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Use of clay mineral (montmorillonite) for reducing poultry litter leachate toxicity (EC_{50})

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Abstract

Poultry litter (PL) has useful nutrients and is therefore used as manure. In addition to N, P and K, PL also contains some heavy metals (As, Cd, Cu, Mn, Pb and Zn), antibiotics, antioxidants, mold inhibitors and other organic compounds. Poultry litter aqueous leachate (PLL) has been shown to be toxic to many organisms; PLL is more toxic than the aqueous leachate of other animal manures used on agricultural soils. Clayey soils are known to retain toxic heavy metals. The objective of this study was to measure the change in toxicity (EC_{50}) of PLL on the addition of clay mineral—montmorillonite. A significant reduction (124%) in toxicity of the clay poultry litter leachate (CLL) after 7 days was observed compared to the toxicity of the PLL alone after 1 day. This indicates that some of the toxic components of the litter were adsorbed by the clay.

Keywords: Poultry litter; Toxicity; Clay mineral; Montmorillonite; EC_{50}

1. Introduction:

More than 8.3 billion broiler chickens (*Gallus gallus domesticus*) were raised in the United States in 2001; Maryland currently ranks seventh in poultry production in the nation [1]. Poultry production results in significant manure generation. Broiler chickens are typically raised for 7 weeks, and 12.16 kg of manure per bird is produced during this period [2]. Nearly 800,000 tons of poultry litter (PL) is generated annually on the Eastern Shore of MD [3]. Currently the most cost-effective disposal method for PL is as an organic fertilizer. PL is a mixture of manure, bedding material, wasted feed, and feathers. PL used on agricultural fields improves soil fertility and aeration, and increases water-holding capacity. PL consists of approximately 4.1% N, 1.4% P, and 2.1% K [4]; PL also contains 37, 20, 390, 655, 35, and 377 ppm of As, Cd, Cu, Mn, Pb and Zn, respectively [5]. Copper and iron are added to poultry feed to prevent anemia; selenium is added to prevent oxidative damage to cells; and zinc and manganese are added to ensure correct eggshell deposition and feather growth [6]. Arsenic compounds such as roxarsone (3-nitro-4-hydroxyphenylarsonic acid) are used in poultry production to control coccidial intestinal parasites [7]. The small amounts of these heavy metals used in poultry feed are nontoxic to poultry, and are generally excreted unchanged from the birds.

Other chemical compounds present in PL include xanthophylls, antibiotics, antiprotozoals, antioxidants, mold inhibitors, probiotics, polychlorinated phenols, tetrachlorodibenzo-*p*-dioxin and hormones [8–11]. The risk associated with the use of PL as a fertilizer depends in part on soils' ability to adsorb the added metals and other toxicants [5]. Soils on the Eastern Shore of MD are generally coarse; therefore, groundwater supplies are potentially at risk from soluble contaminants in the litter. With intense rainfall, runoff waters from poultry-amended soils may reach the Chesapeake Bay and result in disruption of aquatic life [12].

Sources of toxicity within PL include metabolic waste, topical pesticides, feed additives, and bedding material contaminants [2]. Poultry litter aqueous leachate (PLL) toxicity is attributed to ammonia and heavy metals; PLL has been shown to be more toxic than the leachate of other animal manures using marine luminescent organisms (*Photobacterium phosphoreum*) [13]. Soils containing clay minerals

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(illite, montmorillonite, or vermiculite) can fix appreciable quantities of NH_4^+ and heavy metals; the surface charge of clay minerals can be utilized in the adsorption/absorption of pollutants [14]. The objective of this study was to measure the change in toxicity (EC_{50}) of PLL on addition of clay mineral—montmorillonite.

2. Experimental methods

Poultry litter (approximately 4 kg) was collected randomly from the University of Maryland Eastern Shore poultry house in plastic bags. The sample was air-dried, homogenized and sieved (2 mm). For PLL preparation, PL (1 g) was mixed with montmorillonite (0.0, 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 g) and deionized water (100 ml). Na-montmorillonite clay was used in this study (Clay Minerals Repository, Department of Geology, University of Missouri, Columbia, MO). The mixtures were agitated on a rotary shaker at 200 rpm. At the end of 1 or 7 days, the mixtures were centrifuged (30 min at 3000 rpm) and the supernatant was analyzed for toxicity immediately or refrigerated at 4 °C.

Toxicity (EC_{50}) analysis of the supernatant was done using the Microtox Analyzer 500 following Microtox Acute Toxicity basic test procedures [2,15]; higher EC_{50} values indicate reduction in toxicity. Data reduction was carried out using computer software supplied by the manufacturer. Analyses were performed in triplicate and mean values were used for statistical analysis (LSD).

3. Results and discussion

No change in PLL's conductivity, salinity, total dissolved solids or dissolved oxygen concentration was observed on mixing with montmorillonite for 1 or 7 days A very small increase (7.4–7.5 after 1 day, and 8.5–8.6 after 7 days) in the pH of the PLL was recorded on mixing with the clay mineral (Na-montmorillonite).

The toxicity (EC_{50}) of PLL was found to be 2.67 g/L (Table 1). The toxicity of PLL has been reported (EC_{50} or $LC_{50} = 2.9$ g/L) earlier using both "Microtox" and *Ceriodaphnia* assays [13]. The small increase in toxicity (in the present study) can be attributed to change in composition of the feed and the number of flocks raised per batch of litter [16]; microorganisms are not known to contribute to PLL toxicity [17]. Effects of pH on "Microtox" toxicity vary but are not significantly changed in the pH range of 6.8–8.6 [18]. Clay mineral—montmorillonite (0.05–0.5 g) in aqueous (100 ml) leachate did not show any toxicity.

On the addition of montmorillonite (day 1), a reduction in the toxicity of PLL (Table 1) was observed; the amount of reduction was directly proportional to the amount of the added clay mineral (Fig. 1). A significant reduction (day 1) in toxicity was observed with all the clay concentrations. Clay minerals are aluminum silicate sheets with particle size <2 μm diameter. The relatively small particle size and sheet-like structure give clay minerals a very large surface area. Clay minerals possess a negatively charged surface caused by a combination of broken bonds, surface-growth defects and cation substitutions within the lattice [14]. This negative charge can result in an ion fixation process where toxic components such as NH_4^+ and/or heavy metal ions from PL may substitute for interlayer cations (Ca^{2+}, Mg^{2+}, Na^+, and H^+; mostly Na^+ in this case) in the expanded lattice of clay minerals [14,19,20], thus reducing the toxicity of PLL. An increase in pH (as indicated above) can also reduce the solubility of the heavy metals thus further reducing the toxicity.

Table 1
Changes in toxicity of poultry litter with different concentrations of clay after 1 and 7 days mixing

Clay Concn. (g)	Day 1 EC_{50} (g/L)	Day 7 EC_{50} (g/L)
0.0	2.67 ± 0.11 a	4.23 ± 0.18 a
0.05	3.05 ± 0.11 b	4.59 ± 0.09 b
0.1	3.50 ± 0.15 c	5.02 ± 0.07 c
0.2	3.90 ± 0.05 d	5.45 ± 0.17 d
0.3	4.30 ± 0.09 e	5.61 ± 0.06 d
0.4	5.26 ± 0.03 f	5.84 ± 0.01 e
0.5	5.58 ± 0.04 g	5.99 ± 0.05 e

Values after ± denote the S.D. of three replicates. Values followed by the same letter are not significantly different from each other ($P < 0.05$) according to LSD mean comparison test. LSD values for 1- and 7-days means equal 0.17 and 0.19, respectively.

The toxicity of the PLL decreased from 1 to 7 days by 58% (Table 1). Toxicity reduction of PLL with time has also been reported earlier [17]; phenol also shows reduction in toxicity with time [21] as some organic compounds are amenable to degradation transformation with time. After 7 days, the toxicity of the CLL was further reduced compared with 1-day toxicity data. Again, a linear relationship between clay concentration and reduction in toxicity was observed (Fig. 1). Increased mixing time could increasingly exchange the toxic components of the PL with Na^+, and the release

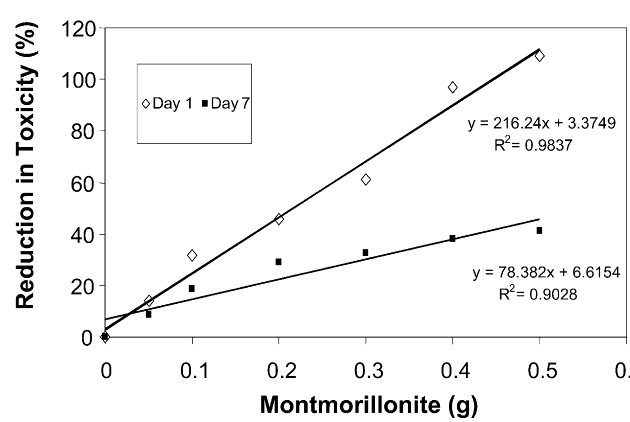

Fig. 1. Reduction in toxicity of poultry litter with montmorillonite after 1 or 7 days mixing.

of the alkaline metal from the clay may reduce the toxicity [12], and increase the pH as noted above. The reduction in toxicity of the PL mixed with 0.5 g clay after 7 days compared to the toxicity of the PLL alone after 1 day was 124%. This indicates that some of the toxic components of the litter were adsorbed by the clay.

4. Conclusions

Clayey soils are known to retain toxic heavy metals [22]; in situ immobilization has the potential to remove metal ions from solution and/or to stabilize metals in soils and clays [23]. The results of this study suggest that the toxic effects of poultry litter may be reduced in agricultural soils by the addition of montmorillonite clay. The clay added to the litter spread on coarse soils may also prove beneficial in other ways [20].

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