The Role of Zinc Compound Residues in the Environmental Degradation of Prevulcanised Natural Rubber Latex Films

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The effect of residual diethyldithiocarbamate on the environmental degradation of prevulcanised natural rubber latex films was studied in the presence of zinc oxide and a conventional phenolic antioxidant. The films were buried in a clayey soil at 40% field capacity and retrieved after 24 and 48 weeks. The overall degradation was significantly greater for films without zinc oxide and without Wingstay L[®], as well as for films prepared at longer vulcanisation process times. Films prepared with zinc oxide and Wingstay L[®] showed generally smooth surfaces after 24 weeks, compared to films prepared without zinc oxide or Wingstay L[®] that had cracks and voids across the surfaces. The concentrations of residual dithiocarbamates remaining could offer an explanation as to why some of the film pieces were slow to degrade, despite the higher degree of crosslinking formed.

Key words: zinc compound residues; environment; degradation; prevulcanised NR; latex films; phenolic antioxidant; Wingstay L[®]; dithiocarbamates; crosslinking

Zinc dithiocarbamates are widely used as accelerators for the vulcanisation of natural rubber (NR) latex. During the prevulcanisation of NR latex, the concentration of free zinc dialkyldithiocarbamate accelerator has been shown to fall linearly with the time of vulcanisation[1]. Furthermore, zinc oxide (ZnO) has an influence upon the disappearance of the free zinc dithiocarbamate and hence the concentration of residual zinc dithiocarbamate which is known to act as an effective antioxidant in enhancing the resistance of latex films to oxidative degradation. It is believed that the addition of zinc as ZnO during the vulcanisation process reactivates the vulcanisation intermediates while capturing any unreactive accelerator as residual. The objective of the present study is to investigate the effect of residual zinc diethyldithiocarbamate (ZDC) on the environmental degradation of prevulcanised NR latex films, in the absence and presence of a conventional phenolic antioxidant, Wingstay L[®] [2,2'-dicyclopentylene-bis-(4-methyl-6-t-butyl-phenol)].

MATERIALS AND METHODS

Preparation of Samples of Vulcanised Latex Mixes

A commercial high ammonia (HA) latex concentrate, preserved with 0.7 % ammonia, was used. The following latex formulations

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were employed for the preparation of vulca-nised latex mixes (*Table 1*). Each of the latex compounds (A, B, C and D) were vulcanised at 70ºC in a single batch (*ca.* 11 kg wet). Samples of vulcanised latex mixes (1.5 kg each) were withdrawn at time intervals of 0, 1, 2, 3, 4, 6 and 8 hours and were labelled as follows:

Sample Reference	Time of vulcanisation, h
A1, B1, C1, D1	0
A2, B2, C2, D2	1
A3, B3, C3, D3	2
A4, B4, C4, D4	3
A5, B5, C5, D5	4
A6, B6, C6, D6	6
A7, B7, C7, D7	8

Preparation of Latex Dipped Films

Coagulant dipped films were prepared from vulcanised latex mixes with the following conditions:

Formers:	Glass plates
	(152 mm length ×
	102 mm width)
Total solids content of compound:	50%
Calcium nitrate in	
Industrial Methylated Spirit:	10%
Latex dwell time:	30 s
Wet-gel leach:	70ºC/5 min
Slurry dip (corn-starch, 10%):	10 s

Latex films were air-dried for a minimum of 24 h prior to stripping.

Determination of Crosslink Density of Latex Vulcanisates

Accurately weighed samples (*ca.* 0.1 g), which were die-cut from latex dipped films, were immersed in toluene (20 mL – 30 mL) in stoppered bottles at ambient temperature for 48 h. At the end of this period, surface solvent was removed with filtered paper and the sample weighed in a stoppered weighing bottle. Toluene was removed by drying at room temperature, for a period of not less than 3 days, to a constant weight. The crosslink concentration was determined, using the modified Flory-Rehner equation[2].

Film Thickness

The mean film thickness was determined with a Mitutoyo digital thickness gauge (accuracy of 0.001 mm).

Microscopy

Samples were cut to approx. 4 mm × 4 mm with arbitrary identification as top and bottom of the sample. They were later coated with gold prior to examination by a JEOL Model JSM-5300 scanning electron microscope. The pictures were captured using INCA Energy software to a magnification of 1000X.

Soil Burial Tests

Pre-weighed film cut into 9 cm × 7.5 cm rectangular pieces from the different treatment combinations were buried in nylon-net mesh bags (38 µm pore size) in a clayey soil (37 kg soil / container) as previously described[3]. Soils were limed to pH 6.5, supplied with nutrients (100 mg N and 150 mg P kg^{-1} soil) and kept at 25ºC and 40% field capacity. The film pieces were retrieved after 24 and 48 weeks, washed in running water and dried prior to weighing.

Effect of Zn Compounds on Microorganism Growth

The effects of ZnO and $ZnSO_4.7H_2O$ on growth of a selected number of environmental

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TABLE 1. LATEX FORMULATIONS EMPLOYED FOR PREPARATION OF
VULCANISED LATEX MIXES

Ingredients	Mix quantity (parts by dry weight)			
	A	B	C	D
High-ammonia (HA) latex (60%)	100	100	100	100
Sulphur (50%)	1	1	1	1
Zinc diethyldithiocarbamate (50%)	1	1	1	1
Zinc oxide (50%)	–	–	0.2	0.2
Wingstay L® (40%)	–	1	–	1

microorganisms (bacteria, fungi and a microalgae) were studied (*Table 8*). These microorganisms were isolates from soil-degraded gloves, earth rubbers, compost sludge, latex and rubber effluent treatment ponds, and from our culture collection[4]. Bacteria and microalgae were grown on Tryptic Soy Agar (Difco, USA) and fungi on Potato Dextrose Agar (Difco, USA).

Calculated volumes of stock solutions were incorporated into molten agar held at 45ºC to give final concentrations of the compounds ranging from 0, 8, 40, 80, 402, 803, 1606, 2409, 3212 and 4015 µg/mL of the metal. ZnO powder (4 g) was suspended in 2g sodium 2-naphthalene sulfonate (SNS) dispersant and made up to 100 mL with water. Growing bacteria from an exponential phase in nutrient broth precultures were streaked on to the surface of the dried plates and fungi from subcultured agar plates were transferred on small pieces of adherent agar (4 mm^2), in duplicates. A control series without the additives were used to compare the extent of growth of these organisms on solid media. The plates were incubated at 30ºC and examined after 5 days. The extent of growth of the soil microflora were recorded and the minimal inhibitory concentrations (MIC) determined. The MIC is defined as the lowest concentration that causes no visible growth.

RESULTS

Soil Burial Tests

In general, the mass losses of the prevulcanised latex films were small even after 48 weeks of soil burial (*Tables 2, 3*). Averaged over all treatments, the percentage of initial weights retained ranged from 71%–98% after 24 weeks, and from 50%–93% after 48 weeks. The slow degradation rates were due to the thicker film pieces (range, 0.32 mm – 0.36 mm, *Table 4*) compared to thinner pieces used in several earlier studies.

The main effects of ZnO, Wingstay L® (WSL) and vulcanisation in latex (PV) times in affecting the degradation of the film pieces were highly significant ($P < 0.001$), and so were the interaction effects (ZnO X WSL, ZnO X PV time, $P < 0.001$; WSL X PV time, $P < 0.05$).

Thus, when averaged over all treatments, the overall degradation was significantly greater for films without ZnO (mean; -ZnO = 70.5%, +ZnO = 87.4%), and for films without WSL (mean; -WSL = 76.0%, +WSL = 81.8%), as well as for films at longer PV times (mean; 0h = 85.6%, 1h = 80.7%, 2h = 81.6%, 3h = 81.6%, 4h = 79.3%, 6h = 74.3%, 8h = 69.4%), but the strong interaction effects necessitate discussing the data individually for specific cases.

TABLE 2. MEAN PERCENT INITIAL WEIGHT REMAINING OF
NR LATEX FILMS AFTER 24 WEEKS OF SOIL BURIAL

Pre-vulcanisation time, h	% initial weight remaining*			
	-ZnO		+ZnO	
	-WSL	+WSL	-WSL	+WSL
0	92.9 b-d	93.3 b-d	94.8 bc	92.9 bd
1	93.3 b-d	93.3 b-d	94.8 bc	94.7 b
2	93.5 b-d	95.7 bc	94.1 bc	94.3 b-d
3	89.4 de	95.4 bc	94.3 bc	93.2 b-d
4	89.0 de	91.8 cd	94.3 bc	94.2 bc
6	80.2 f	87.1 e	94.7 bc	92.6 b-d
8	70.7 g	86.9 e	98.4 a	92.7 b-d

*Means of 3 replications. Values within a row, column or across rows and columns within the body of the table not followed by common letters are significantly different (P<0.05). Analyses based on an arcsin √% transformed data. Significance: ZnO, P<0.001; WSL, P<0.001; Time, P<0.001; ZnO X WSL, P<0.001; ZnO X Time, P<0.001; WSL X Time, N.S.; ZnO X WSL X Time, P<0.001; CV = 1.5%.

TABLE 3. MEAN PERCENT INITIAL WEIGHT REMAINING OF
NR LATEX FILMS AFTER 48 WEEKS OF SOIL BURIAL

Pre-vulcanisation time, h	% initial weight remaining*			
	-ZnO		+ZnO	
	-WSL	+WSL	-WSL	+WSL
0	82.4 c-e	82.0 c-e	88.7 bc	89.4 b
1	66.9 gh	75.6 ef	90.9 b	89.3 b
2	67.9 gh	78.8 de	87.1 bc	92.6 a
3	71.3 fg	76.4 ef	87.8 bc	90.8 b
4	63.2 h	76.0 ef	90.0 b	88.1 bc
6	57.0 i	70.4 fg	83.8 b-d	85.8 bc
8	50.1 j	68.6 g	77.0 ef	81.8 c-e

*Means of 3 replications. Values within a row, column or across rows and columns within the body of the table not followed by common letters are significantly different (P<0.05). Analyses based on an arcsin √% transformed data. Significance: ZnO, P<0.001; WSL, P<0.001; Time, P<0.001; ZnO X WSL, P<0.001; ZnO X Time, P<0.001; WSL X Time, P<0.05; ZnO X WSL X Time, N.S.; CV = 2.6%.

In treatments without ZnO, degradation was greater in films without WSL for several PV time samples after 48 weeks (1, 2, 4, 6, 8 h) (*Table 3*). In the presence of WSL, only film samples from the two longer PV times (6, 8 h) showed lower mass retention from the 0 h treatment.

In film treatments with ZnO, degradation was not different for all PV times (with one exception, 2h PV time) between films containing WSL or without, at 48 weeks (*Table 3*). For treatments without WSL, the longest PV time (8h) resulted in a greater degradation than all other PV times (0 h – 6 h).

TABLE 4. THICKNESS OF NR LATEX FILMS

| Pre-vulcanisation time, h | Thickness (mm)* | | | |
| | -ZnO | | +ZnO | |
	-WSL	+WSL	-WSL	+WSL
0	0.33	0.33	0.32	0.30
1	0.36	0.34	0.35	0.31
2	0.36	0.32	0.32	0.34
3	0.35	0.34	0.33	0.31
4	0.35	0.33	0.36	0.33
6	0.38	0.33	0.33	0.33
8	0.36	0.34	0.35	0.32

*Means of 6 replicates; total of 18 determinations from each replicate.

Microscopy

Film samples prepared without ZnO, with or without WSL, or with ZnO but without WSL generally showed cracks and voids across the surfaces after 24 weeks (*Figures 1a, b and c*). However, films prepared with ZnO and WSL showed surfaces that were generally smooth (*Figure 1d*).

Crosslink Density of Latex Vulcanisates

Increasing the vulcanisation process time increased the crosslink density of the latex films (*Table 5*). This was due to the 'sulfuration' process associated with the insertion of polysulphidic linkages in between the discrete polymer molecules to form a network. Storing the dry film samples at room temperature further increased the formation of crosslinks, and was probably due to the high reactivity of the allylic polysulphidic linkages and the tendency of interchanging reactions occuring in the network structure.

In the absence of the antioxidant but with the presence of Zn, the sulphur-accelerator intermediates generated would bind to more sites enhancing sulphur crosslinking reactions. The antioxidant in this case acts as a radical scavenger, providing electrons to close any free radical reaction occurring within the sulphur linked network. When averaged over all treatments, films with ZnO had significantly higher crosslinks than films without (*e.g.* unaged film mean; -ZnO = 3.057, +ZnO = 3.507). Irrespective of ZnO, films without WSL had higher crosslinks than films containing the antioxidant (*e.g.* unaged film mean without ZnO; -WSL, 3.190; +WSL, 2.923; and with ZnO; -WSL, 3.659, +WSL, 3.354).

ZDC and WSL Content

In treatments without ZnO, all films from the longer PV times (4 h – 8 h) had low or undetectable (<10 p.p.m.) levels of free carbamates but this was not the case when ZnO was present (*Table 6*).

Although oxidation associated reactions in rubber would have consumed some of the phenolic antioxidant, the films in this study eluted similar amounts of the extracted antioxidants indicating that a minimal amount of oxidative ageing occured under these circumstances (*Table 7*).
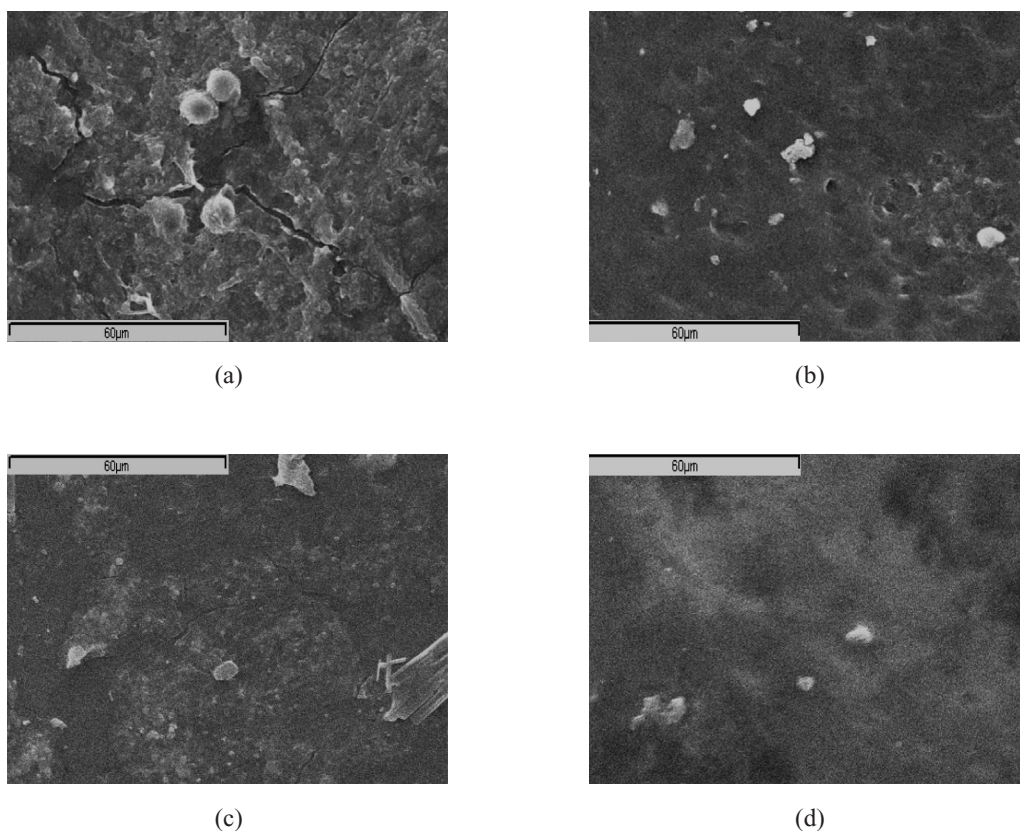
Figure 1. The effect of soil burial on the surface appearance of film pieces (after 24 weeks) examined by SEM: (a) without ZnO, without WSL, (b) without ZnO, with WSL, (c) with ZnO, without WSL, and (d) with ZnO, with WSL. Scale bar = 60 μm.

Effect of Zinc Compounds on Micro-organism Growth

Of the 63 bacterial isolates tested, only 10 (16%) were susceptible to Zn as ZnO at <100 μg/mL while 42 (67%) were able to tolerate Zn above 1000 μg/mL (*Table 10*). On the other hand, all bacteria tested were able to tolerate Zn as $ZnSO_4.7H_2O$ above 100 μg/mL, with 56 (89%) growing above 1000 μg/mL. The fungi, except for yeasts, were tolerant to levels below 4015 μg/mL Zn in both forms. The microalgae was most susceptible to Zn in both forms.

DISCUSSION

The small mass losses even after 48 weeks of soil burial was attributed to the thicker films used (range, 0.32 mm – 0.36 mm). It is already known that thinner latex films (0.15 mm) degraded faster than thicker films within the same moduli[4,5], and that the use of thinner films would have been more appropriate in studying the subject. In the present study, there was little correlation observed ($r^2 = 0.01$) between mass losses and crosslink densities at this film thickness. Degradation from a physical disintegration

TABLE 5. NETWORK CROSSLINK DENSITY OF LATEX FILMS PREPARED FROM
VULCANISED LATEX MIXES

| Pre-vulcanisation time, h | Crosslink density (X 10^{-5} g mol/g rubber)* | | | |
| | -ZnO | | +ZnO | |
	-WSL	+WSL	-WSL	+WSL
(a) 0-time				
0	2.060	2.095	2.921	2.333
1	2.982	2.836	3.321	2.993
2	3.114	2.791	3.571	3.189
3	3.326	2.726	3.691	3.488
4	3.473	3.073	3.777	3.618
6	3.861	3.628	3.981	3.974
8	3.516	3.310	4.349	3.882
(b) 24 weeks				
0	2.466	2.318	2.993	2.993
1	3.058	2.733	3.629	3.617
2	3.184	2.787	3.376	3.474
3	3.487	3.128	3.692	3.764
4	3.600	3.298	3.712	3.839
6	3.786	3.561	3.955	3.921
8	3.650	3.368	3.885	4.123
(c) 48 weeks				
0	2.594	2.383	2.875	3.237
1	3.353	2.975	3.727	3.775
2	3.347	2.965	4.140	3.888
3	3.444	3.066	3.526	4.016
4	3.647	3.207	3.147	4.190
6	3.921	3.253	2.970	4.198
8	3.788	3.413	4.229	4.187

*Values are means of 3 determinations.

effect was evident from the formation of cracks and pores observed on the film surfaces. In fact, the higher the crosslinking density, the harder the material became that made it more susceptible to disintegration. In treatments without ZnO, films with or without WSL at several higher PV times that degraded faster showed higher crosslink densities than films at 0-time (*Table 5*). In treatments with ZnO but without WSL, the longest PV time (8 h) also resulted in a greater degradation than all other PV times (0 h – 6 h). For all these films, the common denominator was the low presence of dithiocarbamate residues, indicating its role in inhibiting environmental degradation. There also exists the possibility that the metal dithiocarbamate accelerators used are also peroxide-decomposing antioxidants that may synergise with the phenolic antioxidants in inhibiting biodegradation[6,7].

The generally accepted pathway in any rubber sulphur (S) vulcanisation begins with the formation of an active sulphurating agent (the S-rich Zn accelerator complexes) from reaction of the vulcanisation ingredients (accelerators, activator) and S[8,9]. The very reactive sulphurating agent reacts with the

TABLE 6. THE CONCENTRATIONS OF FREE ZINC DIETHYLDITHIOCARBAMATE IN LATEX
FILMS PREPARED FROM VULCANISED LATEX MIXES

| Pre-vulcanisation time, h | Free ZDC (p.p.m.) | | | |
| | -ZnO | | +ZnO | |
	-WSL	+WSL	-WSL	+WSL
(a) 0-time				
0	5696	3917	3904	4881
1	3435	1439	3870	1950
2	2390	1358	3902	3885
3	1385	565	3302	5527
4	544	125	2268	4374
6	0	0	3176	4224
8	0	0	2406	4090
(b) 24 weeks				
0	1712	4623	5399	1805
1	1311	4829	7411	1735
2	1933	4539	4918	1644
3	1100	3221	4969	1430
4	285	875	5616	1759
6	166	39	4799	1832
8	14	20	4554	972
(c) 48 weeks				
0	2030	1947	1941	2472
1	1082	850	1572	2361
2	925	1162	1448	1919
3	537	954	1476	2103
4	552	567	1571	2058
6	42	27	1169	1542
8	22	23	1319	1022

rubber polymer to yield a crosslink precursor, and these precursors form initial crosslinks which contain a relatively large number of S atoms in the bridge. In addition, Zn complexes have been identified to possess antioxidant action by catalytically decomposing the peroxides that occur in the radical-chain oxidation reaction. The final rubber network is due to ensuing crosslink shortening, which may be accompanied by crosslink degradation, as well as oxidative modification of the rubber polymer. The reaction process, once a crosslink is formed, involves desulphuration and decomposition that eventually forms the network vulcanisates.

The network structure depends on the vulcanising system, and thus the decrease in the amount of accelerator in films without ZnO. Thus, during S accelerated vulcanisation of NR latex, the concentration of free ZDC falls linearly with time of sulphur prevulcanisation, but the presence of ZnO reduces the rate of its disappearance from the reaction system. This was observed with the samples prepared at different times. Shumane and co-workers[10] attributed the increase in crosslinking rate in rubber samples with ZnO to the ability of ZnO trapping free carbamates to form residual ZDC.

TABLE 7. THE CONCENTRATIONS OF WINGSTAY L® IN LATEX FILMS PREPARED FROM
VULCANISED LATEX MIXES

| Pre-vulcanisation time, h | Wingstay L® (%) | | | |
| | -ZnO | | +ZnO | |
	-WSL	+WSL	-WSL	+WSL
(a) 0-time				
0	–	0.54	–	0.41
1	–	0.55	–	0.49
2	–	0.49	–	0.50
3	–	0.47	–	0.49
4	–	0.47	–	0.47
6	–	0.47	–	0.47
8	–	0.40	–	0.47
(b) 24 weeks				
0	–	0.35	–	0.60
1	–	0.37	–	0.35
2	–	0.40	–	0.45
3	–	0.40	–	0.44
4	–	0.55	–	0.45
6	–	0.50	–	0.40
8	–	0.80	–	0.44
(c) 48 weeks				
0	–	0.40	–	0.50
1	–	0.40	–	0.45
2	–	0.40	–	0.50
3	–	0.25	–	0.50
4	–	0.42	–	0.55
6	–	0.40	–	0.49
8	–	0.40	–	0.50

Blackley[1] had shown that the rate of insertion of crosslinks in the rubber phase is slightly enhanced by the presence of ZnO. This was observed with the samples prepared at the different times, and in the soil burial studies, degradation of latex films was greater in treatments with or without ZnO at the two longest vulcanisation times where the amount of residual dithiocarbamates in the films was almost negligible.

We have shown in a previous study using the same organisms on agar media that ZDC is a very potent bacterial (but not fungal) inhibitor and that concentrations greater than 200 μg/mL (36 μg/mL Zn) were sufficient to inhibit growth of most soil microbes[4]. Of the bacteria tested previously, a small number (16%) could still grow at the highest level of ZDC supplied (~1810 μg/mL Zn), even though 50% of the bacteria were not able to withstand >18 μg/mL Zn. In the present study, all the bacterial isolates could tolerate very high levels of the soluble $ZnSO_4.7H_2O$ but bacterial tolerance to ZnO appeared to be lower, implying that anions may play a role in growth inhibition in a multiphase environment. It had been established in solution culture that the MIC of *Eschericia coli* grown in a mineral salts medium for Zn, Cu, Ni and Co was 1.0 mM (~65 μg/mL Zn as $ZnCl_2$)[11,12]. In another study, growth inhibition of *E. coli* was found to

TABLE 8. EFFECT OF Zn AS ZnO AND $ZnSO_4.7H_2O$ ON MICROBIAL GROWTH

Isolate	Species	Minimal inhibitory concentration (µg/mL)	
		ZnO	$ZnSO_4.7H_2O$
(a) Bacteria			
#2	Unidentified	80	4015
#25	*Burkholderia glumae*	1606	4015
#31	*Pseudomonas stutzeri*	1606	2409
#34	*Staphylococcus aureus*	3212	4015
	subsp. *aureus*		
#45	Unidentified	4015	4015
#46	*Alcaligenes faecalis*	4015	4015
	subsp. *faecalis*		
#51	*Staphylococcus arlettae*	3212	4015
#55/1	*Strenotophomonas maltophilia*	4015	4015
#55/2	*Bacillus cereus/thuringiensis*	1606	1606
#60	*Pseudomonas aeruginosa*	4015	4015
#63	*Aeromonas veronii* DNA group 10	4015	4015
#66	*Xanthomonas campestris*	1606	4015
#68	*Rahnella aquatilis*	402	3212
#70	Unidentified	80	3212
LB103	*Rahnella aquatilis*	803	4015
LB105	*Enterobacter cloacae*	1606	3212
LB106	*Pseudomonas oryzihabitans*	80	803
WG18NF	*Hafnia alvei*	4015	4015
RMM1	*Cellulomonas* sp.	1606	3212
TL3	*Pseudomonas fluorescens*	4015	4015
7NSK2	*Pseudomonas aeruginosa*	4015	4015
ATCC9027	*Pseudomonas aeruginosa*	4015	4015
BK1	*Pseudomonas putida*	80	803
ET1	Unidentified	80	803
ET4	*Streptomyces* sp.	803	4015
BP	*Bacillus polymyxa*	1606	4015
433	*Bacillus subtilis*	1606	4015
ATCC 6633	*Bacillus subtilis*	80	803
518	*Agrobacterium liquefaciens*	4015	4015
512	*Agrobacterium guttatus*	402	1606
ATCC 11842	*Lactobacillus bulgaricus*	1606	4015
A	*Pantoea stewartii* subsp. *stewartii*	80	803
F	*Buttiaauxella warmboldiae*	4015	4015
G	*Microbacterium terregens*	4015	4015
I	Unidentified	2000	4015
J	*Pasteurella pneumotropica*	80	803

TABLE 8 (CONT.). EFFECT OF Zn AS ZnO AND $ZnSO_4.7H_2O$ ON MICROBIAL GROWTH

Isolate	Species	Minimal inhibitory concentration (µg/mL)	
		ZnO	$ZnSO_4.7H_2O$
K	*Burkholderia glumae*	80	803
L	*Sinorhizobium meliloti*	4015	4015
P	*Ochrobactrum anthropi*	2000	4015
C1	Unidentified	402	4015
C2	*Neisseria canis*	803	4015
#432	*Microcyclus flavus*	803	1606
#582	*Flavobacterium aquatile*	1606	4015
#581	*Chromobacterium violaceum*	1606	4015
#585	*Serratia marcescens*	4015	4015
#589	*Agrobacterium radiobacter*	2000	4015
#373	*Bacillus cereus*	4015	4015
#385	*Micrococcus* sp.	4015	4015
#413	*Corynebacterium* sp.	80	1606
#366	*Bacillus megaterium*	402	1606
#370	*Nocardia citrea*	4015	4015
#377	*Agrobacterium tumefaciens*	803	4015
#501	*Acinetobacter anitratum*	4015	4015
#512	*Achromobacter guttatus*	402	4015
#518	*Aeromonas liquefaciens*	4015	4015
#459	*Brevibacterium acetylicum*	402	4015
#420	*Erwinia* sp.	4015	4015
#700	*Vibrio proteus*	4015	4015
#519	*Nocardia* sp.	4015	4015
#598	*Klebsiella aerogenes*	4015	4015
#685	*Xanthomonas* sp.	4015	4015
#573	*Eschericia* sp.	4015	4015
#630	*Citrobacter freundii*	4015	4015
(b) Fungi			
RO	*Rhizopus oligosporus*	4015	4015
AO	*Aspergillus oryzae*	4015	4015
WT1	*Penicillium* sp.	4015	4015
767	*Fusarium monoliforme*	4015	4015
1049	*Fusarium solani*	4015	4015
Bakers yeast	*Saccharomyces cerevisiae*	80	803
(c) Algae			
ALG	*Chlorella* sp.	80	803

*Levels tested; 0, 8, 40, 80, 402, 803, 1606, 2409, 3212 and 4015 p.p.m. Zn

be 163 µg/mL[13]. Filali[14] isolated metal-resistant bacterial species from contaminated waste water and found that the MICs of *Klebsiella pneumoniae* and *Proteus mirabilis* to ZnSO$_4$ was 131 µg/mL Zn. In soil metabolic profile studies, accumulation of Zn up to 2000 mg/kg did not decrease metabolic biodiversity in the microbial community, although pollution-induced community tolerance (PICT) was detectable at soil Zn concentrations above 300 mg/kg[15]. In this study, the calculated amount of Zn in latex films containing both ZDC and ZnO (treatments C, D with 1 p.h.r. ZDC and 0.2 p.h.r. ZnO) averaged 3311 µg/mL, whereas films without ZnO averaged 1757 µg/mL. Since much of the carbamate residuals remained in gloves prior to disposal in soils[16], such high initial levels of the metal would be encountered by soil microbes compared to those used in laboratory cultures. However, the concentrations of metals and organometallic compounds in natural habitats may be reduced because metal toxicity is heavily influenced by environmental conditions, and binding to environmental constituents determines the biological availability of a metal in a system[17–19]. In soils, metal cations can be strongly bound by organic materials (humic, fulvic acids, proteins) and by clay particles to such an extent that they become unavailable to organisms or plants.

Many microorganisms in water, soil and industrial waste, in responding to selective pressures from metal-containing environments, demonstrate resistances to a variety of metal ions *via* genes located on chromosomes, plasmids or transposons encoding specific resistances and resistance systems[11,20–23]. At high concentrations, essential (required nutrients) and non-essential (no biological role) metals can damage cell membranes, alter enzyme specificity, disrupt cellular functions, and damage the structure of DNA. Examples of essential nutrient metals are cobalt [Co(II)], copper [Cu(II)], nickel [Ni(II)] and zinc

[Zn(II)]. Zinc is a component in such a variety of enzymes and DNA-binding proteins that life seems impossible without this redox-inactive former of tight complexes[11]. Human activities have created environments of high selection for metals, and there are six metal resistance mechanisms presently known, *i.e.* exclusion by permeability barrier, intra- and extra-cellular sequestration of the metal by protein binding, active transport efflux pumps, enzymatic detoxification of the metal to a less toxic form, and reduction in the sensitivity of cellular targets to metal ions[21,24,25]. In the case of the highly-reputable metal ions Zn(II), Cd(II) or Co(II), active transport or efflux systems is the prominent genetically determined mechanism used by microorganisms to export toxic metals from their cytoplasm to the external environment, for *e.g.* the plasmid-encoded *cad* system in *Staphylococcus aureus* and *czc* system in *Alcaligenes eutrophus*, and a third chromosomally-encoded system[21]. The *czc* operon contains several genes and encodes four proteins which make up an effluent pump complex. Another detoxification mechanism is intracellular sequestration by protein binding, and metals commonly sequestered within the cytoplasm are Cd (II), Cu(II) and Zn(II) to prevent exposure to essential cellular components. Electron-dense bodies of compartmentalised metals (Zn) have been observed in fungi after growth in a medium containing Zn. Adsorption of Cd (II), Cu(II) and Zn(II) on surfaces of microbial cells, living or dead, is made possible by complexation with polygalacturonic acids as a constituent of the outer layers of bacterial cells. In the fungi *Neocosmospora vasinfecta* and *Candida utilis*, Zn binds to the negatively-charged groups on the hyphal surfaces[17]. In fact, such a combination of features make microorganisms attractive candidates for decontamination of polluted soils and effluents.

The phenomenon of microbial resistance to metal toxicity is relevant to microbial ecology

especially in relation to the degradation of compounded rubbers. Although Zn can be tolerated by microbes, its anionic form plays a central role in growth inhibition of soil microbes. In this soil degradation study on thick pieces of latex films, the concentrations of residual dithiocarbamates remaining could offer an explanation as to why some of the film pieces were slow to degrade, despite the higher degree of crosslinking formed.

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