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Impact of bilge water on seed germination and seedling growth of some vegetable plants

D. I. Olorunfemi^{1*}, I. E. Agbozu² and O.O. Osazuwa³

¹Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria

²Department of Environmental Science, Federal University of Petroleum Resources, Effurun, Nigeria

³Environmental Science Unit, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

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ABSTRACT: In this study, the effect of bilge water on the germination and seedling growth of seeds of okra (*Abelmoschus esculentus*), tomatoes (*Solanum lycopersicum*) and garden egg (*Solanum aethiopicum*) at different concentrations (100, 75, 50, 25% v/v) was investigated. Bilge water was found to inhibit the germination of all the seed types used. The percentage germination, radicle length and plumule length of the seedlings decreased significantly ($p < 0.05$) with increase in bilge water concentration. Seedlings cultivated in the wastewater developed toxicity symptoms but more chlorotic and necrotic regions were observed at higher concentrations. *S. lycopersicum* was least tolerant while *A. esculentus* was the most tolerant to the bilge water. The data obtained from physicochemical analysis of the wastewater revealed that it was acidic (pH 5.11) and most of the heavy metallic contents were higher than allowable FEPA limits for effluent discharge. The results suggest that germination inhibition and poor seedling growth are probably due to strong tendencies of the plant species to accumulate the trace and heavy metals in the growth medium.

Key words: *Abelmoschus esculentus*, *Solanum lycopersicum*, *Solanum aethiopicum*, bilge water, germination, seedling growth.

Introduction

Sea water and fresh water can find its way to the bilge wells due to leakage in the pipe lines, leaky pump and valve glands, from machinery, propulsion system, over flowing of tanks and even due to accidental spills. All these substances get accumulated in the bilge wells and the mixture formed is known as bilge water (Karakulski, *et al.*, 1998).

Bilge water can be described as the liquid that accumulates at the lowest compartment of the ship known as the bilge. Bilge water can be found in almost any vessel. Depending on the design and function of the ship, bilge water a mixture of variety of substances such as fresh water, sea water, sludge, lubricants, urine, oil, detergents, solvents, chemicals, various salts, pitch and metals such as arsenic, copper, chromium, lead and mercury. Oil that often leak from the engine, machinery spaces from engine maintenance activities can mix with water in the bilge.

Bilge water is one of the causes for marine pollution. Untreated bilge water can contain oil, gasoline, solvents, detergents, chemicals, and more and when discharged in to the sea can negatively affect biodiversity, food, and health in coastal areas, oceans and waterways (Singh and Song, 2006). Bilge water may also contain waste and pollutants that are high in oxygen demanding materials and other chemicals.

*To whom all correspondence should be addressed.

In Nigeria, bilge water is believed to be discharged into the environment without due regard to safety of plant and animal life which could constitute a significant source of pollution of the environment. We are not aware of any data available literature on the impact of bilge water on germination and seedling growth of plants. The objective of this study was therefore, to ascertain the level of toxicity or otherwise and perceived consequences, if any, of bilge water on the seed germination and seedling growth of okra (*Abelmoschus esculentus*), tomatoes (*Solanum lycopersicum*) and garden egg (*Solanum aethiopicum*).

Materials and Methods

Bilge Water Source: The bilge water stock sample used for the study was collected from *M/V Theka* (a multipurpose vessel carrying dry cargo, 6301 gross tonnage 132,2m long, IMO number 9259020, built March 2003, Flag: Antigua & Barbuda). The vessel was berthed at canal in Warri Port on the 24th March 2011. Different concentrations (25, 50, and 75%) of the wastewater were prepared by further dilution of the stock solution.

Plant Materials: The seeds of *Abelmoschus esculentus*, *Solanum lycopersicum* and *Solanum aethiopicum* were obtained from Oba Market, Benin City. The seeds were sorted, cleaned and tested for viability using the floating method (Idu and Olorunfemi, 1988). 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.

Germination Studies: 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 received 30 seeds of *A. esculentus*, *S. lycopersicum* and *S. aethiopicum* 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 so affected were removed to avoid contamination of other seeds. The seeds were observed for germination each day and 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. The measurements were made for root and shoot length with a meter rule. Necrotic and chlorotic regions were observed using Swift Stereomicroscope with an eyepiece graticule of 10x magnitude.

Statistical Analysis: Data were expressed as Mean \pm Standard Error of Mean (SEM). Differences between the control and the different concentrations of the bilge water were analyzed statistically by using Duncan Multiple Range (DMRT). P values of <0.05 were considered to be statistically significant. All statistical analyses were carried out using SPSS®14.0 statistical package.

Results and Discussion

Results of various physical and chemical characteristics of the bilge water are presented in Table 1. The wastewater was acidic (pH5.11) and most of the heavy metallic contents were higher than allowable FEPA limits for effluent discharge. It was evident from the values that parameters such as nitrates, phosphates, chlorides, iron, exceeded FEPA (1991)/USEPA (1999) permissible limits.

Table 1: Mean (\pm SE) values of some physical and chemical characteristics of bilge water sample obtained from *M/V Theka* at Warri Port

Parameter	Bilge water	FEPA ^a	USEPA ^b
pH	5.11	6-9	6.5-8.5
BOD ₅ @ 20°C	6.2	50	-
Turbidity (FTU)	15	-	-
Total Hardness	227	-	0-75
Conductivity (μ S/cm)	1,400	-	-
TDS	0.04	2000	500
Ammonia	0.05	-	-
Nitrates	99.5	20	10
Sulphates	107.5	500	250
Phosphates	100.5	5	-
Chlorides	970.3	600	250
Sodium	120.3	-	-
Calcium	110.5	200	-
Potassium	119.3	-	-
Magnesium	130.1	-	-
Copper	0.5	<1	0.009
Manganese	3.9	5	0.05
Lead	0.3	<1	0.003
Iron	5.7	20	0.30
Cadmium	0.1	<1	0.002
Chromium	1.4	-	-
Silver	0.2	-	-
Nickel	0.3	<1	0.005
Zinc	11.6	<1	0.12

Values are means of 3 replicates

All values are in mg/l except conductivity (μ S/cm), turbidity (NTU) and pH with no unit.

BOD: Biochemical oxygen demand

TDS: Total dissolved solids

^aFederal Environmental Protection Agency (1991). Permissible limits for effluent discharge into surface water

^bUnited States Environmental Protection Agency (1999). National recommended water quality criteria – correction

The mean values of growth parameters of the 12 days old seedlings of *A. esculentus*, *S. aethiopicum* and *S. lycopersicum* in various bilge water concentrations respectively are presented in Tables 1-3. The data show that germination of all the seed types were seriously affected by varying doses of applied bilge water. The greater the concentration of the bilge water, the less the percentage germination. The longest roots were produced in the control

for all seed types while the shortest roots were produced in the seedlings of *S. lycopersicum* grown on 100% bilge water. There were marked statistically significant differences ($p<0.05$) in root and shoot lengths of this vegetable plant compared to those of *A. esculentus*, and *S. aethiopicum* at all bilge water concentrations.

Table 1: Mean values (\pm S.E.) of growth parameters of 12 days old seedlings of *Abelmoschus esculentus* in various bilge water concentrations

Bilge water Concentration	Germination (%)	Seedling length (cm)		Shoot/root ratio	Necrotic region (mm)	Chlorotic region (mm)
		Radicle	Plumule			
0 (Control)	81	3.46 \pm 0.11	6.28 \pm 0.01	1.77 \pm 0.04	0.10 \pm 0.00	0.10 \pm 0.00
25	76	3.37 \pm 0.17	6.04 \pm 0.24	1.79 \pm 0.11	1.50 \pm 0.01	1.00 \pm 0.01
50	56	3.15 \pm 0.06	5.31 \pm 0.15	1.69 \pm 0.18	2.50 \pm 0.01	2.30 \pm 0.01
75	41	2.22 \pm 0.13	5.23 \pm 0.17	2.35 \pm 0.25	6.90 \pm 0.05	2.50 \pm 0.02
100	28	1.24 \pm 0.12	4.24 \pm 0.16	3.40 \pm 0.14	8.30 \pm 0.06	2.80 \pm 0.01

Table 2: Mean values (\pm S.E.) of growth parameters of 12 days old seedlings of *Solanum aethiopicum* in various bilge water concentrations

Concentration of solution	Germination (%)	Seedling length (cm)		Shoot/root ratio	Necrotic region (mm)	Chlorotic region (mm)
		Radicle	Plumule			
0 (Control)	73	3.55 \pm 0.16	6.25 \pm 0.02	1.76 \pm 0.11	0.20 \pm 0.00	0.10 \pm 0.00
25	43	3.52 \pm 0.11	4.26 \pm 0.11	1.21 \pm 0.16	2.00 \pm 0.00	6.70 \pm 0.04
50	35	3.24 \pm 0.03	3.34 \pm 0.08	1.03 \pm 0.15	12.50 \pm 0.01	8.50 \pm 0.02
75	24	3.15 \pm 0.05	3.02 \pm 0.02	0.95 \pm 0.01	14.50 \pm 0.01	11.50 \pm 0.01
100	11	2.45 \pm 0.11	2.19 \pm 0.06	0.72 \pm 0.12	15.30 \pm 0.01	12.30 \pm 0.01

Table 3: Mean values (\pm S.E.) of growth parameters of 12 days old seedlings of *Solanum lycopersicum* in various bilge water concentrations

Concentration of solution	Germination (%)	Seedling length (cm)		Shoot/root ratio	Necrotic region (mm)	Chlorotic region (mm)
		Radicle	Plumule			
0 (Control)	76	3.12 \pm 0.11	6.05 \pm 0.05	1.94 \pm 0.07	0.2 \pm 0.00	0.1 \pm 0.00
25	34	2.94 \pm 0.06	3.52 \pm 0.10	1.19 \pm 0.13	3.5 \pm 0.00	7.1 \pm 0.03
50	24	2.24 \pm 0.04	2.41 \pm 0.09	1.08 \pm 0.07	15.6 \pm 0.01	13.1 \pm 0.02
75	18	1.86 \pm 0.09	1.70 \pm 0.23	0.91 \pm 0.01	17.3 \pm 0.03	15.2 \pm 0.01
100	8	1.21 \pm 0.03	1.01 \pm 0.03	0.83 \pm 0.01	19.4 \pm 0.01	16.9 \pm 0.01

There was also a considerable statistically significant ($p < 0.05$) effect of bilge water on the shoot/root ratio in all the seedlings (Tables 1-3). This ratio was greatest for varying concentrations of the wastewater on seedlings of *A. esculentus* and least for *S. lycopersicum*. The effect of the wastewater on the seedling health of the plants (as revealed by stereoscopic microscopic observance) is presented in Tables 1-3. Generally, increased concentrations of the wastewater 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. lycopersicum* and least on those of *A. esculentus*. In the same vein, the development of chlorotic regions followed similar trend.

The toxicity of bilge water is evident on seed germination and seedling growth of *A. esculentus*, *S. aethiopicum* and *S. lycopersicum*, a phenomenon which could be attributable to the presence of heavy metals in the wastewater. This observation is consistent with earlier reports (Olorunfemi *et al.*, 2011) on the effect of ballast water (another wastewater) on the germination profiles of some cereals. The significant reduction and delay in seed germination could also be due to decrease in water uptake at higher level of salinity of the bilge water (Table 1) in view of toxicity of high osmotic pressure of the seedling medium or due to toxicity of the high concentration of chloride ions alone (Khan and Sheikh, 1976, Hayward and Wadleigh, 1949). These observations are consistent with other reports on similar effects in a number of other plant species (Walley *et al.*, 1974).

The bilge water also caused the necrosis and chlorosis of shoots of seedlings of *A. esculentus*, *S. aethiopicum* and *S. lycopersicum* and the severity was concentration and specie dependent. Similar effects have been reportedly attributed to the action of heavy metals like Cr, Cu and Co on the chloroplast of plants (Barcelo *et al.*, 1988, Chatterjee and Chatterjee, 2000).

The results obtained from this study suggest that germination inhibition and poor seedling growth of the vegetable plants used are probably due to the acidity, high salinity and strong tendencies of the plant species to accumulate the trace and heavy metals in the bilge water in their cell sap or because of the failure of sub-cellular organelles to adjust high osmotic potential of cell sap (Walter, 1955, Bernstein, 1961, Meiri *et al.*, 1970). However, flooding of the wastewater could result in improved growth of the plants.

References

- Barcelo J., Vazquez M.D. and Poschenrieder C. 1998. Structural and ultra-structural disorders in cadmium treated bush bean plants (*Phaseolus vulgaris* L.). *New Phytologist*, 108: 37-49.
- Bernstein, L. 1961. Osmotic adjustment of plants to saline media, 1. Steady state. *American Journal of Botany*, 48: 909-918
- Chatterjee, J. and Chatterjee, C. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution*, 109: 69-74.
- Federal Environmental Protection Agency (FEPA) 1991. S1.8 National Environmental Protection (Effluent Limitations) Regulations 1991 as cited by Odiete (1991). **In:** Environmental Physiology of Animals and Pollution, Published by Diversified Resources Ltd., Lagos, Nigeria. pp. 157-219.
- Heyward, H.E. and Wadleigh, C.H. 1949. Plant Growth on Saline and Alkali Soils. *Advances in Agronomy*. Academic Press Inc., New York 1: 38
- Idu, M. and Olorunfemi, D.I. 1998. Germination of the seeds *Afromomum melegueta*, K. Schum (Zingiberaceae). *Journal of Applied Science*, 1: 111 – 118.
- Karakulski, K., Morawski W.A. and Grzechulska, J. 1998. Purification of bilge water by hybrid ultra-filtration and photocatalytic processes. *Purification Technology*, 25:163–173.
- Khan, S.S. and Sheikh, K.H. 1976. Effects of different level of salinity on seed germination and growth of *Capsicum annum*. *Biologia*, 22:15-25
- Meiri, A., Mor, E. and Mayber, A.P. 1970. Effects of time exposure to salinity on growth water status and accumulation in bean plants. *Annals of Botany*, 34: 383-391.
- Olorunfemi, D.I., Duru, E.C. and Okieimen, F.E. 2011. Comparative germination profiles of some arable crops in ballast water. *Bioscience Biotechnology Research Communications*, 4(2): 127-133
- Singh G. and Song, L. (2006) Influence of sodium dodecyl sulfate on colloidal fouling potential during ultrafiltration, *Colloids Surf. A: Physicochemical Engineering Aspects*, 281: 138-146.
- United States Environmental Protection Agency (USEPA) 1999. National recommended water quality criteria – Correction: EPA 822/Z-99-001, USEPA, Washington DC.
- Walley, Y.A., Khan, M.R. and Bradshaw, A.D. (1974). The potential for evolution of heavy metals tolerance in plants. 1. Copper and zinc tolerance in *Agrostis tenuis*. *Heredity*, 32: 309-319.
- Walter, H. (1955). The water economy and hydrature of the plants. *Annual Review of Plant Physiology*, 6: 239-252.