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Determination of chlorophyll *a* and total phosphorus abundance in organic manured fish ponds

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Abstract. An experiment was conducted for 6 weeks (between August and September 2010) with 4 treatments (T1: poultry manure, T2: cattle manure, T3: poultry and cattle manure and T4: control-no manure) assigned to 8 concrete ponds at random to determine chlorophyll *a* and total phosphorus abundance in organic manured fish ponds. Results of the study suggest that T1, T2, and T3 had an effect on chlorophyll *a* and total phosphorus abundance in organic manured ponds. The final mean average for chlorophyll *a* ranged from 0.0105mg/m^3 to 0.045mg/m^3 and that of total phosphorus ranged from 0.024mg/50mL to 0.522mg/50mL. Chlorophyll *a* and phosphorus were positively correlated with a coefficient of 0.92 and it was significant ($p \leq 0.05$). However, weekly performance of chlorophyll *a* and total phosphorus was higher in T3 compared to other treatments. The treatments also showed differences in the number of plankton species available. Phytoplankton species in T3 increased from 888 in week three to 933 in week six. However, the zooplankton species reduced from 10 in week three to 4 in week six compared to other treatments. These results indicate that animal manure varied in influencing the production of chlorophyll *a*, planktons and phosphorus. The variation may be attributed to feed nutritive value of the animals to which they were fed. Farmers should therefore be encouraged to use a combination of poultry and cattle manure to obtain maximum benefits in increasing chlorophyll *a* and total phosphorus.
Key Words: determination, chlorophyll *a*, total phosphorus, organic manure, plankton and fish ponds.

Introduction. Most small scale fish farmers in Zambia have embraced the practice of aquaculture or pond fish farming over the years utilizing different manures as nutrient supplement (Mwale et al 2007). In Zambia, organic manures are readily available and are of cheap source compared to inorganic fertilizers. The use of organic manure for pond fertilization has become one of the most important management operations in recent years by small scale fish farmers, basically to increase biological productivity of fish ponds, thereby helping to raise their standard of living (Sevilleja et al 2001; Hobab 2009). Their wide acceptance lies in their ability to increase chlorophyll *a* and phosphorus abundance in organic manured ponds. The presence of chlorophyll *a,* a principal pigment in all green plants in an aquatic environment indicates the amount of phytoplankton and monitoring its abundance in aquatic environment is a direct way of knowing phytoplankton growth in fish ponds. Chlorophyll *a* abundance in aquatic environment is correlated to the presence of phosphorus a limiting element of biological productivity (Arrignon 1998).

There are several ways that phosphorus enters the water body and one of them is by organic manure decomposition. Organic manure in ponds decomposes and releases phosphorus which stimulates the growth of planktons and increase nutrient availability, leading to increased water quality and fish growth (Sevilleja et al 2001).

The study was conducted in concrete ponds, to determine chlorophyll *a*, total phosphorus and different species of plankton availability in organic manured fish ponds.

Material and Method. An experiment was conducted at the National Aquaculture Research and Development Centre (NARDC), Mwekera in Zambia, using eight (8) outdoor concrete ponds measuring 3m x 3.75m x 1m depth. Each pond was filled with 11,250L of borehole water, at the same time inoculated with 50L of phytoplankton water from another pond which was intended to activate borehole water to speed up phytoplankton production. A constant water depth of 0.25m was maintained throughout the experimental period. Fresh poultry and cattle manure were used with the standard manure application rate of 3kg of poultry manure per week/100m^2 and 10kg of cattle manure per week/100m^2 according to FAO (1994) over a period of six weeks (from August to September 2010). Combined manures were done in parts: 1 part for poultry, 3 parts (approximately 10kg) for cattle per 100m^2/week (Table 1).

Table 1

Treatment and value of organic manure applied to experimental ponds

Treatments	*Value of applied manure*
T1 (poultry)	4.05kg
T2 (cattle)	13.5kg
T3 (poultry and cattle)	13.5kg of both poultry and cattle done in parts: 1part of poultry and 3 parts for cattle.
T4 (control)	No manure applied

Treatments were randomly assigned to experimental ponds. Dissolved oxygen, water temperature and pH were monitored once per week using a water quality checker. Sampling for both chlorophyll *a* and phosphorus was done once per week in each pond with analysis of all samples being done on the same day at the Copperbelt University Biology Laboratory to avoid contamination and degradation.

Chlorophyll *a* was analyzed using spectrophotometry method as prescribed by HP (2000). During sampling, 1000mL of water from each treatment was filtered at a depth of 10cm and from the filtered water of each pond; 50mL was collected in the opaque bottle for phosphorus determination. Ascorbic Acid Method was used to find total phosphorus as recommended by APHA (1998).

A plankton net was passed through each pond on the third and sixth week of the experimental period and 20mL of the water that remained in the net was collected and a drop (0.05mL) was put on the microscope slide to determine the different species present.

A Completely Randomized Design (CRD) was used with four treatments (T1 = poultry manure, T2 = cattle manure, T3 = combination of poultry and cattle (C+P) and T4 = no manure (control)), each replicated two times.

GenStat Discovery, 3rd Edition was used to analyze the data while Microsoft excel was used for graphical presentation of results. Data collected on chlorophyll *a* and total phosphorus were analyzed using one way Analysis of Variance (ANOVA). Treatment means showing significant differences at $p \leq 0.05$ were separated using the Least Significant Differences (LSD). Interactions of treatments were done to find particular trend and relationship between variables.

Results. Mean chlorophyll *a* determined during the experimental period is summarized in Table 2 and Figure 1, which shows variation in all treatments.

There was significant differences ($p \leq 0.05$) in mean chlorophyll *a* of animal manure among the treatments in percentage with T1 (3.2%), T2 (2.3%), T3 (4.5%) and T4 (1.05%). Differences of chlorophyll *a* among the treatments were observed between T1 and T2, T1 and T4, T2 and T4, and T3 and T4 with differences of 0.009, 0.0215, 0.0125 and 0.0345, respectively, using Least Significant Differences (LSD) at 5% level of significant.

Table 2

Mean values of chlorophyll *a* in mg/m^3 over 6 weeks experimental period

Treatments	*Final mean values of chlorophyl a in mg/m^3*
T1	0.032
T2	0.023
T3	0.045
T4	0.011

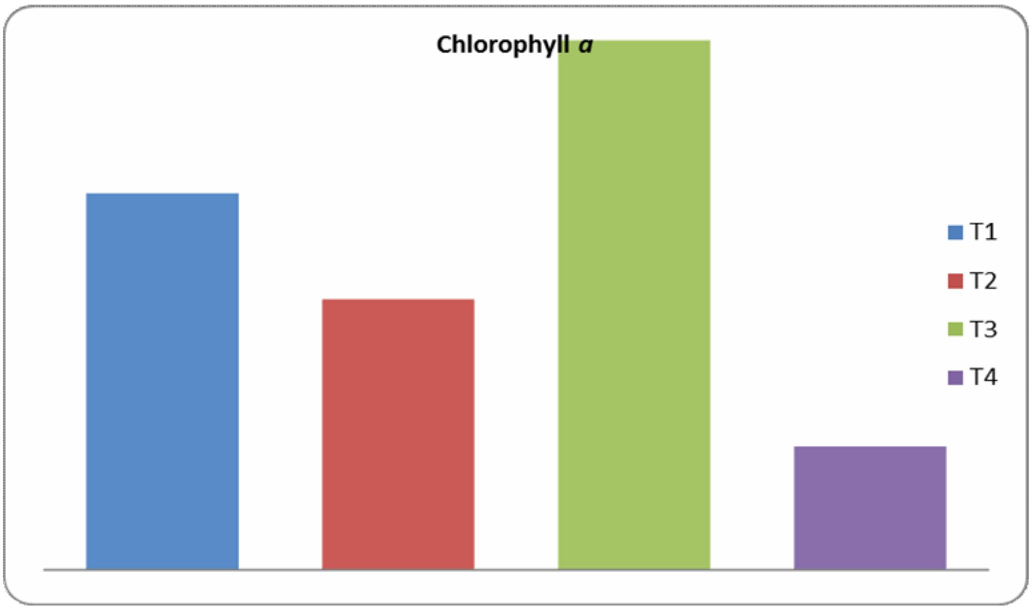

Figure 1. Chlorophyll *a* variation in treatments over 6 week's experimental period.

Weekly performance of animal manure towards chlorophyll *a* production was seen to vary tremendously in each pond over 6 weeks after manure application during the experimental period. It was further observed that treatment 3 (T3) performed better than the rest as shown in Figure 2.

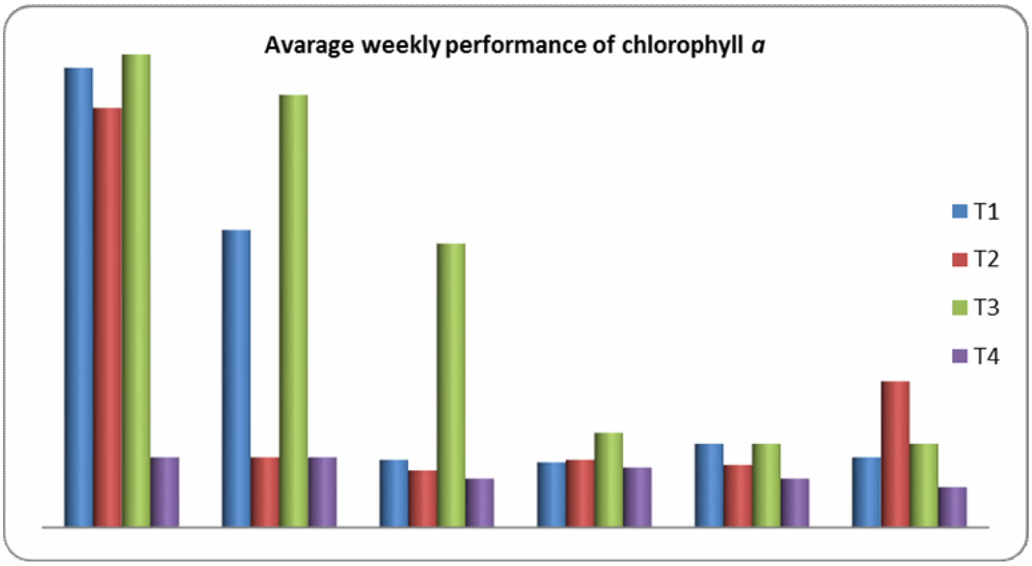

Figure 2. Average weekly performance of chlorophyll *a*.

Animal manure had a significant effect ($p \leq 0.05$) on total phosphorus among treatments. Treatment 3 (T3) was the highest (52.2%), followed by T1 (47.65%) then T2

(9.5%) and lastly T4 (2.4%). Least Significant Difference (LSD) at 5% level of significance was used to separate mean differences between the treatments with T1 and T2 (0.3815), T1 and T4 (0.4525), T2 and T4 (0.071) and T3 and T4 (0.4975), respectively.

The average performance of animal manure towards phosphorus production was seen to vary among the treatments with T3 performing better than the rest of other treatments (see Figure 3 and Table 3).

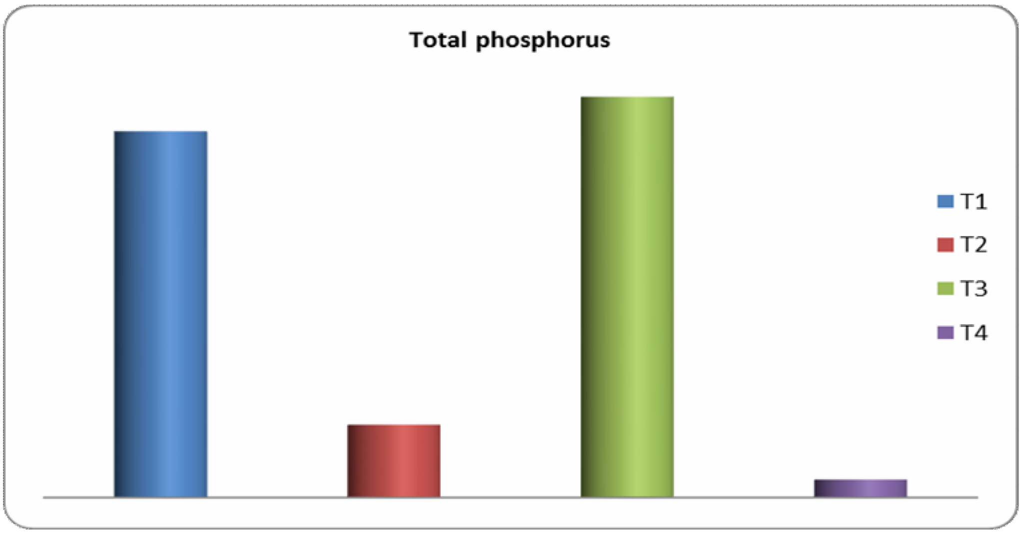

Figure 3. Phosphorus variation in treatments over 6 week's experimental period.

Table 3

Mean values of total phosphorus over 6 weeks experimental period

Treatments	*Final mean values of total phosphorus*
T1	0.477
T2	0.095
T3	0.522
T4	0.024

The result in Figure 4 shows the responses of the treatments in increasing phosphorus production.

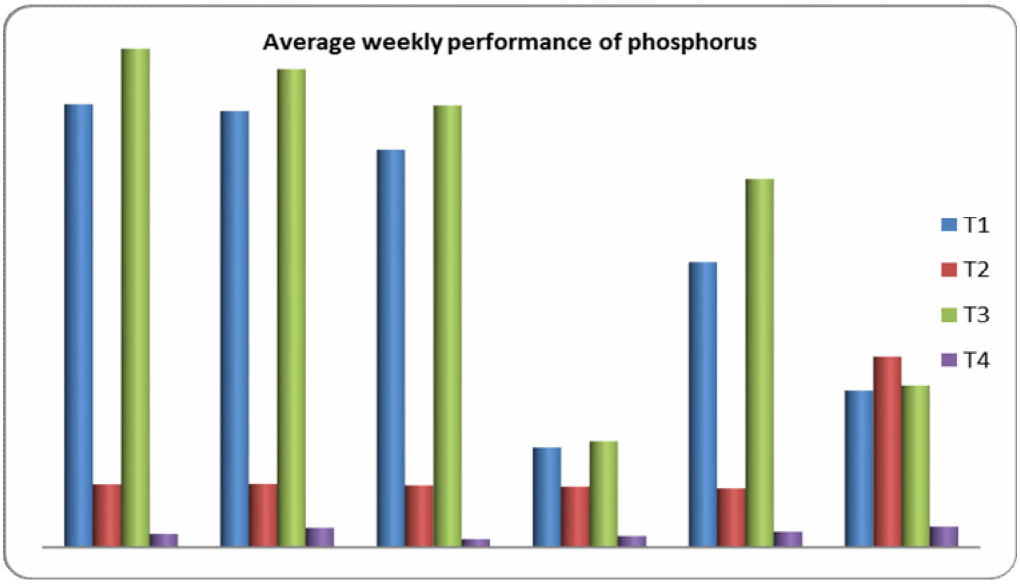

Figure 4. Average weekly performance of phosphorus.

The effect of animal manure showed a strong direct linear relationship between chlorophyll *a* and phosphorus. The correlation coefficient of phosphorus and chlorophyll *a* was 0.92 compared to the standard coefficient of correlation which is 1. This showed that any increase in phosphorus had an effect on chlorophyll *a* production in organic manured ponds.

The results of plankton analysis which was done in the third and sixth week showed significant differences in the composition of both phytoplankton and zooplankton in each treatment. The results showed that phytoplanktons were steadily increasing from week three up to week six with T3 and T1 having the highest number. Poultry manure was assumed to have the highest nutrient content as compared to cattle manure. Therefore, a combination of the two resulted in an increase in phytoplankton production, a performance attributed to high presence of phosphorus and chlorophyll *a* production as observed in T3.

In contrast, zooplanktons decreased between week three and week six. The decrease was attributed to the treatments used. For example, T2 was observed to have more of zooplankton than phytoplankton as compared to T1 which had a higher number of phytoplankton than zooplankton, while T3 had higher numbers of phytoplankton than zooplankton. This was also attributed to food substitute of zooplankton than depending on only phytoplankton. Tables 4 and 5 show the number of both phytoplankton and zooplankton counted in one drop of plankton water.

Table 4

Number of Phytoplankton species observed in organic manured ponds

Weeks	*Number of phytoplankton species counted*			
	T1	T2	T3	T4
3	124	164	888	72
6	232	105	933	14

Table 5

Number of Zooplankton species observed in organic manured ponds

Weeks	*Number of zooplankton species counted*			
	T1	T2	T3	T4
3	55	7	10	14
6	0	13	4	3

The mean water temperature values were in the range of 23.58°C to 23.59°C throughout the experimental period. The correlation between chlorophyll *a* and temperature was 0.39 while that of phosphorus was 0.33 showing a positive although not a strong correlation.

However, there was strong correlation between pH and chlorophyll *a* of 0.86 and that of phosphorus was 0.63. The correlation coefficient between chlorophyll *a* and phytoplankton was positive with 0.856. However, the correlation between chlorophyll *a* and zooplankton was found to be 0.269. Meanwhile, there was no correlation between phytoplankton and zooplankton.

Throughout the experimental period, the mean dissolved oxygen (DO) was in the range of 7.41mgL^{-1} and 7.73mgL^{-1}. Dissolved oxygen was significant in relation to pond performance. Treatment 3 had the lowest mean (7.42mgL^{-1}) DO while treatment 4 had the highest (7.73mgL^{-1}).

Discussion. The results of the present study suggest that treatments (T1, T 2, and T3) had an effect on chlorophyll *a* and total phosphorus abundance in organic manured ponds. The organic manure applied to the ponds was able to release nutrients required by chlorophyll *a*, which suggested that organic manure have positive influence in increasing chlorophyll *a* and phosphorus as shown by the amount applied in each treatment. The presence of different species of plankton further suggested that nutrient concentration in T1, T2 and T3 impacted the pond differently. These results favour the use of a combination (T3) of poultry and cattle manure which showed consistence in both chlorophyll *a* and phosphorus. These results indicate that animal manure varied in influencing the production of chlorophyll *a*, planktons and phosphorus. Treatments T1 and T3 were effective in influencing greater abundance of chlorophyll *a*, plankton and phosphorus at the rate used. The variation may be attributed to feed nutritive value of the animals to which they are fed and because of the fact that fresh manure used contained all the ingredients before much micro activities of decomposition had used up all the nutrients. These findings were in agreement with Das et al (2005) and Muhammad (2008) who reported that poultry manure do influence phosphorus in pond waters to stimulate the growth of plankton and made a significant contribution towards planktonic productivity of fish pond.

Treatment two (T2) alone could not effectively increase chlorophyll *a* and phosphorus abundance in organic manured ponds, which may be attributed to the type of feed that ruminants are subjected to, such as grass that contains high roughages that are low in nutritive value. However, it was further observed that T2 had more phytoplankton count in week three compared to T1. Another interesting observation made indicated a reduction in number of phytoplanktons from 164 in week three in T2 to 105 in week six compared to T1 with 124 in week three and 232 in week six. Zooplankton in T2 were seen to increase from 7 to 13 in week three and week six, respectively, while in T1 it decreased from 55 to 0 in week three and week six, respectively. A combination of poultry and cattle manure (T3) was seen to increase phytoplankton production compared to T2 and T1 alone. The phytoplankton increased from 888 to 933 in week three and week six, respectively, while zooplankton decreased from 10 to 4 in week three and week six, respectively. Treatment 1 recorded a reduction of zooplankton in week 3 from 55 to zero in week 6, while treatment 3 recorded a reduction in week 3 from 10 to 4 in week 6 while treatment 4 recorded a reduction in week 3 from 14 to 3 in week 6. This change may be attributed to reduction in dissolved oxygen and low nutrient levels as observed in treatment 4 with high oxygen but low zooplankton.

The variation in the plankton performance can be attributed to many factors: in week three T2 had decomposed at a faster rate as compared to T1. However, in week 6 T2 decreased because the nutrient values were reducing while T1 maintained its nutrient value thereby supporting phytoplankton growth. In terms of zooplankton, it was observed that T2 supported the growth of more zooplankton as compared to phytoplankton there by reducing the number of phytoplankton as zooplanktons ate phytoplankton. The zooplankton in T1 decreased gradually as they died when the environment was becoming favourable to encourage proliferation of phytoplankton. The high presence of zooplankton in T3 was due to the influence of T2. The decrease in zooplankton encouraged more of the phytoplankton production making T3 the best as it was able to balance its pond ecosystem.

The biomass of zooplankton is low in aquatic environment with very low nutrient or organic matter content as observed in T4. When organic matter content increases and the transparency increases, under these condition phytoplankton and bacteria propagate quickly and stimulate the development of a zooplankton fauna. However, high propagation of bacteria in ponds produces high carbon dioxide that depletes dissolved oxygen as was observed in T3 having the lowest mean dissolved oxygen. Zooplanktons take a considerable time to develop and stabilize. The quality and distribution of zooplankton depend on the phytoplankton biomass and water temperature. When water temperature is lower then the zooplankton will be lower. Based on this study, it was observed that T3 had the lowest zooplankton compared to T2. According to Bwala & Omoregie (2009), the diversity of zooplankton is not only dependent on feeding on phytoplankton alone but they substitute by feeding other available feed like bacteria which give room to phytoplankton reproduction and unlike in a situation when zooplankton are dependent on only phytoplankton alone they then tend to reduce the number of phytoplankton species in ponds.

Ekpenyong (2000) reported that chlorophyll *a* is correlated to phytoplankton as increases in nutrient abundance in ponds give rise to increase in zooplankton production which in turn, reduces the phytoplankton biomass through grazing and as such chlorophyll *a* is reduced. In this experiment, the mean chlorophyll *a* were found to be 45mg/m^3 in T3, 32mg/m^3 in T1, 23mg/m^3 in T2 and 10.5mg/m^3 in T4. This was attributed to nutrient availability in ponds; while in control chlorophyll *a* was lower as expected because of lower nutrient availability.

The mean phosphorus was abundant in T1 with 0.4765mgL^{-1};T3 had 0.5215mgL^{-1}, T2 with 0.095mgL^{-1} and T4 with the lowest of 0.024mgL^{-1}. The phosphorus was within the range where it could support pond activities. Increase in phosphorus abundant in T3 was a result of continuous decomposition of animal manure and availability of nutrient abundant in poultry manure which was augmented with cattle manure. Based on this study, zooplankton and phytoplankton were negatively correlated. The possible explanation could be that the prominent relationships between phytoplankton and zooplankton in the ecosystem were that at certain time of the experiment the zooplankton actively reduced the number of phytoplankton, when the ecosystem had other substitute like bacteria and fungi they substituted and left the phytoplankton to flourish well.

The results of the weekly performance of both the chlorophyll *a* and phosphorus showed an increase during the first 3 weeks and a drop in the last 3 weeks with treatment 3 having 78%, 70%, 58%, and 25% in week 1, 3, 5 and 6, respectively, as compared to T1 with 70%, 65%, 45% and 25% in weeks 1, 3, 5 and 6 of phosphorus. The weekly performance of chlorophyll *a* were seen to be decreasing with increase in weeks with T3 having 9%, 5%, 2% and 1% and T1 with 9%, 1%, 2% and 1% for week 1, 3, 5 and 6, respectively. This could be due to the decomposition of manure affected by several factors like change in temperature and pH affected by dissolved oxygen. Temperature affects the decomposition rate of manure. Much of nutrient was reducing as weeks were increasing. However, it can be seen that T3 showed consistence compared to other treatments, with low dissolved oxygen although not critical but able to support pond ecosystem.

Conclusion. The results from the study demonstrate that animal manure have the ability to be used as organic fertilizer in fish ponds to encourage the primary production of phytoplankton and nutrient availability of phosphorus in Zambia. The effectiveness of the treatments was observed between treatment 1 and treatment 3. Animal manure produced mean chlorophyll *a* in increasing order of 5%, 3%, 2% and 1% in T3, T1, T2 and T4, respectively. In terms of phosphorus production, T3, T1, T2 and T4 had 52%, 48%, 10% and 2% respectively.

However, from the study, it was observed that phytoplankton species varied when counted in a microscope. It was observed that T1 had an increase from 124 species counted to 232 species in week 3 and week 6. Treatment 2 had a drop of phytoplankton species from 164 to 105 while T3 had an increase from 888 species to 933 species and the T4 had a drop as well from 72 species to 14 species. From the study, T2 and T4 were seen to perform badly in increasing phytoplankton. However, it was observed that zooplankton species decreased in T1 from 55 in week three to 0 in week six and T2 increased from 7 species to 13 whereas T3 had a drop as well from 10 species to 4 and in T4 there was a drop in species from 14 to 3 in week three and week six, respectively.

Based on the outcome of animal manure treatment, it can be concluded that chlorophyll *a,* plankton and total phosphorus are abundant in animal manure. On the other hand, the performance of treatment 1 and treatment 3 showed a strong relationship because of high significant in encouraging the presence of chlorophyll *a* and total phosphorus.

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