Column study to assess the mobility of Salinomycin in soil environment

ABSTRACT

Aim: Salinomycin sodium ($C_{42}H_{69}NaO_{11}$) is a polyether ionophore commonly used in poultry industries to prevent coccidial infections and promote growth.Salinomycin sodium (SAL-Na) has high toxicity and possesses the potential to induce fatality upon ingestion, inhalation, or dermal absorption;thus, it is crucial to evaluate its fate in the soil environment.The column studywas conducted at laboratory condition to examine the behavior of the Salinomycin sodium and their mobility potential to move to the surface and groundwatersin soils with sandy and loamy sand textures.

Sample location: Agricultural soils with no previous history of exposure to salinomycin were collected from the Macdonald Campus Farm of McGill University in Ste-Anne de Bellevue, Quebec, Canada. In the current study, two types of soils are assessed,i.e.,HOM-sand and LOM-loamy sand. HOM-sand soil was a Dalhousie sandy soil with high organic matter (HOM-sand).

Results: Soil column leaching experiments indicated that the SAL-Na was undetected in sandy and loamy sand soil leachate. This indicates that the amount added to each column was not leached off the soil fractions as it is strongly sorbed. Compared to sterile soil, nonsterile soil has more movement of SAL-Na. The leachate obtained from the soil column, which had a hydraulic conductivity of 75%, exhibited a greater concentration (0.48 mg/L) of SAL-Na when passed through a phosphate buffer. Additionally, the mobility of SAL-Na was shown to be higher in the nonsterile soil.About 35% of SAL-Na was found in leachate of sandy soil and 20% in loamy sand soils.

Key Words:SAL-Na;column study;mobility; soil contamination

1. INTRODUCTION

Veterinary medicines are given to livestock to treat disease and protect their health.After the application of the drug, these substances may be metabolized, and a mixture of the parent compound and metabolites will then be excreted in the urine and feces(Keenum *et al*.,2022) [14].Release of veterinary medicines to the environment occurs directly, through the treatment of animals on pasture and indirectly, *via* the application of treated animals' manure to land.

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Some antibiotic compounds have the potential to leach through soil or with surface runoff during rain events and contaminate local groundwater 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*.,2012) [2].Agriculturally derived antibiotics have also been identified in surface water in Colorado, USA. (Yang and Carlson, 2003[25]; Cha *et al*., 2005[4]; Keenum *et al*., 2022)[14].A US Geological Survey study on the occurrence of pharmaceuticals in surface waters identified several antibiotics that are not used for human therapy in the US (Kolpin *et al*.,2002) [17].Antibiotics have been detected worldwide in soils, surface water, groundwater, and sediments (Kim and Carlson, 2006[16]; Rabolleand Spliid,2000) [20]. The detection of these antibiotics in soil may have the potential to contaminate the surface water.

The duration of antibiotic persistence in the terrestrial environment varies, spanning from less than a day to many weeks or even months. This variability is largely influenced by factors such as temperature and the chemical composition of the antibiotic (Gaskins *et al*.,2002) [10].Depending on the degradation rate and the sorptive properties, the parent substance or its metabolites may reach the aquatic environment through surface runoff or leaching through the soil profile.Key chemical properties such as water solubility, soil pH, volatility, and sorption influence soil antibiotic transport.

The antibiotic SAL-Na tested in the present experiment has been extensively used in poultry industries to prevent coccidiosis(Lefebvre *et al*.,2006) [19].Salinomycin is also known to increase the rate of weight gain, thus enhancing productivity (Khan *et al*., 2008)[15]. Salinomycin is a naturally occurring monocarboxylic polyether antibiotic produced by a strain of *Streptomyces abbes* (ATCC – 21838). The 454 tons of salinomycin active ingredientis used in the USA.BIO-COX-120G containing 12% SAL-Na as an 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 2004) [9].Salinomycin is also approved for use as a cattle feed additive (BIO GRO) in the range of 5-10 g/ton of complete feed.A radio-labeled (C^{14}) study reported the SAL-Na persistence in the food chain organisms with moderately low biodegradability (European Food Safety Authority 2005) [8].SAL-Na is resistant to aerobic degradation (Khan *et al*., 2008) [15].

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The polyether ionophores are toxic to many bacteria, protozoa, fungi, and higher organisms. their three-dimensional confirmation creates a highly hydrophobic exterior and hydrophilic interior, enabling the binding of one or more cations.The lipophilic nature allows ready penetration of cell membranes, enabling uncontrolled influx and or efflux of selected ions, such as potassium and sodium, from the cell.This osmotic interference often leads to cell death(European Food Safety Authority 2005) [8].Antibiotics present in soil cause a reduction in microbial biodiversity and potentially influence the growth and enzyme activity of existing bacterial communities via biomass production and nutrient transfer (Grenni*et al*.,2018) [11]. Factors that may affect degradation for tylosin include organic matter content, pH, moisture, temperature, oxygen status, and soil texture (Cycoń*et al*., 2019[6]; Christian *et al*. 2003) [5]. Antibiotics are widely used in veterinary medicine. In animals, they are metabolized and partially excreted unchanged, which leads to their release into the environment and bioaccumulation in animal products. Human consumption of products containing antibiotics poses a threat to health and causes the development of antibiotic resistance. (Lavrukhinaa*et al*.,2022) [18].

There is limited information available on the fate and mobility of SAL-Na in the soil environment(Graber *et al*., 1990[12]; Yeager and Halley, 1990[26]; Sadeghi *et al*., 2000[21]).Laboratory soil columns have the opportunity for increased replication compared to field and semi-field research because to the ease of collecting and managing several tiny cores. Additionally, manipulating tiny soil cores allows for simpler investigation of certain processes. Soil column leaching experiments are often used as a laboratory-scale technique to evaluate the environmental behavior of substances. This work aimed to examine the sorption, desorption, and mobility characteristics of Salinomycin sodium in various agricultural soils.

2. MATERIALS AND METHODS

2.1 Chemicals

Analytical reagent grade, sodium phosphate (Mono and dibasic), methanol, ammonium hydroxide, acetic acid, and salinomycin (pure) were purchased from Sigma. The stock solution of salinomycin (pure from Sigma Aldrich) was prepared by dissolving 10 mg salinomycin in 10mL of methanol (CH_3OH) and stored at 4°C.Standard solutions were freshly prepared by diluting the stock with methanol in the vial. These standards were used for the preparation of

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calibration curves. The Salinomycin sodiumwas extracted from the commercial feed material. The structure of SAL-Na is shown in Figure 1, and selected properties are given in Table 1.

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

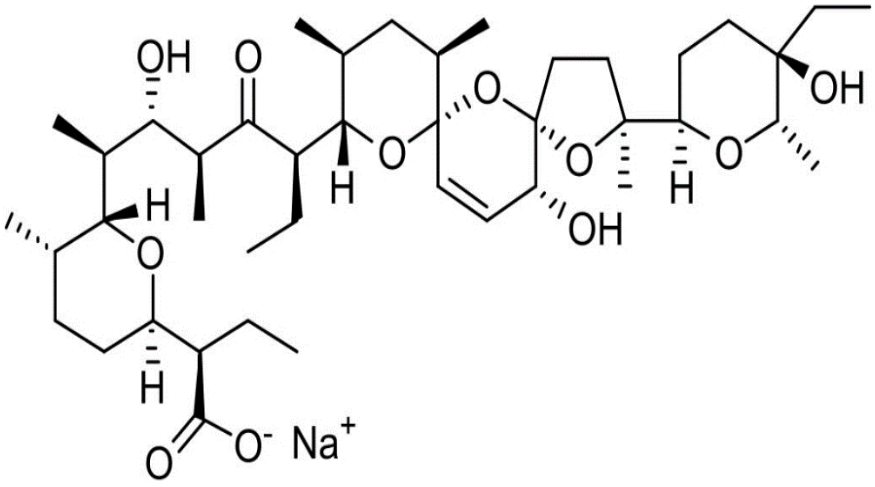

Fig. 1 Chemical structure of SAL-Na

2.2 Extraction of SAL-Na

Fifty grams of feed material was weighed into a 250mL conical flask, and100 mL of methanol was added into each flask.The mixture was mixed well for 24 hours in a mechanical shaker. The methanol was filtered through a Whatman no.1 filter and evaporated overnight. Dried SAL-Na was dissolved in known (100mL) methanol and further diluted to check the final concentration of SAL-Na in HPLC-CAD (Charged Aerosol Detector).

2.3 Soil Characterization

Agricultural soils with no previous history of exposure to salinomycin were collected from the Macdonald Campus Farm of McGill University in Ste-Anne de Bellevue, Quebec, Canada. In the current study, two types of soils are assessed,i.e.,HOM-sand and LOM-loamy

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sand. HOM-sand soil was a Dalhousie sandy soil with high organic matter (HOM-sand). Dalhousie soil was developed from lacustrine material deposited as a thick covering. It is slightly alkaline. The LOM-loamy sandsoil was Chicot loamy sand, having low organic matter (LOM-loamy sand). This soil is formed from thin alluvial material. All the soils were air-dried, passed through a 2-mm sieve, and stored in polyethylene containers at room temperature for further analysis. Physical and chemical properties of the soils were determined using standard methods: pH in a 2:5 soil:water slurry (Jackson, 1973) [13], organic carbon by wet digestion with $K_2Cr_2O_7$ and H_2SO_4(Walkley and Black,1934) [24],texture (by hydrometer method), and Cation Exchange Capacity (CEC) by Atomic Adsorption Spectrometer. The various soil characteristics are listed in Table 2 and 3.

Table 2: Physio-chemical characteristics of soil

Soil type	**OC (%)**	**TOC (%)**	**OM (%)**	**Bulk density (g/cc)**	**pH**	**Sand (%)**	**Silt (%)**	**Clay (%)**
Loamy sand	3.7	4.93	8.5	1.23	6.8	77.5	17.5	5
Sand	3.9	5.2	8.36	1.33	6.7	92.5	2.5	5

Table 3: Chemical characteristics of soil in cmol (+)/kg

Soil Type	**Ca**	**Mg**	**K**	**Na**	**Mn**	**Fe**	**CEC**
Loamy sand	7.02	1.15	0.27	0.15	0.03	0.02	8.64
Sandy	2.426	0.07	0.053	0.133	0.01	0.02	2.686

2.4 pH adjustment

The initial pH of clay, loamy sand, and sandy soils was 6.7, 6.8, and 6.9.All three soils were equilibrated with 1M concentrations of HCL and NaOH.Adjustment of the desired pH (4 and 9) was made eight times over 20 days till the pH of the soil was stabilized.Excess liquid from each soil sample was drained after pH adjustment, and soils were allowed to air dry for 48h. The soils were pulverized, rechecked for pH, and used for sorption studies(Sassman and Lee, 2007) [22].

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2.5 MobilityStudy-Flow rate

Beforethe experiment, all columns were wetted from the bottom by capillary rise witha rising water level gradually.Once saturation had been reached, the water content of each column was measured.Solute contents were stabilised by implementing a downward flushing technique utilizing deionized water within the column. Hydraulic conductivities were calculated by Q=KiA (K=Q/iA, A=area, i=length).The saturated hydraulic conductivity of sandy and loamy sand soils was 251cm/day and 82.6cm/day. The flow rates were fixed based on 10%, 25%, and 75% of hydraulic conductivity.The pore volumes (Volmer and Lock,1998) [23] of sandy and loamy sand soils were 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 and 134 mL for loamy sand 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).

2.6 Soil Column

Sandy and loamy sand 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), with three columns for sterile and nonsterile 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 9cm with soil.The columns were pre-wetted with 120 mL of deionized water and equilibrated for 24 h before application of the antibiotic.SAL-Na,4 mg/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 and loamy sand soil, 10%(68h), 25%(27.4h) and 75%(9.14h).The eluted liquid was collected at each 2.5 pore volume and analyzed for SAL-Na by HPLC-CAD, a newer detector.The column was split into three 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,leachate was analyzed for SAL-Na, and the soil wasextracted and analyzed for the same.

2.7 Soil Extraction

The soil fractions were extracted with phosphate buffer (pH7) to determine the SAL-Na residues retained in the soil column.To each 3cm fraction of the column depth, 50 mL of

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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 an amber glass vial and diluted with an equivalent amount of phosphate buffer before analysis.

2.8 Analysis of SAL-Na in supernatant

The filtered supernatants were analyzed for salinomycin using HPLC CAD (Charged Aerosol Detector) equipped with a C18 column. An elution gradient with methanol (80%), water (13%), ammonium hydroxide, and acetic acid buffer (7%) (pH 5), and a flow rate of 1mL/min was followed. Salinomycin concentration was determined using calibration curves.

2.9 S**tatistical analysis**

Results obtained in this study were analyzed by two-way ANOVA and Multiple comparison tests(least significant difference) using MATLAB version 7.8.

3. RESULTS AND DISCUSSION

3.1 Mobility of SAL-Na in soil columns

The leachate of many soil columns, operating at hydraulic conductivities of 10% and 25% (Table 4), did not exhibit detectable levels of the more strongly sorbed SAL-Na. The initial pore volume, namely 2.5 pore volume, exhibited decreased concentrations of 0.5µg/L and 0.45µg/L in sterile sandy soil. Conversely, nonsterile sandy soil had slightly elevated concentrations of 0.75µg/L at 25% hydraulic conductivity, while no detectable concentrations were seen at 10% hydraulic conductivity. This finding suggests that the quantity introduced into each column remained within the soil fractions (Fig.2) for 10% and 25% hydraulic conductivities.

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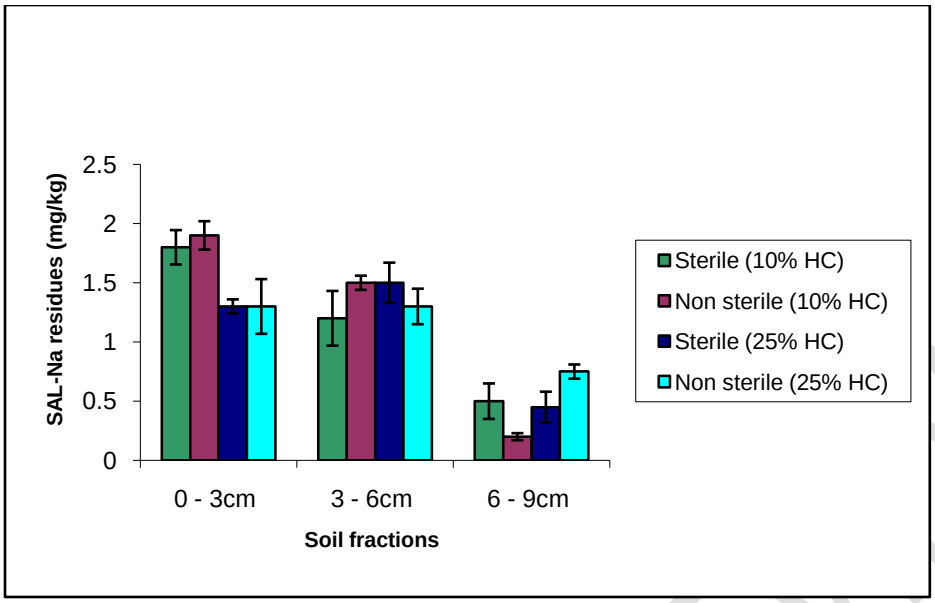

Fig. 2 Distribution of Salinomycin Sodium residues in sandy soil column

Leachate collected from sandy soil columns passed with water at 75% hydraulic conductivity showed very low concentrations of salinomycin (0.12µg/L in sterile soil and 0.35µg/L in nonsterile soil). Compared to sterile soil, SAL-Na mobility was high in nonsterile soil, but SAL-Na was not detected in leachate from 5 to 25 pore volumes. This shows that SAL-Na has less mobility in pure deionized water.The concentration of salinomycin in leachate collected from the sandy soil column passed with phosphate buffer at 75% hydraulic conductivity wasnegligible (0.18ng/L from sterile soil and 0.05ng/L from nonsterile soil) at five pore volumes.But from 10 pore volumes SAL-Na,the leachate hadhigher amounts(0.28mg/L in sterile and 0.40mg/L in nonsterile soil).When compared to sterile,nonsterile had maximum (0.40mg/L) concentration and movement at ten pore volumes, and from 15-25 pore volumes, sterile soil (0.48mg/L) had more mobility than nonsterile soil (0.32mg/L).At 25 pore volume again, the concentration declined to 0.13mg/L insterile and 0.10mg/L in nonsterile soil (Table 4).

Table 4:SAL-Na concentration in leachate collected at different pore volumes

Loamy sand (sterile)	**Pore volumes**					
	1	5	10	15	20	25
Leachate with water(75%HC)	0.23ng/L	nd	nd	0.1ng/L	nd	nd
Leachate with buffer(75%HC)	nd	0.3ng/L	0.029mg/L	0.32mg/L	0.2mg/L	0.2mg/L
Leachate with water(10%HC)	0.001ng/L	nd	nd	nd	nd	nd
Leachate with water(25%HC)	0.9ng/L	nd	nd	nd	nd	nd

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Non-sterile						
Leachate with water(75%HC)	nd	nd	nd	nd	nd	nd
Leachate with buffer (75%HC)	nd	0.45ng/L	0.033mg/L	0.47mg/L	0.8mg/L	0.3mg/L
Leachate with water(10%HC)	0.01ng/L	nd	nd	nd	nd	nd
Leachate with water(25%HC)	0.3ng/L	nd	nd	nd	nd	nd
Sandy soil (Sterile)						
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
Non-sterile						
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 triplicates, nd-not detectable

The amount of Sal-Na in sandy soil fractions was presented in Figures 2and 3.The soil had more concentration than leachate because SAL-Na was tightly sorbed to the soil.At 10% hydraulic conductivity, both sterile and nonsterile soils have more concentrations at the top (0-3 cm) (1.8 to 1.9 mg/kg), and the bottom (6-9cm) soil has lower concentrations, 0.5mg/kg in sterile and 0.2 mg/kg in nonsterile soil (Fig.2). At 25% hydraulic conductivity, the top sterile soil (0-3cm)had a lower concentration (1.3 mg/kg) than the middle (3-6cm) (1.5 mg/kg), and again bottom soil (6-9cm) showedthe minimum concentration(0.45 mg/kg to 0.75mg/kg) for sterile and nonsterile soil respectively. There was a significant ($P<0.05$) difference observed between 10% and 25% hydraulic conductivity in the top (0-3cm) soils, and no significant difference was observed in other middle, bottomlayers, sterile, and nonsterile soils.

At 75% hydraulic conductivity, the sterile sandy soil column passed with water showeda lower (0.93 mg/kg) concentration at the top (0-3cm) and a higher concentration at the bottom (6-9cm) (1.57 mg/kg), but nonsterile soil showed maximum concentration at the top (0-3cm) (1.5 mg/kg)(Fig.3).These results showed after continuous dilution, there may be chances of

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movement in sterile soil. The sandy soil column passed with buffer at 75% hydraulic conductivity showed a lesser concentration (0.48 mg/kg) in the topsoil (0-3cm) than in the bottom (6-9cm) soil.The nonsterile soil had a greater concentration in the uppermost layer (0-3cm) with a value of 0.72 mg/kg compared to the sterile soil at the same depth. The lower layers of both sterile and nonsterile soils had greater concentrations (Fig.3). At a hydraulic conductivity of 75% (buffer), statistically significant variations ($P<0.05$) were seen between the top (0-3cm) and middle (3-6cm) layers when comparing soils that had been passed with water. However, no significant difference was found in the bottom (6-9cm) layers.

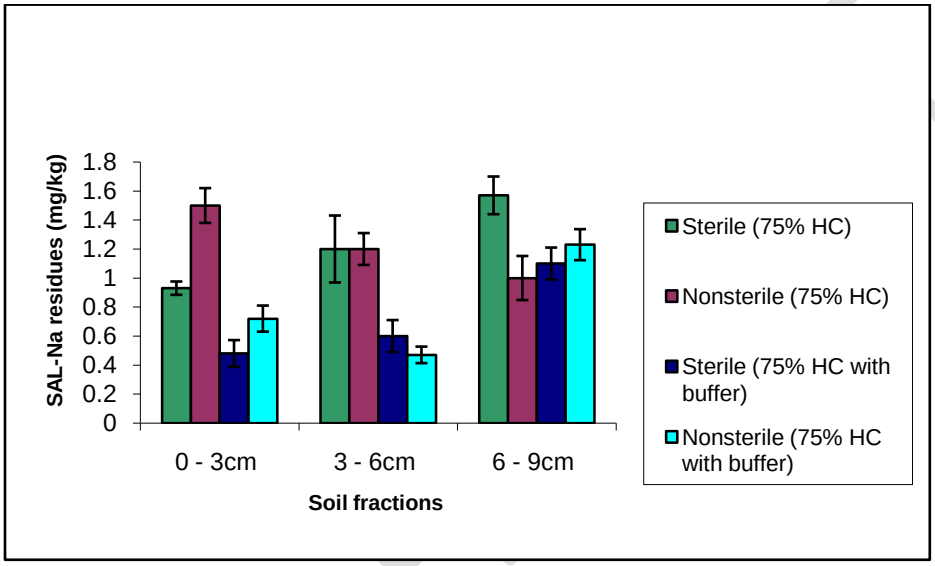

Fig. 3 Distribution of Salinomycin Sodium residues in sandy soil column at 75% hydraulic conductivity

The concentration of leachate collected from loamy sand soil at 10% hydraulic conductivity was negligible (0.001ng/L) in sterile and nonsterile soil.At 25% hydraulic conductivity,the soil had a leachate concentration of 0.9ng/L in sterile soil and 0.3ng/L in nonsterile soil.Aslight increase in movement was observed in 25% hydraulic conductivity rather than 10% hydraulic conductivity with water.When compared to loamy sand soil, sandy soil has more movement.

At 75% hydraulic conductivity, the first 2.5 pore volumes showed negligible 0.23ng/L and 0.1ng/L, and SAL- Na was not detected upto 25 pore volumes in both sterile and nonsterile soil.Soil column passed by phosphate buffer at 5 and 10 pore volumes were 0.3ng/L and 0.029 mg/L in sterile soil, 0.45ng/L, and 0.033ng/L in nonsterile soil.When compared to sterile and

nonsterile, it has more movement.The higher (0.47mg/L) mobility was observed in nonsterile at 15 pore volumes than in sterile (0.3mg/L).The same trend was observed in 20 and 25 pore volumes (Table 4).

The top (0-3cm) loamy sandsoil showed a higher concentration (2.4 mg/kg) in sterile concentration than sandy soil. Loamy sand soil also showed very low concentration in the bottom (6-9cm). Nonsterile top (0-3cm) soil has a lower concentration (1.8mg/kg) than sterile soil at 10% hydraulic conductivity (Fig.4).At 25% hydraulic conductivity,non-sterile soil has a higher concentration of topsoil (1.8 mg/kg) than sterile soil (1.35 mg/kg).Again, the same trend in distribution (bottom less) was observed in 25% hydraulic conductivity in both soils(Fig.4). Top and bottom soils at 10%hydraulic conductivity showed significant ($P<0.05$) difference with 25% hydraulic conductivity.

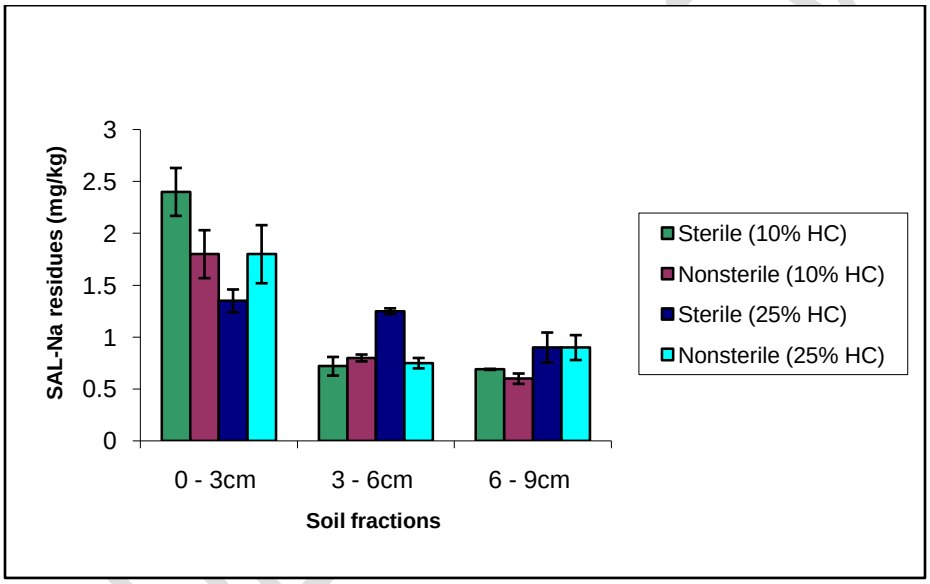

Fig. 4 Distribution of Salinomycin Sodium residues in loamy sand soil column

At 75% hydraulic conductivity,the sterile top (0-3cm) soil column passed with water had more concentration (0.8mg/kg) than nonsterile (0.5 mg/kg).The bottom (6-9 cm) had less (1.2 mg/kg) concentration than the middle soil (1.75 mg/kg) (Fig.5).The soil column passed with phosphate buffershowed a very low concentration in the top (0-3cm) soil (0.9 mg/kg in sterile) than the middle and bottom soil at 75% hydraulic conductivity.Nonsterile soil has shown a very low SAL-Na (0.24 mg/kg)(0-3cm) than sterile soil (0.4mg/kg) (Fig. 5).Loamy sand soil had higher concentrations in all depths than sandy soil.Thus, higher SAL-Na movement was observed in sandy soils with phosphate buffer.Multiple comparisons (LSD) analysis showed that

there was a significant (P<0.05) difference was observed between the movement of SAL-Na in sandy and loamy sand soils.The mobility of veterinary medicines in the manure behavior is different from soil to soil, which may be due to variations in the degradation mechanism, the degradation mechanism in the soil is most probably due to aerobic degradation.Most of the time, these antibiotics are non-extractable (Carlson and Mabury, 2006) [3].

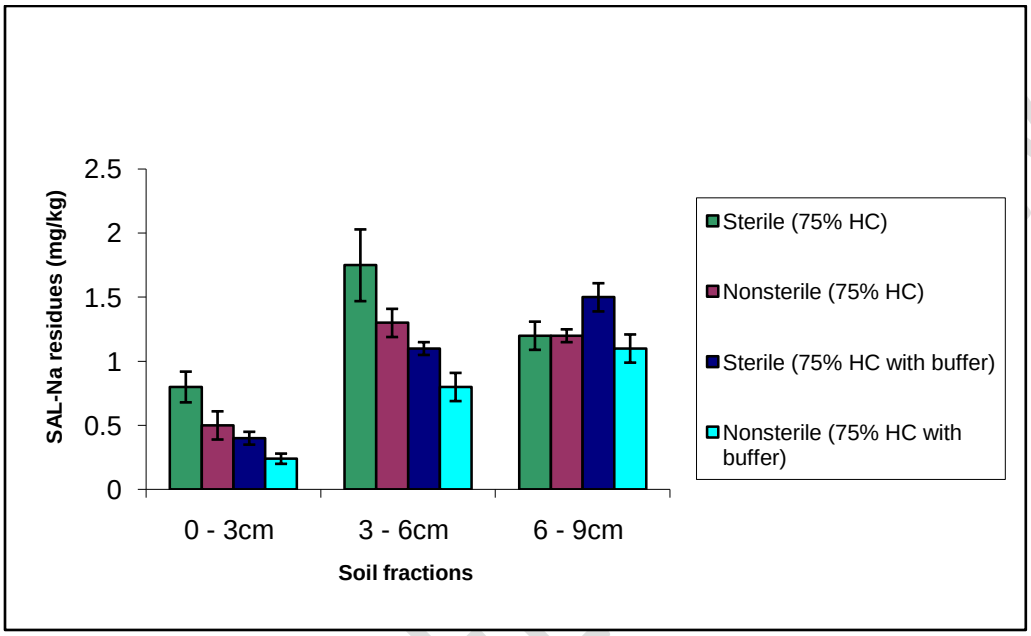

Fig. 5 Distribution of Salinomycin Sodium residues in loamy sand soil column at 75% hydraulic conductivity

Physical sealing of the soil with antibiotics or change in pH as a result of manure addition alters the medicines' speciation of fate. The movement of these contaminants to groundwater can depend on different environmental and hydrogeological factors such as land use, soil properties, geological and hydrogeological properties and climate (Essaid *et al*., 2015)[7]. While in transport, contaminants are subject to several complex physical, chemical and biological transformation processes that can provide attenuation, depending on the pathway taken. (Based on the physicochemical properties of the anticoccidials (mostly highly hydrophobic, with high organic carbon sorption coefficients), the most important of these environmental factors are soil and Quaternary deposit properties (such as pH, texture, structure, organic content, permeability and thickness), with adsorption to soil likely to be a significant attenuation process as these contaminants move through the unsaturated zone to groundwater (Alonso *et al.*, 2019)[1].

3.2 Future implications

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Subsequent investigations in the field of study may further advance the current research by delving into the enduring consequences of SAL-Na on the overall quality of soil and its possible ramifications on the development of plants. In addition to investigating the sorption, desorption, and transport of SAL-Na in soil, researchers can examine the influence of other environmental factors, such as fluctuations in pH levels or microbial populations. In addition, developing analytical techniques may enhance detection sensitivity, enabling the identification of even more diluted amounts ofsalinomycinsodium in both soil samples and leachates. Ultimately, the findings of this research have the potential to provide valuable insights for formulating laws and regulations about the use and disposal of SAL-Na, to mitigate its ecological ramifications.

4. CONCLUSION

Salinomycin sodium (Sal-Na) is a chemical of high toxicity often used in the poultry sector, and its behavior within soil ecosystems is of considerable importance. This current study examined the behavior of the SAL-Na and theirmobility potential to move to the surface and groundwatersin soils with sandy and loamy sand textures.The results suggest that SAL-Na exhibits high attachment to soil fractions, hence minimizing the potential for leaching. The mobility of SAL-Na exhibited greater levels in the nonsterile soil, suggesting that the presence of microorganisms may influence the transport dynamics. In summary, this work offers significant insights into the fate and dynamics of SAL-Na in soil, therefore contributing to the development of efficient management approaches aimed at mitigating the environmental hazards linked to its use.

Data availability statement

All the required data are available within the manuscript

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