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Germination and Seedling Growth of *Zea mays* L., *Sorghum bicolor* L. and *Pennisetum americanum* L. Under Varying Concentrations of Bilge Water

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Abstract
Corn (*Zea mays* L.) [SUWAN =1-SR 063802 (yellow maize) and T.Z.R. (white maize)], guinea corn (*Sorghum bicolor* L.) and millet (*Pennisetum americanum* L.) tolerance to bilge water of varying concentrations [25, 50, 75, 100% (v/v)] was assessed at germination and seedling growth stages. Results obtained from physicochemical analysis of the bilge water revealed that it was slightly acidic (pH 5.91) and the concentration of Zn, Ni and Pb in the wastewater were higher than the National Environmental Standards and Regulation Enforcement Agency (NESREA) and United States Environmental Protection Agency (USEPA) limits for effluent discharge. The wastewater was found to inhibit the germination of all the seed types used and were concentration-dependent, for instance, treatment of *S. bicolor* seeds with 25% bilge water produced only 52% germination at 96 h while 28% germination was recorded for seeds treated with the stock at the same time interval. Length of radicle and plumule of the seedlings decreased with increase in bilge water concentration. The longest roots (44.6±0.44 - 19.6±0.40 cm) were produced in the control for all the seed types while the shortest roots (11.3±0.36 cm) were produced in the seedlings of *S. bicolor* grown on the undiluted bilge water. All the seedlings cultivated in the wastewater developed toxicity symptoms but more chlorotic and necrotic regions were observed at higher concentrations. *S. bicolor* was least tolerant while *P. americanum* was the most tolerant to the wastewater. Germination inhibition is attributed primarily to the acidity, trace and heavy metals contents of the bilge water. The results show that cereals were suitable for evaluating the effects of the wastewater in plant growth bioassays.

Key words: Bilge water, Germination, *Zea mays* L., *Sorghum bicolor* L., *Pennisetum americanum* L.

Introduction

Bilge water is a mixture of variety of substances such as fresh water, sea water, sludge, lubricants, urine, oil, detergents, solvents, chemicals, various salts, pitch and metals (e.g. arsenic, copper, chromium, lead and mercury) that accumulates at the lowest compartment of the ship known as the “bilge”.

The International Convention for the Prevention of Pollution from Ships (MARPOL 73/78) is an international agreement to achieve the complete elimination of global pollution of the marine environment by oil and other harmful substances as well as the minimization of accidental discharges of such substances [1]. Although Nigeria is a signatory to the Memoranda of Understanding on Port State Control in the West and Central African sub-region, the MOU has been ineffective to reduce, control and prevent marine pollution in the country [2], a direct consequence being that wastewaters from oil tankers are discharged into the nation’s environment with outright disregard to MARPOL 73/78. Elsewhere, a number of cruise lines have been charged with environmental violations by discharging untreated oily bilge water directly into the ocean which can damage marine life [3].

Data obtained from the physicochemical analysis of bilge water from an earlier study revealed that it was acidic and most of the heavy metals were higher than allowable FEPA limits for effluent discharge. However,

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the wastewater also contains micro-nutrients which are beneficial for plant growth and metabolism [4]. Wastewaters produced as a result of commercial activities, if properly re-channelled into positive uses, could alleviate the problem of shortage of water for irrigation as it does not only conserve valuable water resources but also takes advantage of the nutrients contained in effluent for plant growth.

Seed germination is a critical stage that ensures reproduction and controls the dynamics of plant populations, thus it is a critical test of probable crop productivity. Presently, we are not aware of any reports in literature on the agro-potential or toxicity of bilge water in cereals. In view of such perspectives, the present investigation was conducted to evaluate the impact of different concentrations of the wastewater on seed germination and primary stages of growth of cereal crops of economic importance: corn (*Zea mays* L.) [SUWAN =1-SR 063802 (yellow maize) and T.Z.R. (white maize)], guinea corn (*Sorghum bicolor* L.) and millet (*Pennisetum americanum* L.). It would be of interest if there is an optimum dilution level for bilge water that would enhance the germination and hence be of any useful irrigation purpose.

Materials and Methods

Collection of Bilge Water

The bilge water used in this experimental study was collected freshly from *Green Nova*, a multipurpose vessel which hailed from Mauritania and berthed at canal in Warri Port in Delta State in 2011. Different concentrations (25, 50, and 75%) of the wastewater were prepared by further dilution of the stock solution.

Plant Materials

Maize seeds (*Zea mays* L.) yellow variety (SUWAN = 1-SR 063802, streak resistant) and white variety (T.Z.R.) were obtained from Edo State Agricultural Development Programme, Ogba Road, Oko Village, near Benin City while guinea corn (*Sorghum bicolor* L.) and millet (*Pennisetum americanum* L.) seeds were procured from Lagos Street, Benin City. The seeds were sorted, cleaned and tested for viability using the floating method [5]. The viable seeds were then surface sterilized in 5% sodium hypochlorite solution for 10 minutes before use, to avoid fungal contamination and thereafter washed thoroughly with deionized water.

Physicochemical Analysis

The bilge water was analysed for a number of standard physicochemical properties, including total dissolved solids (TDS), total hardness, sulphates, phosphates, nitrates, biochemical oxygen demand (BOD), and dissolved oxygen (DO) according to methods described by APHA [6]. Ten metals namely Pb, Cu, Hg, Cd, Cr, Fe, Zn, Al, Ni, and Mn were analysed in the water samples according to standard analytical methods [6,7] using an atomic absorption spectrophotometer (AAS) (PerkinElmer A Analyst 100). The metal standards were prepared to known concentrations, labelled, and kept inside plastic bottles that were pre-washed with concentrated nitric acid and distilled water. The absorbance of the standards, bilge water samples and control was taken in triplicates. Graphs of the concentrations against the absorbance of each of the standards for the metals were plotted. Thereafter, the concentrations of the metals in the wastewater were interpolated from their respective graphs.

Germination Studies

Germination studies followed the procedures used in previous studies [4]. Forty-five Petri dishes (8 cm diameter) were washed with deionized water and lined with filter paper (Whatman No. 1) for germination study. Each Petri dish contained 30 seeds of *Z. mays*, (white and yellow varieties), *S. bicolor* and *P. americanum* and 20 ml of test solution.

Treatments comprised of control (deionized water), 25, 50, 75, and 100% of bilge water.

The Petri dishes were arranged in a completely randomized block design with three replicates of test solution for each plant material. The experiment was conducted in a growth chamber at 25°C, 12 hours light and 12 hours dark period, (illumination of 2500 lux, Philips T2 40W/33 lamp). Test solutions were added when necessary to keep the double-layered filter papers moist. The filter papers were examined daily for fungal infection and seeds affected were removed to avoid contamination of other seeds. The seeds were observed for germination each day and percentage germination were recorded. Seeds were considered germinated when both radicle and plumule had emerged to about 0.2 cm.

For seedling experiment, 20 pre-germinated seeds were transferred to 30 plastic beakers filled with 100 mL of bilge water of the different concentrations as used for germination experiment. An equal quantity of polystyrene beads was added to each beaker. This experiment was arranged in a complete randomized block design and each block contained three replicates for each concentration of the test solution. The beakers were aerated during the course of seedling development. Seedlings were allowed to grow for 12 days after which they were taken out from the solutions and washed carefully. Root and shoot lengths were measured with a meter rule. Necrotic and chlorotic regions were observed using Swift Stereomicroscope with an eyepiece graticule of 10x magnitude.

Statistical Analysis

Data were analyzed statistically by using Duncan Multiple Range (DMRT). All statistical analyses were carried out using SPSS®14.0 statistical package.

Results

Table 1 shows a comparison in the physicochemical parameters of bilge water obtained from *Green Nova* in Warri Port compared to permissible limits for national [8,9] and international [10] regulatory bodies. It can be seen that the pH of the wastewater was slightly acidic and the concentration of some of the heavy metallic contents (Zn, Ni and Pb) were higher than NESREA and USEPA limits for effluent discharge. Some micronutrients (K, Ca, S, Cu, Mn) were also present in appreciable quantities.

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Table 1: Some Physicochemical Parameters of bilge water Sample Obtained from *Green Nova* in Warri Port

Parameter	Results	[8]FEPA	[9]NESREA	[10]USEPA
Colour	Light grey	NS	-	-
pH	5.91	6-9	6-9	6.5-8.5
$BOD_5$20°C	6.2	50	50	250
Turbidity	15 units	NS	-	-
Total Hardness	227	NS	-	0-75
Conductivity	1400	NS	-	-
Total Alkalinity	2.55	NS	-	-
TDS	0.04	2000	500	500
Ammonia	0.05	NS	1	-
Nitrates	99.5	20	10	10
Sulphates	107.5	500	250	250
Phosphates	100.5	500	2	-
Chloride	970.3	600	250	250
Sodium	120.3	NS	-	-
Calcium	110.5	200	-	-
Potassium	119.3	NS	-	-
Magnesium	130.1	NS	-	-
Copper	0.5	<1	0.5	0.009
Manganese	3.9	5	0.2	0.05
Lead	0.3	<1	0.05	0.003
Iron	5.7	20	-	0.30
Aluminium	ND	NS	-	-
Cadmium	0.1	<1	0.02	0.002
Chromium	1.4	NS	0.05	-
Silver	0.2	NS	-	-
Nickel	0.3	<1	0.05	0.005
Zinc	11.6	<1	-	0.12

Values are means of 3 replicates. All values are in mg/L except conductivity (µS/cm), turbidity (NTU) and pH with no unit.
BOD: Biochemical oxygen demand.
TDS: Total dissolved solids

The germination profiles of seeds of the cereals: *Zea mays* (yellow), *Zea mays* (white), *Sorghum bicolor* and *Pennisetum americanum* at different bilge water concentration are presented in Figures 1-4. Compared with the control, percentage germination of seeds of the cereals decreased with increase in concentration. Unlike the seeds of *S. bicolor* and *P. americanum* which germinated at the 12 h, seeds of *Z. mays* (white and yellow varieties) germinated slowly at 18 h. However, at the 96 h, germination rose to about 82% at lower concentrations of the wastewater. On the other hand, seeds of *P. americanum* were more or less unaffected by the wastewater; attaining about 86% germination at 24 h at all concentrations and control. Seeds of *S. bicolor* were the most affected by the wastewater treatment, for instance, treatment with 25% bilge water produced only 52% germination at 96 h while 28% germination was recorded for seeds treated with the stock at the same time interval.

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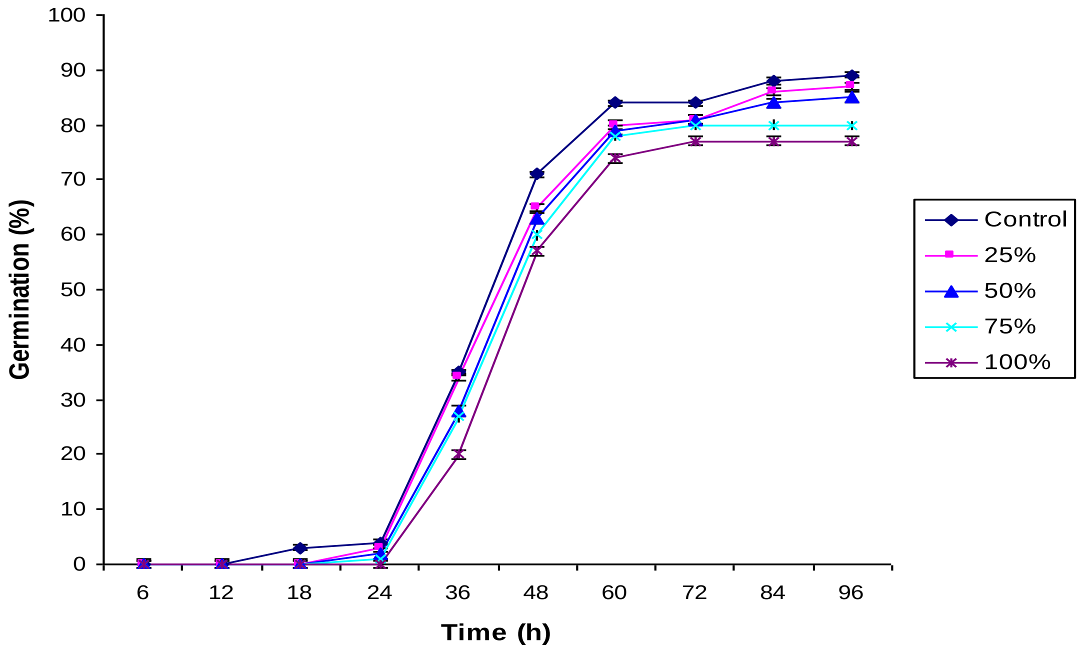

Fig 1: Percentage germination of seeds of *Zea mays* (white variety) at different time periods and bilge water concentrations.

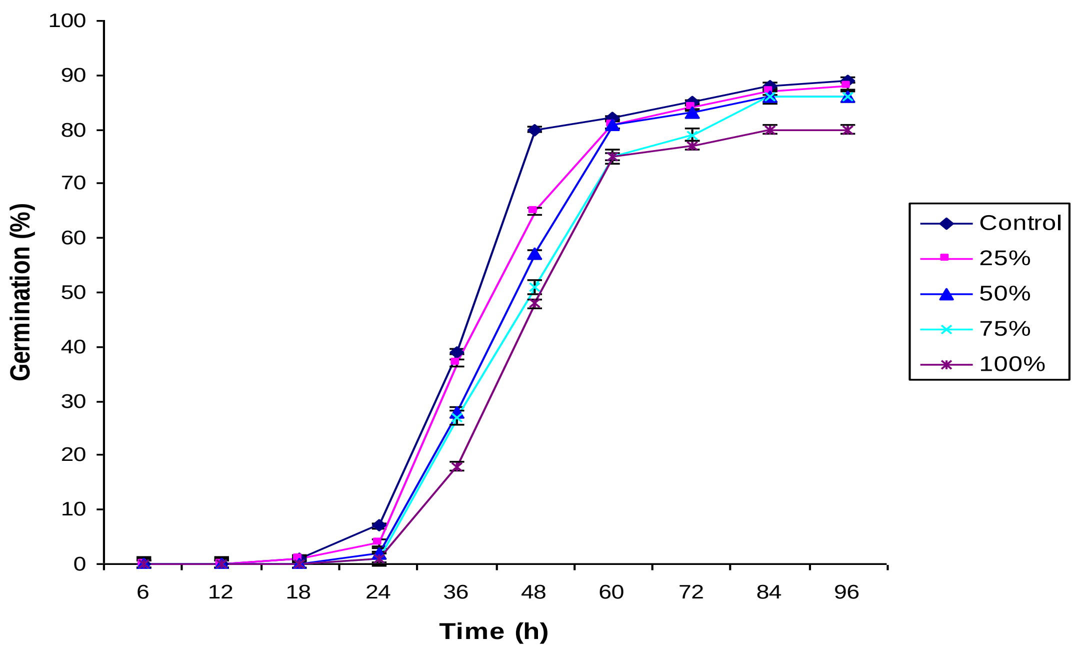

Fig 2: Percentage germination of seeds of *Zea mays* (yellow variety) at different time periods and bilge water concentrations.

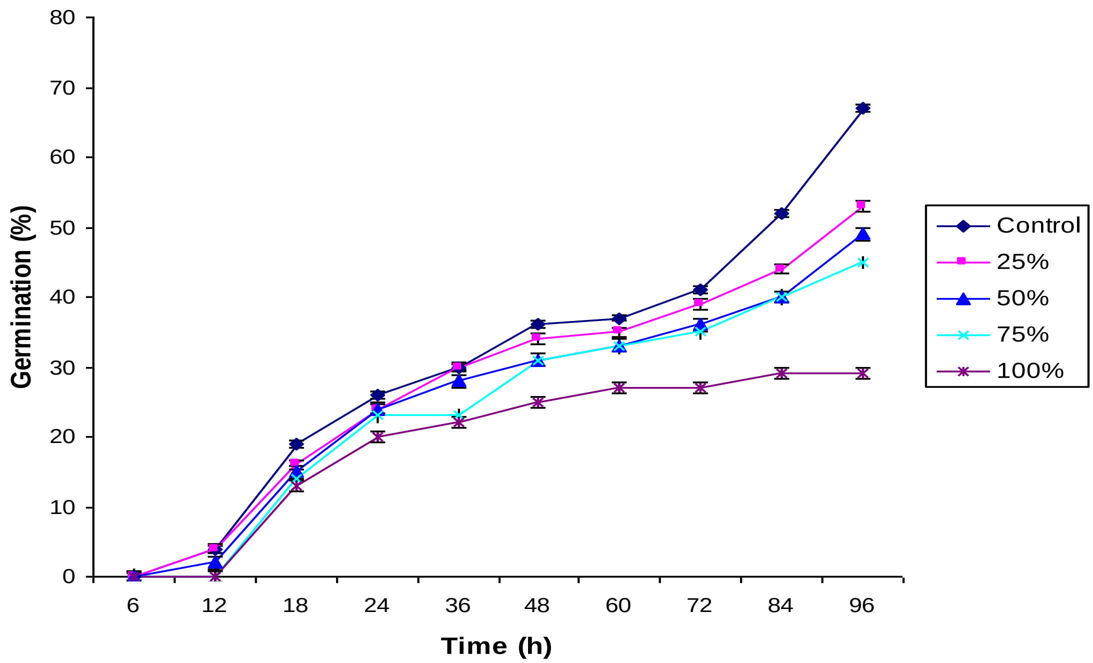

Fig 3: Percentage germination of seeds of *S. bicolor* at different time periods and bilge water concentrations.

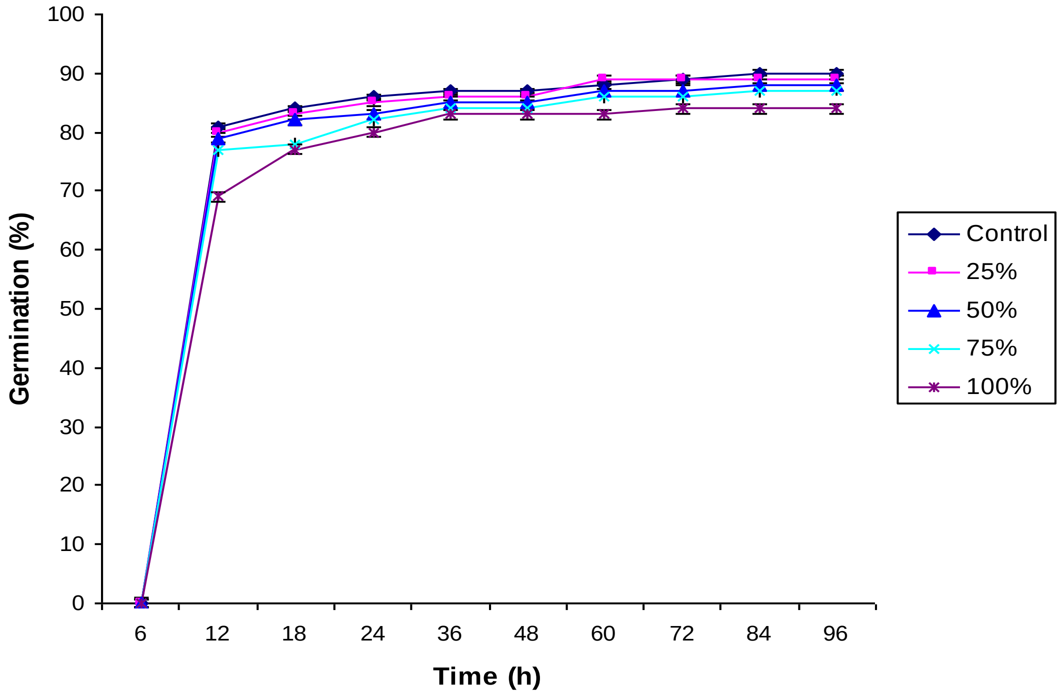

Fig 4: Percentage germination of seeds of *P. americanum* at different time periods and bilge water concentrations.

In particular, *P. americanum* was found to be most tolerant with the undiluted wastewater with a percentage germination of 82% at the 96 h while the seeds of *S. bicolor* gave the least percentage germination of 25% at the same period (Fig. 5).

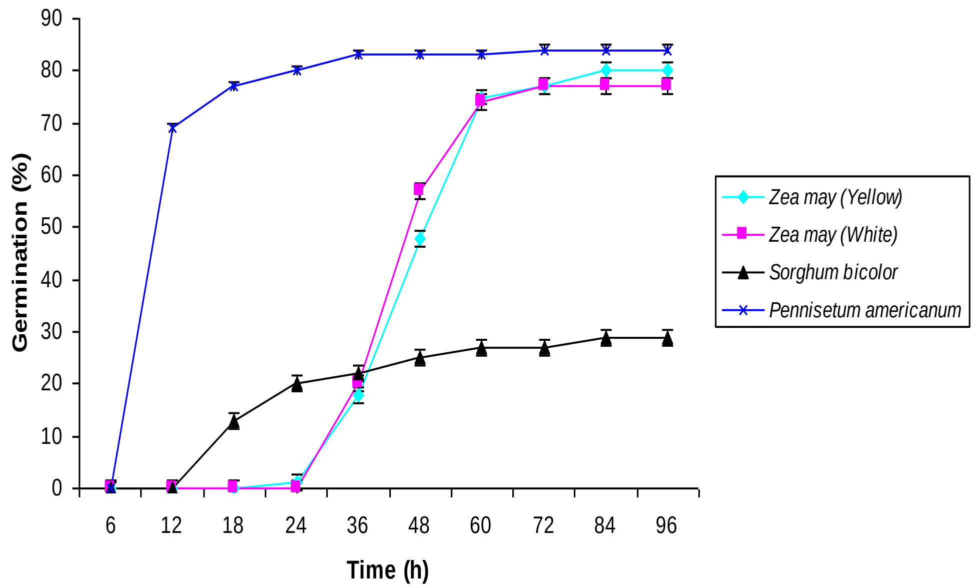

Fig 5: Percentage germination of seeds of *Z. mays*, *S. bicolor* and *P. americanum* cultivated in 100% bilge water at different time periods.

The effect of the wastewater on the mean length and health (as revealed by stereoscopic microscopic observance) of the 12 day old seedlings of *Z. mays* (white and yellow varieties), *S. bicolor* and *P. americanum* is presented in Tables 2-5. The longest roots were produced in the control for all seed types while the shortest roots were produced in the seedlings of *S. bicolor* grown on 100% bilge water. There were marked statistically significant differences ($p<0.05$) in root and shoot lengths of the cereal compared to seedlings of *Z. mays* and *P. americanum* at all bilge water concentrations. In the same vein, high concentrations of the bilge water caused significant ($p<0.05$) necrotic regions in the seedlings than lower concentrations. The damage to seedling health was most pronounced in seedlings of *S. bicolor* and least on those of *P. americanum*. A similar trend was observed with the development of chlorotic regions in the seedlings.

Table 2: Effect of Bilge Water on the Plumule Length (cm) of 12 Day Old Cereal Seedlings at different Concentrations

Concentration of bilge water (%)	*Z. mays (Yellow)*	*Z. mays (White)*	*S. bicolor*	*P. americanum*
0 (Control)	22.3 ± 0.30 [a]	27.6 ± 0.48 [a]	19.6 ± 0.40 [a]	44.6 ±0.44 [a]
25	19.6 ± 0.41 [a]	24.3 ± 0.49 [a]	17.3 ± 0.41 [a]	39.3 ± 0.54 [a]
50	19.0 ± 0.51 [a]	20.3 ± 0.44 [b]	15.3 ± 0.39 [b]	31.0 ± 0.50 [b]
75	15.6 ± 0.40 [b]	19.6 ± 0.41 [c]	14.3 ± 0.38 [b]	28.3 ± 0.46 [b]
100	13.6 ± 0.38 [c]	17.6 ± 0.40 [c]	11.3 ±0.36 [c]	20.6 ±0.42 [c]

Values with similar alphabets along the same column are not significantly different from each other

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Table 3: Effect of Bilge Water on the Radicle Length (cm) of 12 Day Old Cereal Seedlings at different Concentrations

Concentration of bilge water (%)	*Z. mays (Yellow)*	*Z. mays (White)*	*S. bicolor*	*P. americanum*
0 (Control)	50.6 ± 0.48 [a]	54.6 ± 0.46 [a]	26.6 ± 0.52 [a]	71.3 ±0.49 [a]
25	48.3 ± 0.51 [a]	43.0 ± 0.49 [a]	22.6 ±0.58 [a]	63.0 ±0.56 [a]
50	43.3 ±0.52[b]	40.0 ± 0.51 [b]	19.6 ± 0.55 [b]	55.6 ± 0.55 [b]
75	41.3 ± 0.50 [b]	39.3 ±0.47 [b]	19.0 ± 0.56 [b]	50.3 ± 0.58 [b]
100	34.0 ±0.49 [c]	33.0 ± 0.43 [c]	15.6 ±0.52 [c]	44.6 ± 0.56 [c]

Values with similar alphabets along the same column are not significantly different from each other.

Table 4: Effect of Bilge Water on the Necrotic Region (mm) of 12 Day Old Cereal Seedlings at different Concentrations

Concentration of bilge water (%)	*Zea mays (Yellow)*	*Zea mays (White)*	*S. bicolor*	*P. americanum*
0 (Control)	0.10±0.00	0.10±0.00	0.1 ± 0.00	0.10 ± 0.00
25	2.00±0.00	2.00±0.00	3.6 ± 0.00	1.00 ± 0.01
50	10.50±0.01	10.50±0.01	14.3 ± 0.01	1.50 ± 0.01
75	13.00±0.00	12.0±0.01	16.3 ± 0.01	5.70 ± 0.02
100	13.00±0.00	12.20±0.00	18.6 ± 0.01	6.10 ± 0.02

Table 5: Effect of Bilge Water on the Chlorotic Region (mm) of 12 Day Old Cereal Seedlings at different Concentrations

Concentration of Bilge water (%)	*Zea mays (Yellow)*	*Zea mays (White)*	*Sorghum bicolor*	*Pennisetum americanum*
0 (Control)	0.10 ± 0.00	0.10 ± 0.00	0.1 ± 0.00	0.10 ± 0.00
25	6.10 ± 0.00	6.40 ± 0.04	7.1 ± 0.03	1.00 ± 0.00
50	6.20 ± 0.02	6.80 ± 0.02	14.0 ± 0.02	2.00 ± 0.00
75	8.50 ± 0.01	10.00 ± 0.00	16.0 ± 0.01	2.40 ± 0.01
100	9.20 ± 0.01	10.20 ± 0.00	17.0 ± 0.01	2.60 ± 0.01

Discussion

Seed germination is a critical stage that ensures reproduction and controls the dynamics of plant populations, thus it is a critical test of probable crop productivity. The impact of wastewaters on seed germination of a number of plants has been well documented. Lower effluent concentrations of brackish water [11], sugar factory effluents [12], drug factory effluents [13] and textile effluents [14] were found to support seed germination while, at higher effluent concentrations, germination efficiencies were difficult and retarded. This observation was attributed to increase in the osmotic pressure as a result of total salts in the wastewater.

The physicochemical analysis of the bilge water used in this study reveals that it was acidic and relatively hard with high amounts of chloride. There was significant reduction and delay in the germination of white and yellow varieties of *Z. mays* and *S. bicolor* while seeds of *P. americanum* were more or less unaffected by the undiluted wastewater. This observation is in agreement with earlier reports that the ability of seeds to germinate under high osmotic pressure differs with variety as well as species of plants [15].

Although the bilge water used in this study contains micro-nutrients which are beneficial for the growth and metabolism of the plants, it also has appreciable amounts of heavy metals. Accumulation of toxic heavy metals leads to stress conditions in the plant system by interfering with the metabolic activities and physiological functioning of the plants. Heavy metals are known to cause membrane damage, structural disorganization of organelles, impairment in the physiological functioning of the plants and ultimately growth retardation [16,17,18,19]. The toxicity of bilge water on the seed germination and necrotic and chlorotic effects on seedling shoots of the cereals investigated in this study could be attributable to the presence of heavy metals in the wastewater. Similar effects on seed germination and growth impairment reportedly attributable to heavy metal contamination have been reported [4,20,21].

In conclusion, this study has shown that bilge water contains toxic substances, particularly heavy metals, which could restrict the usefulness of the wastewater for irrigation purposes unless it is properly treated.

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