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Growth performance and phytoremediation ability of *Azolla pinnata* in produced water

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ABSTRACT: In the present study, effect of produced water on the growth of *Azolla pinnata* was observed and the phytoremediating ability of the plant was also exploited. *A. pinnata* was grown in produced water concentrations of 0% (control), 5%, 10%, 15%, 20% and 25%. The plant exhibited reduced growth rate in a concentration dependent order. Toxicity symptoms of produced water on the plant include chlorosis, frond disintegration and eventual death. Produced water exposure resulted in less than 20% growth inhibition in 5- 15 % treatment concentrations. The optimum removal efficiency concentration of produced water by *A. pinnata* was at 10- 20%. It was revealed that *A. pinnata* has low potential for improving the quality of produced water at high concentrations. This study exposed the need for proper produced water treatment and strict monitoring to ensure compliance with standards set by regulatory bodies before its discharge to surface water to mitigate the environmental impacts. © JASEM

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Key Words: produced water, heavy metal, aquatic macrophytes.

Petroleum is a major source of revenue for many countries and its production has been described as one of the most significant in the twenty-first century (Oliveira et al., 2005). Despite its importance, petroleum is produced with large volume of water, with wastewater accounting for more than 80 % of liquid waste (Azetsu- Scott et al., 2007), as high as 95 % in aged oilfields (Kaur et al., 2009). Produced or formation water is water from underground formations brought to the surface during oil or gas production. The water has been in contact with hydrocarbon-bearing formations, and thus it contains some of the chemical characteristics of the formations and the hydrocarbons. There is a wide variation in the level of its composition due to geological formation, lifetime of the reservoir and the type of hydrocarbon produced (Joel et al., 2010).

As the production age of the well increases, the oil production decreases and the water production increases. In Nigeria, it is a known fact that much of this waste produced water is dumped in the environment especially during drilling operations through discharge pipelines to streams or the sea (Onajake and Abanum, 2012).

The relative amount of hydrocarbon contributed to the aquatic environment by oilfield produced water is so small, however, the numerous inorganic and organic constituents dissolved in the produced water can be potentially or actually far more hazardous than the crude oil itself (Pritchard 1979). Unlike oil which forms a slick, produced water readily mixes with flowing water after discharge (Collins, 1980).

The ecological health of many river systems is threatened by the numerous inorganic and organic constituents dissolved in the produced water and the accumulation of these contaminants in the aquatic environments. As such, many freshwater river systems have been classified as unfit for human consumption by the Federal Environmental Protection Agency.

Various floating aquatic macrophytes have been proposed as agents of choice for phytoremediation of wastewater because they are fast growing, adapt easily to various conditions and can tolerate a wide pH range (4.5- 8.3) (Satapathy et al., 2014) and easy maintenance. Among them, water fern (*Azolla pinnata*) has shown a remarkable effectiveness in phytoremediation (Mazumder and Parikh 2015).

The present study investigates the growth performance and phytoremediation potential of *A. pinnata* on produced water. Studies of this nature can help us determine whether this plant can be utilized for the purpose of phytotechnology of produced water.

MATERIALS AND METHODS

The aquatic macrophyte used for this experiment was *A. pinnata.* R. Br. It was collected from Ikpoba Hill wetland area in Benin City. Edo State. The produced water used for this study was collected from a Flow Station in Edo State, Nigeria. Twenty (20) fronds of *A. pinnata* were placed in each experimental bowl which contained 1000 ml of control (produced water free) medium and 5%, 10%, 15%, 20% and 25% concentrations of produced water. Increase or

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decrease in plant number was noted and morphological changes of the plants were observed. Plant samples from all the treatments were carefully harvested after ten (10) days for physicochemical analysis. The experiment was set up in triplicates for each concentration.

Relative growth rate was monitored after 10-day test period using this equation

$$\mu = \left(\frac{\ln Ntn - \ln Nt0}{tn - t0}\right)$$

Where Nt_n was fresh weight at day 10 and Nt_0 was fresh weight at day 0 and (t_n- t_0) is the experimental time change (days) (William and Hendrik 2002).

The percentage inhibition was calculated for the test plants on day 10 of the experiment using the formula:

$$\%\ \text{Inhibition} = 100 - \left(\frac{\text{Measured biomass}}{\text{Theoritical biomass}}\right) * 100$$

Where biomass measured is the treatment plant number and theoretical biomass is the control plant number on termination

Measurement of each parameter was taken at the end of the experiment. The equation below was used to determine the removal efficiency (%) on day 10 based on the replicates averages of 0%, 5%, 10%, 15%, 20% and 25% of the Fe, Pb, Zn, Cd, Phosphate, Nitrate, Sulphate and THC analysis (Shafi et al.,2015).

$$\text{Removal efficiency} = \frac{C^1 - C^0}{C^0}$$

Where C^0 = initial value of water quality parameter C^1 = value of water quality parameter on termination of the experiment.

Physiochemical analysis was carried out according to standard methods for examination of water and waste water (APHA, 1998).

Data were presented as mean value ± standard error. Comparison of mean values was made by Analysis of Variance (ANOVA), followed by Duncan's multiple range test (DMRT) at a significance level of $p = 0.05$ using SPSS package, 16th edition.

RESULTS AND DISCUSSION

The mean number of plants at every 2 day interval till termination of the experiment is shown in Figure 1. Increase in produced water concentration caused a decrease in plant number. On day 6, control (29.667±3.21,) had comparably higher number of plants than other treatments. On termination of the experiment, number of plants in control (36.35±4.25) and 5% (36.70±5.84) produced water concentration were higher compared to 20% (13.40±2.08) and 25 % (3.22±0.57) produced water concentrations.

Statistically, it was observed that *A. pinnata* exposed to produced water showed no significant difference ($p > 0.05$) between the control and concentrations 5%, 10% and 15% on the 2nd and 4th day while significant difference ($p < 0.05$) occurred between the control and concentrations 20% and 25%. On the 6th day, significant difference ($p < 0.05$) was observed between the control and the various concentrations.

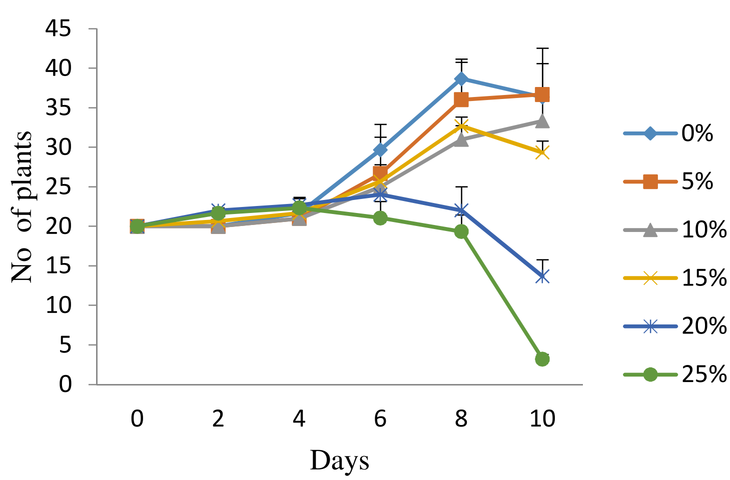

Fig 1: Biomass of *A. pinnata* in produced water concentrations

Growth rate of *A. pinnata* was highly hindered by the produced water as shown in Figure 2. The plant had reduced survival in 20% and 25% concentrations. At the end of the experimental period, fresh weight of the plant in 15%, 20% and 25% produced water concentrations had the lowest growth rate when compared with control, 5% and 10% produced water concentrations.

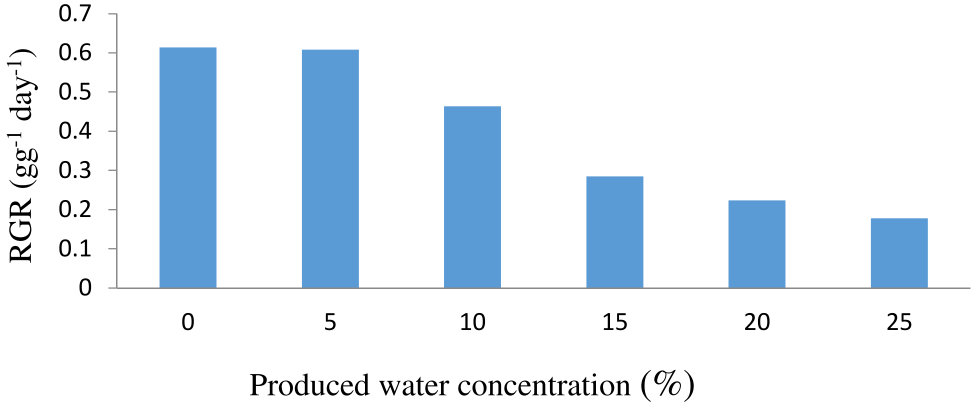

Fig 2: Growth rate of fresh biomass of *A. pinnata* in concentrations of produced water on termination

After 10 days of produced water treatment, percentage inhibition of plant growth was 62% and 98% respectively at 20% and 25% produced water concentrations, when compared to the control (Figure 3). However, no inhibition was noticed at 0% concentration. Generally, percentage inhibition increased with increasing concentration of produced water.

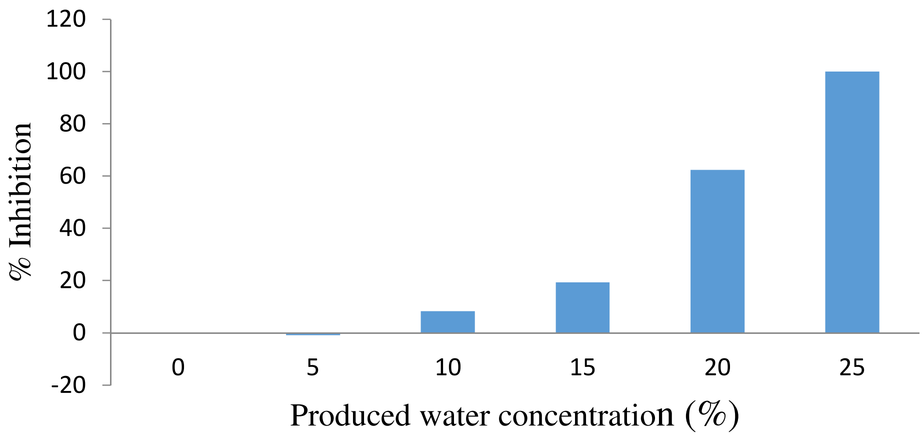

Fig 3: Percentage inhibition of produced water to *A. pinnata*

The optimum removal efficiency concentration of produced water by *A. pinnata* was from 10- 20% as shown in Table 1. Lead and Iron had highest removal efficiency of 82% and 66% respectively at 20% produced water concentrations.

Table 1: Percentage removal efficiency of heavymetals and nutrient from produced water by *A. pinnata*.

Produced Water Concentration (%)	Percentage Removal Efficiency (%)						
	Fe	Pb	Zn	Cd	PO_4	NO_3	SO_4
0	31	13	5	11	33	15	6
5	42	35	10	23	39	63	8
10	56	66	10	36	53	54	12
15	75	61	32	44	45	37	14
20	82	66	20	41	44	40	18
25	73	57	6	39	42	27	15

The results presented here indicate that *A. pinnata* density was concentration and time dependent. This is maintained by the fact that increase in produced water concentration was seen to cause a decrease in number of plants as the time of exposure increased. The result indicates that produced water concentrations (20-25%) had a negative effect on plant growth. The hindering effect of produced water on *A. pinnata* growth may be as a result of the toxic effect of low molecular weight aromatic compounds classified as dissolved oil, and the less soluble PAHs and heavy alkyl phenols also present in produced water as dispersed oil (Hudgins, 1994). A study by Ajao (1985) showed high concentration of pollutants retard growth and increased sensitivity of plants to other stress, decrease plant growth by interfering with plant carbon allocation and root symbiosis (Azeez and Sabbar, 2012) and even degradation of chlorophyll. Umudi (2011) reported that produced water is very contaminated and when the concentration of the salts, heavy metals and solid particles are above tolerance limit of the plant, they could be very toxic to the plant leading to significant reduction in plants number.

Relative growth rate of *A. pinnata* decreased in the presence of produced water in a concentration dependent manner. Growth inhibition is a common response to heavy metal stress and is also one of the most important agricultural indices of heavy metal tolerance Srinivasan et al., (2014). Chlorosis and necrosis are some of the visible symptoms indicating severe metal toxicity (Apel and Hirt, 2004). This result is in accordance with the findings of Vecchia et al., (2005) who concluded that decreased plant growth might be associated with the inhibition of mitotic index noticed with heavy metal treatment.

Produced water was stressful to *A. pinnata,* this is due to the excess of nutrients and ions which can be beneficial to plant growth but in excess could be toxic and affect the growth of plant.

Removal efficiency of *A. pinnata* showed the plant has different absorption potential for each metal with higher affinity for iron and lead and lower affinity for cadmium and zinc. *A. pinnata* has the potential to be used for absorption of iron and lead at high concentration of 25% produced water concentration. The use of *A. pinnata* as a phytoremediation agent has also been reported by Shafi et al, 2015.This plant has been able to develop some internal mechanisms that allow the uptake, tolerance and accumulation of high concentrations of heavy metals that would be toxic to other plants (Gautam et al., 2014).

Conclusion: Aquatic ecosystem contaminated with large quantity of organic pollutants is a considerable threat to the environment that can have diverse effects on organisms and water quality. *A. pinnata* is capable of eutrophication and bioremediation as it is able to reduce phosphate and nitrate significantly. The present findings showed that produced water reduced the growth rate of *A. pinnata* at higher concentrations. Furthermore, it can be suggested that *A. pinnata* can be used for the phytoremediation of low- level iron and lead removal in produced water.

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