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Changes in Poultry Litter Toxicity with Time

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With recent concerns about consumption of red meat and the advancements in poultry breeding, production of poultry (and litter) is increasing nationwide. The shortages and higher costs associated with energy and fertilizers have increased interest in the use of poultry litter as manure. Mean crude protein values of 14, 23 and 30% have been reported for litter on which 1, 2 or 3 successive flocks were raised, respectively (Bandel et al. 1974; Perkins et al. 1964). A ton of dry poultry litter contains about 80 lbs of nitrogen, 50 lbs of phosphate and 40 lbs of potash; samples of poultry litter analyzed by Kunkle et al (1981) also contained 2.4% calcium, 2.65% potassium, 319 mg/kg copper, 35 mg/kg arsenic, 34 mg/kg lead, and smaller amounts of cadmium, mercury and selenium. Composition of the poultry litter varies depending on the number of flocks raised, type of ration fed, the base material used underneath, frequency of cleaning, application of ammonia control and other chemicals and many other factors. The Delmarva Peninsula on the Eastern Shore of Maryland ranks 4th in the nation in poultry and litter production (500 million chickens with an economic value of about $1.2 billion annually). The amount of litter produced is approximately 5.5 tons per 1,000 birds per year. Land application (and or disposal) of this litter may result in pollution of groundwater (Ritter & Chirnside 1984). With rainfall, surface water run-off from land on which poultry litter has been applied (or disposed of) reaches the Chesapeake Bay from this region. The Biochemical Oxygen Demand and the Chemical Oxygen Demand for the poultry litter have been reported to be as high as 24,000 to 255,000 ppm, respectively (Perkins et al. 1964). Leachate from this litter can severely disrupt the aquatic life of any water body and cause fish kills. In addition to these chemicals there

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are micro-organisms (bacteria and viruses) and antibiotics present in the poultry litter.

The die-off rate of fecal organisms from poultry litter was not dependant on soil type or litter application (Crane et al. 1980). Chlorotetracycline, used in poultry feed, was absorbed by soil colloids and/or combined with other organic materials (Warman & Thomas 1981). There is no information in the literature on the toxic effects of these chemical and microbiological pollutants from poultry litter. The study of the toxic effects of a multitude of substances, as present in the poultry litter, is a complex issue; conventional aquatic toxicity tests using fish may require long time periods of up to 4 wk. Recently, the Microtox toxicity system has been used for the determination of aquatic toxicity for a variety of substances (Ribo 1987; Sanchez et al. 1988). This bioassay method uses the marine luminescent bacterium (Photobacterium phosphoreum) and measures the changes in light emission of these bacteria on exposure to a toxic substance. It measures the effective concentration of the toxic substance that results in 20%, 50% or 80% decrease in light output (EC_{20}, EC_{50}, or EC_{80}). The EC_{50} values compare favorably with the traditional LD_{50}; this method is now accepted by EPA (1987) as one of the bioassay techniques for toxicity monitoring. The objective of this study was to measure the changes in EC_{50}, using poultry litter aqueous extracts, with time to simulate varying rainfall durations.

MATERIALS AND METHODS

Litter, from poultry houses, after raising 14 flocks, along with the base materials (saw dust and wood shavings) was air dried, pulverized and sieved (5mm) to remove the larger materials. A small amount (5 g) of the sieved material was equilibrated with 1 L of distilled water in 4 flasks each. The flasks were hand shaken periodically. At the end of 1, 6, 12 or 24 hours the supernatant liquid was centrifuged at 2,500 rpm for 30 min. The centrifuged liquid was then used for Microtox testing as outlined in the manual (Beckman Instruments 1982); data reduction (for EC_{50} values) was carried out using the computerized software supplied by the manufacturer after applying the necessary color correction as recommended. The Microtox reagent (bacteria) was prepared fresh, and used within 1 hr of reconstitution for each experiment, to get the optimum light output in every case. All the experiments were repeated 3 times and mean values were used for

statistical analyses. Experiments were also conducted after subjecting the litter-extract to ultra-violet radiation and boiling. Conductivity, redox potential and pH for all the extracts were also measured. Chemical analyses were done following the standard procedures (APHA 1985).

RESULTS AND DISCUSSION

Results of chemical analyses of the two poultry litter extracts are presented in Table 1; heavy metals (cadmium, lead, arsenic) concentrations were low (< 50 ppb) and as a result trends of changes in concentration with extraction time could not be determined accurately. The total volatile solids - TVS fraction (about 70% of the total dissolved solids - TDS) indicates a high concentration of volatile organic and inorganic materials. Kjeldahl-N, NH_3, and PO_4 increased with extraction time as the extracts became more acidic. The redox potential decreased suggesting a reduction of the oxidation state of metal ions to a lower stable state, which in some cases may result in reduced solubility (Bockris, 1988) and thus toxicity.

Table 1. Changes in chemical composition of poultry litter aqueous extracts with time.

Composition ppm	Sample 1		Sample 2	
	1 hr	24 hr	1 hr	24 hr
TDS	744	878	956	1006
TVS	480	648	534	656
PO_4	37	45	47	55
Kjeldahl-N	90	210	90	130
NH_3	38	39	23	27
pH	8.2	7.0	8.4	6.8
Conductivity (umohs/cm)	600	770	700	850
Redox Pot. mv	150	90	140	70

The average coefficient of variation (CV) for the EC_{50} values within any litter sample used in this study, determined in triplicate, was less than 15%. Higher CVs (18-27%) have been reported for this bioassay method using pure chemicals (Bulich et al. 1984). Samples of poultry litter (from different poultry houses) showed larger CVs because of the varying compositions and thus toxicities. The changes in the toxicity of the poultry litter, given as EC_{50} values, for 5 min and 15 min incubation times are given in Table 2. No significant

changes in pH and conductivity were noticed for the various extracts even after exposure to UV radiation or to boiling. The optimum pH, for highest sensitivity of the bacteria in the Microtox assay, is 6.7 (Qureshi et al. 1984); the pH for the litter extract varied from 6.8 -8.4. Effects of pH on `Microtox' toxicity vary with the toxicant but are not significantly changed in the pH range of 6.8 - 8.4 (Yates & Porter 1984); Qureshi et al. (1984) recommend that the samples be assayed by the microtox test without pH adjustment to maintain their stability and integrity. The conductivity of the litter extracts ranged from 6.0-8.5 x 10^2 µmhos. The amount of the litter (toxicant) required for the EC_{50} values reported here did not show any statistically significant change (except for sample 1, 24 hr extract) with the change in incubation time, from 5 to 15 min; this is in agreement with the observations of Qureshi et al. 1984, who along with others have used the 15 min EC_{50} data for assaying samples containing metal ions.

Table 2. Changes in EC_{50} values of poultry litter aqueous extracts with time.

Time hr	EC_{50} (ml) Sample 1		Sample 2	
	5 min	15 min	5 min	15 min
1	14.04[a]	12.02[a]	4.63[a]	3.21[a]
6	25.72[a]	21.65[a]	3.92[a]	3.93[a]
12	32.94[a]	22.78[a]	4.41[a]	4.79[a]
24	72.20[b]	47.4[b]	31.01[b]	30.90[b]

Each mean represents the average of 3 replicates. Values followed by the same letter are not significantly different from each other at $P < 0.05$ according to the Duncan's Multiple Range and Waller-Duncan K-ratio t-tests.

The amount of the toxicant needed for an EC_{50} value with 5 min incubation time is more than with 15 min incubation time as is normally the case with studies using these bacteria. The toxicity of poultry litter decreased (higher EC_{50} values mean more toxicant is needed) as the time of extraction increased. The toxicity values were significantly different from each other for the 1 hr versus 24 hr extract at $P < 0.05$ according to the Duncan Multiple and Waller-Duncan K-ratio t-test. This change can result from the presence of metals and/or organics (Qureshi et al. 1984). Further, the litter extract being a mixture of many

different chemicals may show a synergistic, antagonistic or a simple additive response with time. Given enough time, the presence of one metal ion species may reduce another metal to a more or less toxic form (Qureshi et al. 1984). Metal ions and organic pollutants are known to exihibit complex interactions in bioassays of this nature; increasing the concentration of one pollutant may change a synergistic response to antagonistic (Babich & Stotzky 1986). Organic compounds (tryptone, yeast, cysteine, humic acid, succinic acid) and inorganic cations (calcium, magnesium) are known to reduce the toxicity of lead. In addition, many organic chemicals, including phenols, show reduced toxicity with time (Beckman 1980). Bulich (1984) also showed a reduction in extractable toxicity using 5 waste-soil mixtures with time; he suggested that the water soluble organics from the waste were amenable to degradation transformation in the soil. This reduction in extractable toxicity (with time) is different from the bioaccumulation of a toxicant by fish during prolonged exposures or the detoxification of the compounds by nitrobacter activity as time progressed (Wang & Reed 1984).

The EC_{50} values of the extracts did not change on subjecting the extracts to UV radiation, proving that microorganisms are not playing a role in the toxicity. The toxicity was significantly reduced, to half of its original value, on boiling the extract for 15 min (and after making up the original volume with distilled water) which can be due to the possible changes in PO_4^{-3} and heavy metal toxicity on boiling (Babich & Stotzky 1986).

From this study it can be concluded that leachate from poultry litter exhibits toxicity to marine organisms but the toxicity decreases with increase in leaching time.

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