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Original article

Assessing the prevalence of veterinary antibiotics and associated potential ecological risk in dryland soil, manure, and compost: A case study from Saudi Arabia

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ABSTRACT

Application of manure and compost can result in the accumulation of veterinary antibiotics in soil, subsequently posing ecological risks. The occurrence and potential ecological risk assessment of nine antibiotics (4 tetracyclines, 3 sulfonamides, and 2 macrolides) residues in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia are reported in this study. A total of 36 samples (20 soil, 8 manure, and 8 compost) were collected and their physiochemical characteristics were analyzed. Large variabilities were observed in organic matter, pH, electrical conductivity, and nutrient status. In the soil samples obtained, three textural groups were identified: sandy loam, loamy sand, and loam. Overall, the mean concentrations of all detected antibiotics measured were lower ($<50\ \mu g\ kg^{-1}$) in all matrices (soil, manure, and compost). However, the mean concentrations of tetracycline (TC), doxycycline (DC), oxytetracycline (OT), and sulfamethoxazole (SMZ) were 97.96, 183.29, 101.24, and 69.58 $\mu g\ kg^{-1}$, respectively, in manure samples, and 49.59, 93.26, 74.21, and 18.32 $\mu g\ kg^{-1}$, respectively, in compost samples. The concentrations of antibiotics were highest in manure samples, then compost, and finally soil. The calculated risk quotient (RQ) values for the majority of the studied antibiotics were <0.1, indicating no to low adverse ecological effects. However, the higher RQ values for OT and DC suggested possible adverse ecological effects of these compounds in soil.

1. Introduction

Antibiotics have extensively been utilized in livestock industry as growth promoters, therapeutics, and prophylactics (Ramaswamy et al., 2010). It has been estimated that the global antibiotics consumption has crossed 63,151 tons per annum (Al-Wabel et al., 2020). However, animals are unable to completely absorb these drugs in their body and ~70% of the drugs are released into the environment through excretion (Ahmad et al., 2019). Recently, antibiotics contamination has increasingly become an agricultural and environmental problem which are of major concern to the public and scientists worldwide (Almajed et al., 2021; Wu et al., 2015). The application of sludge and manure as soil amendment, as well as irrigating the fields with reclaimed water could possibly introduce the antibiotics into soil resources (Wu et al., 2015). The persistence of antibiotics in the environment varies with type of compound and conditions. Thus, antibiotics may accumulate in the soil over time when the input rates exceed dissipation rates. This is evident in a study conducted on the sulfamethoxazole and ciprofloxacin contamination in Mexican soils, where it was demonstrated that these two chemicals could accumulate in the soil as a sequestered form over a period of 20 years during long-term irrigation with untreated wastewater (Dalkmann et al., 2012). Antibiotics residues in the environment may hinder the germination of seed, retard the growth of crops, and up take in plant, subsequently posing a great risk to soil ecosystem (Gros et al., 2019). These veterinary antibiotics can also be transferred from soil to crops. The accumulation of antibiotics in plants grown under manure-amended soils system has widely been reported

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(Miller et al., 2016). Therefore, there is a greater possibility of transferring the antibiotics accumulated in crops to humans through the food chain.

An increased resistance against tetracycline has been found in the arable soil with long-term application of fresh manure and compost, while the dominant tetG genotypes shared strong homology with pathogenic bacteria (Peng et al., 2015). The harmful impacts of antibiotics residues in soil, water, terrestrial organisms, and food as well as the emergence and spread of resistant microorganisms are reported in many studies (Ahmed et al., 2015). However, most of these studies did not consider the implications of antibiotics in terms of the risk to soil ecosystems. Therefore, the adverse impacts of antibiotics residues on human health and ecosystem need to be explored.

As a paramount agricultural area in the Kingdom of Saudi Arabia (KSA), veterinary antibiotics are extensively used in Al-Kharj city, Riyadh province where various animal husbandry farms are situated which include cow, camel, sheep, and chicken farms. These farms generate a substantial amount of animal manure. However, the existence of antibiotics and the potential ecological risks of manures and compost in this area have not been explored yet. Therefore, current study was conducted to investigate the existence and concentrations of some selected antibiotics in the representative soil, manure, and compost samples in the Al-Kharj region, Riyadh Province, KSA, and to evaluate the potential ecological risks. The results from this research will render worthwhile information on antibiotic residues in animal manures and composts to use when deciding whether it is necessary to treat animal manure and compost before being applied as soil additives.

2. Materials and methods

2.1. Study area

Al-Kharj city, which is located 77 km south of Riyadh at around 24°8′54″N and 47°18′18″E, was surveyed to collect soil, manure, and compost samples. Al-Kharj area contains a desert climate with a higher temperature (23 °C to 45.5 °C) and receives low precipitation (67 mm annually). Al-Kharj is famous for its agricultural and dairy production. According to an estimate, about 80% of the dairy products produced in the KSA come from this area, which subsequently produces huge quantities of manure. Additionally, there are several composting facilities in the area which are transforming manure into compost.

2.2. Sample collection and preparation

The sites for soil, manure, and compost sampling located in the Al-Kharj area are shown in Table 1. Composite soil samples were collected from surface (0–15 cm) and sub-surface (15–30 cm) layer from 10 sites using auger and stored in rinsed plastic containers. The composite manure samples were collected from 8 different locations in the Al-Kharj area. Likewise, 8 composting facilities were visited and composite samples of compost were collected. In this way, a total of 36 samples were collected from the study area. Approximately 1 kg each of manure and compost samples were collected in acetone rinsed plastic containers. All samples were kept in an ice-box and transferred to the laboratory, where they were stored in dark at −10 °C till further analyses.

2.3. Characterization

The pH and electrical conductivity (EC) of the samples were determined in a 1:2.5 (w/v) ratio suspension in deionized water. Soil organic matter was determined by following the method of Walkley-Black (Hesse, 1971). Cation exchangeable capacity (CEC), $CaCO_3$, exchangeable cations (Na, K, Mg, and Ca), phosphorus (P) and nitrate (NO_3^-) in all the samples were analyzed by following the standard protocols. Soil available phosphorus (P) and heavy metals in all the samples were extracted with AB-DTPA. Thereafter, the concentration of P was analyzed by using a UV/VIS spectrophotometer (Lambda EZ 150, PerkinElmer, USA), while heavy metals were determined with an Inductivity coupled plasma optical emission spectrophotometer (PerkinElmer Optima 4300 DV, USA). The particle size distribution of the soil samples was analyzed by using Malvern Mastersizer 2000 equipped with Hydro 2000S. The proximate analyses of manure and compost samples were performed by following the standard methods (ASTM D1762-84: ASTM, 1989).

Table 1
Coordinates of the sampling sites for the collection of soil, manure, and compost samples in Al-Kharj, Saudi Arabia.

Sample type	Site No.	Latitude	Longitude
Soil	S-1	N24° 15.375	E047° 28.489
	S-2	N24° 15.370	E047° 28.459
	S-3	N24° 15.381	E047° 28.474
	S-4	N24° 13.539	E047° 37.391
	S-5	N24° 13.538	E047° 37.332
	S-6	N24° 14.488	E047° 29.881
	S-7	N24° 11.966	E047° 32.432
	S-8	N24° 11.961	E047° 32.439
	S-9	N24° 11.995	E047° 25.386
	S-10	N24° 11.999	E047° 25.384
Manure	M-1	N24° 11.978	E047° 32.426
	M-2	N24° 11.983	E047° 25.374
	M-3	N24° 11.988	E047° 25.411
	M-4	N24° 14.481	E047° 29.886
	M-5	N24° 13.524	E047° 37.358
	M-6	N24° 11.978	E047° 32.428
	M-7	N24° 19.348	E047° 26.975
	M-8	N24° 19.513	E047° 26.802
Compost	C-1	N24° 11.966	E047° 32.448
	C-2	N24° 11.978	E047° 32.466
	C-3	N24° 11.983	E047° 25.355
	C-4	N24° 12.204	E047° 26.905
	C-5	N24° 14.481	E047° 29.838
	C-6	N24° 13.527	E047° 37.321
	C-7	N24° 13.529	E047° 37.383
	C-8	N24° 15.370	E047° 28.462

2.4. Extraction and quantification of antibiotics

2.4.1. Sample preparation

Specifically, tetracycline (TC), doxycycline (DC), oxytetracycline (OT), and chlortetracycline (CTC) were selected as representatives for tetracyclines, sulfadiazine (SDZ), sulfathiazole (STZ), and sulfamethoxazole (SMZ) as representatives for sulfonamides, and tylosin (TL) and erythromycin (EMC) as representatives of macrolides. The samples were ground and sieved through a 75 μm aperture to get homogenized particle size. Thereafter, 1.0 g sample was mixed with 20 mL of McIlvain buffer (625 mL of 0.2 M Na_2HPO_4 and 1000 mL of 0.1 M citric acid) and 200 μL of 5% Na_2EDTA and then shaken at 200 rpm for 20 min. Then the sample was withdrawn and centrifuged at 4000 rpm for 15 min. The supernatant was collected in amber-colored glass bottles. In this way, two more extracts were also collected and combined.

2.4.2. Solid-phase extraction

HLB cartridges (3 mL/60 mg) were activated by eluting with 3 mL methanol, 3 mL 0.5 N HCl, and 3 mL of HPLC grade water. Then, samples were loaded under 40 psi of vacuum at ~2 mL min^{-1} flow rate using Teflon tubes. Then, the cartridges were rinsed with 3 mL HPLC grade water and dried for 30 min under vacuum. 40 μL of internal standard (1 mg L^{-1} Simeton) was added to each 15 mL

Table 2
Percent recovery, limit of detection (LOD) in μg kg^{-} and limit of quantification (LOQ) in μg kg^{-} for the veterinary antibiotics in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia.

Antibiotics	Abbreviation	Percent recovery	LOD	LOQ
Tetracycline	TC	127.89 ± 4.56	36.06	42.78
Doxycycline	DC	79.16 ± 8.45	47.71	51.05
Oxytetracycline	OT	72.59 ± 7.21	26.44	33.69
Chlortetracycline	CTC	121.36 ± 6.88	35.48	39.98
Sulfadiazine	SDZ	87.58 ± 5.98	25.77	33.26
Sulfathiazole	STZ	91.56 ± 3.69	37.32	43.25
Sulfamethoxazole	SMZ	67.47 ± 4.55	42.70	51.67
Tylosin	TC	116.64 ± 8.77	38.14	46.79
Erythromycin	EMC	124.85 ± 7.19	32.26	38.31

collection vial. The antibiotics were then eluted from the HLB cartridges into the collection vials with 2.5 mL of methanol (twice) with a flow rate of 2 mL min^{-1}. The extracts in the collection vials were mixed on a vortex mixer and evaporated to 150 μL by gentle heating at 50 °C under N_2 atmosphere. 250 μL solution consisting of 0.1% formic acid and 25% methanol in ultrapure water was added to the extracts. Finally, the extracts were filtered using 0.45 μm syringe filters and stored in amber-colored HPLC vials for further analyses. All the samples were prepared and extracted thrice including blanks.

2.4.3. Antibiotics quantification

High-performance liquid chromatography (HPLC; Prominence-i, LC-2030C, Shimadzu, Japan), with a reversed-phase Raptor C18 column was used for antibiotics quantification. Calibration curves (R^2 > 0.99) were constructed between antibiotics concentrations versus absorbance by using standards. Standards and spiked samples were also run as unknown samples for quality assurance.

The separation of tetracycline antibiotics was proceeded by using three mobile phases i.e., methanol, acetonitrile, and 0.1 M oxalic acid (pH = 3.0). The analyses were performed in a gradient system with mobile phase composition of 7:8:85 (methanol:acetonitrile:oxalic acid) for 0.0–15 min, then 10:20:70 (methanol:acetonitrile:oxalic acid) for 15–20 min. The column temperature was set to 35 °C, flow rate to 0.5 mL min^{-1}, and λ to 354 nm. For the separation of macrolides, 0.05 M KH_2PO_4 solution (pH = 3.0) and acetonitrile were used as mobile phases. The analyses were performed in a gradient system with 30 °C of column temperature, 0.5 mL min^{-1} flow rate, and 270–354 nm of wavelength. The sulfonamide antibiotics were separated by using phosphoric acid

Table 3
Descriptive analyses of the selected physio-chemical properties of soil, manure, and compost as well as proximate attributes of manure and compost samples collected from Al-Kharj, Saudi Arabia.

Source	Parameter	Unit	Mean	Median	Min.	Max.	SD	C.V.	Skew	Kurtosis
Soil (*n* = 20)	pH	–	8.01	8	7.42	8.46	0.26	3.21	−0.63	0.48
	EC	dS m^{-1}	4.51	3.6	1.41	8.9	2.23	49.49	0.92	−0.4
	OM	%	1.04	0.56	0.11	3.8	1.09	105.42	1.6	1.29
	CEC	cmol kg^{-1}	13.84	12.07	6.07	29.06	5.73	41.41	1.01	0.65
	Available P	mg kg^{-1}	2.23	2.32	1.06	3.15	0.63	28.27	−0.18	−1.3
	NO_3^-	mg kg^{-1}	12.45	11.32	3.02	25.46	5.45	43.73	0.66	0.11
	Ca	meq L^{-1}	3.83	3.19	0.88	7.65	2.24	58.44	0.25	−1.18
	Mg	meq L^{-1}	3.83	3.53	2	6.6	1.31	34.16	0.74	0.3
	Na	meq L^{-1}	9.76	9.56	3	14.55	2.73	27.97	−0.3	0.4
	K	meq L^{-1}	58.16	45.45	4.82	112.15	33.04	56.81	0.3	−0.86
Manure (*n* = 8)	pH	–	7.97	7.97	7.04	8.71	0.5	6.26	−0.4	−0.12
	EC	dS m^{-1}	5.29	4	1.35	10.72	3.79	71.53	0.41	−1.5
	OM	%	14.34	13.66	10.17	19.14	3.41	23.75	0.19	−1.3
	CEC	cmol kg^{-1}	71.09	74.55	29.47	108.83	33.37	46.94	−0.14	−1.71
	Available P	mg kg^{-1}	693.66	340.03	107.47	1970.8	772.22	111.33	1.07	−0.73
	NO_3^-	mg kg^{-1}	16.04	15.51	10.56	22.75	4.86	30.28	0.33	−1.39
	Ca	meq L^{-1}	9.25	8.5	0	26	7.98	86.25	1.01	0.66
	Mg	meq L^{-1}	26.5	25	19.5	39	6.85	25.83	0.65	−0.76
	Na	meq L^{-1}	47.71	36.22	14.78	102.83	34.49	72.3	0.82	−0.95
	K	meq L^{-1}	36.41	41.16	1.76	69.29	27.29	74.96	−0.16	−1.57
	Moisture	(%)	13.5	13.34	4.47	18.79	4.26	31.57	−1.04	0.79
	Volatiles	(%)	31.38	33.39	21.42	39.71	7.71	24.55	−0.27	−1.64
	Ash	(%)	28.6	29.42	11.73	45.09	11.63	40.67	−0.09	−1.19
	Resident matter	(%)	26.52	25.77	16.14	40.74	8.95	33.74	0.32	−1.24
Compost (*n* = 8)	pH	–	7.96	8.24	7.06	8.53	0.61	7.63	−0.49	−1.54
	EC	dS m^{-1}	7.33	8.02	3.83	10.78	2.42	32.99	−0.35	−0.97
	OM	%	8.46	8.17	1.57	13.51	4.2	49.64	−0.19	−1.05
	CEC	cmol kg^{-1}	39.46	26.85	21.07	101.43	27.32	69.25	1.7	1.41
	Available P	mg kg^{-1}	1084.6	997.98	454.14	1933.3	559.08	51.55	0.26	−1.4
	NO_3^-	mg kg^{-1}	28.41	16.55	6.71	72.96	25.92	91.23	1.1	−0.66
	Ca	meq L^{-1}	12.56	12	5	20	5.38	42.84	0.03	−1.32
	Mg	meq L^{-1}	17.25	17.5	3.5	27.5	8.18	47.43	−0.31	−0.99
	Na	meq L^{-1}	59.04	73.07	14.07	93.7	29.43	49.84	−0.48	−1.35
	K	meq L^{-1}	47.7	49.09	14.58	72.75	22.18	46.5	−0.38	−1.24
	Moisture	(%)	16.33	15.91	9.68	21.39	4.21	25.78	−0.18	−1.3
	volatiles	(%)	31.58	32.16	19.74	38.57	6.05	19.15	−0.79	−0.16
	Ash	(%)	30.05	31.13	12.91	41.8	8.51	28.32	−0.8	0.33
	Resident matter	(%)	22.04	21.37	10.2	40.39	9.3	42.19	0.79	0.03

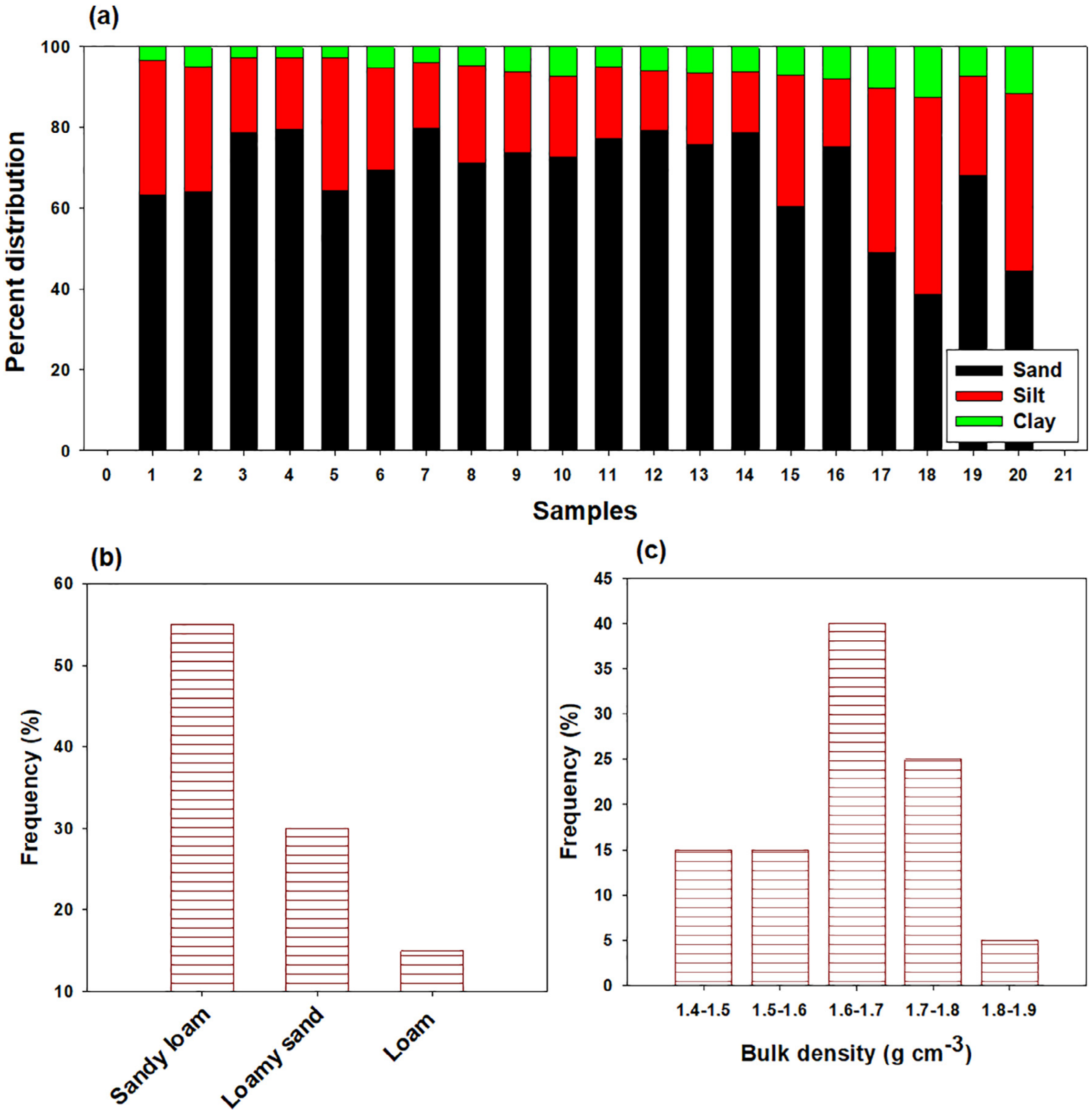

Fig. 1. Percent distribution of soil particles (a), as well as frequency of soil texture (b) and bulk density (c) for the soil samples collected from Al-Kharj, Saudi Arabia.

(pH = 3.0) and acetonitrile as mobile phases. A gradient system consisting of 7:93 acetonitrile:phosphoric acid was maintained for an initial 5.0 min, then switched to 30:70 of acetonitrile:phosphoric acid from 5.0 to 18 min, and held up to 25 min. Thereafter, the acetonitrile:phosphoric acid ratio was switched to 7:93 during 25–30 min and held up to 35 min. The analyses were performed in a gradient system with 35 °C of column temperature, 0.5 mL min^{-1} flow rate, and 210–354 nm of wavelength.

The limit of detection (LOD) and limit of quantification (LOQ) were measured as the lowest point of the calibration curve with a signal-to-noise ratio of >3 (Kim and Carlson, 2007), while percent recovery was calculated by using Eq. (1) (Table 2).

$$Recovery(\%) = \frac{detected\ response\ of\ spiked\ sample\ before\ SPE}{detected\ response\ of\ spiked\ sample\ after\ SPE} \times 100 \tag{1}$$

2.5. Potential ecological risk

The potential ecological risk was evaluated by following the methods of Zhang et al. (2015). The risk quotient (RQ) to estimate the potential ecological risk for different contaminants in the environment was estimated by using Eqs. (2)–(4).

$$RQ = \frac{PEC}{PNEC} \tag{2}$$

$$PEC = \frac{C \times m\left(1 - \frac{w}{100}\right)}{\rho \times 10 \times depth} \tag{3}$$

$$PNEC = \frac{TOX}{AF} \tag{4}$$

where *PEC* is predicted environmental concentration of antibiotic (μg kg^{-1}), *PNEC* is predicted no-effect concentration of antibiotic (μg kg^{-1}), *C* is the antibiotic concentration (μg kg^{-1}), *m* is the annual application rate of fertilizer (t h^{-1}), *w* is moisture content of manure and compost sample (%), *ρ* is the soil density (kg m^{-3}), *TOX* is the toxicity of antibiotic to soil microorganism obtained from the literature (μg kg^{-1}), and *AF* is assessment factor (1000 for acute toxicity and 100 for chronic toxicity). The samples with RQ of <0.01 were considered as non-toxic, whereas, the samples with RQ values

Table 4
Descriptive analyses for the total metal/metalloids contents (mg kg^{-1}) in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia.

Source	Heavy metal	Mean	Median	Min.	Max.	SD	C.V.	Skew	Kurtosis
Soil (*n* = 20)	As	8.58	9.15	1.67	14.00	3.68	42.82	−0.47	−0.98
	Cd	ND	ND	ND	ND	ND	ND	ND	ND
	Co	22.11	21.83	15.17	33.00	4.51	20.40	0.81	0.44
	Cr	55.38	51.34	21.10	81.64	16.01	28.90	−0.11	−0.20
	Cu	18.03	17.58	8.17	28.64	5.84	32.39	0.09	−0.87
	Fe	11365.00	11188.00	7923.30	16657.00	2495.10	21.95	0.81	−0.03
	Mn	189.76	181.58	112.15	336.83	65.60	34.57	0.96	−0.02
	Mo	ND	ND	ND	ND	ND	ND	ND	ND
	Ni	67.02	53.92	33.46	188.64	39.59	59.08	2.19	3.88
	Pb	ND	ND	ND	ND	ND	ND	ND	ND
	Zn	42.90	36.50	14.78	139.54	29.84	69.56	2.32	4.63
Manure (*n* = 8)	As	1.26	0.08	0.00	6.00	2.23	177.31	1.46	0.54
	Cd	ND	ND	ND	ND	ND	ND	ND	ND
	Co	7.30	7.90	3.14	11.50	3.38	46.25	−0.11	−1.56
	Cr	54.72	25.17	14.87	189.83	60.17	109.96	1.62	1.26
	Cu	29.25	19.58	6.17	64.00	22.76	77.82	0.49	−1.47
	Fe	2860.60	2318.30	1577.80	6076.70	1466.40	51.26	1.45	0.95
	Mn	89.88	62.08	35.76	204.50	60.33	67.12	0.96	−0.49
	Mo	0.46	0.00	0.00	2.50	0.87	188.60	1.88	2.06
	Ni	48.00	41.83	25.33	101.17	25.70	53.54	1.16	0.27
	Pb	ND	ND	ND	ND	ND	ND	ND	ND
	Zn	67.76	36.37	20.33	214.33	69.14	102.04	1.41	0.51
Compost (*n* = 8)	As	1.00	0.50	0.00	4.67	1.58	158.11	1.75	1.83
	Cd	ND	ND	ND	ND	ND	ND	ND	ND
	Co	11.25	11.83	6.33	16.17	3.30	29.32	−0.09	−1.13
	Cr	64.15	48.33	26.67	186.17	50.65	78.95	2.03	2.57
	Cu	47.23	46.92	29.83	64.00	10.95	23.19	−0.04	−0.88
	Fe	5781.90	5896.70	4146.70	7061.70	1006.00	17.40	−0.46	−0.96
	Mn	150.27	137.33	120.67	195.17	31.29	20.82	0.46	−1.49
	Mo	1.56	0.67	0.00	5.17	1.96	125.17	0.85	−0.72
	Ni	51.04	43.42	29.17	110.33	25.59	50.14	1.74	1.83
	Pb	ND	ND	ND	ND	ND	ND	ND	ND
	Zn	95.06	72.08	52.50	190.00	51.23	53.89	0.98	−0.59

of 0.01–0.1 had lower risk, 0.1–1.0 had medium risk, and >1.0 had high risk.

2.6. Data analyses

The residual concentrations of the antibiotics in samples were statistically analyzed using Microsoft Excel and Statistics 8.01 program. Descriptive statistics were used to calculate the minimum, maximum, mean, skewness, kurtosis, and coefficient of variation (CV) of the data.

3. Results and discussion

3.1. Physiochemical characterization of the collected samples

Descriptive analyses of physiochemical properties of soil, manure, and compost samples are shown in Table 3. Soil samples indicated slightly alkaline and saline nature of the soil as indicated with pH and EC values. Overall, higher values of CV and lower skewness depicted more variability and more symmetry. The variation in soil EC, OM, and CEC could be due to the degradation and mobility of certain salts and organic compounds by compost and manure waste dumped in the surroundings. Owing to soil calcareousness, a reduced amount of mean P was found, while, mean NO$_3^-$ contents were higher. Some cations (Ca, Mg, Na, K) were detected in the normal range in collected soil samples, while higher CV values showed a diverse distribution of these cations among analyzed soil samples. Chemical analyses of studied manure and compost samples detected pH, EC, and CEC in a similar range while in comparison with compost, higher percentage of OM was found in manure. Both manure and compost were found enriched in available P contents, while compost possessed a comparatively higher amount

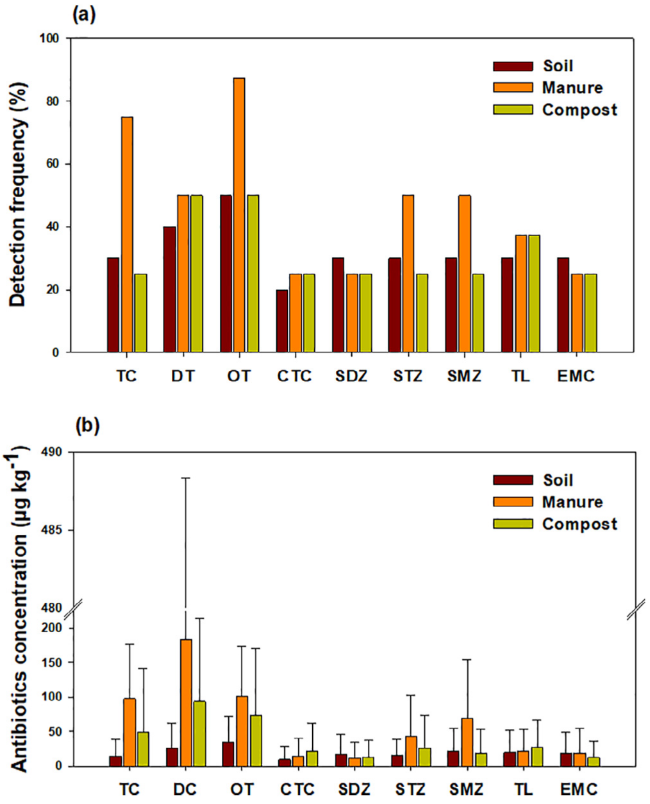

Fig. 2. Detection frequency (a) and average concentrations (b) of tetracycline (TC), doxycycline (DC), oxytetracycline (OT), chlortetracycline (CTC), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMZ), tylosin (TL), and erythromycin (EMC) in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia.

Table 5
Descriptive analyses for the antibiotics (μg kg^{-}) in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia.

Source	Antibiotics	Mean	Median	Min.	Max.	SD	C.V.	Skew	Kurtosis
Soil (*n* = 20)	TC	14.94	0.00	0.00	62.20	23.87	159.83	1.01	−0.79
	DC	26.54	0.00	0.00	85.65	34.88	131.44	0.66	−1.31
	OT	34.52	24.78	0.00	381.50	37.34	108.17	0.30	−1.58
	CTC	9.35	0.00	0.00	49.40	19.21	205.54	1.51	0.31
	SDZ	18.00	0.00	0.00	62.69	28.24	156.90	0.88	−1.21
	STZ	15.33	0.00	0.00	58.60	24.19	157.83	0.92	−1.09
	SMZ	21.27	0.00	0.00	88.95	34.19	160.74	1.03	−0.72
	TL	19.92	0.00	0.00	78.95	31.61	158.70	0.96	−0.96
	EMC	18.60	0.00	0.00	81.56	30.01	161.36	1.07	0.57
Manure (n = 8)	TC	97.96	100.32	0.00	201.01	78.01	79.64	−0.08	−1.49
	DC	183.29	29.26	0.00	750.25	305.01	166.41	1.19	−0.44
	OT	101.24	85.22	0.00	249.00	71.72	70.85	0.90	0.62
	CTC	14.23	0.00	0.00	62.10	26.50	186.18	1.19	−0.52
	SDZ	12.05	0.00	0.00	55.45	22.65	187.93	1.25	−0.29
	STZ	43.18	25.54	0.00	171.05	59.29	137.32	1.31	0.72
	SMZ	69.58	34.81	0.00	198.46	85.23	122.50	0.61	−1.26
	TL	22.19	0.00	0.00	67.44	30.86	139.09	0.57	1.61
	EMC	18.93	0.00	0.00	88.45	35.70	188.62	1.28	−0.20
Compost (n = 8)	TC	49.59	0.00	0.00	206.25	91.93	185.36	1.16	−0.64
	DC	93.26	20.82	0.00	270.25	119.94	0.60	0.57	−1.52
	OT	74.21	23.77	0.00	255.40	96.65	130.65	0.84	−0.68
	CTC	20.89	0.00	0.00	112.00	41.56	198.96	1.58	0.91
	SDZ	13.11	0.00	0.00	53.95	24.29	185.27	1.16	−0.65
	STZ	25.99	0.00	0.00	109.46	48.21	185.51	1.17	−0.62
	SMZ	18.32	0.00	0.00	86.99	34.70	189.44	1.30	−0.09
	TL	27.66	0.00	0.00	85.41	38.89	140.58	0.62	−1.50
	EMC	12.57	0.00	0.00	51.15	23.28	185.20	1.16	0.66

TC: tetracycline, DC: doxycycline, OT: oxytetracycline, CTC: chlortetracycline, SDZ: sulfadiazine, STZ: sulfathiazole, SMZ: sulfamethoxazole, TL: tylosin, and EMC: erythromycin.

of NO_3^- (Idrees et al., 2018). A major diversity was found in manure and compost samples concerning the distribution of cations, available P, and certain chemical characteristics. Moisture and volatile contents were higher in manure and compost, while resident carbon was lower to medium with a maximum value of 40.74% and 40.39% in manure and compost, respectively. In all collected soil samples, sand particles were found in a significantly higher percentage followed by silt and clay particles (Fig. 1a). Sandy loam texture was dominant, followed by loamy sand and loam (Fig. 1b). Majority of the samples shown bulk density in the range of 1.6–1.7 g cm^{-3} (Fig. 1c).

3.2. Prevalence of metal/metalloids in the collected samples

Table 4 shows the descriptive analyses of metal/metalloid contents in soil, manure, and compost samples. Most of the metals were below the maximum allowable limit, while, the concentrations of iron (Fe) and manganese (Mn) were higher in studied soil, manure, and compost samples followed by zinc (Zn), nickel (Ni), and chromium (Cr). Fe contents were the highest in soil samples i.e., 7923.30–16,657.00 mg kg^{-1}. Among observed metal/metalloids, Mn was ordered second-highest metal in soil with the mean value of 189.76 mg kg^{-1} which is counted under the critical limits of 400 mg kg^{-1} as suggested by FAO/WHO (2001). The mean Cr concentration was 55.38 mg kg^{-1}, which might has accumulated due to leaching from topsoil area, sanitary landfills, and some anthropogenic activities (Rafique et al., 2021).

A similar trend of heavy metals concentration was found in manure and compost samples. Among all observed metals in manure and compost, Fe was found in the highest concentration in manure (1577.80–6076.70 mg kg^{-1}) and compost (4146.70–7061.70 mg kg^{-1}) followed by Mn, Zn, Cr, and Ni. Heavy metals concentration in animal manure usually depends on the type of feed consumed by animals and feed efficiency for animal nutrition aspect. Heavy metals may persist un-degradable in manure and compost and therefore, long term application results in their accumulation is soil (Alvarenga et al., 2015).

3.3. Residues of antibiotics in collected samples

The detection frequency and mean concentrations of nine veterinary antibiotics detected in soil, manure, and compost samples collected from Al-Kharj are shown in Fig. 2. All the antibiotics were detected in the soil, manure, and compost samples with detection frequencies in the range of 25%–87.5%. The detection frequencies for all the antibiotics were higher in manure samples, as compared to the soil and compost samples. OT showed a higher frequency in manure samples, followed by TC, STZ, and SMZ. Overall, the mean concentrations of all the antibiotics were higher in manure samples, indicating their excretion from the animals without complete absorption. The concentrations of the antibiotics in compost samples were relatively lower, indicating the degradation of some of the antibiotics during the composting process due to higher temperatures (Zhou et al., 2020). Soil samples exhibited lower concentrations of all the antibiotics, which could be due to the dilution as well as degradation of the antibiotics after the application of manure and compost (Yang et al., 2016). It was observed that the mean concentrations of DC were higher in both manure and compost samples, followed by OT and TC. Overall, the mean concentrations of all the tested antibiotics in all matrices (soil, manure, and compost) were below 50 μg kg^{-1}, with exception of TC, DC, OT, and SMZ.

The descriptive analyses for the studied antibiotics in soil, manure, and compost samples are shown in Table 5. Soil samples contained the highest mean concentration of OT (34.52 μg kg^{-1}) with a maximum concentration of 381.50 μg kg^{-1}, followed by DC (26.54 μg kg^{-1}), whereas, the concentrations of SDZ, SMZ, TL, and EMC are comparable (18.00–21.27 μg kg^{-1}). The values of SD for all soil samples were in the range of 19.21 to 37.34. The higher CV values (108.17%–205.54%) for the studied antibiotics in the soil

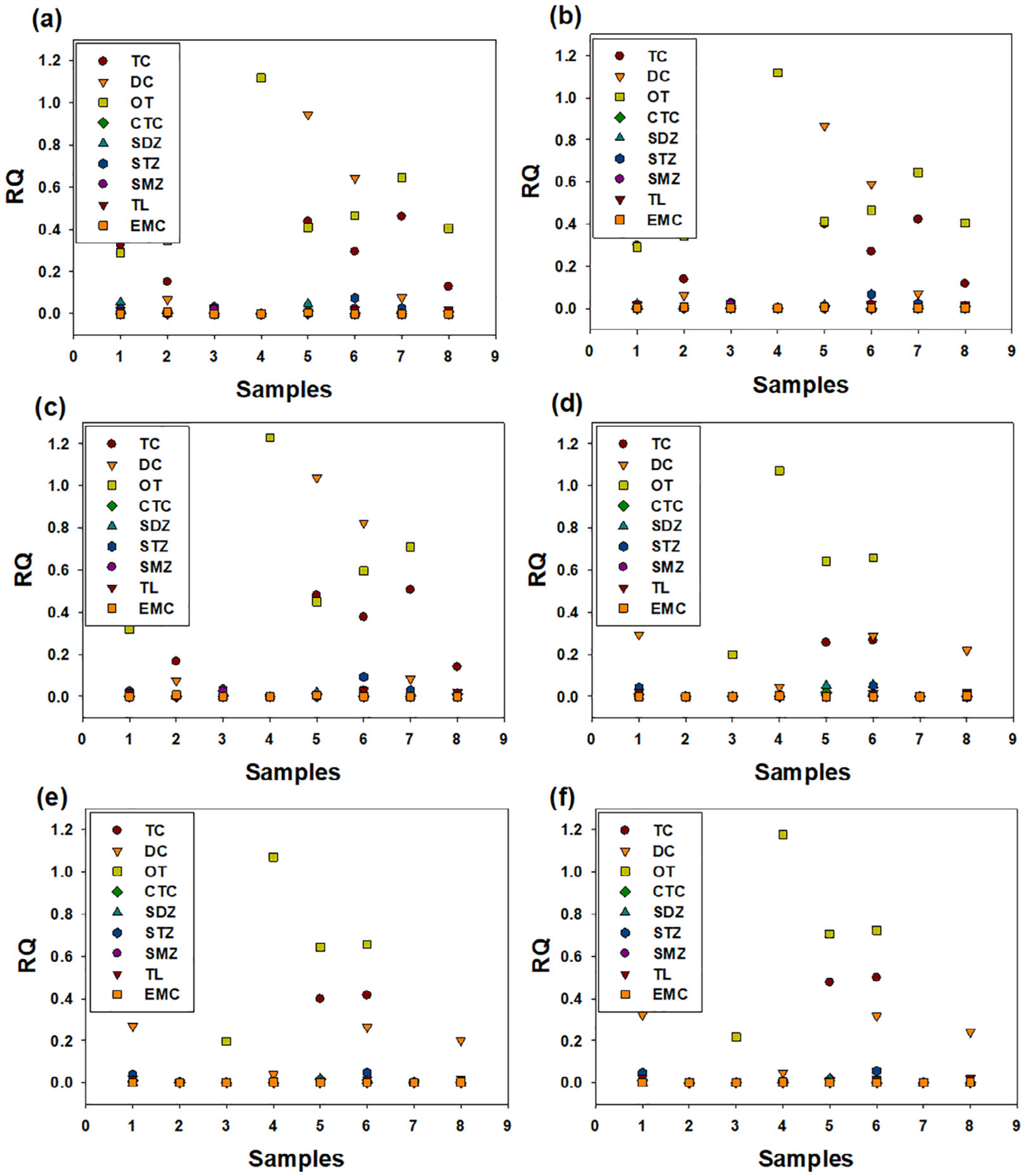

Fig. 3. Risk quotient (RQ) of tetracycline (TC), doxycycline (DC), oxytetracycline (OT), chlortetracycline (CTC), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMZ), tylosin (TL), and erythromycin (EMC) calculated for manure application to sandy loam soil (a), manure application to loamy sand soil (b), manure application to loam soil (c), compost application to sandy loam soil (d), compost application to loamy sand soil (e), and compost application to loam soil (f).

samples indicated a huge variability in the data. The skewness of the data was closer to zero with negative kurtosis, except for EMC. For the manure samples, the mean concentration of DC was higher (183.29 µg kg^{-1}), followed by OT (101.24 µg kg^{-1}) and TC (97.96 µg kg^{-1}), while SDZ and CTC were the lowest among the studied antibiotics (12.05 and 14.23 µg kg^{-1}, respectively). Alike soil samples, the calculated CV values were higher (70.85%–188.62%), suggesting the higher variability in the data. Further, the data was slightly skewed as shown by the values closer to zero. The kurtosis for OT, STZ, and TL was negative, while it was positive for the rest of the studied antibiotics. Akin to the manure samples, the mean concentration of DC (93.26 µg kg^{-1}) was higher in compost samples as compared to other studied antibiotics, followed by OT (74.21 µg kg^{-1}) and TC (49.59 µg kg^{-1}). The mean concentrations of SDZ (13.11 µg kg^{-1}) and EMC (12.57 µg kg^{-1}) were the lowest in compost samples, as compared to the other studied antibiotics. Similar to the soil and manure samples, the data for the studied antibiotics in compost samples had a higher variability (CV = 130.65%–198.96%), with a slightly normal distribution (skewness = 0.55–1.58), and negative kurtosis. Overall, the mean concentrations of TC, DC, OT, SMZ, and STZ were higher in manure samples, while the mean concentrations of CTC and TL were higher in compost samples and that of SDZ was higher in soil samples. The higher concentrations of tetracycline and sulfonamide antibiotics in manure samples have already been reported in various parts of the world (Wang et al., 2017). For instance, the reported concentrations of SDZ in Malaysia is 23,000 µg kg^{-1}, Denmark is 2000 µg kg^{-1}, and China is 800 to 46,370 µg kg^{-1}, which is far higher compared with the detected SDZ concentration in this study (Wang et al., 2017). Likewise, the reported maximum concentrations of OT, TC, and CTC in swine manure in different countries were in the range of 15,680–173,200 µg kg^{-1}, 4000–136,000 µg kg^{-1}, and 11,630–172,900 µg kg^{-1}, respectively (Pan et al., 2011; Wang et al., 2017). Thus, it can be seen that relatively lower concentrations of residual OT, TC, CTC, and SDZ in manure were detected in the current study.

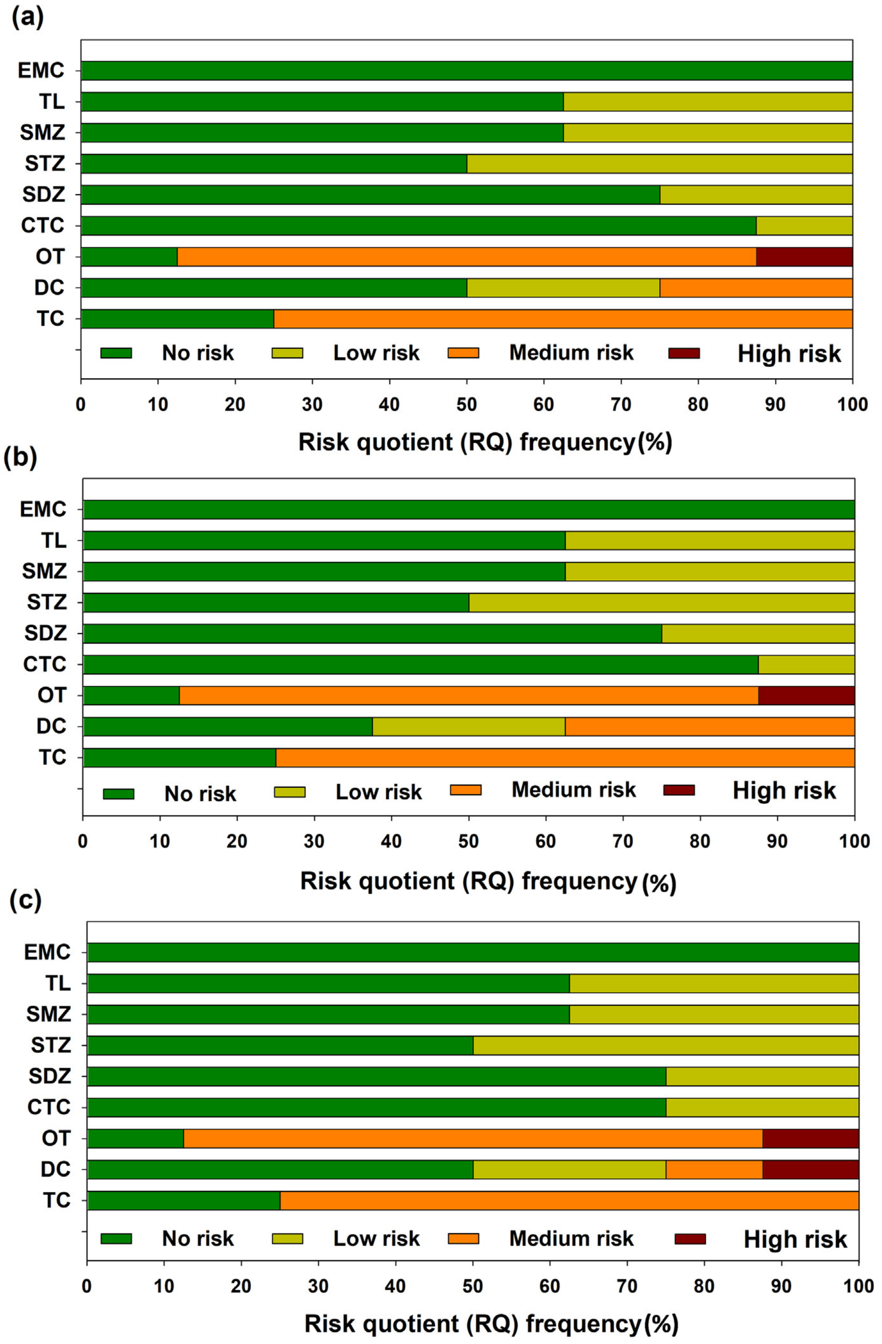

Fig. 4. Frequency of risk quotient (RQ) for tetracycline (TC), doxycycline (DC), oxytetracycline (OT), chlortetracycline (CTC), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMZ), tylosin (TL), and erythromycin (EMC) for manure application to sandy loam soil (a), loamy sand soil (b), and loam soil (c).

Composting is considered to remove/degrade the prevailing antibiotics from the manure; however, the presence of various antibiotics has already been reported even after the composting process (Zhou et al., 2020). Though the concentrations of many antibiotics were lower in compost as compared to the manure; however, CTC, TL, and SDZ were even higher than that of manure. Higher concentrations of these antibiotics could impose harmful impacts on the ecosystem and human health if applied as soil amendments. The prevalence of these antibiotics in soil has already been reported as a result of the direct or indirect application of manure or compost to agricultural lands (Hamscher et al., 2002; Li et al., 2011). Nevertheless, the concentrations of the antibiotics in soil were relatively lower in the current study as compared to the other reported concentrations around the world.

3.4. Potential environmental risk assessment

The risk quotient (RQ) was estimated through the ratio of measured environmental concentration (MEC) to the predicted no-effect concentration (PNEC) (European Commission, 2003). The values of the PNEC used in this study were taken from the literature (Zhou et al., 2020; Chen et al., 2018; Thiele-Bruhn and Beck, 2005). Overall, the calculated RQ values were larger for the manure samples, than that of the compost samples (Fig. 3). The RQ values for OT were higher (0–1.228) in all the samples, followed by DC (0–1.034), while the lowest RQ values were found for EMC (0–0.007). The calculated RQ values for sandy loam and loamy sand soil were comparable, whereas, these values were higher for the loam soils.

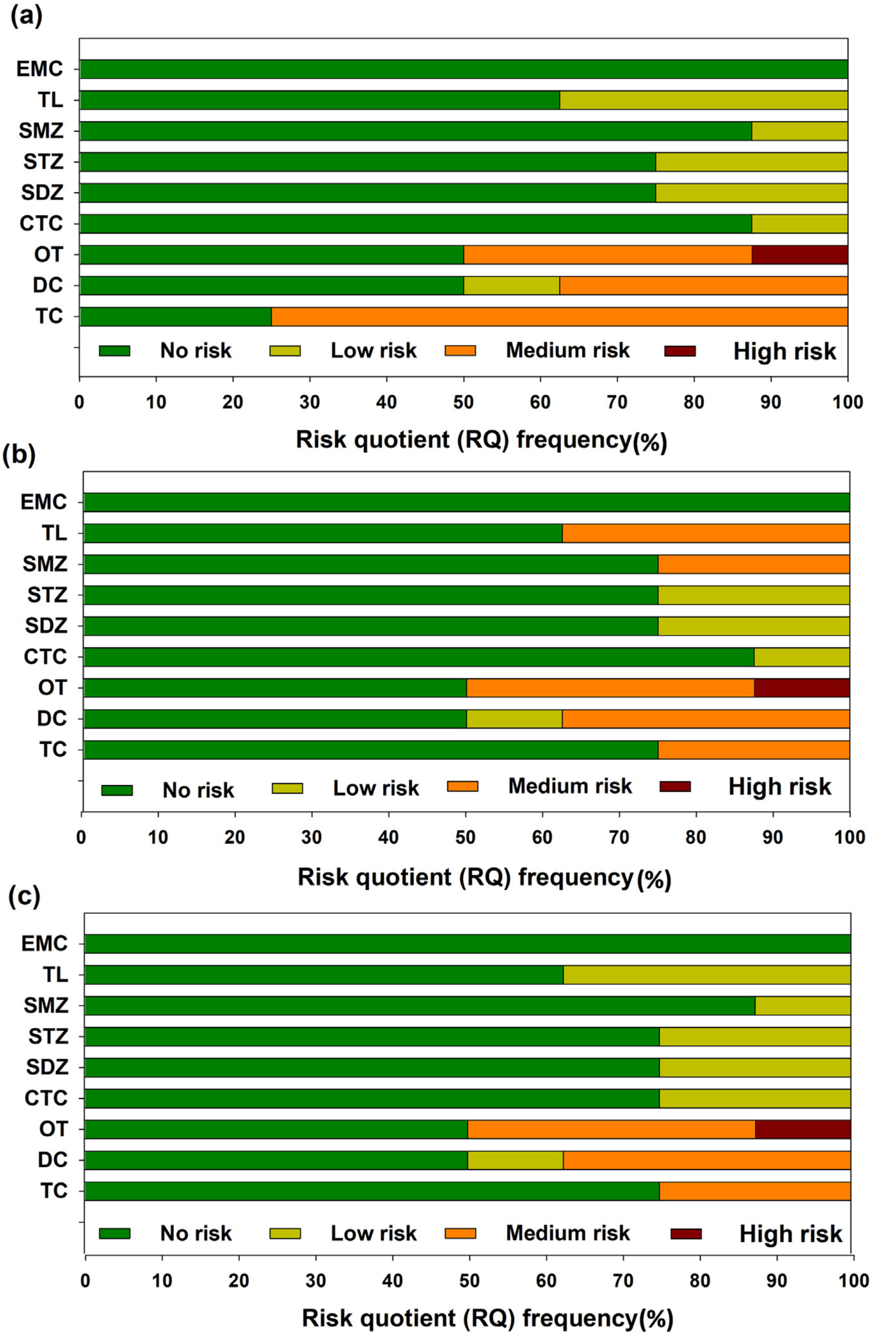

Fig. 5. Frequency of risk quotient (RQ) tetracycline (TC), doxycycline (DC), oxytetracycline (OT), chlortetracycline (CTC), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMZ), tylosin (TL), and erythromycin (EMC) compost application to sandy loam soil (a), loamy sand soil (b), and loam soil (c).

For better understanding, the RQ values were classified into 4 categories i.e., no risk (<0.01), low risk (0.01–0.1), medium risk (0.1–1.0), and high risk (>1.0). Fig. 4 represents the RQ frequencies for manure addition to three differently textured soil. For majority of the samples, the higher proportions were in the category of no or low risk. Overall, CTC, SDZ, STZ, TL, and EMC were not seen to pose serious ecological risks as predicted by the lower RQ values (<0.1); however, OT, DC, and TC were seen to possess medium to high ecological risks. The RQ values for OT were above 1.0 for 12.5% samples in sandy loam, loamy sand, and loam soil, indicating higher ecological risks. Likewise, 12.5% of samples exhibited a higher ecological risk of DC contamination in loam textured soils. The proportions of samples for posing medium ecological risks of OT, DC, and TC were 75%, 25%, and 75% for sandy loam soils, 75%, 37.5%, and 75% for loamy sand soils, and 75%, 12.5%, and 75% for loam soils, respectively. Previously, Qian et al. (2016) reported lower to medium ecological risks of tetracyclines owing to their higher concentrations in manures. Alike, the calculated RQ frequencies for compost addition to different soils are presented in Fig. 5. Similar to the manure amendments, the majority of the samples showed low to no ecological risks after compost addition into the soil. CTC, TC, STZ, SDZ, SMZ, TL, and EMC generated RQ values below 0.1, except for OT and DC in loamy sand soil. Among all the studied antibiotics, 12.5% of samples had shown possible ecological risk due to OT in all three types of soils. Possible medium risk due to OT, DC, TC, TL, and SMZ was in the range of 25%–75% as shown by the RQ values of 0.1–1.0. Therefore, these results suggested that the majority of the studied antibiotics were not posing adverse ecological effects; whereas, possible adverse effects of OT and DC can be expected after application of compost and manure in soil. Therefore, long-term application of manure and compost containing tetracyclines should be avoided (Zhou et al., 2020). However, multi-antibiotics toxicity and the effects of other co-existing contaminants were not investigated in this study, which if considered, could significantly increase the potential of ecological risk (Chen and Zhou, 2014). The potential ecological risks due to manure application were higher as compared to the compost application, suggesting that compost could be relatively a safer amendment than manure (Zhang et al., 2015). Therefore, attention should be given to the administration of antibiotics in veterinary farms, as well as the treatment and application of manure and compost to the soil. Moreover, further studies are required to develop methods to reduce/degrade antibiotics in manure and compost-based products.

4. Conclusion

The residues of antibiotics in soil, manure, and compost samples collected from Al-Kharj, Saudi Arabia were investigated in this study. Results demonstrated that antibiotics residues were higher in manure samples, followed by compost, and soil. Tetracycline (TC), doxycycline (DC), oxytetracycline (OT), and sulfamethoxazole (SMZ) were in higher concentrations in manure (up to 183.29 $\mu g\ kg^{-1}$) and compost (up to 93.26 $\mu g\ kg^{-1}$). The estimated risk quotient (RQ) values suggested that the majority of the studied antibiotics were not posing adverse ecological effects. However, OT and DC indicated a higher ecological risk, while TC indicated medium ecological risk in soil. Thus, special attention should be given to the use of manure and compost in agroecosystem to avoid any adverse ecological impacts. Moreover, further studies are needed to understand the transformation and fate of various antibiotics in the soil after manure and compost addition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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