

# Inhibition of nitrification in soil by metal diethyldithiocarbamates

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**Abstract:** Nitrification acts as a key process in determining fertilizer use efficiency by crops as well as nitrogen losses from soils. Metal dithiocarbamates in addition to their pesticidal properties can also inhibit biological oxidation of ammonium (nitrification) in soil. Metal [M = V(III), Cr(III), Mn(II), Fe(III), Ni(II), Cu(II), Zn(II) and Co(II)] diethyldithiocarbamates (DEDTC) were synthesized by the reaction of sodium diethyldithiocarbamate with metal chloride in dichloromethane/water mixture. These metal diethyldithiocarbamates were screened for their ability to inhibit nitrification at different concentrations (10 µg/g soil, 50 µg/g soil and 100 µg/g soil). With increasing concentration of the complex, capacity to retard nitrification increased but the extent of increase varied for different metals. At 100 µg/g soil, different complexes showed nitrification inhibition from 22.36% to 46.45%. Among the diethyldithiocarbamates tested, Zn(DEDTC)<sub>2</sub> proved to be the most effective nitrification inhibitor at 100 µg/g soil. Manganese, iron and chromium diethyldithiocarbamates also proved to be effective nitrification inhibitors than the others at 100 µg/g soil. The order of percent nitrification inhibition in soil by metal diethyldithiocarbamates was: Zn(II) > Mn(II) > Fe(III) > Cr(III) > V(III) > Co(II) > Ni(II) > Cu(II).

**Keywords:** nitrification inhibitor diethyldithiocarbamate; metal complexes; nitrogenous fertilizers; pesticides

## Introduction

Nitrification is a process in which ammonium form of nitrogen is converted into nitrate form. Nitrogen use efficiency in terms of plant uptake is generally low and varies greatly under different soil and cropping conditions. Most fertilizer N applied to soils is in the form of ammonium- or ammonium producing compounds such as urea, and is usually oxidized rapidly to nitrate by nitrifying microorganisms in soils. Application of nitrogen fertilizers more than optimum levels leads to low nitrogen recoveries and greater nitrogen accumulation in the soil profile. The accumulated nitrate-N is susceptible to losses through leaching and may become a contaminant of ground and surface water. Nitrates when ingested in food or water may be reduced to nitrites by bacteria in mouth and gut (Shrivastava, 2000). If nitrite-containing water is utilized for drinking purposes, it can react with secondary amine present in the human body and may form carcinogenic nitrosoamines (Wu, 1983; Shrivastava, 1995). Recent concerns about environmental effects of intensive fertilizers has created widespread interest in the possibility of reducing these effects through the use of compounds that will retard nitrification of ammonium or ammonium yielding fertilizers in soils and thereby reduce the contribution of fertilizer N to nitrate contamination of ground and surface waters (Bundy, 1973; Bijay-Singh, 1995).

Metal dithiocarbamates are already in use as pesticides (Malik, 1999). Besides pesticidal activity, some work has been reported on the soil nitrification inhibition capacity of metal carbamates and their thioanalogs. Sodium diethyldithiocarbamate inhibited nitrification up to 12 per cent at 10 ppm concentration after 14 d of incubation (Bundy, 1973). Nishihara (Nishihara, 1962) reported that soil fumigant, Vapam, inhibit both ammonia and nitrate oxidation. According to Stratton *et al.* (Stratton, 2002), Captan, a soil fungicide can inhibit nitrification to some extent without affecting the plant growth. The purpose of the present work was to synthesize some transition metal diethyldithiocarbamates and to investigate their effectiveness as nitrification inhibitors in soils.

## 1 Materials and methods

Metal [M = V(III), Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II)] diethyl-dithiocarbamates were synthesized by the reaction of sodium diethyldithiocarbamate with metal sulphate/

chloride in dichloromethane/water mixture(Arora, 2003) .

The soil used for present investigation was crushed and passed through 2 mm sieve. Soil was analyzed for pH, electrical conductivity and organic carbon content. The organic carbon content was determined by wet digestion method of Walkley and Black(Walkley, 1934) .

To study the effect of metal complexes as nitrification inhibitors, 10 g portions of air-dried, sieved soils were taken in plastic vials and treated with 1ml of solution containing 2 mg of nitrifiable nitrogen as ammonium sulphate. The stock solutions/suspensions(500 ppm) of metal complexes were prepared and these were serially diluted to obtain 250 ppm and 50 ppm. Two milliliter solution/suspension of the metal complex was applied to 10 g soil sample. Each treatment was replicated thrice. Soils were established at field capacity moisture regime. The vials were covered with parafilms having holes for aeration, weighed and incubated at 25 ± 1 °C for 21 d. Weights were checked weekly for moisture loss during incubation and were compensated by adding water. Control was kept along with the treatments .

After 21 days, soil samples were analyzed for KCl-extractable ammonium-N and nitrate-N to estimate the extent of nitrification. The contents in the vials were shaken with known volume of 2 mol/L KCl for one hour. The suspensions were filtered and the filtrates were analyzed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> forms of nitrogen following microkjeldahl method(Keeney, 1966) . A blank without soil was also carried out. The amount of ammonium and nitrate nitrogen was calculated using following formula:

$$14 \cdot 1000 \cdot a \cdot b / c \cdot 1 / d \cdot e \cdot 10^6,$$

where *a* is the normality of H<sub>2</sub>SO<sub>4</sub> used (N); *b* is the volume of KCl used for extraction + moisture content of soil(ml); *c* is the weight of soil taken(g); *d* is the volume of KCl extract used for estimation of nitrogen(ml) and *e* is the volume of sulphuric acid used for NH<sub>4</sub><sup>+</sup> -N/NO<sub>3</sub><sup>-</sup> -N (ml) .

The percent nitrification inhibition was calculated using the formula;

$$(C - S) / C \cdot 100,$$

where *C* is the nitrification in control and *S* is the nitrification in treatment .

2 Statistical analysis

Analysis of variance was based on following statistical model.

$$X_{ij} = m + g_i + b_j + e_{ij}, \quad i = 1, 2 \cdots p, \quad j = 1, 2, \cdots R,$$

where, *X<sub>ij</sub>* is the observations in *i*th treatment *j*th replication; *m* is the overall mean; *g<sub>i</sub>* is the effect of *i*th treatment; *b<sub>j</sub>* is the effect of *j*th replication; *e<sub>ij</sub>* is the random error component; *p* is the number of treatments; *r* is the number of replications .

The analysis of variance based on this mathematical model is given in Table 1 .

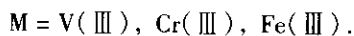
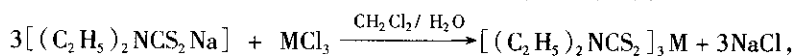
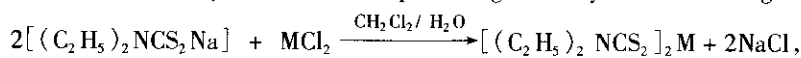
Table 1 The analysis of variance

Source	d . f .	Sum of squares	Mean squares	F ratio
Replications	<i>r</i> - 1	$\sum_{j=1}^r X_{.j}^2 / p - X^2 . . / rp$	<i>M<sub>r</sub></i>	$F = (r - 1) / (p - 1) (r - 1) = M_r / M_e$
Treatment	<i>p</i> - 1	$\sum_{i=1}^p X_{i.}^2 / r - X^2 . . / rp$	<i>M<sub>g</sub></i>	$F = (p - 1) / (p - 1) (r - 1) = M_g / M_e$
Error	( <i>p</i> - 1) ( <i>r</i> - 1)	$\sum_{i=1}^p \sum_{j=1}^r X_{ij}^2 - X_i^2 / r - \sum_{j=1}^r X_{.j}^2 / p + X^2 . . / rp$	<i>M<sub>e</sub></i>	

Notes: *X<sub>ij</sub>*, *p* and *r* are same as defined earlier; *X<sub>i</sub>* = *i*th treatment total; *X<sub>j</sub>* = *j*th replication total, and *X . .* = total of *rp* experimental units; Standard error( *S . E .* ) = √2 × error mean square/ number of replications; LSD = *S . E .* × *t* - ratio

3 Results and discussion

Metal diethyldithiocarbamates were synthesized by displacement reaction of sodium diethyldithiocarbamate with the corresponding metal chloride in dichloromethane/water mixture. The organic layer was dried over anhydrous sodium sulphate to get the crystals of resulting metal diethyldithiocarbamate.



The molecular formulae of the complexes were deduced from elemental analysis and were characterized by IR-spectroscopic studies. Relevant data are given in Table 2.

Table 2 Analytical data of metal diethyldithiocarbamates

Complex	Melting point, °C	Specific conductance, s/m	Elemental analysis						IR-spectral data, cm <sup>-1</sup>			
			% of metal		% of carbon		% of nitrogen		C-N	C-S	N-C	S-S
			Calculated	Experimental	Calculated	Experimental	Calculated	Experimental				
V(DEDTC) <sub>3</sub>	61—63	1.60 × 10 <sup>-3</sup>	10.28	9.09	36.37	37.41	8.49	8.90	1466	976	1138, 1196, 1263	
Cr(DEDTC) <sub>3</sub>	62—64	2.34 × 10 <sup>-3</sup>	10.49	10.03	36.30	37.23	8.47	8.93	1459	989	1159, 1227, 1269	
Mn(DEDTC) <sub>2</sub>	68—70	3.14 × 10 <sup>-3</sup>	15.67	14.95	34.19	35.22	7.98	8.81	1459	966	1159, 1196, 1270	
Fe(DEDTC) <sub>3</sub>	250—52	3.53 × 10 <sup>-3</sup>	11.20	10.85	36.00	36.46	8.40	8.49	1458	983	1153, 1203, 1267	
Co(DEDTC) <sub>2</sub>	263—65	3.58 × 10 <sup>-3</sup>	16.60	16.06	33.81	34.97	7.99	8.12	1458	990	1133, 1212, 1266	
Ni(DEDTC) <sub>2</sub>	230—32	3.52 × 10 <sup>-3</sup>	16.52	16.01	33.84	33.89	7.90	7.83	1459	988	1148, 1203, 1271	
Cu(DEDTC) <sub>2</sub>	194—96	2.42 × 10 <sup>-3</sup>	17.66	17.19	33.38	33.48	7.79	7.68	1459	999	1143, 1204, 1269	
Zn(DEDTC) <sub>2</sub>	170—72	3.22 × 10 <sup>-3</sup>	18.01	18.91	33.24	33.26	7.76	7.90	1459	991	1143, 1202, 1269	

Notes: DEDTC = diethyldithiocarbamate

Nitrification acts as key process in determining fertilizer use efficiency by crops as well as nitrogen loses from soils. Even under the best circumstances no more than two thirds of the nitrogen added as fertilizer or biological nitrogen fixation can be utilized by crop plants or in the soil. Metal diethyldithiocarbamates can influence biological oxidation of ammonia in soils. Metal diethyldithiocarbamates were tested for their role in inhibiting the microbiological process of nitrification in the soil. The fate of nitrogen compounds present in the soil depends on a number of factors such as temperature, moisture content, pH and soil texture, cropping conditions, microorganisms present in the soil, nitrification-denitrification processes etc. Nitrogen present in soil is mainly lost as ammonia volatilization, denitrification and leaching. Excessive nitrification leads to the accumulation of nitrates and results in leaching from soil to ground water (Shrivastava, 1988; Olaniya, 1977). On the other hand, reduced nitrification leads to accumulation of ammonium nitrogen, which is lost via volatilization. Therefore, a balance between ammonium and nitrate nitrogen is imperative.

Metal dialkyldithiocarbamates were tested as soil nitrification inhibitors. The characteristics of the soil selected for study were: pH (7.8), organic carbon content (0.35%) and electrical conductivity (0.25 s/m). The soil samples were

Table 3 Percent nitrification inhibition in soil by metal diethyldithiocarbamates

Complex	Amount of metal diethyldithiocarbamate applied		
	10 µg/g soil	50 µg/g soil	100 µg/g soil
V(DEDTC) <sub>3</sub>	6.69	25.27	33.32
Cr(DEDTC) <sub>3</sub>	7.17	33.41	40.37
Mn(DEDTC) <sub>2</sub>	5.90	17.25	41.65
Fe(DEDTC) <sub>3</sub>	2.80	05.80	41.07
Co(DEDTC) <sub>2</sub>	5.30	09.22	30.80
Ni(DEDTC) <sub>2</sub>	7.81	09.73	23.77
Cu(DEDTC) <sub>2</sub>	2.67	02.70	22.36
Zn(DEDTC) <sub>2</sub>	8.96	11.11	46.25
LSD (P = 0.05)	1.15	0.75	0.80

Note: DEDTC = diethyldithiocarbamate

treated with metal complexes of diethyldithiocarbamate (DEDTC) ligand namely vanadium diethyldithiocarbamate [V (DEDTC)<sub>3</sub>], chromium diethyldithiocarbamate [Cr (DEDTC)<sub>3</sub>], manganese diethyldithiocarbamate [Mn (DEDTC)<sub>2</sub>], ferric diethyldithiocarbamate [Fe (DEDTC)<sub>3</sub>], cobalt diethyldithiocarbamate [Co (DEDTC)<sub>2</sub>], nickel diethyldithiocarbamate [Ni (DEDTC)<sub>2</sub>], copper diethyldithiocarbamate [Cu (DEDTC)<sub>2</sub>] and zinc diethyldithiocarbamate [Zn (DEDTC)<sub>2</sub>] at three different concentrations i.e. 100 µg/g soil, 50 µg/g soil and 10 µg/g soil for studying nitrification inhibition. The data pertaining to nitrification inhibition capacity of different complexes is given in Table 3. Data revealed that with increase in the concentration of metal complex applied, there is an increase in the nitrification inhibition capacity. The complexes exhibited a maximum inhibition at 100 µg/g soils, although different complexes registered nitrification inhibition from 22.36% to 46.25%. Zinc diethyldithiocarbamate was most effective in inhibiting nitrification (46.45%), followed by manganese diethyldithiocarbamate (41.65) which was at par with ferric diethyldithiocarbamate (41.07). Chromium diethyldithiocarbamate inhibiting 40.37% nitrification was statistically same as the ferric complex. For other metal complexes nitrification inhibition varies from 22.36% to 33.32%. The order of per cent nitrification inhibition in soil by metal diethyldithiocarbamates is Zn(II) > Mn(II) > Fe(III) > Cr(III) > V(III) > Co(II) > Ni(II) > Cu(II). In addition to nitrification inhibition property, these complexes possess well known fungicidal (Arora, 2003; Kamble, 2000) and insecticidal (Zorc, 1999) properties, and hence can be better exploited as agrochemicals. However, more investigations will be needed to investigate other environmental consequences of these compounds.

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