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# Changes in Poultry Litter Toxicity with Simulated Acid Rain

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The Delmarva Peninsula on the Eastern Shore of Maryland ranks 4th in the nation in poultry production and generates 9,500 metric tons of poultry manure/ litter per day (North 1984; USDA 1987). The poultry litter contains many macro and micro nutrients and is an excellent source of fertilizer. The litter also contains antibiotics, heavy metals (copper, zinc, lead, arsenic, cadmium, manganese), hormones and many micro-organisms. Land application of this litter has been the only means of its utilization and disposal. With rainfall, surface water run-off (leachate), from land on which litter has been applied, reaches the Cheasapeake Bay from this region. This leachate with its high organic and inorganic salt contents and high biochemical oxygen demand can severely disrupt the aquatic life and cause fish kills. Poultry litter aqueous extract has been shown to be toxic to marine organisms (photobacterium phosphoreum); the extract's $EC_{50}$ values increase (thereby showing a decrease in toxicity) with increase in leaching time (Gupta & Krishnamurthy 1990). The objective of this research was to study the effect of simulated acid rain (pH 3, 4 & 5) on the toxicity of poultry litter extracts.

## MATERIALS AND METHODS

Experimental design and approach (for litter samples, aqueous extract preparation, chemical analyses of the extract, toxicity assessment, replicates, data reduction and statistical computations) as discussed earlier (Gupta & Krishnamurthy 1990) were used in this study. Briefly, 5g of the air dried, pulverized, sieved (5 mm) litter sample was mixed with 1 L of deionized or acid rain water in each of 4 flasks. At the end of 1, 6, 12 or 24 hr the supernatant liquid was centrifuged and used for 'Microtox' bioassay with photobacterium phosphoreum (Beckman 1982). Simulated

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acid rain was prepared by adding a mixture of 6.5 ml sulphuric acid and 3.5 ml nitric acid to deionized water till the desired pH (3, 4 or 5) was reached. Control samples (deionized water, acid rain water with no poultry litter - pH 3, 4 or 5) were also bioassayed. All samples were analyzed in triplicate. Poultry litter was also analyzed for pH, conductivity, redox potential and nutrients using standard methods (APHA, 1985).

## RESULTS AND DISCUSSION

The bioluminescent marine organisms (photobacterium phosphoreum) used for this bioassay retained 97±2% of their light output when exposed to deionized water (pH 6.4), 88±2% with acid rain pH 5, 78±2% with pH 4 and only 8±2% with pH 3. The $EC_{50}$ values for acid rain pH 3, 4 and 5 were 30, 280 and 818 ml, respectively, suggesting that pH 6.4 is non-toxic, pH 5 and 4 are increasingly more toxic and pH 3 is very toxic.

Changes in pH, conductivity and redox values for the poultry litter extracts with acid rain water of pH 3, 4 or 5 are shown in Table 1 and changes in chemical composition of poultry litter extracts with deionized water with time are given in Table 2. Concentration of nitrate and nitrite did not show any change with extraction time; the amounts of both ammonia and phosphate increased by about 15%.

Table 1. Changes in pH, conductivity and redox potential of poultry litter extracts.

| | pH of the acid rain | | |
|---|---|---|---|
| | 3 | 4 | 5 |
| pH | | | |
| 1 hr | 7.15 | 8.2 | 8.7 |
| 24 hr | 6.73 | 7.6 | 7.8 |
| Conductivity (μmhos/cm) | | | |
| 1 hr | 700 | 734 | 744 |
| 24 hr | 775 | 768 | 764 |
| Redox potential (mv) | | | |
| 1 hr | 102 | 70 | 58 |
| 24 hr | 74 | 50 | 46 |

The $EC_{50}$ values for the various poultry litter extracts increased significantly (at $p < 0.05$) only after 24 hr extraction (Table 3). Increasing $EC_{50}$ values reflect decreasing toxicity. The $EC_{50}$ values for the 1 hr litter extracts, with deionized water, were approximately 10 times less than those of the 24 hr extract. Acid rain (pH 3) alone is as toxic as the litter extract (24 hr) with deionized water of pH 6.4.

Table 2. Changes in chemical composition of poultry litter-deionized water extract with time.

| Composition (ppm) | 1 hr | 24 hr |
|---|---|---|
| Total dissolved solids | 956 | 1006 |
| Total volatile solids | 534 | 656 |
| Total phosphate | 47 | 55 |
| Kjeldahl-N | 90 | 130 |
| Ammonia-N | 23 | 27 |
| Nitrate-N | 10 | 11 |
| Nitrite-N | 1 | 1 |

Table 3. Effect of acid rain on $EC_{50}$ (15 min) of poultry litter extracts (ml used).

| Extraction Time (hr) | pH 3 | pH 4 | pH 5 | pH 6.4 |
|---|---|---|---|---|
| 01 | $5.9^{b,x}$ | $3.9^{b,x}$ | $5.6^{b,x}$ | $3.9^{b,x}$ |
| 06 | $7.4^{b,x}$ | $6.8^{b,x}$ | $6.5^{b,x}$ | $3.2^{b,x}$ |
| 12 | $13.0^{b,y}$ | $6.5^{b,x}$ | $4.8^{b,x}$ | $4.7^{b,x}$ |
| 24 | $35.1^{a,y}$ | $25.3^{a,y}$ | $24.5^{a,y}$ | $30.9^{a,y}$ |

Means with the same letter are not significantly different at $P < 0.05$ using Duncan's Multiple Range Test; a,b represent columns comparison; x,y shows rows comparison.

The changes in toxicity showed a similar pattern when litter was extracted with simulated acid rain. The toxicity of acid rain (pH 4 or 5 ) with poultry litter (Table 3) was always higher than the toxicity of acid rain alone, but the toxicity decreased as the time of extraction increased. The poultry litter extracts with acid rain pH 3, 4 , 5 or with deionized water did not show significant differences in toxicity, except after 12 hr extraction with acid rain pH 3. The regression analyses of toxicity ($EC_{50}$) with time (or pH) showed a second order reaction kinetics (Fig. 1), with a correlation coefficient ($r^2$) of 0.99 and 0.93, respectively. Acid rain (pH 3) was more toxic than its mixture with litter (24 hr extract); this antagonistic behavior can result from the observed increase in phosphate (Table 2) with extraction time and acidity.

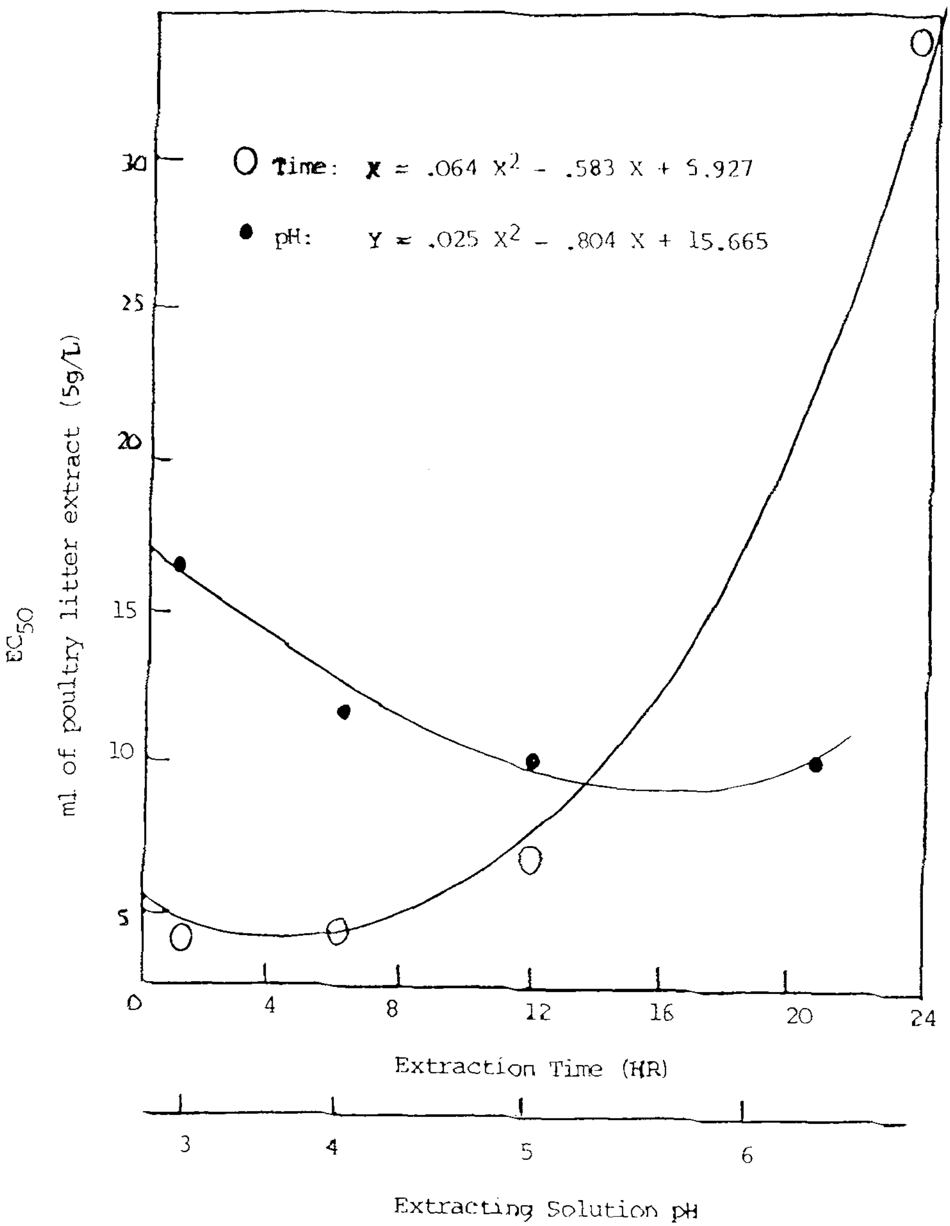


Figure 1. Changes in poultry litter extract toxicity with time and pH.

Vasseur et al. (1986) reported a large reduction in zinc toxicity in the presence of phosphate at all pH levels and an increase in the toxicity of some organic substances (phenols) in the presence of phosphate in acidic medium. Frank et al. (1988) found various polycholorophenols from preservatives in the wood shavings used as the base material in litter. This suggests that both the heavy metals and the organic compounds could be contributing to the toxicity of the litter extracts. The pH of all the extracts decreased with an increase in the time of extraction (Table 1). Beaubien & Jolicoeur (1984) found that the activity of various microorganisms remained within 10% of the optimal value at pH 6.5, between pH 5 - 8, in response to many heavy metals. The pH affects the chemical speciation of heavy metals , particularly the divalent ones; with increasing pH complex hydroxylated species of the metals are formed. Babich & Stotzky (1986) reported increased toxicity of bacteria and fungi to cadmium on increasing the pH from 5 - 9 due to the formation of $CdOH^{+}$. The pH also affects the complexation and binding of metals to organic constituents in the growth medium; as pH is increased complexation of most heavy metals to both inorganic and organic constituents is also increased. Vasseur et al. (1986) have shown that zinc and cadmium were less toxic in the presence of calcium, using photobacterium phosphoreum. Increased acidity, as well as increased extraction time, of the litter could also release more calcium,thus reducing the toxicity of heavy metals. The decrease in redox potential (Table 1) with an increase in extraction time also points to a reduction both in the toxicity of metals from a reduced oxidation state and their solubility (Bockris, 1988). A reducing environment can enhance the microbial conversion of sulphates to sulfides resulting in the precipitation of the heavy metals (Babich et al. 1982).

Litter extract is a complex mixture of many different chemicals. Metal ions and organic pollutants are known to exhibit complex interactions. Increasing the concentration of organic compounds (yeast, cysteine, humic acid) can reduce the toxicity of metal ions (Beckman 1980). Many organic chemicals, such as phenol, are known to exhibit reduced toxicity with time. A reduction in toxicity may then take place with an increase in extraction time or a decrease in pH. From these observations it can be concluded that increasing acidity of the extracting water reduced the toxicity of the leachate from poultry litter.

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