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Full Length Research Paper

Effect of soil pH on sorption of salinomycin in clay and sandy soils

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Salinomycin is polyether ionophore, commonly used in poultry industry for the prevention of coccidial infections and promotion of growth. A large amount of the administered antibiotic is excreted as parent compound, eventually reaching agricultural lands. This makes it imperative for researchers to understand the behavior of the compound in soil environment by conducting sorption-desorption studies. In this study, sorption of salinomycin was measured in four agricultural soils, a clay soil with low organic matter content (LOM), a clay soil with high organic matter content (HOM), a sandy soil with HOM, and a loamy sandy (LOM) soils, at three pH levels, namely 4, 7 and 9. Desorption studies was carried out using the batch equilibration technique. It was observed that more than 98% salinomycin was strongly sorbed by all soils, irrespective of the soil organic matter content or soil pH. The sorption of salinomycin to the sandy soil marginally increased as the pH decreased, while the sorption to the two clay soils marginally increased as the pH increased. Desorption of salinomycin with methanol over a 72 h period was < 0.2% of the amount added; however, it was > 70% with a phosphate buffer (pH 7). Since the phosphate buffer would mimic, to some extent, the quality of water flowing through field soils containing various salts, it was concluded that salinomycin could pose significant threats to both shallow ground water and surface water bodies.

Key words: Salinomycin, sorption, pH, desorption, environmental pollution, phosphate buffer.

INTRODUCTION

Antibiotics have been detected worldwide in soil, surface water, ground water, and sediment (Kolpin et al., 2002; Christian et al., 2003; Kim and Carlson, 2006). The persistence of antibiotics in terrestrial environments ranges from less than one day to weeks or even months, depending primarily on temperature and the chemical structure of the antibiotic (Rabolle and Splid, 2000). According to the rate of degradation and the sorptive properties, the parent substance or its metabolites may reach aquatic environments through surface runoff or leaching through the soil profile. Key chemical properties, such as water solubility, soil pH, volatility, and sorption, influence antibiotic transport in soils. Typical manure may also contain high levels of ammonia that would tend to increase the pH of soil solution, thus affecting the sorption of certain compounds.

Salinomycin is a naturally occurring, monocarboxilic, polyether, and antibiotic, elaborated by a strain of *Streptomyces abbes* (ATCC – 21838). Salinomycin is used for the prevention of coccidiosis in broiler chickens. The highest usage in USA is estimated to be 454 tons of salinomycin (active ingredient). Salinomycin is also approved for use as a cattle feed additive (BIO GRO) in the range of 5 - 10 g ton^{-1} of complete feed. Once administered, it can persist in organisms. A study conducted using radio labelled (C^{14}) reported that the salinomycin persists in food chain organisms, with moderately low biodegradability (EIA, 1987). Salinomycin is resistant to aerobic degradation. Its relatively low aqueous solubility (3.4 mg ml^{-1}) limits its availability for biological degradation, causing it to persist for longer periods in the environment (European Food Safety Authority (EFSA), 2004).

The potential antibacterial effect of salinomycin in the environment would be against gram positive bacteria, at

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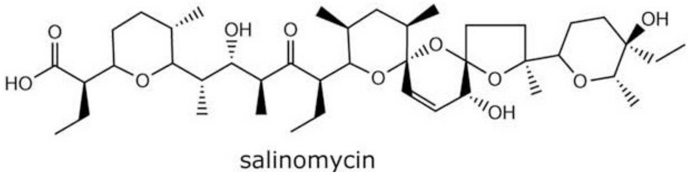

Figure 1. Structure of salinomycin.

Table 1. Characteristics of salinomycin.

Salinomycin	Free Acid
Molecular formula	$C_{42}H_{70}O_{11}$
Molecular weight	750
Melting point (C°)	112.5 – 113.5
P_{ka}	6.4
Water solubility	3.4 mg mL^{-1} readily soluble in methanol
Stability	Unstable in acidic condition stable in alkali condition

[a] P_{ka} = acid dissociation constant.

levels of 0.39 µg ml^{-1} and above. It inhibits bacterial protein synthesis through the attachment to 9 transfer RNA binding sites on the 30 s ribosomal unit (Cater et al., 2000). It could change microbial populations in the environment. This, in turn, could undermine the ability of microbes to degrade other pollutants such as pesticides (Boxall et al., 2003). This could also have deleterious effects on important chemical cycles, such as nitrification and denitrification (Frestegard et al., 1997). Salinomycin is unstable under acidic conditions and stable under alkaline conditions (EFSA, 2004); therefore, its soil sorption is expected to be pH-dependent. Currently, information about sorption of salinomycin to soil is not readily available. Therefore, the objective of this study was to investigate the sorption behavior of salinomycin in four agricultural soils at three different pH levels (4, 7 and 9).

MATERIALS AND METHODS

Chemicals

Only analytical reagent grade or high purity chemicals were used in this study. Sodium phosphate (Mono and di basic), methanol, ammonium hydroxide and acetic acid were purchased from Sigma. The salinomycin standard was obtained from Sigma Aldrich Company. The structure of salinomycin is shown in Figure 1 and selected properties are given in Table 1. The stock solution of salinomycin was prepared by dissolving 10 mg salinomycin in 10 ml of methanol (MeOH), and stored at 4°C. Standard solutions were freshly prepared by diluting the stock solution with methanol in vials.

Soil characterization

Four 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. Bearbrook soil is a dark-brown to grayish-brown clay having granular structure. Generally free carbonate is absent in upper part of this soil. Soils having high organic matter (HOM-clay) and low organic matter (LOM-clay) were collected from this soil series. The third 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 fourth soil 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 2 mm sieve, and stored in plastic 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 (Trivedy and Goel, 1986), organic carbon by wet digestion with $K_2Cr_2O_7$ and H_2SO_4 (Walkey and Black, 1934), texture (by hydrometer method), and cation exchange capacity (CEC) by atomic adsorption spectrometer. The various soil characteristics are listed in Tables 2 and 3.

pH adjustment

The initial pH of the two clay (LOM and HOM), loamy sand and sandy soils were 6.7, 6.4, 6.9 and 6.7, respectively, (Table 2). All four soils were equilibrated with 1 M concentrations of HCL and NaOH. The pH was adjusted eight times over 20 days to stabilize at desired levels of 4 and 9. Excess liquid from each soil sample was drained after pH adjustment, and soils were allowed to air dry for 48 h. Soils were pulverized, rechecked for pH, and used for sorption studies.

Sorption

The sorption experiments were performed with four different concentrations of salinomycin, 0.5, 5, 10 and 25 mg L^{-1}, in methanol. The UV-radiated sterile soils were used for sorption studies in order to minimize the effect of microbial growth and activity on the results. 20 mL of salinomycin solution was

Table 2. Physio-chemical characteristics of soils used in this study.

Soil type	OC (%)	TOC (%)	OM (%)	pH	Soil Texture		
					Sand (%)	Silt (%)	Clay (%)
Clay	4.31	5.7	9.88	6.7	30	20	50
Clay	5.35	7.13	12.3	6.4	10	20	70
Loamy sand	2.25	3	5.17	6.9	87.5	5.1	7.5
Sand	3.9	5.2	8.96	6.7	92.5	2.5	5

[a] OC:Organic carbon, TOC:Total organic carbon, OM:Organic matter.

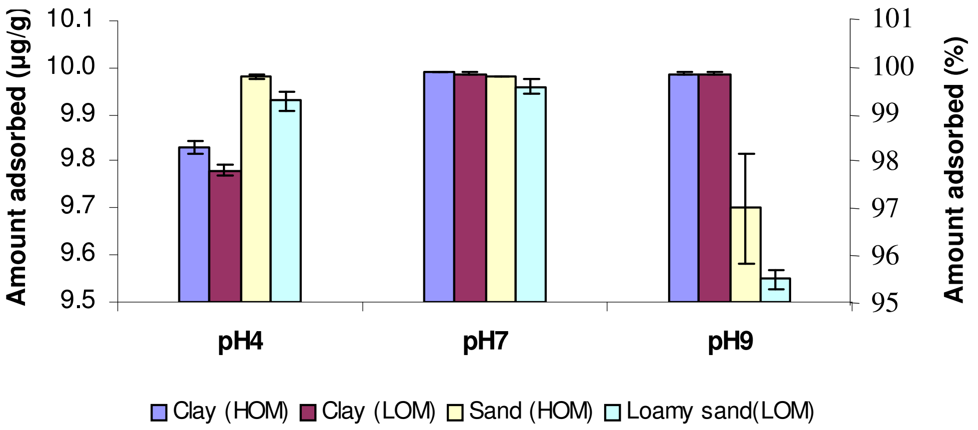

Figure 2. Effect of pH on adsorption of salinomycin at 10 µg/g soil.

added to the 1 g of sterile soil in a 100 ml conical flask to obtain final concentrations of 10, 100, 250, and 500 µg g^{-1} of soil. Moreover, 20 ml of methanol was added to 1 g of soil for the blank, and 20 ml of each concentration of salinomycin solutions were kept in flasks, without soil, as controls. All samples were agitated for 24 h on a rotary shaker. After centrifugation at 3000 g for 30 min, supernatants were transferred to glass vials, filtered through 0.45 µm filters, and subsequently analyzed by HPLC-CAD (Charged Aerosol Detector), to determine the amount of salinomycin remaining in solution. The sorbed salinomycin concentration was calculated using the following equation:

$$S = \frac{\mathrm{Vaq}\ (\mathrm{Ci} - \mathrm{C})}{\mathrm{Ms}} \qquad (1)$$

Where S is salinomycin sorbed to soil (µg g^{-1}), V_{aq} is the initial volume (L), M_s is the soil mass (g), C_i is the initial concentration (µg L^{-1}) and C is the concentration remaining in the solution phase after equilibrium (µg L^{-1}).

As will be evidenced by the results presented later on, the four soils used in this study fully adsorbed the antibiotic. Therefore, it became necessary to carry out further sorption tests by increasing antibiotic concentrations in order to make sure that the sorption capacity of soil is reached and there is antibiotic left in the solution phase, and, more importantly, to rule out any experimental errors or extremely fast chemical degradation (no microbial degradation was possible because all soils were sterilized before use). The high-concentration sorption tests were done on the sandy soil only. Since this soil had HOM, it was decided to purchase a LOM-sand from a commercial outfit (Reno Department, Pointe Claire, Quebec, Canada) and to include it in the sorption/desorption study. The highest concentration used was 5,000 µg g^{-1}. An equal amount (20 ml) of methanol was added to the soil, and the flasks were agitated for 24 h, centrifuged, as previously described, and this procedure was repeated up to 72 h.

Analysis of salinomycin

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

Statistical analysis

Results obtained in this study were analyzed using two-way ANOVA and multiple comparisons least significant difference (LSD). These tests were done using MATLAB, version 7.8.

RESULTS AND DISCUSSION

The sorption of salinomycin in two clays, loamy sand and sandy soils at three different pH levels is presented in Figures. 2, 3, 4 and 5 for 10, 100, 200, and 500 µg g^{-1} initial concentrations, respectively. Figure 2 shows that,

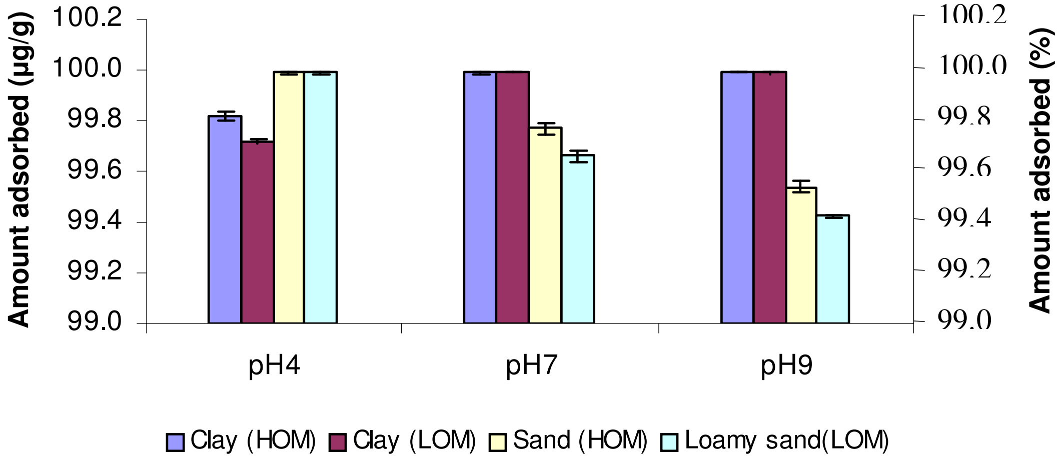

Figure 3. Effect of pH on adsorption of salinomycin at 100 µg/g soil.

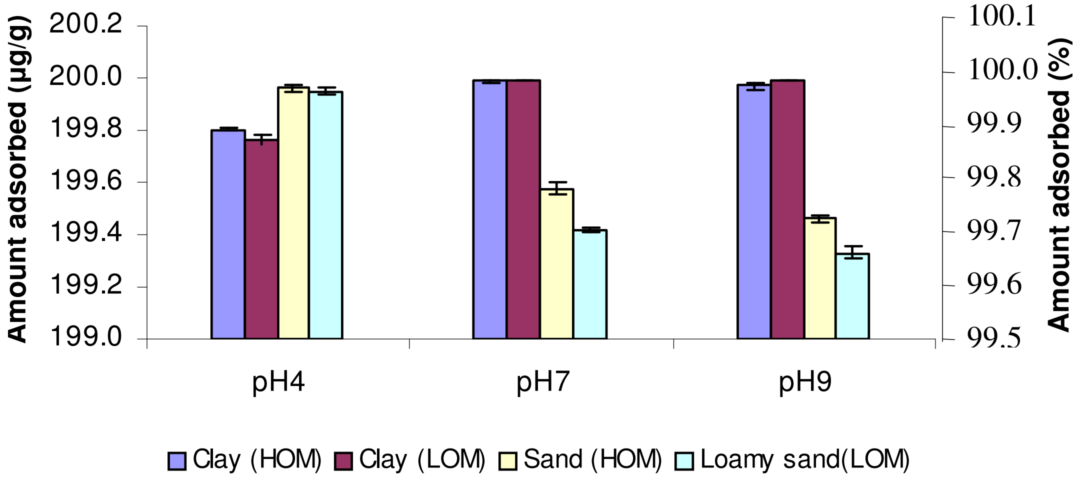

Figure 4. Effect of pH on adsorption of salinomycin at 200 µg/g soil.

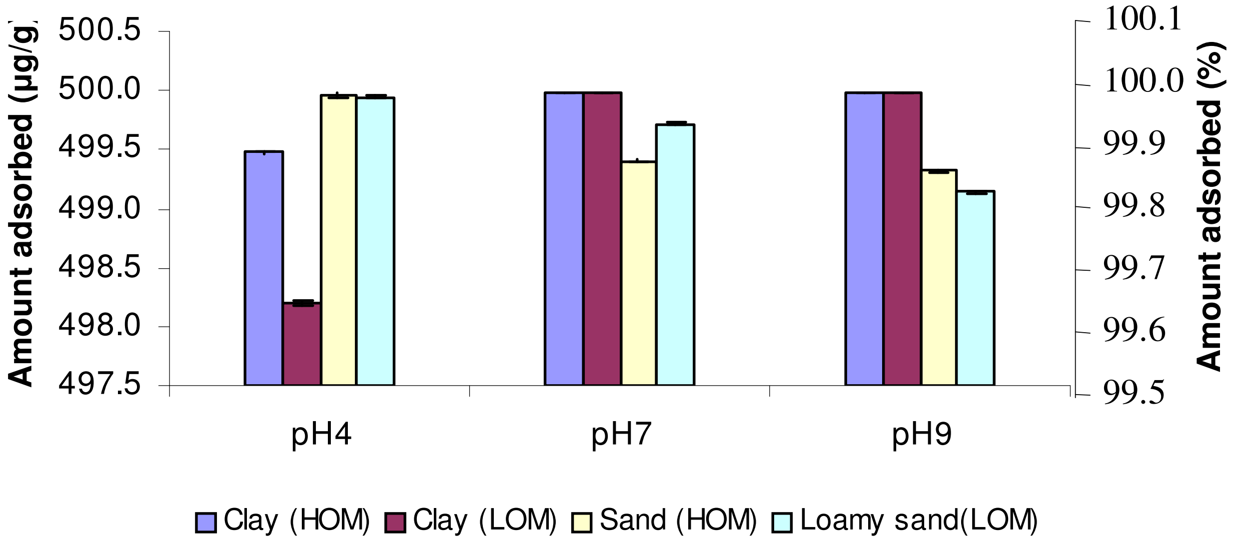

Figure 5. Effect of pH on adsorption of salinomycin at 500 µg/g soil.

Table 3. Chemical characteristics of soils used in this study (in cmol(+) kg^{-1}).

Soil Type	Ca	Mg	K	Na	Mn	Fe	CEC
Clay (HOM)	12.77	3.03	0.31	0.213	0.05	0.0166	16.4
Clay (LOM)	16.87	5.95	0.453	0.233	0.03	0.02	23.553
Sand (HOM)	3.323	1.02	0.12	0.06	0.01	0.02	4.656
Loamy sand (LOM)	2.426	0.07	0.053	0.133	0.01	0.02	2.686

[a] LOM-Low organic matter content; HOM-High organic matter content, CEC-Cation Exchange Capacity.

at 10 µg/g concentration, the sorption of salinomycin was higher (99.3~99.8%) in the sandy and loamy sandy soils, irrespective of organic matter content, at pH 4 and 7. It was slightly lower (95.5 - 97.0%) for the same at pH 9. Multiple comparison LSD analysis showed salinomycin sorption in HOM-clay to be significantly different ($P < 0.05$) from that in LOM-clay, LOM-loamy sand, and HOM-sandy soils. In the sandy soil, at 100 µg g^{-1} concentration, the sorption of salinomycin was 100% at pH 4, followed by 99.7-99.8% at pH 7 and 99.4-99.5% at pH 9 (Figure 3). At 200 and 500 µg g^{-1} levels also, the trend was similar, although, sorption was slightly higher (Figures 4 and 5). This shows that salinomycin is strongly sorbed to the sandy soil; more than 95% of its initial amount is sorbed, irrespective of the organic matter content or pH level. One noticeable observation is that the amount sorbed to both sandy and loamy sandy soils was relatively small at pH 9 with 10 µg g^{-1}, as compared to the other pH levels. It was also observed that sorption was relatively higher in the soil with the higher organic matter content, as compared to the soil with the lower organic matter content. A significant difference ($P < 0.05$) was observed between HOM-clay and LOM-clay soils at 200 µg g^{-1}, and no significant difference was observed in the sandy and loamy sandy soils with low and high organic matter content.

In the two clay soils, at 10 µg g^{-1} level, the sorption was slightly lower (97.8 - 98.3%) at pH 4, as compared to the other pH levels (99.9%) (Figure 2). A similar trend was also observed for the other three concentrations (100, 200 and 500µg g^{-1}), although 100% sorption was observed at pH 7 and 9 (Figures 3, 4 and 5). The results show that salinomycin was also strongly sorbed to the clay soils, irrespective of the organic matter content. However, at pH 4, the sorption of salinomycin was higher in the HOM-clay soil; the sorption was quite high at pH 7 and 9 for both levels of organic matter content. A clear contrast was observed between the sandy and clay soils at 10 µg g^{-1} concentrations. The sorption was lowest in the case of clay soils at pH 4, whereas it occurred at pH 9 in sandy soils. A significant difference ($P < 0.05$) was observed between pH 4, 7 and 9 in HOM- and LOM-clays and HOM-sandy and LOM-loamy sandy soils.

Sorption decreased with increasing pH in sandy and loamy sandy soils but not in the two clay soils. As the clay soils have high OM content (9.88 - 12.3%), when pH increases, the humic molecules could have deprotonated, thereby increasing their negative charge. Also, aluminum ions that are initially complexed to the organic matter could have started to hydrolyze and eventually precipitated as aluminium hydroxide. These processes would also contribute to the increase in negative charge. Thus, clay soils have a higher pH-dependent CEC. To some extent, the increasing exposure of negative charge on humic molecules with increasing pH may over-compensate the declining positive charge of salinomycin.

Theoretically, the maximum sorption of salinomycin on the variable charge of organic matter surfaces would occur at a pH value close to the P_{ka} value (acid dissociation constant). However, there may still be a substantial sorption, even at pH values which are several units higher than P_{ka}, due to selective sorption of charged molecules by the organic matter (Berglof et al., 2002). Higher sorption of antibiotics on clay-rich soils has also been reported previously (Rabolle and Splid, 2000; Loke et al., 2002; Kumar et al., 2004). Thus, higher antibiotic adsorption on clay soils is mainly due to their greater exchange capacity because of the higher clay content and organic matter. Loke et al. (2002) also reported that binding of salinomycin was influenced by ionic binding to divalent metal ions such as Mg^{2+} and Ca^{2+}. In clay loam soils, these ions were higher than in the sandy soils, thus this may have contributed to the higher sorption (Table 3). Organic matter was found to have a small effect on the sorption of salinomycin, and it was evident from all four soils (Figures 2, 3, 4, and 5). All four soils used in this study were field soils and they had relatively high organic matter content (Table 2). Therefore, it was decided to investigate sorption behavior of salinomycin under low organic matter content conditions. To this end, construction sand was purchased from a local store with 0.3% organic matter content. Again, the antibiotic sorption was found to be very high. It was decided to use even higher initial concentrations, that is, 5,000 and 10,000 µg g^{-1}), but still most of the antibiotic was sorbed. This may be due to salinomycin's greater ability to coordinate with inorganic cations in solution, resulting in increased hydrophobicity of salinomycin. This effectively neutralizes the carboxylic acid's negative charge. Also, salinomycin might interact with cations on soil exchange sites through cation bridging. To estimate the saturation point of salinomycin sorption in LOM-sandy soil, a very

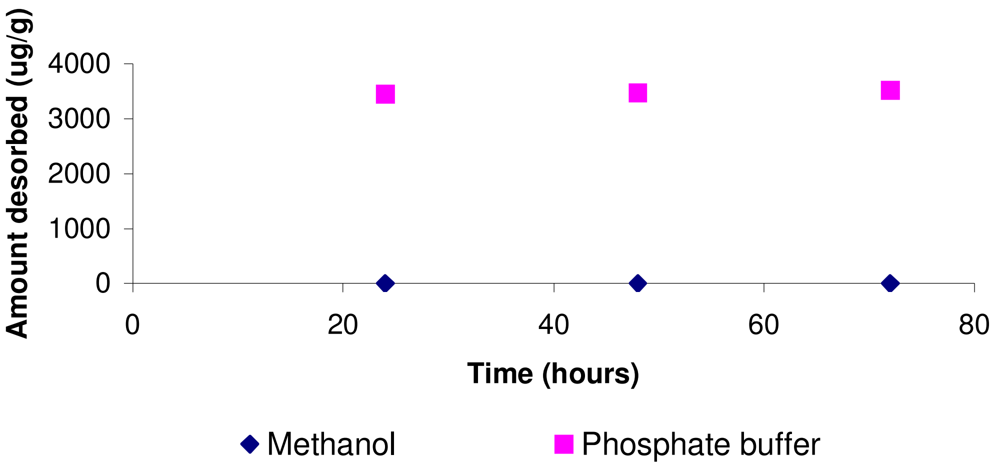

Figure 6. Desorption of salinomycin in sandy soil.

high initial concentration of 50,000 µg g^{-1} was used on the sandy soil since it was the least adsorbent amongst the four soils tested. It was found that nearly 35,000 µg g^{-1} was sorbed in 24 h. This indicates the sandy soil has very high sorption capacity for salinomycin.

Tolls (2001) reported that veterinary antibiotic sorption to soil cannot be predicted from compound K_{ow} (Otanol-water partition coefficient) and soil organic carbon (OC) content. Sithole et al. (2003) suggested that antibiotic interaction with soil organic matter occurs as a result of binding to divalent cations, ion exchange interactions, and hydrogen bonding between acidic groups in humic acids and polar groups on salinomycin. Binding to divalent metal cations has also been suggested to explain salinomycin sorption in both marine and manure sediments. In addition, the carbonyl and either oxygen atoms can coordinate with metal ions, resulting in some degree of encapsulation (Volmer and Lock, 1998; Paulus et al., 1998)). This coordination gives ionophores the unique ability to transport metal ions across cell membranes as either undissociated acids or neutral complexes and gives the molecule a largely hydrophobic exterior (Shen and Bradbelt, 2000; Paulus et al., 1998; Miao et al., 2003). Salinomycin has a higher affinity for Na^+, K^+, Ca^{2+} and Mg^{2+} with a strong preference for sodium (EFSA, 2004).

Desorption of salinomycin was also performed in HOM-sandy soil and the construction sand (LOM) at higher concentration (5000 µg g^{-1}) using methanol. The desorption results are shown in Figure 6. While desorption was performed over a 72 h period, it was observed that only < 0.2% of the added amount desorbed from the soil (Figure 6). This suggests that once salinomycin is sorbed on to these soils, its leaching to lower depths may not occur. However, since the desorption was being done with deionized water which may not be a good representative of rainwater making its way through the soil profile, it was decided to use a phosphate buffer for desorption. The percolating water will tend to dissolve several salts, commonly present in field soils, (especially phosphates) this condition is well mimicked by the phosphate buffer solution.

Shen and Bradbelt (2000) have explained the mechanism of desorption using a phosphate buffer. The polar functionality of the antibiotic molecule, such as salinomycin, is oriented towards the cation at the centre, while the outer solvent-accessible portion of the molecule is largely hydrophobic (Dobler, 1981; Sobott et al., 1997). Also, the carboxylic acid or ester groups, in addition to the tetrahydropyran or tetrahydrofuran groups in the backbone of ionophores like salinomycin can undergo anion exchange with soil to have a considerable anion exchange capacity, especially the high organic matter content soils. Therefore, highly selective phosphate anions are capable of desorbing salinomycin. The results from our study showed that, out of 5,000 µg g^{-1} salinomycin, 3,450 µg g^{-1} had desorbed from the construction sand with the phosphate buffer after 24 h. When the desorption period was extended to 72 h, there was a minimal further increase in desorption - a total of 3,525 µg g^{-1} was desorbed (Figure 6). This showed that although, salinomycin is strongly sorbed to soil, it could also readily move into underground aquifers and water bodies under field conditions.

Conclusions

Sorption of salinomycin in clay and two sandy soils was very high (> 98%). It also tended to slightly increase with increasing soil organic matter and clay contents. The influence of pH on the sorption of salinomycin in various soils was quite different. Sorption on the clay soil marginally increased as the pH increased, while sorption on the sandy and loamy sandy soil decreased as pH increased. If the rainwater were without any salts, salinomycin will strongly sorbed on to soil, with very little chances of it leaching into ground water. However, it was shown in this study that a phosphate buffer (pH 7) easily desorbed very tightly sorbed salinomycin. Since actual rainwater would contain dissolved salts, there is a high likelihood that it may desorb salinomycin, thus posing a greater threat to groundwater and, to some degree, surface water contamination.

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