Effects of some trace elements on urea hydrolysis in soils

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Summary

The aim of this work was to evaluate iron, copper, and zinc ions as inhibitors to control urea hydrolysis, biosynthesis of urease, and ammonia evolution in some Spanish soils treated with urea.

The main results obtained in this investigation can be summarized as follows.
— These ions inhibited the urease activity by 7-20 % when applied at 20 μg g^{-1} of soil.
— The inhibitory effect was most pronounced in light-textured soils. The cupric ion, in this respect, was the most effective one.
— These elements did not inhibit microbial production of urease in two soils tested, even when applied at 20 μg g^{-1} of soil.
— Compared with zinc and ferrous ions, the cupric ion markedly retarded urea hydrolysis and reduced the loss of urea-N as ammonia, in one soil tested.

Introduction

The rapidly increasing importance of urea as a nitrogen fertilizer in world agriculture has emphasized the need for research to find methods of reducing some of the problems encountered in its use as a fertilizer.

One approach to reduce the problems associated with the use of urea as a fertilizer is to find agents to retard its hydrolysis when applied to soils. Any compound proposed to inhibit urease activity in soils must be cheap, effective and safe to use. For effectiveness, the inhibitor must dissolve and move with the fertilizer urea when its granules are applied to the soil. This approach has received considerable attention, and many compounds have been tested and patented as inhibitors of urea hydrolysis in soils (Anderson, 1962, 1969; Besekau et al., 1974;

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Bremner & Douglas, 1971a; Geissler et al., 1970; Hyson, 1963; Peterson & Walter, 1970; Sor, 1968, 1969; Sor et al., 1968, 1971; Tomlinson, 1967). Most of these inhibitors are inorganic or organic compounds, previously shown to be potent inhibitors of urease activity in vitro.

Peterson & Walter (1970), however, claim that urea hydrolysis in soils can also be retarded through addition of compounds that function as anti-metabolites towards urease-producing micro-organisms. In these cases the inhibitory effect on urea hydrolysis in soils is based on controlling the production of (extracellular) urease by soil micro-organisms.

Daif & Badr El-Din (1979) studied the effect of some heavy metals on urease activity in vitro using pure jack bean urease. They reported that use of iron, copper, and zinc to delay urea hydrolysis is possible. Consequently, the purpose of the work reported here was to evaluate iron, copper, and zinc as inhibitors to control urea hydrolysis, microbial production of urease, and ammonia evolution in soils treated with urea.

Materials and methods

The soils used were surface (0-30 cm) samples, taken in the Calamonte area near Mérida in the province of Badajoz, Spain. They were selected to obtain a wide range in properties (Table 1). Before use, each soil sample was air-dried and crushed to pass a 2-mm screen. In the analyses reported in Table 1, particle-size distribution was determined by pipette analysis (silt and clay) after destruction of organic matter by hydrogen peroxide and dispersion with sodium pyrophosphate, sand was determined by sieving, calcium carbonate equivalent by the Piper rapid titration method (Hesse, 1971), organic carbon by the Kurmies me-

Table 1. Characteristics of the soils used in the investigation.

Soil No	Sand (%)	Silt (%)	Clay (%)	Textural grade[1]	CaCO$_3$ equiv. (%)	Organic carbon (%)	Total nitrogen (%)	pH	CEC (meq/ 100 g)	Urease activity[2]
1	89	9	2	S	0.0	0.43	0.02	4.9	2.6	247
2	88	8	4	LS	0.0	0.22	0.02	4.3	2.1	247
3	81	14	5	LS	0.0	0.41	0.05	5.9	2.6	238
4	63	28	9	SL	1.0	0.45	0.05	5.8	9.7	258
5	49	42	9	L	4.0	0.79	0.07	7.4	9.7	250
6	30	58	12	SiL	6.2	0.87	0.08	7.4	34.1	269
7	59	16	25	SCL	0.0	0.57	0.04	5.1	16.5	268
8	35	29	36	CL	0.0	1.69	0.07	7.3	29.6	268

[1] S = sand, LS = loamy sand, SL = sandy loam, L = loam, SiL = silty loam, SCL = sandy clay loam, CL = clay loam.
[2] μg of urea hydrolysed g^{-1} of soil h^{-1} (37 °C).

thod, total nitrogen by a semi-micro Kjeldahl procedure, pH by a glass electrode in a 1:2.5 (w/v) suspension of $CaCl_2$ 0.01 M, CEC by percolation with ammonium acetate 1 M, buffered at pH 8.2 for calcareous soils or at pH 7.0 for non-calcareous soils. Urease activity was determined by the non-buffer method, described by Zantua & Bremner (1975).

Analyses of 1:5 (w/v) soil-water extracts showed these soils to be almost free of water-soluble portions of Fe, Cu, and Zn. It should be pointed out that, in general, heavy metal activities in soils are not characterized by their water-soluble concentrations. These analyses, however, were performed in order to determine the exact initial concentrations of these metallic ions. In all experiments Fe, Cu, or Zn was applied as the sulphate of the bivalent cation.

To study the effects of these ions on urea decomposition in soil, samples of air-dried soil, containing 5 g of oven-dry material, were placed in 100-ml glass bottles. Sufficient water was added to bring the total volume of water in each sample to 2 ml. The samples were then treated with 1 ml of water containing 2 mg of urea alone or 2 mg of urea plus either 25, 50, or 100 μg of the metal tested. The bottles were stoppered and incubated at 25 °C. After 24 or 48 hours, the soils of duplicate bottles from each treatment were extracted with 50 ml of a 2 M KCl solution, containing 250 μg of phenylmercuric acetate (PMA, an effective urease inhibitor), and the extracts were analysed for urea by the colorimetric procedure described by Douglas & Bremner (1970).

A study of the effects of these metallic ions on the production of urease by soil micro-organisms was performed in only two soils (Nos 3 and 5 from Table 1) where samples of air-dried soil, containing 5 g of oven-dry material, were placed in 100-ml glass bottles and treated with 1 ml of water containing either 10 mg of glucose alone or 10 mg of glucose plus 100 μg of metal. Water was added to bring the total volume to 2 ml per sample. The bottles were stoppered and incubated at 30 °C. After 24 and 48 hours duplicate bottles were removed from the incubator, and urease activity in the incubated soil samples was assayed by the non-buffer method described by Zantua & Bremner (1975).

To investigate the effects of ferrous, cupric, and zinc ions on the volatilization of ammonia from urea-treated soil, portions of air-dried soil, containing 10 g of oven-dry material of soil No 3 (Table 1) were placed in 250-ml French square bottles and treated with 2 ml of water containing either 10 mg of urea-N alone or 10 mg of urea-N plus 200 μg of metal. Water was added to bring the total volume to 3 ml per sample. The bottles were fitted with an aeration device having an acid trap for absorption of ammonia evolved during incubation which was maintained at 20 °C (Bremner & Douglas, 1971b). After 14 days duplicate bottles were removed from the incubator, and the ammonium in the acid traps was determined (Bremner & Edwards, 1965). The incubated soil was analysed for urea, soluble + exchangeable ammonium, and nitrate + nitrite (Douglas &

Bremner, 1970; Bremner & Keeney, 1966).

Most experiments and analyses were performed in triplicate. The results reported in this paper are the mean values.

Results and discussion

Urease activity in the different soils
In comparison with data presented by Zantua & Bremner (1975), urease activity is very high in all soils (Table 1). Probably the buffering capacities of the soils are not sufficient to prevent high pH values of the soil suspensions after urea decomposition. It is known that urease activity can be increased under alkaline conditions. (Bremner & Mulvaney, 1978). The small differences in urease activity in the different soil samples (Table 1) suggest that the pH had reached such a high value that urease was inactivated within the 5-hour incubation period. Combination of different rates of urea hydrolysis and different moments of urease inactivation can yield values for urease activity which do not deviate significantly.

Retardation of urea hydrolysis
The results of the effects of ferrous, cupric, and zinc ions on urea hydrolysis in the eight experimental soils are presented in Table 2. It is striking that in all soils the amounts of urea hydrolysed were very high and only slightly correlated with organic matter content and texture. On the average, 77 and 85 % of the added urea was already hydrolysed after 24 and 48 hours, respectively. These observations suggest that the soil-dependent rate of urea decomposition can only be detected during the first hours. In course of time, differences in the amounts of urea hydrolysed are thus eliminated. For that reason, inhibitory effects as reported in Table 2 are probably somewhat disguised. In most cases, the lower concentrations of metallic ions had a very slight or even no effect on retardation of urea hydrolysis. The effectiveness of these ions was obvious only when applied at a concentration of 20 μg g^{-1} of soil with variations in the effect of each ion from one soil to another and in the effect of different ions in each special soil. The results indicate that the inhibitory effect of metallic ions is most pronounced in light-textured soils and smaller in the heavier ones. This behaviour may be due to adsorption of these ions in the heavy-textured soils and may, thus, suggest that their potential values as urease inhibitors are greatest in light-textured soils.

Results obtained by many investigators (Bahadur & Chandra, 1962; Daif & Badr El-Din, 1979; Hughes et al., 1969; Shaw, 1954; Shaw & Raval, 1961; Toren & Burger, 1968) have shown that urease isolated from plants or micro-organisms is inactivated by very small amounts of Fe^{2+}, Cu^{2+}, Zn^{2+}, and other heavy

Table 2. Effect of ferrous, cupric, and zinc ions on urea hydrolysis (μg hydrolysed g^{-1} of soil, 25 °C) in soils during 24 and 48 hours.

Metallic ion[1]	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Soil 7	Soil 8
24 hours after incubation								
None	252	284	274	286	284	349	364	379
Fe (5 μg)	251	280	263	283	280	348	.353	372
Fe (10 μg)	250	280	262	281	280	348	347	369
Fe (20 μg)	218	228	239	268	258	325	331	349
Cu (5 μg)	251	278	272	276	278	346	339	371
Cu (10 μg)	247	265	268	275	265	335	335	371
Cu (20 μg)	213	220	234	258	253	318	315	347
Zn (5 μg)	246	273	262	280	273	341	343	365
Zn (10 μg)	244	272	259	280	272	339	331	364
Zn (20 μg)	220	232	235	258	255	326	316	352
48 hours after incubation								
None	269	323	278	326	323	398	399	400
Fe (5 μg)	267	316	277	326	316	396	398	399
Fe (10 μg)	267	325	271	323	315	396	395	399
Fe (20 μg)	243	267	271	309	300	392	392	394
Cu (5 μg)	269	313	273	323	313	395	399	399
Cu (10 μg)	264	313	266	317	313	392	388	399
Cu (20 μg)	235	246	254	293	292	387	387	394
Zn (5 μg)	265	314	269	323	314	398	397	400
Zn (10 μg)	264	311	265	322	311	398	394	399
Zn (20 μg)	245	262	265	303	299	393	379	396

[1] Amount of metal added per gram of soil is indicated in parentheses.

metals. Data obtained by Bremner & Douglas (1971a) and Daif & Badr El-Din (1979) and the results of the present work indicate that the amounts of heavy metal ions needed to cause substantial inhibition of soil urease were much larger than the amounts needed for equivalent inhibition of pure jack bean or soya bean urease. Long-term application of such ions at high concentrations may be, however, undesirable because of their potential phytotoxic properties, especially on light-textured soils.

The inhibitory effects of 20 μg g^{-1} of soil of either ferrous, cupric, or zinc ions were usually comparable to, and sometimes greater than those reported by Bremner & Douglas (1971a), who used concentrations of 50 μg g^{-1} of soil. This could suggest that the degree of contact of the metallic ion with urea is one of the ' factors determining the effectiveness of the inhibition. If this is true, it may be possible to use these metallic ions at much lower concentrations. At this moment the authors are aiming at finding a reliable method to evaluate such metals, especially for the way of mixing them manufacturally with urea.

Table 3. Effect of ferrous, cupric, and zinc ions on urease activity in two soils treated with glucose.[1]

Soil No	Metallic ion	Urease activity after ... h of incubation at 30 °C		
		0	24	48
3	None	238 ± 0.9	257 ± 6.0	260 ± 4.9
	Fe	238 ± 1.4	257 ± 2.6	260 ± 4.4
	Cu	239 ± 4.0	257 ± 4.6	261 ± 5.7
	Zn	239 ± 3.4	257 ± 5.8	261 ± 6.4
5	None	250 ± 2.1	270 ± 4.0	272 ± 5.7
	Fe	249 ± 5.5	269 ± 4.7	272 ± 2.0
	Cu	250 ± 4.2	269 ± 6.8	272 ± 2.2
	Zn	250 ± 6.9	270 ± 7.1	273 ± 2.4

[1] 2 mg of glucose g^{-1} of soil with or without 20 μg of metal g^{-1} of soil.
[2] μg of urea hydrolysed g^{-1} of soil h^{-1} (37 °C).

Microbial production of urease

To study the effects of the metallic ions on the production of urease by soil micro-organisms, the urease activity in two soils (Nos 3 and 5 from Table 1) was determined after incubation of soils with glucose. This compound was added to stimulate production of urease by soil micro-organisms. The results presented in Table 3 show that urease activity was only slightly stimulated after application of glucose in the control treatments. This suggests that addition of a carbon source to soils, in which inorganic nitrogen is practically absent, is not sufficient to stimulate bacterial growth substantially within 48 hours. Ferrous, cupric, and zinc ions did not affect microbial production of urease, even when applied at a concentration of 20 μg g^{-1} of soil.

From these results and those of the previously described experiment the conclusion seems to be justified that retardation of urea hydrolysis in soils in response to addition of ferrous, cupric, or zinc ions was not achieved by controlling the biosynthesis of urease, but by inhibiting the activity of (the extracellular fraction of) this enzyme present in the soil before treatment.

It is generally accepted that critical concentrations of heavy metals such as iron, copper, and zinc in the leaves, associated with nutrient deficiency symptoms in higher plants, are valid almost universally, regardless of climatic conditions or soil type, as long as the sampling techniques and analytical methods are comparable throughout. On the other hand, the values associated with phytotoxicity will vary over a rather wide range, since reduction in growth and appearance of symptoms will vary strongly with light intensity, temperature, moisture stress, salt composition, textural grade, acidity and organic matter content of the soil. Since the highest concentration of metallic ions used in our experiments (20 μg g^{-1} of soil) did not affect the activity of ureolytic micro-organisms, it can be postulated that such a concentration probably does not injure plants but, perhaps,

may even promote their growth. This postulation is based on the widely accepted theorem that *plants are more tolerant than micro-organisms*. We think, however, that more knowledge is necessary about the effects of long-term application of these metals on plant growth before leaving our earlier mentioned statement that application of such ions at these concentrations is undesirable in principle.

Ammonia volatilization and nitrification
Table 4 shows the results of a comparison of the effects of metallic-ion applications (20 μg g^{-1} of soil) on the recovery of urea-N as urea, soluble + exchangeable ammonium, ammonia, and nitrate + nitrite, when a loamy sand (soil No 3 from Table 1), treated with urea, was incubated at 20 °C for 14 days.

It can be seen that, compared with zinc and ferrous ions, the cupric ion markedly retarded urea hydrolysis and reduced volatilization of ammonia, while more nitrate + nitrite accumulated in the soil. The superiority of the Cu treatment in retarding urea decomposition, presumed to be due to the reaction of cupric ions with the amide groups of urea and/or urease, can be explained by postulating the formation of organo-metal complexes which are more stable than those formed in case of either ferrous of zinc treatments. These relatively stable complexes may be considered as the limiting factor which restricts the urea hydrolysis rate. This assumption is supported by the values of residual urea as represented in Table 4.

It is well known that ammonium ions in solution may be adsorbed by soil colloids, or transformed to nitrate or ammonia (Chu & Knowles, 1966). In addition, equilibria exist between adsorbed ammonium, ammonium in solution and free ammonia. Consequently, any increase in the production of ammonium ions will give rise to the evolution of gaseous ammonia. In this connection, Ernst & Massey (1960) reported that free ammonia is *always* produced following the formation of ammonium carbonate in the course of urea hydrolysis in the soil. The very high ammonium concentrations prevented the onset of the nitrification process in the zinc and ferrous treatments. The deviating behaviour of the cu-

Table 4. Effect of ferrous, cupric, and zinc ions on transformations of urea to free ammonia, soluble + exchangeable ammonium, and nitrate + nitrite after incubation of urea treated soil No 3 from Table 1) for 14 days at 20 °C.

Metallic ion per g of soil	Recovery of urea-N (%)				
	as urea	as NH_3	as NH_4^+	as $NO_3^- + NO_2^-$	total
None	16.0 ± 0.5	18.8 ± 0.9	62.0 ± 1.3	1.0 ± 1.0	97.8
Fe 20 μg	18.3 ± 0.8	19.0 ± 1.2	60.0 ± 1.5	1.0 ± 0.8	98.3
Cu 20 μg	30.5 ± 2.8	9.2 ± 1.1	42.1 ± 1.7	17.0 ± 1.0	98.9
Zn 20 μg	20.2 ± 0.9	16.0 ± 1.0	60.8 ± 7.7	1.0 ± 1.1	98.0

pric ion with respect to ammonia evolution can be due to the formation of Cu-NH$_3$ complexes and hence to suppression of substrate inhibition in the nitrification reactions. As copper is known as an essential cofactor in nitrifying enzymes, it is not excluded that addition of copper stimulates the formation of nitrite by *Nitrosomonas* species, despite of the high ammonium concentration.

Acknowledgements

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