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Fate and Transport of Salinomycin Sodium in Sandy soil

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Salinomycin sodium (BIO COX) is a polyether ionophore, commonly used in the poultry industries for the prevention of coccidial infections and promotion of growth. Salinomycin sodium (SAL-Na) is very toxic, and may be fatal, if swallowed, inhaled or absorbed through the skin than many other antibiotics, thus evaluating its fate in the soil environment is of importance. Mobility of SAL-Na was measured in sandy soil. Soil column leaching experiments indicated that the strongly sorbed SAL-Na was not detected in the leachate of sandy soils, indicating that the amount added to each column was not leached off the soil fractions. When compared to the sterile soil, non sterile soil has more movement of SAL-Na. Leachate collected from the soil column (75% hydraulic conductivity) passed with phosphate buffer showed higher concentration (0.48 mg/L) of SAL-Na and the movement was also observed higher in non sterile soil. About 35% of SAL-Na was found in leachate of sandy soil.

Key words: SAL-Na, Soil, Mobility, Environmental consequences

Salinomycin is a naturally occurring mono carboxilic polyether antibiotic produced by a strain of *Streptomyces abbes* (ATCC – 21838). The antibiotic, SAL-Na tested in the present experiment has been extensively used in poultry industries to prevent coccidiosis (Gaskins *et al*., 2002; Lefebvre *et al*., 2006). Salinomycin is also known to increase the rate of weight gain, thus enhancing productivity (Khan *et al*., 2008). The highest usage in USA is estimated to be 454 t of salinomycin active ingredient. BIO-COX-120G containing 12% SAL-Na as active substance is effective as coccidiostat for chickens to enhance fattening at a dose range of 50-70mg SAL-Na/kg of complete feed.(European Food Safety Authority (EFSA), 2005). Agriculturally derived antibiotics have also been identified in surface water in Colorado, USA. (Yang and Carlson., 2003; Cha *et al*., 2005). A US Geological Survey study on the occurrence of pharmaceuticals in surface waters identified a number of antibiotics that are not used for human therapy in the US (Kolpin *et al*., 2002). Antibiotics have been detected worldwide in soils, surface water, ground water and sediments (Kolpin *et al*., 2002; Kim and Carlson, 2006). The relatively high frequency of detection of these agricultural antibiotics may be indicative of the potential for surface water contamination by these chemicals.

The persistence of antibiotics in the terrestrial environment ranges from less than one day to weeks or even months depending primarily on temperature and the chemical structure of the antibiotic (Rabolle *et al*., 2000). Depending on the rate of degradation and the sorptive properties, the parent substance or its metabolites may reach the aquatic environment through surface run-off or leaching through the soil profile. Key chemical properties such as water solubility, soil pH, volatility and sorption influence the antibiotic transport in soils.

Antibiotic compounds used for the veterinary purpose have the potential to leach through soil or with surface run-off during rain events and contaminate local ground water and surface waters. For example, multiple classes of antibacterial compounds have been reported in surface and groundwater samples collected proximal to pig and poultry farms in the USA (Campagnolo *et al*., 2002). There is limited information available on the fate and mobility of SAL-Na in the soil environment. (Robolle and Spliid, 2000). Laboratory soil columns offer the potential for more replication than field and semi field studies as it is easier to collect and manage multiple small cores. Hence this study aims to investigate the fate and transport of SAL-Na in sandy and loamy sand soil through soil column experiments.

Material and Methods

Analytical reagent grade or high purity chemicals were used. Sodium phosphate (Mano and di basic), methanol, Ammonium hydroxide and Acetic acid were purchased from Sigma. The salinomycin sodium was extracted from commercial feed material. The selected properties of SAL-Na is given in Table 1. The stock solution of salinomycin (pure from Sigma Aldrich) was prepared by dissolving 10 mg salinomycin in 10mL of methanol (MeOH) and stored at 4°C. Standard solutions were freshly prepared by diluting the stock solution with methanol in vial. These standards were used for preparation of calibration curves and cross checked with commercial SAL-Na.

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Extraction of SAL-Na

About 50g each of the feed material was weighed into 250mL (4) conical flask and100 mL of methanol was added into each flask. The mixture was shaked for 24h in a mechanical shaker. The methanol was filtered through a What man no.1 filter and allowed to overnight for evaporation. Dried SAL-Na was dissolved in known (100mL) methanol and further diluted to check the final concentration of SAL-Na using HPLC-CAD.

Soil characterization

Macdonald campus agricultural sandy soils (upland) no history of exposure to salinomycin were used in the experiment. The soil samples were allowed to air dry, passed through 2-mm sieve and stored in plastic containers at room temperature for further analysis. Physical and chemical properties of soils were determined using standard methods. pH using a 2:5 soil: water slurry (Trivedy and Goel, 1986), organic carbon by wet digestion with $K_2Cr_2O_7$ and H_2SO_4 (Walkey and Black, 1934), texture (Bouyoucos, 1936) and Cation Exchange Capacity (CEC) by AAS (Atomic Adsorption Spectrometer). Sandy soil with a bulk density of 1.33 g/cc and organic carbon (3.9%) is used for assessing the fate and transport of salinomycin sodium.

Mobility study-flow rate

Prior to experiment, all columns were wet from the bottom up by capillary rise with raising water level gradually. Once saturation had been reached, the water content of each column was measured. Solute contents were then stabilized by flushing deionized water downwards through the column. Hydraulic conductivities were calculated by Q=KiA (K=Q/iA, A=area, i=length). The saturated hydraulic conductivity of sandy soils was, 251cm/day. The flow rates were fixed based on 10, 25 and 75% of hydraulic conductivity. The pore volumes (Blake and Hartge, 1986) of sandy was calculated from the bulk density (Table 2), particle density (2.65 mg/m^3)) of the soil and volume (49.95cc) of the column. Five pore volumes (124mL for sandy soil) were collected at 10 and 25% of hydraulic conductivity and 25 pore volumes were collected at 75% hydraulic conductivity with deionized water and phosphate buffer 0.1M (pH7)

Soil column

Sandy soils were used to prepare the soil columns for the column leaching experiments. Air dried soil was manually packed into a plastic column measuring 12 x 2.6 cm (inside diameter) at the rate of three columns for sterile and non sterile soils. Glass wool was placed in the bottom of the columns to prevent leaching of soil particles and the columns were packed to a height of approximately 9 cm with soil. The columns were pre wet with 120 mL of deionized water and equilibrated for 24 h before application of the antibiotic. SAL-Na, 4mg/L solution was applied to the tube, fixed above the column and allowed to pass through the column at flow rates of sandy soil, 10% (21.4h), 25%(4.2h) and 75%(2.86h) hydraulic conductivity. The eluted liquid was collected at each 2.5 pore volumes and analyzed for SAL-Na by HPLC-CAD a newer detector. The column was split into 3 layers top (0-3cm), middle (3-6cm) and bottom (6-9cm). The soil was gently removed from the columns for extraction and SAL-Na analysis. To check the movement, at 75% hydraulic conductivity, phosphate buffer (pH) was also passed through the column and the leachate was analyzed for SAL-Na and the soil was also extracted and analyzed for the same.

Soil extraction

For determination of the SAL-Na residues retained in the soil column, the soil fractions were extracted with phosphate buffer (pH7). To each 3cm fraction of the column depth, 50 mL of phosphate buffer was added in a 150 mL conical flask and the mixture was agitated for 12 h in a rotary shaker. Soil particulates were removed by centrifugation at 3500 rpm for 30 min. Five µL of supernatant was transferred to amber glass vial and diluted with an equivalent amount of phosphate buffer before analysis.

Analysis of salinomycin in supernatant and soil

The filtered supernatants were analyzed for salinomycin using HPLC CAD (Charged Aerosol Detector), equipped with C18 column and an elution gradient with methanol (80%), water (13%), ammonium hydroxide and acetic acid buffer (7%) (pH 5) and flow rate of 1mL/min was used. Method detection limits, SAL-Na concentration, using HPLC-CAD was determined by preparing the calibration curves. Two way ANOVA and multiple comparison (LSD) was done using MATLAB version 7.8

Results and Discussion

The more strongly sorbed SAL-Na was not detected in the leachate of many of the soil columns ran at 10 and 25% hydraulic conductivity. The first (2.5 pore volume) showed lower (0.5 µg/L and 0.45 µg/L in sterile sandy soil) and non sterile showed slightly higher (0.75 µg/L) concentration at 25% hydraulic conductivity and not detected at 10% hydraulic conductivity (Table 2 and Fig.1). At 75% hydraulic conductivity, sterile sandy soil column passed with water showed lower (0.93 mg/kg) concentration at top (0-3cm) and higher concentration at bottom (6-9cm) (1.57 mg/kg), but non sterile soil showed maximum concentration at top (0-3cm) (1.5 mg/kg) (Fig. 2). These results showed, after continuous dilution, indicates that there may be chances of movement in sterile soil. The sandy soil column passed with buffer at 75% hydraulic conductivity.

Table 1. Characteristics of salinomycin sodium

Salinomycin	Na Salt
Molecular Formula	$C_{42}H_{69}O_{11}$ Na
Molecular Weight	772
Melting Point (C)	140 – 142
p_{Ka}	6.4
Water Solubility	3.4 mg/mL readily soluble in methanol
Stability	Unstable in acidic condition stable in alkali condition

Table 2. SAL-Na concentration in leachate collected at different pore volumes

Sandy soil (sterile) / pore volume	1	5	10	15	20	25
Leachate with water(75% HC)	0.12µg/L	ND	ND	ND	ND	ND
Leachate with buffer(75% HC)	ND	0.18ng/L	0.28mg/L	0.48mg/L	0.34mg/L	0.13mg/L
Leachate with water (10% HC)	0.5µg/L	ND	ND	ND	ND	ND
Leachate with water(25% HC)	0.45 µg/L	ND	ND	ND	ND	ND
Sandy soil (Non sterile) / Pore volumes	1	5	10	15	20	25
Leachate with water (75% HC)	0.35µg/L	ND	ND	ND	ND	ND
Leachate with buffer(75% HC)	ND	0.05ng/L	0.40mg/L	0.32mg/L	0.28mg/L	0.1mg/L
Leachate with water(10% HC)	ND	ND	ND	ND	ND	ND
Leachate with water(25% HC)	0.75µg/L	ND	ND	ND	ND	ND

Note: Values are average of three replications, ND-not determined: HC-Hydraulic conductivity

showed lesser concentration (0.48 mg/kg) in top soil (0-3cm) than bottom (6-9cm) soil. Non sterile soil had

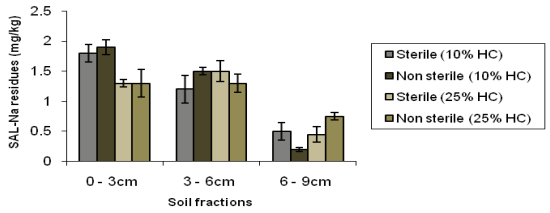

Fig.1. Distribution of SAL-Na residues in sandy soil column

higher concentration at the top (0-3cm) (0.72 mg/kg) than the top sterile soil. The bottom layers of both sterile and non sterile soils had higher concentrations. (Fig.2).

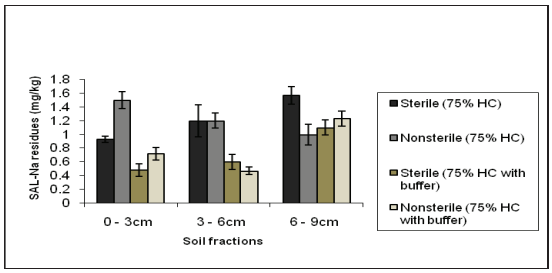

Fig.2 Distribution of SAL-Na in sandy soil column at 75% hydraulic conductivity

At 75%hydraulic conductivity (buffer), top (0-3cm) and middle (3-6cm) showed significant ($P<0.05$) difference with soils passed with water and no significant difference observed in bottom (6-9cm) layers. This indicated that the amount added to each column was left in the soil fractions (Fig 1 and 2) at 10, 25 and 75% of hydraulic conductivity.

Conclusion

This study concludes the sorption, desorption behaviour of the SAL-Na and the potential for the compound to move to surface and groundwater. Although SAL-Na is strongly, sorbed to soil, if contaminated water containing phosphates reach the site, SAL-Na could move to aquifers and water boides.

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