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Effect of Ballast Water on the Growth of Two Aquatic Macrophytes (*Azolla pinnata* R.Br and *Lemna paucicostata* Hegelm)

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ABSTRACT: The study investigated the effect of ballast water on the growth of two aquatic macrophytes, *Azolla pinnata* R.Br. and *Lemna paucicostata* Hegelm. The ballast water used for this study was sourced from MTCRARROCH vessel which berthed in Koko Port. The macrophytes were grown at different concentrations (25%, 50%, 70% and 100%) of ballast water, using distilled water as control (0%). The experiment lasted for twelve (12) days. There was increased growth in both macrophytes with increasing concentration of ballast water. There was an increase in the growth of both macrophytes at all concentrations from the beginning to the end of the experiment. Of both test macrophytes, *L. paucicostata* was more tolerant to ballast water. The ballast water was acidic (pH 6.7) and with high electrical conductivity (1790 μS/cm). Iron, zinc, lead and cadmium were within the permissible limits of Standard Organization of Nigeria and the World Health Organization. Water quality parameters such as pH, electrical conductivity, total dissolved solids, salinity, dissolved oxygen, biochemical oxygen demand, NO_3, SO_4, PO_4, Fe, Pb, Zn, Cd; chlorophylls *a* and *b*, removal efficiency, percentage inhibition and yield were determined. Most of the water quality parameters of ballast water for the test macrophytes were significant.

Keywords: *Azolla pinnata*, *Lemna paucicostata,* Ballast water, Macrophytes, Bioremediation

Introduction

Ballast water is mostly sea water, sometimes containing sediments held in tanks and cargo holds of ships to increase stability and maneuverability during transit. Ballast water is taken into a tank from one body of water and discharged into another body of water and this can introduce invasive species of aquatic life (Carlton and Geller1993; Ruiz *et al*., 2000).Control of the discharge of ballast water is an international issue handled by the International Maritime Organization (IMO). Internationally, there has been slow but steady progress towards international legal binding controls on ballast water. When evaluating ballast water treatment options, a number of general factors must be considered including cost, enforcement, the effectiveness of the method and the risk the treatment method may pose to human health and the environment. Ballast water is used to balance the ship as it uses up fuel during a long voyage (Casale, 2002).

Discharge of ballast water from oil tankers could constitute significant portion of the oil and other impurities introduced into the aquatic environment each year with consequences for both animal and plant life. It is believed that a marine species invades a new environment somewhere in the world every nine weeks (Ruiz *et al*. 2000).

In Nigeria, ballast water is indiscriminately discharged into the aquatic environment due to lack of enforced International Maritime Organization (IMO) regulations (Olorunfemi *et al.,* 2012). Apart from the threat that comes with the movement of marine life, there are also issues pertaining to overexploitation of living marine resources and physical alteration and destruction of marine habitats **(**Hegazy *et al*., 2012; Ruiz *et al*., 2000). The objectives of the study were to investigate the effect of ballast water on the morphology of the aquatic macrophytes and determine the physical and chemical composition of the ballast water.

Materials and methods

The ballast water used for this study was sourced from MTCRARROCH vessel which berthed in Koko Port in Burutu Local Government Area of Delta State on the 4th of April 2013. The total number of ballast tanks was 20 while the number of tanks in the ballast was 5 and one of the tanks was exchanged after the vessel deballasted. The macrophytes were grown in different concentrations (0, 25, 50, 75 and100 %) of ballast water, using distilled water as control. The macrophytes were obtained from the Ikpoba river bank. The experiment was laid out in a randomized block design using five (5) treatments with three (3) replicates and each replicate containing 15 bowls with 3 bowls per treatment. Growth parameters (number of *Azolla pinnata* fronds and *Lemna paucicostata* leaflets, the number of roots, were counted every 2(two) days. Fresh and dry weights of the macrophytes were also determined before and after the experiment after which the concentrations of Fe, Cd, Pb and Zn were determined by atomic absorption spectrophotometry. The ballast water together with the control (distilled water) were analysed for a number of standard physicochemical properties, including total dissolved solids(TDS), pH, salinity, dissolved oxygen, biochemical oxygen demand, chlorophyll *a* and *b*, removal efficiency, percentage inhibition, yield, NO_3, SO_4, PO_4, Fe, Pb, Zn, and Cd as described by APHA (1998). Cadmium, iron, zinc and lead were analyzed in the water samples according to standard analytical method (USEPA, 1996) using Atomic Absorption Spectrophotometer (AAS) (HACH 2800 model).

Chlorophyll content determination: was determined at the end of the experiment by extracting fresh leaves in 90% acetone under low light intensity according to Arnon (1949).

Percentage inhibition: was also determined at the end of the experiment according to Phatarpekar and Ansan (2000) represented by the formula below,

$$100 - \frac{\text{Measured biomass}}{\text{Theoretical biomass}} \times \frac{100}{1}$$

Measured biomass = Number of plants of test aquatic macrophytes in ballast water
Theoretical biomass = Number of plants of test aquatic macrophytes in control

Removal efficiency: was determined according to Souza *et al.* (2013) using the formular below,

$$RE = \frac{(W\text{-}C)}{(WC)} \times \frac{100}{1}$$

Where: RE= removal efficiency (%),
WC = initial value of water quality parameter (day 0)
C = value of water quality parameter on day 12

Statistical Analysis: The mean values and the standard error of mean for data obtained from the macrophytes test was calculated. Data was expressed as mean±standard error of mean (SEM). Differences among the mean were determined using ANOVA and paired T-test, while the statistical differences between control and different concentrations of ballast water were analysed using the Dennett post-hoc test ($p>0.05$).All statistical analyses were carried out using the SPSS® 16.0 statistical package.

Results

The growth response of *A. pinnata* to ballast water is represented in Figure 1. There was an increase in the growth of both macrophytes at all concentrations from the beginning to the end of the experiment. The experiment lasted for twelve (12) days

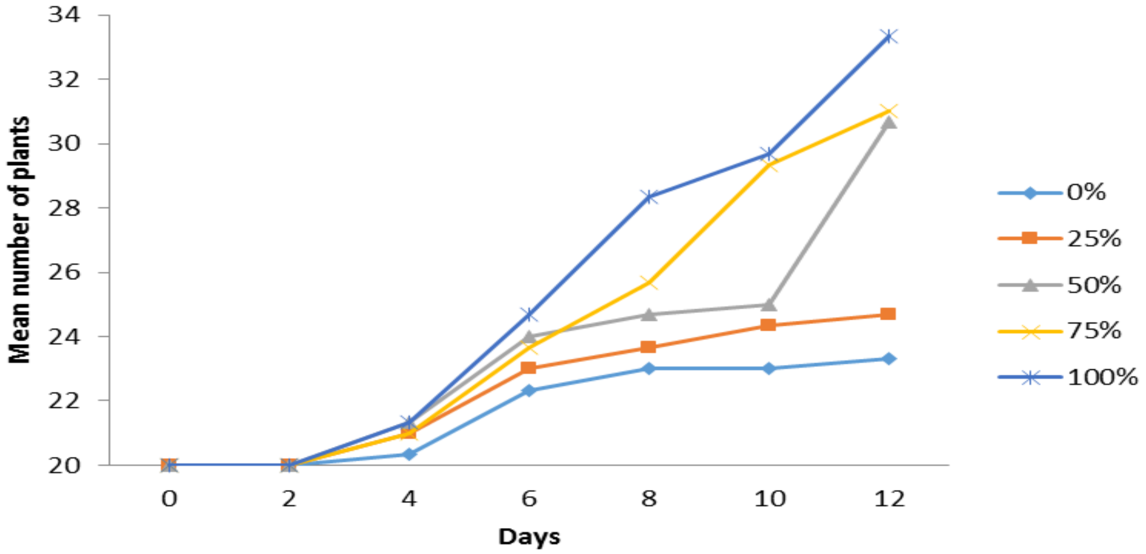

Figure 1: Growth response of *A. pinnata* in ballast water

There was lag phase as no growth was recorded on day 0 and day 2 for *A. pinnata*. Growth was recorded from day 4 to the end of the experiment and this stage can be represents the exponential phase of the test macrophyte. Marginal growth was observed in the control on day 4 and 6. The mean number of plants increased in all the ballast water concentration. There was an increase in the number of plants at increasing ballast water concentration until the termination of the experiment. The least number of plants was observed in 0% (distilled water). Statistically, there was a significant difference ($p<0.05$) on the effect of concentration on the number of plants and there was also a significant difference on the effect of time on the number of plants.

The growth response of *L. paucicostata* to ballast water is represented in Figure 2.

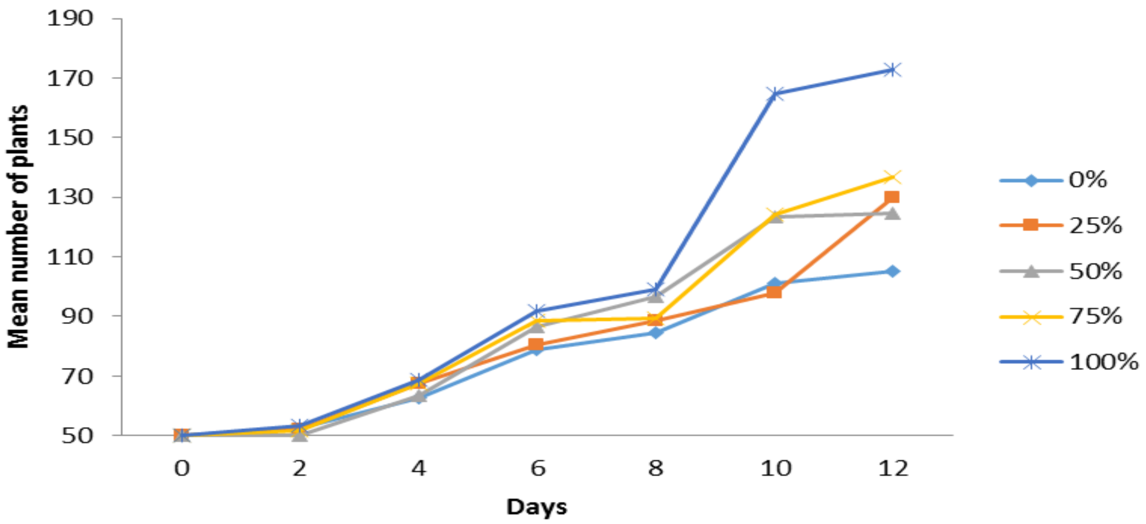

Figure 2: Growth response of *L. paucicostata* to ballast water

No growth was observed on day 0 and day 2(lag phase). An improved growth was observed from day 2 for all the ballast water concentration including the control. Substantial growth increase was observed at all ballast water concentrations until the termination of the experiment. Growth was maximum at 100% ballast water concentration and least at 0% distilled water or control. There was a significant difference ($P<0.05$) on the effect of concentrations on the number of plants and there was also a significant difference on the effect of time on the number of plants while there.

Table 1 shows the various physical and chemical characteristics of ballast water used in this study. The waste water was slightly acidic (pH 6.7) and most of the heavy metals except lead, were within SON and WHO permissible for effluent discharge.

Table 1: Physico-chemical characteristics of ballast water

Parameter	Ballast water value	SON limit (2007)	WHO limit (2011)
pH	6.7	6.5-8.5	6.5-9.2
Electrical	1790	1000	1400
conductivity(μS/cm)	890	500	1200
TDS (mg/L)	6.8	-	<30
TSS (mg/L)	0	5	5
Turbidity (NTU)	0.01	-	-
TOC (%)	164.7	-	300
HCO_3 (mg/L)	12.4	-	-
Hardness (mg/L)	0.36	-	-
PO_4 (mg/L)	3.019	50	50
NO_3 (mg/L)	32.3	100	500
SO_4 (mg/L)	1.61	200	<20
Na (mg/L)	9.10	-	<100
Ca (mg/L)	3.30	0.20	-
Mg (mg/L)	0.35	0.3	1.0
Fe (mg/L)	0.10	3	3
Zn (mg/L)	0.010	0.01	0.01
Pb (mg/L)	0.009	0.003	0.003
Cd (mg/L)	0	-	-
THC (mg/L)	144.1	250	250
Chloride (mg/L)	0.2	-	>6.0
BOD (mg/L)	8.95	-	5
DO (mg/L)	0.81	-	-
Salinity (‰)	-	-	.

Variations in the physicochemical parameters of different ballast water concentrations on the test aquatic macrophytes (*A. pinnata* R. Br. and *L. paucicostata* Hegelm) are presented in Table 2. There was concentration-dependent increase in the phosphates, nitrates, sulphates and four heavy metals namely: Fe, Pb, Zn and Cd. THC was not detected in the test macrophytes at all ballast water concentrations.

Table 2: Variations in the physicochemical parameters of different ballast water concentrations on the test aquatic macrophytes (*A. pinnata* R. Br. and *L. paucicostata* Hegelm) at the termination of the experiment.

Test Macrophytes in Different Concentrations of Ballast Water (%)	Conc. (mg/L)														
	Fe		Pb		Zn		Cd		PO_4		NO_3		SO_4		THC
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
A. pinnata	0.35		0.010		0.10		0.009		0.36		3.019		32.3		
0		0.01		0.000		0.03		0.001		0.15		0.01		14.2	ND
25		0.23		0.010		0.16		0.010		0.22		1.08		15.6	ND
50		0.25		0.013		0.23		0.015		0.27		1.31		18.1	ND
75		0.30		0.020		0.25		0.020		0.30		1.35		19.3	ND
100		0.35		0.023		0.27		0.023		0.36		2.11		22.3	ND
L. paucicostata															
0		0.01		0.000		0.03		0.001		0.15		0.20		12.0	ND
25		0.31		0.015		0.11		0.015		0.22		1.23		22.9	ND
50		0.35		0.020		0.20		0.020		0.32		1.31		24.3	ND
75		0.41		0.023		0.25		0.022		0.31		2.41		27.6	ND
100		0.57		0.024		0.30		0.031		0.35		3.07		30.5	ND

Discussion

Ballast water is one of the primary vectors responsible for the global transport of vegetative and resting stages of aquatic micro-organisms as well as for potentially pathogenic bacteria (Carlton and Geller, 1993; Ruiz *et al*., 2000). Macrophytes differ considerably in their tolerance to the magnitude and kind of pollution (Gopal and Sharmal, 1990). Macrophytes are considered as important components of the aquatic ecosystem not only as food source for aquatic invertebrates, they also act as an efficient accumulator of heavy metals (Liao and Chang, 2004). According to DeBusk and Reddy (2007), the characteristics of aquatic macrophytes as bioremediation agents for waste water treatment include fast growth rate, high biomass production and the ability to accumulate high concentrations of nutrients and heavy metals over a long time exposure with no damage concerns. The characteristic nature of ballast water depends on the source from which it was obtained. As the ballast water concentration increased, the growth rates of the test macrophytes increased until the termination of the experiment. This was probably because there was an increase in the nutrients present in the ballast water concentration for the test macrophytes.

As evident from the results, the effects of wastewater on both aquatic plants varies when compared to one another. *Lemna paucicostata* started having new leaflets on Day 2 but *Azolla pinnata* was delayed until Day 6 before visible growth was observed when they were compared with each other. This may be due to the fact that *L. paucicostata* acclimatized to the ballast water concentrations earlier than *A. pinnata*. Sofia *et al.* (2005) opined that certain physical, chemical and biological properties of water up to an adequate level are good for a plant's health but become toxic at excessive levels that could retard the growth of the plants. The plants of interest in this study are aquatic in nature and may probably have a better defense mechanism for adjusting to wastewater. The reason for increased growth throughout the experiment is because of sustained nutrient supply throughout the duration of the experiment. Also, the test macrophytes had their peak growths in 100% ballast water concentration probably due to the highest concentrations of micronutrients (PO_4, SO_4, NO_3) and micronutrients (Fe, Zn, Cd, Pb) all of which enhance growth significantly.

The lag phase for both test macrophytes delayed for two days probably because they were trying to adapt to the ballast water at the different concentrations. Exponential phase was observed from day 4 to the end of the experiment where the test macrophytes grew luxuriantly due to the concentration of macronutrients present in the ballast water at different concentrations. The removal efficiency of the test macrophytes was zero (0) in distilled water. The macronutrients present in the different ballast water concentration were removed drastically except for sulphate which increased as the ballast water concentration increased.

The test macrophytes responded to the different ballast water concentration from day to day 12. There was branching in *A. pinnata* and new leaflets in *L. paucicostata* from day 4 to the end of the experiment. Spores were present in all the concentrations of ballast water except the distilled water for *A. pinnata* on day 8 while browning was noticed in some leaflets in *L. paucicostata*. Spores were observed on day 10 for *L. paucicostata* which gave rise to new leaflets.

In conclusion, ecofriendly measures should be employed and implemented to protect our aquatic environment from living organisms that are endemic to other regions. Many of these anthropogenic mediated transported organisms have affected adversely the abundance and biodiversity of native biota in their receiving environments, often causing significant environmental and economic impacts. It is therefore pertinent that regulatory bodies should ensure that ballast water is adequately treated and should not be discharged indiscriminately into the environment.

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