The L size showed a good RGR of range 0.18, 0.11, and 0.08 (g/g/d) for 7, 14, and 21 days in respective, whereas the M and S size were less than 0.13, 0.08, and 0.06 (g/g/d), respectively (Table 1). The DT of *AM* growth was good in the L size with 3.93, 6.04, and 8.98 days, while the others were more than 5, 9, and 15 days during the period of growing 7, 14, and 21 days, respectively. Meanwhile, the bioconcentration factor (BCF) showed high values of approximately 570 and 1100 for Cu and Zn in the large size, respectively.

3.2 TKN, Crude protein, and TP in biomass of AM

Total Kjeldahl nitrogen (TKN) and crude protein were shown significantly higher in the M and S size ($P \leq 0.05$). TKN was increased in *AM* from (1.65 - 2.41%) and (1.65 - 2.2%), while crude protein was increased from (10.3 - 15.04%) and (10.3 -14.68%) in the M and S size, respectively. Besides, total phosphorous (TP) was increased in the S range from (0.37 - 0.43%) higher than the M (0.37 - 0.4%), while it was decreased in the large size (Table 2).

3.3 The efficiencies of AM for recycling PW5

3.3.1 Temperature, EC, TDS, and TSS of PW5

The temperature in each size (L, M, and S) showed significantly different ($P < 0.05$) within 7 days. The L size showed slightly lower around (30.7 - 28.5 °C), while the M and S sizes were (30.7-29.5 °C). However, it was revealed non-significant differences ($P > 0.05$) in each size within 21 days around 28.8 °C (Table 4). The M size indicated high effectiveness in reducing EC 17% (433 - 361µS/cm), while the S and L sizes were lower approximately 13% (433-366µS/cm) and 10% (433 - 377µS/cm) for 14 days, respectively; however, all sizes decreased efficiency within 21 days (Table 4). Whereas TDS was removed significantly ($P \leq 0.05$) in the M from 259 - 136 mg/L (47.4%) following the S and L ranged from 259 - 260 mg/L (22.7%) and 259-267mg/L (12.6%) for 14 days, respectively; however, the efficacy was declined in all types of surface area within 21 days (Table 4). Meanwhile, TSS was removed effectively in the M ($P \leq 0.05$) about 52% (39 - 19 mg/L), while the S and L were lower than 40% (39 - 23 mg/L) and 15% (39 - 36) within 14 days, separately. In conclusion, all sizes of surface areas were decreased efficiencies in removing contaminants within 21 days (Table 4).

3.3.2 pH, DO, BOD, and COD

All sizes of surface areas showed non-significant differences ($P > 0.05$) in pH and DO parameters during the experiment. pH and DO were kept in balance around (7.4 - 7.8) and (6.9 - 5.8 mg/L) within 14 days, respectively (Table 5). The results of removing BOD found that the surface areas and time were significantly affected ($P \leq 0.05$). The M size was the most effective in reducing BOD around 77.6% (13.5-3 mg/L) within 7 days, whereas the L size was less than 45 %. Furthermore, the S size was still more effective about 68% for 21 days, while the others decreased effectiveness (Tables 5 and 6). The COD removal was shown significantly effective ($P \leq 0.05$) in the S and M size in the respective range between (90.4 - 21.4 mg/L) 76 %and (90.4 - 22.6 mg/L) 75% for 14 days, individually, while the L was less than 20%; however, the L size was the highest efficiency around 64% for 21 days, while the others were less than 9% (Tables 5 and 6).

3.3.3 Nitrate (NO_3^-) and Phosphate (PO_4^{3-})

The different sizes of surface areas and period of the experiment revealed significant differences ($P \leq 0.05$) of NO^{3-} and PO_4^{3-} reduction. The S size was the most effective in removal NO_3^- around 66% (1.04 - 0.39 mg/L), while the others were less than 60% within 14 days (Tables 5 and 6); however, the M and L size showed highly effective in the respective range 64% (1.04 - 0.37 mg/L) and 63% (1.04 - 0.39 mg/L) for 21 days. Moreover, PO_4^{3-} was removed 100% and 72% (1.41 - 0.39 mg/L) in the L and M size, while the S was less than 17% (1.41-1.18 mg/L) within 14 days, respectively (Tables 5 and 6).

3.3.4 Cu and Zn removing from PW5 and increasing in biomass of AM

The efficiency of *AM* in removing Cu from PW5 showed nonsignificant differences ($P > 0.05$) in the three distinct surface areas. The Cu was reduced from PW5 approximately 53% (0.11 - 0.05 mg/L) in the S size, whereas Zn was removed from the L size about 11% (0.23 - 0.21 mg/L) within 21 days (Table 2 and 3). Meanwhile, both Cu and Zn were increased significantly ($P \leq 0.05$) in the biomass of *AM*. The L size showed that the amount of Cu in biomass of *AM* was increased from 49 to 63 mg/kg (22%), while the M and S sizes were less than 58 mg/kg (14%). The amount of Zn was increased highly in the S size about 123% (111 - 249 mg/kg), whereas the M and L size were around 107% (111 - 231 mg/kg) and 111% (111 - 236 mg/kg), respectively, (Table 2 and 3). Besides, the bioconcentration factor (BCF) showed high values around 570 and 1100 of Cu and Zn in the large area, respectively (Figure. 4).

3.4 Characteristics of the raw materials in vermiculture

Cow dung (CD) and Cow dung with *Azolla microphylla* (CDA) showed significant differences ($p \leq 0.05$) in almost all of the parameters including EC, OC, OM, Cu, Zn, and C/N ratio, except pH. The EC was higher in CDA (3.22 mS/cm) than the CD less than (2 mS/cm). OC and OM showed the highest in CDA (23.1%) and (39.9%), and while in CD less than (22%) and (38%), respectively. Besides, heavy metals (Cu and Zn) demonstrated lower in CDA (59.9 mg/kg) and (234.1 mg/kg), while in CD was over than (69 mg/kg) and (244 mg/kg), respectively. Whereas, C/N ratio was higher in CD (26.5) than CDA (19.6) (Table 7).

3.5 The efficiency of AM in vermiculture

3.5.1 Vermicompost and compost quality

The results (Table 8) demonstrated all parameters including pH, EC, OC, OM, Cu, Zn, and C/N ratio in vermicompost (decomposed by earthworm, *Eudrillus ugeniae*) were significantly better (p-value ≤ 0.05) than the compost, non-earthworm. The pH in vermicompost of CD and CDA were (7.9 and 7.87) in respective lower than the compost (8.28 and 7.99). Whereas, the EC showed the highest in vermicompost of CDA (2.95 mS/cm), while the CD was less than (2 mS/cm). Furthermore, the OC was significantly lower in vermicompost of CDA and CD (14.5 % and 14.2%), than the compost (15% and 16.1%), respectively. Whereas the OM showed significantly lower in vermicompost of CDA and CD in respective (25.1% and 24.5%), while the compost over (25% and 27%). The Cu was increased highly in vermicompost of CDA and CD about (59.6 mg/kg and 58.7 mg/Kg), whereas Zn was highest in vermicompost of CDA (334 mg/kg), while the others were less than (302 mg/Kg). Interestingly, the C/N ratio showed the lowest level in vermicompost of CDA (13.5), while the compost and vermicompost of CD over (15).

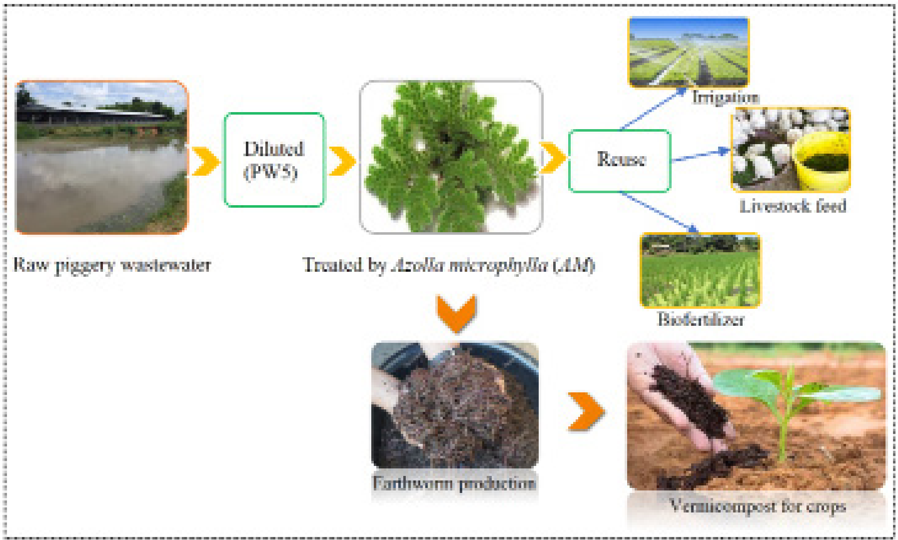

Figure 1. General scheme of the experiment

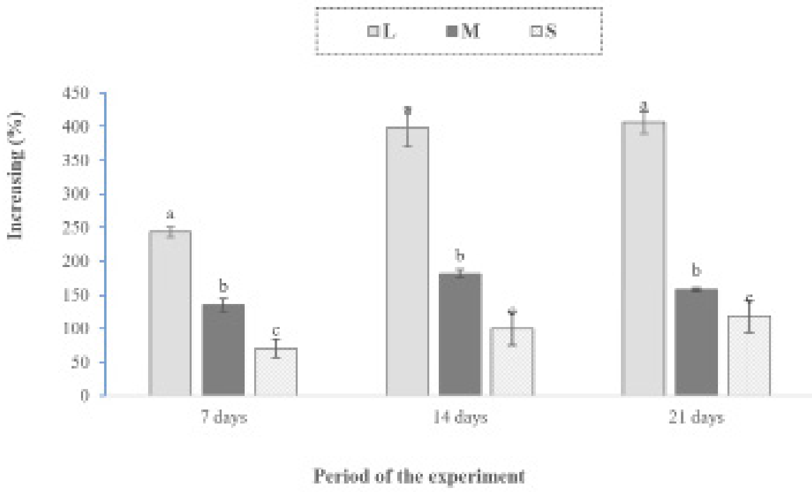

Figure 2. The accumulative growth rate of *AM* in three different surface areas including the large (L, 0.47 m^2), medium (M, 0.22 m^2), and small (S, 0.16 m^2) within 21 days. Remark: error bars indicate standard deviations, with different lowercase letters indicating significant differences at ($p \leq 0.05$)

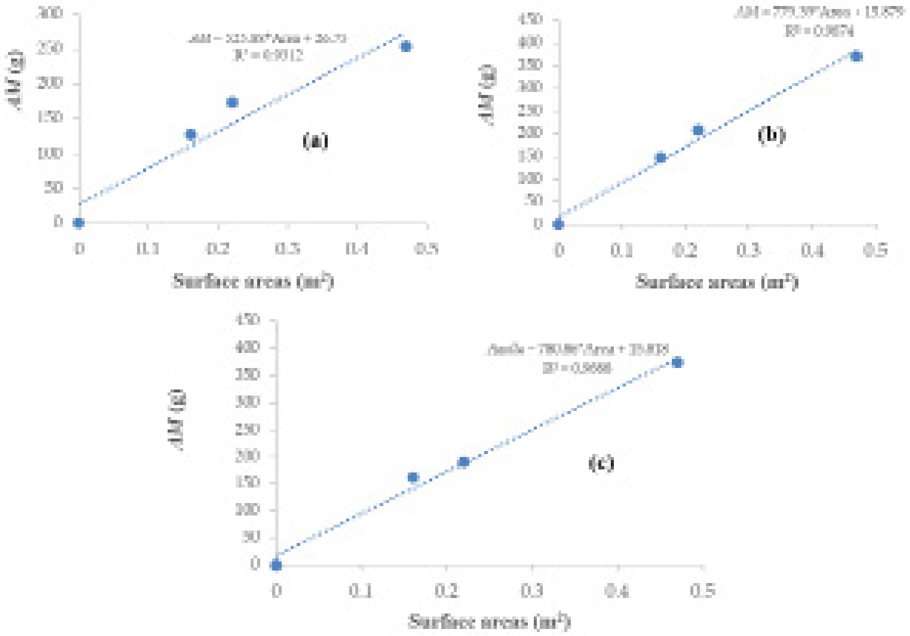

Figure 3. Relationship between the growth of *AM* (fresh biomass) and surface areas during 7 days, 14 days, and 21 days. Remark: (a) Estimation of the biomass of *AM* for 7 days; (b) Estimation of the biomass of *AM* for 14 days; (c) Estimation of the biomass of *AM* for 21 days.

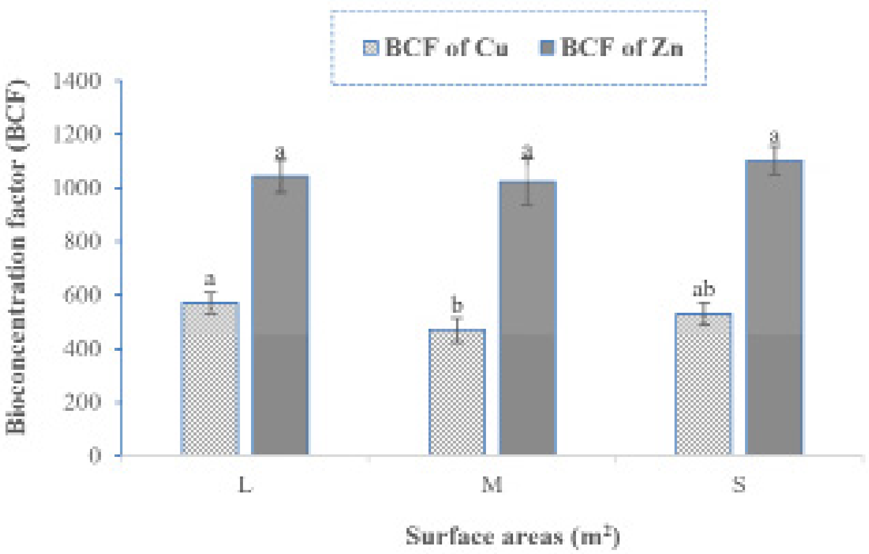

Figure 4. Bioconcentration factor of heavy metal (Cu and Zn) in biomass of *AM* (dried biomass basis) in different surface areas including the large (L, 0.47 m^2), medium (M, 0.22 m^2), and small (S, 0.16 m^2) within 21 days. Remark: error bars indicate standard deviations, with different lowercase letters indicating significant differences at ($p \leq 0.05$).

Table 1. Biomass of *AM*, relative growth rate, and doubling time after the initial introduction of *AM* 74g in PW 5 (60 litters without compensation) in 3 different surface areas (L, M, and S) within 21 days under polyethylene house condition at temperature 32.7 ± 3.5 °C

Parameters	Area (m^2)	Period of time (days) (mean ± SD, n = 3)			
		0 day	7 days	14 days	21 days
*Azolla*_fresh (g)	L	74 ± 0 a	254 ± 5.7 a	370 ± 20.8 a	374 ± 11.8 a
	M	74 ± 0 a	174 ± 6.9 b	208 ± 4.7 b	191 ± 1.4 b
	S	74 ± 0 a	126 ± 10.3 c	148 ± 18.1 c	162 ± 17.8 c
*Azolla*_dry (g)	L	2.81 ± 0 a	9.7 ± 0.22 a	14 ± 0.79 a	14.2 ± 0.45 a
	M	2.81 ± 0 a	6.6 ± 0.26 b	7.9 ± 0.18 b	7.3 ± 0.05 b
	S	2.81 ± 0 a	4.8 ± 0.39 c	5.6 ± 0.69 c	6.2 ± 0.68 c
RGR (g/g/d)	L	-	0.18 ± 0.003 a	0.11 ± 0.004 a	0.08 ± 0.001 a
	M	-	0.12 ± 0.006 b	0.07 ± 0.002 b	0.05 ± 0 b
	S	-	0.08 ± 0.011 c	0.05 ± 0.009 c	0.04 ± 0.005 c
DT (days)	L	-	3.93 ± 0.07 c	6.04 ± 0.2 c	8.98 ± 0.17 c
	M	-	5.68 ± 0.26 b	9.41 ± 0.2 b	15.34 ± 0.12 b
	S	-	9.95 ± 0.18 a	15.62 ± 1.22 a	17.5 ± 1.1 a

Remark: (-) Not changing; (DT) Doubling time; (RGR): Relative growth rate; (PW 5): Piggery wastewater 5% diluted with tap water; (L) Large plastic container = 0.47 m^2; (M) Medium plastic container = 0.22 m^2; (S) Small plastic container = 0.16 m^2; (n=3) Three replicates of each area; (SD) Standard deviation; the letter (a, b, c) Showed the least significant differences ($P \leq 0.05$) (from a to b = high to low; yet the same letter was not difference)

Table 2. Characteristics of chemical parameters in PW 5 and *AM* in 3 different surface areas (L, M, and S) before and after the experiment under polyethylene house condition at temperature 32.7 ± 3.5 shown in mean values and standard deviation

Parameters	Time (days)	Surface areas (m^2) (mean ± SD, n = 3)			CV (%)	LSD (0.05)
		L	M	S		
			PW 5			
Cu (mg/L)	0	0.11 ± 0.01 a	0.11 ± 0.01 a	0.11 ± 0.01 a	9.09	ns
	21	0.06 ± 0.01 a	0.06 ± 0 a	0.05 ± 0 a	8.66	ns
Zn (mg/L)	0	0.23 ± 0.02 a	0.23 ± 0.02 a	0.23 ± 0.02 a	6.65	ns
	21	0.21 ± 0.01 b	0.23 ± 0.01 ab	0.23 ± 0 a	3.57	*
			AM			
Cu (mg/kg)	0	49.75 ± 3.5 a	49.75 ± 3.5 a	49.75 ± 3.5 a	7.09	ns
	21	63.07 ± 3.6 a	51.77 ± 1.8 b	58.35 ± 1.4 a	4.21	*
Zn (mg/kg)	0	111.7 ± 1.08 a	111.7 ± 1.08 a	111.7 ± 1.08 a	0.96	ns
	21	236.1 ± 3.28 b	231.4 ± 3.25 b	249.3 ± 6.78 a	1.98	*
TKN (%)	0	1.65 ± 0.07 a	1.65 ± 0.07 a	1.65 ± 0.07 a	4.24	ns
	21	1.86 ± 0.04 b	2.41 ± 0.02 a	2.2 ± 0.24 a	6.67	*
Crude protein (%)	0	10.3 ± 0.43 a	10.3 ± 0.43 a	10.3 ± 0.43 a	4.17	ns
	21	11.65 ± 0.25 b	15.04 ± 0.12 a	14.68 ± 0.83 a	3.67	*
TP (%)	0	0.37 ± 0.02 a	0.37 ± 0.02 a	0.37 ± 0.02 a	4.68	ns
	21	0.36 ± 0.02 b	0.40 ± 0 b	0.43 ± 0.02 a	4.59	*

Remark: (PW 5) Piggery wastewater 5% diluted with tap water; (L) Large plastic container = 0.47 m^2; (M) Medium plastic container = 0.22 m^2; (S) Small plastic container = 0.16 m^2; (n=3) Three replicates of each area; (CV %): Coefficient of variation; (LSD *) Least significant differences at confidential interval 95% ($p \leq 0.05$); (ns) non-significant differences at 95% ($p>0.05$)

Table 3. Percentage of decrease of chemical parameters from PW 5 and increase in *AM* after the experiment within 21 days under polyethylene house condition at temperature 32.7 ± 3.5 °C

Parameters	Surface area (m^2) (n = 3)			CV (%)	LSD (0.05)
	L	M	S		
	Decreasing from PW 5				
Cu	48.37 ± 6.34 a	49.59 ± 5.26 a	53.79 ± 3.1 a	10.05	ns
Zn	11.21 ± 3.96 a	0	0	52.17	*
	Increasing in *AM*				
Cu	22.19 ± 4.5 a	10.34 ± 0.37 b	13.18 ± 3.68 b	22.07	*
Zn	111 ± 2.18 b	107 ± 4.48 b	123 ± 7.06 a	4.38	*

Remark: (PW 5) Piggery wastewater 5% diluted with tap water; (L) Large plastic container = 0.47 m^2; (M) Medium plastic container = 0.22 m^2; (S) Small plastic container = 0.16 m^2; (n=3) Three replicates of each area; (CV %) Coefficient of variation; (LSD *) Least significant differences at confidential interval 95% ($p \leq 0.05$); (ns): non-significant differences at 95% ($p > 0.05$)

Table 4. Characteristics of physical parameters after initial inoculation of *AM* 74g in PW 5 (60 litters without compensation) with 3 different surface areas (L, M, and S) during the experiment period under polyethylene house condition at temperature 32.7 ± 3.5 °C shown in mean values and standard deviation

Parameters	Area (m^2)	Period of time (days) (mean ± SD, n = 3)			
		0 day	7 days	14 days	21 days
Temperature (°C)	L	30.7 ± 0.3 a	28.5 ± 0.4 b	28.6 ± 0.4 a	28.8 ± 0.2 a
	M	30.7 ± 0.3 a	29.4 ± 0.2 a	28.3 ± 0.1 a	28.7 ± 0.1 a
	S	30.7 ± 0.3 a	29.5 ± 0.1 a	28.5 ± 0.1 a	28.8 ± 0.1 a
EC (µS/cm)	L	433 ± 0.3 a	376 ± 0.7 b	366 ± 4.5 ab	386 ± 8.2 b
	M	433 ± 0.3 a	377 ± 1.2 ab	361 ± 4.3 b	389 ± 2.3 ab
	S	433 ± 0.3 a	386 ± 7.8 a	377 ± 7 a	402 ± 9.7 a
TDS (mg/L)	L	259 ± 2.3 a	203 ± 9.2 b	260 ± 13.9 a	263 ± 9.9 b
	M	259 ± 2.3 a	200 ± 0 b	136 ± 6 b	226 ± 4 c
	S	259 ± 2.3 a	272 ± 8 a	267 ± 15.1 a	294 ± 11.1 a
TSS (mg/L)	L	39 ± 6.1 a	62 ± 6 b	36 ± 6 a	78 ± 2 c
	M	39 ± 6.1 a	50 ± 6 b	19 ± 6.4 b	100 ± 13.1 b
	S	39 ± 6.1 a	85 ± 11.5 a	23 ± 7 b	197 ± 1 a

Remark: (PW 5): Piggery wastewater 5% diluted with tap water; (L) Large plastic container = 0.47 m^2; (M) Medium plastic container = 0.22 m^2; (S) Small plastic container = 0.16 m^2; (n=3) Three replicates of each area; (SD) Standard deviation; the letter (a, b, c): showed the least significant differences ($P \leq 0.05$) (from a to b = high to low; yet the same letter was not difference)

Table 5. Characteristics of chemical parameters after initial inoculation of *AM* 74g in PW 5 (60 litters without compensation) with 3 different surface areas (L, M, and S) during the experiment period under polyethylene house condition at temperature 32.7 ± 3.5 ºC shown in mean values and standard deviation

Parameters	Area (m^2)	Period of time (days) (mean ± SD, n = 3)			
		0 day	7 days	14 days	21 days
pH	L	7.4 ± 0.06 a	7.8 ± 0.17 a	7.8 ± 0.02 a	7.6 ± 0.02 a
	M	7.4 ± 0.06 a	7.8 ± 0.04 a	7.8 ± 0.03 a	7.7 ± 0.03 a
	S	7.4 ± 0.06 a	7.9 ± 0.08 a	7.8 ± 0.03 a	7.7 ± 0.02 a
DO (mg/L)	L	6.9 ± 0.2 a	6.6 ± 0.5 a	5.5 ± 0.1 a	5.8 ± 1.5 a
	M	6.9 ± 0.2 a	7.2 ± 0.2 a	5.8 ± 0.3 a	6.7 ± 0.4 a
	S	6.9 ± 0.2 a	7.2 ± 0 a	5.8 ± 0.1 a	6.3 ± 1.1 a
BOD (mg/L)	L	13.5 ± 0 a	7.5 ± 9.78 a	10.5 ± 0.7 a	16.5 ± 1.26 a
	M	13.5 ± 0 a	3 ± 9.62 b	9 ± 5.25 a	9 ± 3.34 b
	S	13.5 ± 0 a	4.5 ± 2.51 b	10 ± 4.17 a	4.25 ± 2.67 b
COD (mg/L)	L	90.4 ± 8.2 a	69.2 ± 10.7 b	72.5 ± 3.6 a	40 ± 12.4 b
	M	90.4 ± 8.2 a	69.2 ± 17.8 b	22.6 ± 3.6 b	86.3 ± 3.6 a
	S	90.4 ± 8.2 a	154.9 ± 17.8 a	21.4 ± 4.1 b	42.3 ± 8.2 b
NO_3^--N (mg/L)	L	1.04 ± 0.01 a	0.7 ± 0 ab	0.42 ± 0.01 ab	0.39 ± 0.01 b
	M	1.04 ± 0.01 a	0.59 ± 0.02 b	0.51 ± 0.06 a	0.37 ± 0.01 b
	S	1.04 ± 0.01 a	0.8 ± 0.11 a	0.39 ± 0.05 b	0.44 ± 0.03 a
PO_4^{3-} (mg/L)	L	1.41 ± 0.01 a	0.86 ± 0.06 b	0 ± 0 c	0 ± 0 c
	M	1.41 ± 0.01 a	0.74 ± 0.07 b	0.39 ± 0.02 b	0.42 ± 0.04 b
	S	1.41 ± 0.01 a	1.39 ± 0.14 a	1.18 ± 0.16 a	1.23 ± 0.24 a

Remark: (PW 5) Piggery wastewater 5% diluted with tap water; (L) Large plastic container = 0.47 m^2; (M) Medium plastic container = 0.22 m^2; (S) Small plastic container = 0.16 m^2; (n=3) Three replicates of each area; (SD) Standard deviation; the letter (a, b, c) Showed the least significant differences ($P \leq 0.05$) (from a to b – high to low; yet the same letter was not difference)

Table 6. Percentage of removing contaminants by inoculation of biomass of AM 74g at the beginning in PW 5 (60 litters) with 3 different surface areas (L, M, and S) under polyethylene house condition at temperature 32.7 ± 3.5 ºC

Parameters	Time (days)	Efficiencies of *AM* (R %) Surface areas (m^2) (mean ± SD, n = 3)			CV (%)	LSD (0.05)
		L	M	S		
EC	7	13 ± 0.2 a	13 ± 0.3 a	11 ± 1.8 b	8.31	ns
	14	15 ± 1.1 ab	17 ± 1 a	13 ± 1.6 b	8.39	*
	21	11 ± 1.9 a	10 ± 0.6 ab	7 ± 2.2 b	18.3	ns
TDS	7	21.7 ± 2.89 a	22.7 ± 0.69 a	-5.1 ± 2.33 b	16.7	*
	14	-3.9 ± 0.81 c	47.4 ± 2.75 a	0.8 ± 0.77 b	11.63	*
	21	-3.5 ± 0.38 b	12.6 ± 0.95 a	-15.9 ± 1.81 c	-53.57	*
TSS	7	-41 ± 13.9 a	-48 ± 8.1 a	-121 ± 10.9 b	-15	*
	14	15 ± 10.2 b	52 ± 14.9 a	40 ± 13.3 ab	36	*
	21	-84 ± 6.4 a	-213 ± 25 b	-369 ± 23.5 c	-9.08	*
BOD	7	44.4 ± 9.8 b	77.6 ± 2.5 a	66.8 ± 9.6 a	12.83	*
	14	21.2 ± 0.7 b	32.8 ± 4.2 a	25.5 ± 5.3 a	15.8	*
	21	-22.1 ± 1.3 c	32.8 ± 3.3 b	67.6 ± 6.7 a	18.39	*
COD	7	31.1 ± 0.5 a	33.9 ± 14.7 a	-91.2 ± 10.4 b	-19.74	*
	14	19.4 ± 8.1 b	74.9 ± 4.7 a	76.1 ± 5.9 a	12.26	*
	21	64.3 ± 2.7 a	8.3 ± 5.1 b	52.4 ± 12.8 a	19.45	*
NO_3^--N	7	33 ± 0.86 b	43 ± 1.91 a	17 ± 0.15 c	3.92	*
	14	60 ± 0.35 a	51 ± 5.78 b	66 ± 0.35 a	5.74	*
	21	63 ± 0.6 a	64 ± 1.21 a	58 ± 2.6 b	2.74	*
PO_4^{3-}	7	39 ± 4.8 a	48 ± 4.5 a	6 ± 4.9 b	15.24	*
	14	100 ± 0 a	72 ± 1.6 b	16 ± 11.5 c	10.67	*
	21	100 ± 0 a	70 ± 2.5 b	19 ± 13 c	12.15	*

Remark: (-) decrease efficiency; (PW 5) Piggery wastewater 5% diluted with tap water; (L) Large plastic container – 0.47 m^2; (M) Medium plastic container – 0.22 m^2; (S) Small plastic container – 0.16 m^2; (n=3) Three replicates of each area; (R %) Percentage of removing; (CV %) Coefficient of variation; (LSD *) Least significant differences at confidential interval 95% ($p \leq 0.05$); (ns) non-significant differences at 95% ($p > 0.05$)

Table 7. Physicochemical characteristics of raw material including CD and CDA at ratio 1:1

Parameters	CD	CDA	CV (%)	LSD (0.05)
pH	8.02 ± 0.2 a	7.97 ± 0.01 a	1.14	ns
EC (mS/cm)	1.95 ± 0.01 b	3.22 ± 0.09 a	2.27	*
OC (%)	21.7 ± 0.38 b	23.1 ± 0.24 a	1.62	*
OM (%)	37.4 ± 0.66 b	39.9 ± 0.41 a	1.61	*
Cu (mg/kg)	69.9 ± 2.4 a	59.9 ± 0.6 b	3.51	*
Zn (mg/kg)	244.3 ± 1.4 a	234.1 ± 3.3 b	0.99	*
C/N	26.5 ± 2.27 a	19.6 ± 0.16 b	6.36	*

Remark: (CD) cow dung; (CDA) cow dung with *Azolla microphylla* diriving from cultivation by piggery wastewater at 5%; Values in the table are mean of three replicates ± standard deviation; (*) significant difference with confidential interval 95% (p-value ≤ 0.05); (ns) non-significant difference (p-values > 0.05)

Table 8. Comparison nutrient quality between compost (without earthworm) and vermicompost (with the earthworm, *Eudrillus ugeniae*)

Parameters	CD		CDA		LSD (0.05)
	Compost	VC	Compost	VC	
pH	8.28 ± 0.01 a	7.90 ± 0.19 b	7.99 ± 0.11 b	7.87 ± 0.09 b	*
EC (mS/cm)	1.97 ± 0.12 c	2.37 ± 0.16 b	2.82 ± 0.18 a	2.95 ± 0.07 a	*
OC (%)	16.1 ± 0.19 a	14.2 ± 0.17 d	15 ± 0.06 b	14.5 ± 0.21 c	*
OM (%)	27.8 ± 0.33 a	24.5 ± 0.29 d	25.9 ± 0.1 b	25.1 ± 0.37 c	*
Cu (mg/kg)	59.7 ± 0.2 ab	62.1 ± 3.1 a	56.2 ± 2.1 b	58.6 ± 0.8 ab	*
Zn (mg/kg)	301.7 ± 1.5 c	326.5 ± 0.5 b	298.1 ± 5.1 c	334 ± 3.3 a	*
C/N	17.8 ± 0.21 a	15 ± 0.38 c	16.2 ± 0.38 b	13.5 ± 0.32 d	*

Remark: (CD) cow dung; (CDA) cow dung with *Azolla microphylla* diriving from cultivation by piggery wastewater at 5%; Values in the table are mean of three replicates ± standard deviation; (*) significant difference with confidential interval 95% (p-value ≤ 0.05); (ns) non-significant difference (p-values > 0.05)

4. Discussions

The large surface area within 14 days showed better performance for the *Azolla* growth because this fern produces its mat horizontally (Lumpkin and Plucknett, 1980). The much density in the small area causes less productivity, and it would decrease its biomass after completed growth of around 13 to15 days (Khan, 1988). The doubling time (DT) and relative growth rate (RGR) of *AM* were also good in respective (3.93 - 6.04 days) and (0.18 - 0.11 g/g/d) within period experiment 7 days and 14 days. It was slightly good if compare to previous works showed the DT of *AM* growth was about 5.4 days and RGR 0.13 (g/g/d) within 14 days (Arora and Singh, 2003), and (DT, 3.93 days) within 7 days was similar to the previous study (DT, about 3.5 to 6 days) (Arora and Saxena, 2005; Box, 2018). Furthermore, the DT in the L was 8.98 days (for 21 days) which was stayed in the reported range from 7.41 to 9.62 days with the experimental period of 28 days (Kimani *et al.,* 2020). Whereas, the bioconcentration factor (BCF) showed high values of approximately 570 and 1100 for Cu and Zn in the L, respectively. This result was revealed similarly to the previous study (Akhtar *et al.,* 2019). Normally, the BCF values over 1000 were considered as an ideal indicator of useful fern for phytoremediation (Adel *et al.,* 1998). Therefore, *AM* was a good hyperaccumulator of heavy metal that could be used for removing Cu and Zn from PW5. The crude protein in biomass of *AM* after the experiment was approximately 15% compared to preceding studies was about (18 - 20%) in *Azolla* (Datta, 2011; Brouwer *et al.,* 2019).

The temperature in each size (L, M, and S) was shown significantly different ($P < 0.05$) within 7 days. The temperature in the L size showed slightly lower around (30.7-28.5 ^{o}C), while the M and S sizes were (30.7-29.5 ^{o}C) due to the L consisting of a thick mat of *AM* not allowing the surface water to exchange temperature with the atmosphere (Kimani *et al.,* 2020). However, it was revealed non-significant differences ($P > 0.05$) within 21 days in all sizes around 28.8 ^{o}C. The M size indicated high effectiveness in reducing EC 17% (433-361μS/cm); however, all sizes of surface area decreased efficiency within 21 days due to its adult phase (high capacity) around 13 to15 days, afterward, it could die or get lower effectiveness (Khan, 1988). Whereas TDS was removed effectively in the M from 259 - 136 mg/L (47.4%) for 14 days, that was a bit slighter than the previous study (Amare *et al.,* 2018), under restriction levels of wastewater reuse for irrigation (< 2000 mg/L) Saudi Arabia, and (< 1500 mg/L) for Jordan (WHO, 2006), yet the efficacy was declined in all types of surface area within 21 days. Based on the Food and Agriculture Organization (FAO) reported that TDS and EC in water less than 450 mg/L and 0.7 dS/m, respectively, did not impact crop-growing (USEPA, 2012). Meanwhile, TSS was reduced from (39 - 19 mg/L) about 52% in the M within 14 days. This concentration is below the standard of discharging of piggery wastewater in Thailand (PCD, 2016), and effluent reuse for irrigation in Jordan (WHO, 2006). However, all types of surface areas decreased efficiency within 21 days. The efficacy of *AM* was mostly good within 14 days because of its full growth around 13 - 15 days (Khan, 1988). Furthermore, much density of biomass of *AM* could make it die and decompose, which causes the effectiveness to decline after its completed growth. The pH value was kept in balance around (7.4 - 7.6), which is in the range of water irrigation standards permission 6.5 - 8.4, (USEPA, 2012), and DO value was around (6.9 - 6.7 mg/L) also good for all organisms to survive (USEPA, 2006). The S and M sizes were the most effective in reducing BOD by 66% (13.5-3 mg/L) and 77% (13.5 - 4.5 mg/L) individually within 7 days, a bit higher than a study was demonstrated (Amare *et al.,* 2018), while it was similar to previous work (Noorjahan and Jamuna, 2015). All the values of BOD stayed below permission of discharging effluent from piggery farm to public bodies (60 - 100 mg/L) in Thailand (PCD, 2016), the standard of wastewater reuse for crop irrigation in Jordan (30 - 300 mg/L), and limitation of using recycled water for restricted irrigation in Saudi Arabia (< 40 mg/L), and the standard for wastewater reuse (< 20 mg/L) in Kuwait (WHO, 2006). The COD was removed excellently the S area by 76% (90.4 - 21.4 mg/L) within 14 days, comparable to the previous one (Amare *et al.,* 2018). The COD values were lower than the standard of piggery wastewater (not over 100 mg/L) in Thailand (PCD, 2016), and the standard of effluent reuse for irrigation in Jordan, restricted irrigation in Saudi Arabia, and effluent reuse in Kuwait (WHO, 2006). The S size was the most effective in removal NO_3^- within 14 days by 66% (5.22 - 3.99 mg/L). The values of NO_3^- were quite good for crop irrigation, below the standard of irrigation (30 mg/L) (USEPA, 2012). Moreover, $PO4^{3-}$ was removed 100% in the L area within 14 days which is friendly to the environment, not to cause eutrophication. Based on the previous study demonstrated that the concentration of PO_4^{3-} in water is more than 1 mg/L causing high eutrophication (Fadiran *et al.,* 2008). Regarding the percentage of reduction of NO_3^- and PO_4^{3-} was similar to the efficiency of A. filiculoides in removing nitrogen and phosphorous (Amare *et al.,* 2018).

Meanwhile, the Cu was reduced from PW5 to approximately 53% (0.11 - 0.05 mg/L) in the three distinct surface areas, while Zn was removed excellently in the L area about 11% (0.23 - 0.21 mg/L) within 21 days. The amount of Cu and Zn are potential micronutrients for crops to thrive well (Talebi *et al.,* 2019), in case the concentration of Cu and Zn in water is less than 0.2 (mg/L) and 2 mg/L, respectively (USEPA, 2012). Therefore, this result is below the standard of water reuse of the USEPA. The L showed that the concentration of Cu increased in biomass of *AM* 22% (49 to 63 mg/kg), while Zn was increased highly in the S about 123% (111-249 mg/kg) which is higher than A. caroliniana demonstrated in previous work (Morand *et al.,* 2011). Besides, the bioconcentration factor (BCF) showed high values around 570 and 1100 of Cu and Zn in the L, respectively. This result was comparable to the previous study (Akhtar *et al.,* 2019). Normally, the BCF values over 1,000 were considered as an ideal indicator of useful fern for phytoremediation (Arora *et al.,* 2006). Therefore, *AM* was a good hyperaccumulator of heavy metal that could be used for removing Cu and Zn from piggery wastewater. Mostly, the requirement of Zn concentration in leaves for enough growth of the crop is around 15 - 20 mg/kg, and the maximum limitation (threshold level) is approximately 100 - 300 mg/kg of dried leaves (Broadley *et al.,* 2007; Talebi *et al.,* 2019). On the other hand, the concentration of the Cu and Zn in *AM* of this study was also beneath the standard of soil metal (agricultural soil) in China approximately100 mg/kg and 250 mg/kg, respectively (Lixia *et al.,* 2008), and the European Union standard guideline, the amount of Cu and Zn in the soil, was not over in respective 100 mg/kg and 200 mg/kg (Tóth *et al.,* 2016). Therefore, *AM* could be applied as a biofertilizer for increasing soil fertility. Moreover, it was a good source of Cu and Zn for animal feed blending, especially for pig production to eliminate the risk of diarrhea (Shelton *et al.,* 2009). Moreover, the previous research showed that when Cu and Zn concentration were blended in pig feed individually 27 mg/kg and 65 mg/kg could preserve the growth rate and mineral homeostasis, which is still below the standard of commission regulation No. 1334/2003 of the European Union for animal production 25 mg/kg and 150 mg/kg, respectively (Hernández *et al.,* 2008). In furtherance, the appropriate levels of Cu and Zn in pig dietary of Denmark for piglets (175 and 250mg/kg), growing-finishing pigs (35 and 250 mg/kg), and sows (35 and 250 mg/kg), respectively (Poulsen, 1998). Besides, the limitation of Cu and Zn in the diet of various categories of animals were allowed in respective including dairy cattle (35 and 150 mg/kg), piglets under 12 weeks (170 and 150 mg/kg), and broilers (25 and 150 mg/kg) (Jongbloed *et al.,* 2004). As a recommendation, the amount of biomass of *AM* should be calculated following the categories and requirements before applying.

The vermicompost (decomposed by earthworm) revealed better than the compost that was parallel to the previous study (Bhat *et al.,* 2018). The pH in vermicompost of cow dung (CD) and cow dung with *Azolla* (CDA) was (7.9 and 7.87) lower than the compost (8.28 and 7.99). These values were similar to the previous study (Bhat *et al.,* 2015b). Furthermore, the OC was significantly lower in vermicompost of CDA and CD (14.5% and 14.2%), while the compost over (15% and 16%), respectively, due to the efficiency of earthworm in decomposing of organic waste, meanwhile, the OM was lower in vermicompost of CD and CDA around (24.5% and 25.1%) in comparison to compost over 25% due to the efficacy of earthworms (Eudrillus ugeniae) that enabled to break and degrade organic matter to produce vermicast (Julka, 1993; Bhat *et al.,* 2018).

The Cu was increased in vermicompost of CD and CDA about (62.1 mg/kg and 58.6 mg/kg), while the compost was less than (59 mg/kg). Meanwhile, Zn showed highly in vermicompost of CDA around (334 mg/kg) in comparison to the compost less than (302 mg/kg). This increase of Cu and Zn could be because of the mechanism of earthworms in decomposing organic waste, and this outcome was also comparable to the previous ones (Bhat *et al.,* 2015b; Ananthavalli *et al.,* 2019). Interestingly, the C/N ratio was significantly reduced in vermicompost of CDA approximately (13.5), while the vermicompost and compost were more than (15 and 17.8), respectively. This showed that the earthworms' activities in degradation were more effective in the mixed materials of CDA than CD. Furthermore, the vermicompost quality not only could be stored for further use up to 3 months but also could improve the quality as well owing to in the vermicompost consisted of many activities of bacteria and fungi continuing to decompose organic matter (Kleawklaharn and Iwai, 2014).

5. Conclusion

The three different sizes of surface areas including the large (L, 0.47 m^2), medium (M, 0.22 m^2), and small (S, 0.16 m^2), and experimental periods were significantly affected on both biomass productivity of *AM* and contaminants' removal from piggery wastewater 5% (PW5). The biomass of *AM* was produced vastly in the L, and the doubling time (DT) and relative growth rate (RGT) of *AM* were also good in the L within 14 days. Meanwhile, the contaminants were removed well, mostly in the M and S sizes within 14 days. The concentration of contaminants in the PW5 was beneath the standard of discharging wastewater of Thailand and other standards as mentioned in the discussion. Therefore, the PW5 after treatment could be applied for irrigation or other uses. Whereas, the vermicompost provided better quality than traditional compost in the mixed materials of cow dung with *AM* (CDA). Hence, piggery wastewater was a good source for producing biomass of *AM*, while the biomass of *AM* was a good supplemental ingredient for enhancing nutrient quality for crops which could be followed the BCG economy model.

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