Differential response of \textit{Tilapia guineensis} fingerlings to inorganic fertilizer under various salinity regimes

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(Received: 6 April, 2005; Accepted: 08 December, 2005)

\textbf{Abstract:} \textit{The influence of salinity on the response of the estuarine teleost, \textit{Tilapia guineensis} fingerlings to acute toxic effects of inorganic nitrogen-phosphorus-potassium (NPK) (15:15:15) fertilizer was investigated using semi-static bioassay. The toxicity of NPK fertilizer was found to increase significantly with increase in the salinity level from 0.05 \(\%\) to 32.4 \(\%\). The 96 hr \textit{LC}_{50} value at salinity of 32.4 \(\%\) was 0.11mg/l and was found to be significantly \((p < 0.05)\) higher than the toxicity values at any other salinity level of media evaluated. The implication of the findings is that pollution control standards and/or safe limits for brackish water ecosystem should consider variations in salinity regimes for greater relevance and reliability.}

\textbf{Key words:} Response, Tilapia, Toxicity, Fertilizer, Salinity.

\section*{Introduction}

In recent times, several aquacultural researches have been directed towards identifying fishes already intensively farmed that can survive and grow in brackish and/or seawater environment (Chervinski, 1981; Ezenwa, 1984; Chukwu, 1999, 2002). In Africa and other tropical regions, there are vast coastal swamp lands estuaries and lagoon areas of saline soils where there is little or no competition for land use (Satia, 1990). These saline regions of freshwater shortage could be reclaimed for fish culture operations. However, with the current trend of increased industrialisation and urbanisation and use of agrochemicals has come a greater risk of pollution of coastal estuarine lagoons and mangrove swamps.

The use of fertilizer in aquaculture is as important as it is in agriculture. According to Adesiyan (1992) most of the important sources of fertilizer pollution in aquatic environment comes from agricultural lands, liquid effluents from nitrogen-phosphorus-potassium (NPK) fertilizer processing plants, spillage during offloading at jetty and during fertilization and liming. The impact of fertilizers as a pollutant on fisheries has been reviewed by Waldichuk (1974). Perhaps the most important effect of agro-chemical pollutant in the aquatic environment is ecological disruption. Pearson and Betteridge (1977) reported that various kinds of agro-chemical pollutants including fertilizers have different effects on different organisms, the intensity of which depends on the genetic make up of the species, its previous history as well as temperature, pH and salinity. From salinity tolerance experiments by Chervinski (1981) it was observed that \textit{Tilapia guineensis} when exposed to high salinities of order of 14.40 \(\%\) to 32.5 \(\%\) showed signs of stress. Since salinity changes induce physiological responses in fishes, such changes may also influence their susceptibility to toxicants. \textit{Tilapia guineensis} though found in freshwater environment is a brackish water fish distributed along the coastal lagoons and estuaries of West Africa (Pullin and Lowe-McConnel, 1982). As the fish is now considered as a suitable cultivable fish species in brackish water environment, studies on factors affecting its survival are, therefore, imperative in view of the present emphasis on aquaculture development in West Africa as well as in the world, in general. The present study attempts to determine the acute lethal toxicity of NPK fertilizer against fingerlings of \textit{Tilapia guineensis} under varying salinity regimes.

\section*{Materials and Methods}

\textbf{Source and maintenance of test organisms:} \textit{Tilapia guineensis} (Bleeker, 1862) fingerlings of mean standard length 3.38+0.88 cm and body weight 2.45+0.56 g were obtained from Alakotomeji Fish Farm, Badagry in Nigeria. The fish were acclimatized for 14 days in large tanks (30cm x 30m x 70cm), containing dechlorinated and aerated tap water under laboratory conditions (Temperature 30.0 \(\pm\) 2\(^\circ\)C; Relative humidity 78.0+3\%). The fish were fed with pellets of NIOMR\textregistered fish feed (Ufodike and Onusiriuka, 1990), once daily at 3\% of their body weight. Acclimatization of test organisms to laboratory conditions was in accordance with guidelines for bioassay techniques (APHA, 1985).

\textbf{Test compound and media:} The test compound used in this study is the nitrogen – phosphorus-potassium (NPK) 15:15:15 inorganic fertilizer. Sea water was collected from the Lagos Bar Beach, filtered and stored in 25 liters plastic containers.

\textbf{Bioassay:} Acute semi-static bioassays were conducted in the laboratory following the methods of APHA (1985). Graded concentrations of the NPK fertilizer were prepared according to the method of Ufodike and Onusiriuka (1990). A preliminary test was carried out to ascertain the range of concentrations to be used before the final bioassay. Ten fish were exposed to each of the five toxicant concentration (0.80, 0.85, 0.90, 0.95 and
Fig. 1: The influence of salinity changes on the toxicity of NPK fertilizer against *Tilapia guineensis* fingerlings.

1.20 mg/l) and untreated control at each pre-determined salinity regime (0.05 ‰, 14.4 ‰, 28.89 ‰ and 32.4 ‰) in replicates. The pH, temperature, total alkalinity and dissolved oxygen content were measured daily following APHA (1985).

**Quantal/behavioural response:** The behaviour and general conditions of the fish were observed during the bioassay. Mortality was used as a measure of toxicity. Fish were considered dead when they failed to show any sign of movement or response to gentle prodding with sharp object. (Chukwu and Ugbeva, 2003). The fish were examined for mortality at 24, 48, 72 and 96 hr.

**Statistical analysis:** Dose mortality results were analysed with a computer programme using Probit analysis after Finney (1971), the values of LC50 and their confidence limits were obtained and tabulated as adopted by Don Pedro (1989) and used as indices for assessing the susceptibility of the test organisms to the toxicants. The indices of toxicity measurement derived from these analyses were LC50 (median Lethal concentration that causes 50% response [mortality] of exposed organisms), LC95 (Lethal concentration that causes 95% response [mortality] of exposed organisms) and T.F. (toxicity factor for relative potency measurements).

**Results and Discussion**

**Physico-chemical parameters of test solution:** The physico-chemical parameters of the test solutions varied slightly with mean temperature of 25.0±1.35°C, pH 7.8±0.18, alkalinity 25.6±0.38 mg/l and dissolved oxygen content of 9.5±0.14 mg/l. Addition of the toxicants did not significantly alter water quality (F_cal 26.91, F_tab 3.11; DF 5; p < 0.05). The physico-chemical parameters for all the test solutions in this study were found to have fluctuated slightly. According to Boyd and Lichtkoppler (1979) water quality parameters such as dissolved oxygen, pH, hardness and temperature affect the toxicity of chemicals to fish.

**Influence of salinity on acute toxicity of NPK fertilizer against *Tilapia guineensis* fingerlings:** The acute toxicity of NPK fertilizer against *Tilapia guineensis* fingerlings increased steadily with increasing salinity levels from 0.05 ‰ to 32.4 ‰ (Table 1 and Fig. 1). At 32.4 ‰, the toxicity of NPK fertilizer based on derived 96 hr LC50 was 0.111 mg/l which was significantly (no overlap in 95% CL of 96 hr LC50) higher than the toxicity values at any other salinity level of media evaluated (Table 1). Similarly, on the basis of computed toxicity factor (96 hr LC50 value ratios), NPK fertilizer at salinity level of 32.4 ‰ was about two, three and seven times more toxic than salinity levels of 24.8 ‰, 14.4 ‰ and 0.05 ‰ respectively when tested against *T. guineensis* fingerlings (Table 1).

The result of the toxicity test of NPK fertilizer against *Tilapia guineensis* fingerlings showed that NPK fertilizer like most agro-chemical substances is toxic to animals at high concentrations. Furthermore, the 96 hr LC50 value decreased as the time of exposure and salinity of test media increased. For example, the toxicity factor for the 96 hr LC50 at salinity 32.4 ‰ was 7.7 times more toxic than those of other salinity levels of media evaluated in this study. These results suggest that the toxicity of NPK fertilizer to estuarine animals may be synergized by osmotic stress at the salinity extremes.
Table 1: Toxicity of NPK fertilizer against *Tilapia guineensis* fingerlings under salinity regimes.

<table>
<thead>
<tr>
<th>Test media</th>
<th>Treatment</th>
<th>LC50 (95 % FL.) (mg/l)</th>
<th>LC50 (95 % FL.) (mg/l)</th>
<th>LC50 (95%FL.) (mg/l)</th>
<th>Slope</th>
<th>Variance of slope</th>
<th>df</th>
<th>Regression equation</th>
<th>Toxicity factor (T.F2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>24</td>
<td>0.0695(0.800-0.431)</td>
<td>0.946(1.00-0.862)</td>
<td>1.286(1.879-1.146)</td>
<td>12.371</td>
<td>12.769</td>
<td>2</td>
<td>Y=0.299+12.371X</td>
<td>1.00</td>
</tr>
<tr>
<td>Seawater</td>
<td>48</td>
<td>0.749(0.818-0.642)</td>
<td>0.938(0.976-0.901)</td>
<td>1.176(1.366-1.079)</td>
<td>16.813</td>
<td>13.973</td>
<td>3</td>
<td>Y=0.464+16.813X</td>
<td>1.090</td>
</tr>
<tr>
<td>(Salinity</td>
<td>72</td>
<td>0.786(0.810-0.717)</td>
<td>0.886(0.908-0.863)</td>
<td>1.024(1.087-0.973)</td>
<td>26.128</td>
<td>26.128</td>
<td>4</td>
<td>Y=1.378+26.128X</td>
<td>1.060</td>
</tr>
<tr>
<td>0.05%</td>
<td>96</td>
<td>0.774(0.793-0.691)</td>
<td>0.863(0.887-0.840)</td>
<td>1.002(1.061-0.853)</td>
<td>25.555</td>
<td>22.554</td>
<td>4</td>
<td>Y=1.629+25.555X</td>
<td>1.090</td>
</tr>
<tr>
<td>40%</td>
<td>24</td>
<td>0.361(0.475-0.239)</td>
<td>0.661(0.749-0.569)</td>
<td>1.21(1.609-0.990)</td>
<td>6.778</td>
<td>1.680</td>
<td>2</td>
<td>Y=1.129+6.278X</td>
<td>1.00</td>
</tr>
<tr>
<td>Seawater</td>
<td>48</td>
<td>0.173(0.263-0.097)</td>
<td>0.514(0.623-0.417)</td>
<td>1.532(2.447-1.082)</td>
<td>3.482</td>
<td>0.394</td>
<td>3</td>
<td>Y=1.005+3.482X</td>
<td>1.28</td>
</tr>
<tr>
<td>(Salinity</td>
<td>72</td>
<td>0.110(0.200-0.047)</td>
<td>0.445(0.565-0.340)</td>
<td>1.605(3.488-1.124)</td>
<td>2.714</td>
<td>0.301</td>
<td>3</td>
<td>Y=0.954+2.714X</td>
<td>1.48</td>
</tr>
<tr>
<td>14.4%</td>
<td>96</td>
<td>0.034(0.150-0.05)</td>
<td>0.365(0.457-0.287)</td>
<td>1.408(2.253-0.961)</td>
<td>2.812</td>
<td>0.182</td>
<td>4</td>
<td>Y=1.232+2.812X</td>
<td>1.81</td>
</tr>
<tr>
<td>80%</td>
<td>24</td>
<td>0.280(0.356-0.218)</td>
<td>0.590(0.628-0.392)</td>
<td>1.10(1.452-0.912)</td>
<td>2.601</td>
<td>11.284</td>
<td>2</td>
<td>Y=1.926+2.510X</td>
<td>1.00</td>
</tr>
<tr>
<td>Seawater</td>
<td>48</td>
<td>0.192(0.256-0.423)</td>
<td>0.421(0.524-0.862)</td>
<td>1.501(2.351-0.816)</td>
<td>3.204</td>
<td>16.381</td>
<td>1</td>
<td>Y=0.134+5.111X</td>
<td>1.40</td>
</tr>
<tr>
<td>(Salinity</td>
<td>72</td>
<td>0.165(0.299-0.629)</td>
<td>0.340(0.249-0.542)</td>
<td>1.362(1.823-0.34)</td>
<td>4.332</td>
<td>21.262</td>
<td>1</td>
<td>Y=1.334+2.550X</td>
<td>1.74</td>
</tr>
<tr>
<td>24.8%</td>
<td>96</td>
<td>0.142(0.533-0.280)</td>
<td>0.286(0.349-0.439)</td>
<td>1.256(1.553-0.470)</td>
<td>3.334</td>
<td>0.242</td>
<td>2</td>
<td>Y=0.44+2.560X</td>
<td>2.21</td>
</tr>
<tr>
<td>90%</td>
<td>24</td>
<td>0.188(0.452-0.330)</td>
<td>0.435(0.332-0.824)</td>
<td>1.220(0.920-0.901)</td>
<td>7.661</td>
<td>0.561</td>
<td>1</td>
<td>Y=0.434+2.162X</td>
<td>1.00</td>
</tr>
<tr>
<td>Seawater</td>
<td>48</td>
<td>0.159(0.951-0.221)</td>
<td>0.382(0.445-0.931)</td>
<td>1.020(0.820-0.520)</td>
<td>4.660</td>
<td>0.550</td>
<td>2</td>
<td>Y=0.060+1.332X</td>
<td>1.13</td>
</tr>
<tr>
<td>(Salinity</td>
<td>72</td>
<td>0.141(0.630-0.420)</td>
<td>0.426(0.920-0.520)</td>
<td>1.126(0.501-0.62)</td>
<td>2.731</td>
<td>1.330</td>
<td>1</td>
<td>Y=1.824+1.923X</td>
<td>1.66</td>
</tr>
<tr>
<td>32.4%</td>
<td>96</td>
<td>0.138(0.430-0.712)</td>
<td>0.111(0.660-0.450)</td>
<td>1.520(1.201-0.666)</td>
<td>31.242</td>
<td>0.811</td>
<td>3</td>
<td>Y=1.555+3.422X</td>
<td>3.91</td>
</tr>
</tbody>
</table>

T.F1 = Toxicity factor = LC50 of test compound at 24 hr
T.F2 = Toxicity factor = 96 hr LC50 of other salinity regime
96 hr LC50 of most toxic salinity regime
investigated and therefore their sensitivity during the peaks of rainy season and dry seasons.

Earlier workers (Eddy, 1981; Pickering, 1981) have demonstrated the effect of salinity or chloride ion concentration on the susceptibility/tolerance of organisms exposed to pollutants. Bertinger and Huey (1981) reported that progressive increase in environmental chloride concentration from <6.1mg/l to 300 mg/l increased toxicity of nitrate to crayfish Procambarus simulans, Eddy (1981), using Clibanarius africanus as the test organisms had reported that the median lethal toxicity of Escravos crude oil blend was found to decrease with increasing salinity, indicating that the crude oil was more toxic at high salinities than at low salinities. Recent studies by Oyewo and Don-Pedro (2003), have shown that salinity influenced the toxicity of heavy metals to Tilapia guineensis and Nerita senegalensis at salinity levels above 25%.

Symptoms NPK fertilizer toxicity: Tilapia guineensis fingerlings showed sensitivity to the toxicants by an initial increase in restlessness, rate of uncoordinated movement, loss of equilibrium and death. This abnormal behaviour was more pronounced in test media of higher sa linities. The osmotic stress induced by sudden variation in the ambient osmotic concentration of a fish is often associated with net gain or loss or no change of water content depending on the osmotic concentration of the medium (Chukwu, 1999, 2002). Teleost fish osmoregulation is largely the result of integrated transport of ions and water across the gills and through the gut. The osmolal gradient between the fish and its environment is maintained by active transport processes. When the osmotic concentration of the medium is different from that of the animal, the animal undergoes a series of symptoms that are characteristic of the osmotic stress. Symptoms of osmotic stress include increased respiration, increase in electrical resistance, increase in heart rate, increased rate of uncoordinated movement, loss of equilibrium and death. These symptoms may be followed by a return to normal if the fish is exposed to ambient water. The sensitivity of the fish to the toxicants is determined by their ability to adapt to the increased salinity. The sensitivity of the fish to the toxicants is determined by their ability to adapt to the increased salinity. The sensitivity of the fish to the toxicants is determined by their ability to adapt to the increased salinity.

From the result in this study, it is postulated that euryhaline teleost fishes are under physiological stress particularly at the peaks of the dry and wet seasons, and such stress may easily synergise the toxicity of common priority pollutants including inorganic fertilizers. Thus, pollution control standards and/or safe limits for brackish water ecosystem should consider variations in salinity regimes for greater relevance and reliability.

References

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