SUPPORTING INFORMATION

# Evaluation of the antibacterial activity of eco-friendly hybrid composites on the base of oyster shell powder modified by metal ions and LLDPE.

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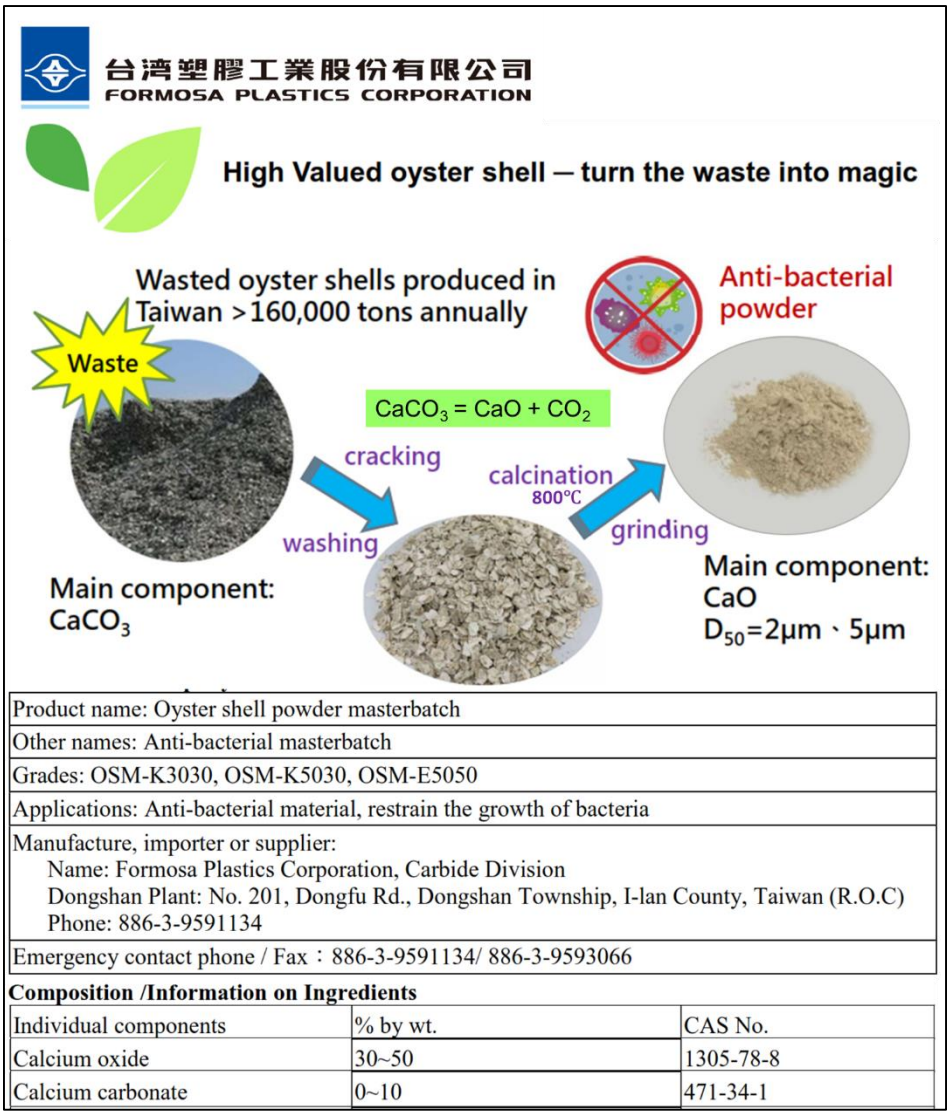


| | | |
|---|---|---|
| Product name: Oyster shell powder masterbatch | | |
| Other names: Anti-bacterial masterbatch | | |
| Grades: OSM-K3030, OSM-K5030, OSM-E5050 | | |
| Applications: Anti-bacterial material, restrain the growth of bacteria | | |
| Manufacture, importer or supplier:<br>Name: Formosa Plastics Corporation, Carbide Division<br>Dongshan Plant: No. 201, Dongfu Rd., Dongshan Township, I-lan County, Taiwan (R.O.C)<br>Phone: 886-3-9591134 | | |
| Emergency contact phone / Fax：886-3-9591134/ 886-3-9593066 | | |
| **Composition /Information on Ingredients** | | |
| Individual components | % by wt. | CAS No. |
| Calcium oxide | 30~50 | 1305-78-8 |
| Calcium carbonate | 0~10 | 471-34-1 |

**Figure S1** Datasheet of oyster shell powder (OSP) masterbatch by Formosa plastics corporation

**Table S1.** Composition of LLDPE-3470 biocomposites.

| Sample name | Composition | | | | |
|---|---|---|---|---|---|
| | LLDPE (gram) | CaO (gram) | CaO-Ag (gram) | CaO-Zn (gram) | CaO-Cu (gram) |
| LLDPE-3470 | 100 | - | - | - | - |
| LLDPE/CaO 1% | 99 | 1 | - | - | - |
| LLDPE/CaO 2% | 98 | 2 | - | - | - |
| LLDPE/CaO Ag 1% | 99 | - | 1 | - | - |
| LLDPE/CaO Ag 2% | 98 | | 2 | - | - |
| LLDPE/CaO Zn 1% | 99 | - | - | 1 | - |
| LLDPE/CaO Zn 2% | 98 | - | - | 2 | - |
| LLDPE/CaO Cu 1% | 99 | - | - | - | 1 |
| LLDPE/CaO Cu 2% | 98 | - | - | - | 2 |

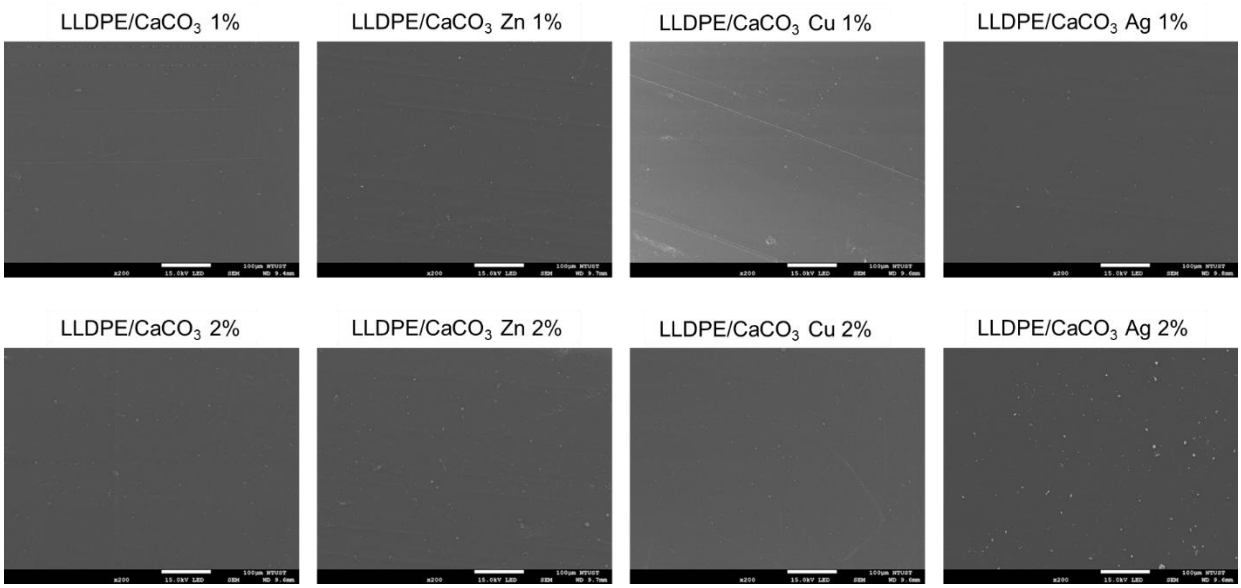


**Figure S2,** SEM surface image of polypropylene test pieces added with different concentrations prepared by calcium carbonate series modified metal ions

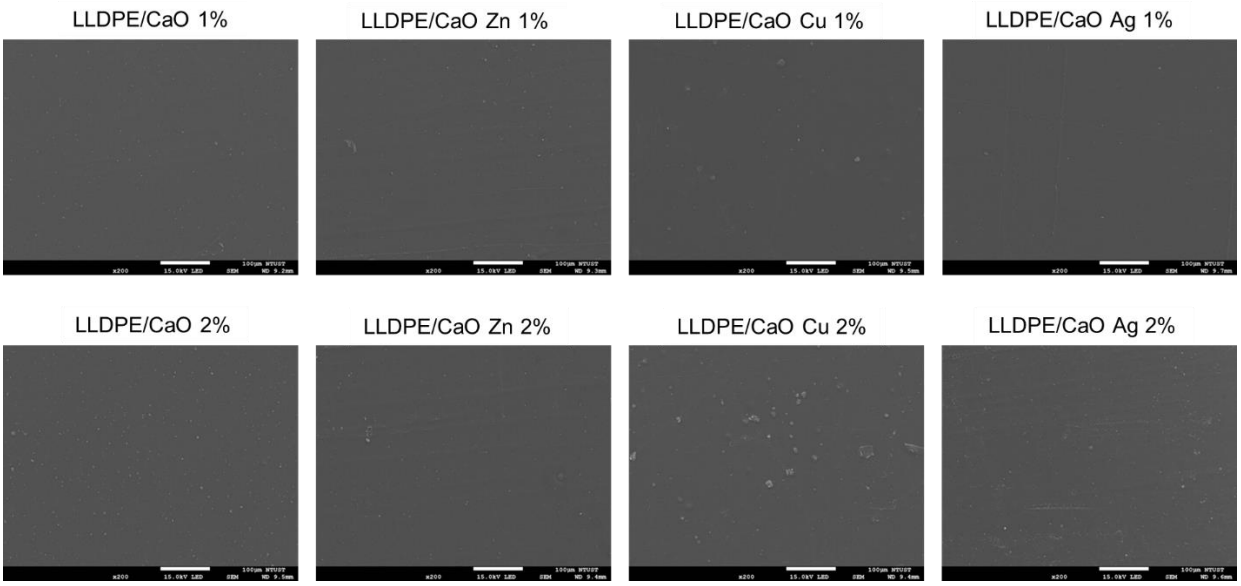


**Figure S3,** SEM surface image of polypropylene test pieces added with different concentrations prepared by calcium oxide series modified metal ions.

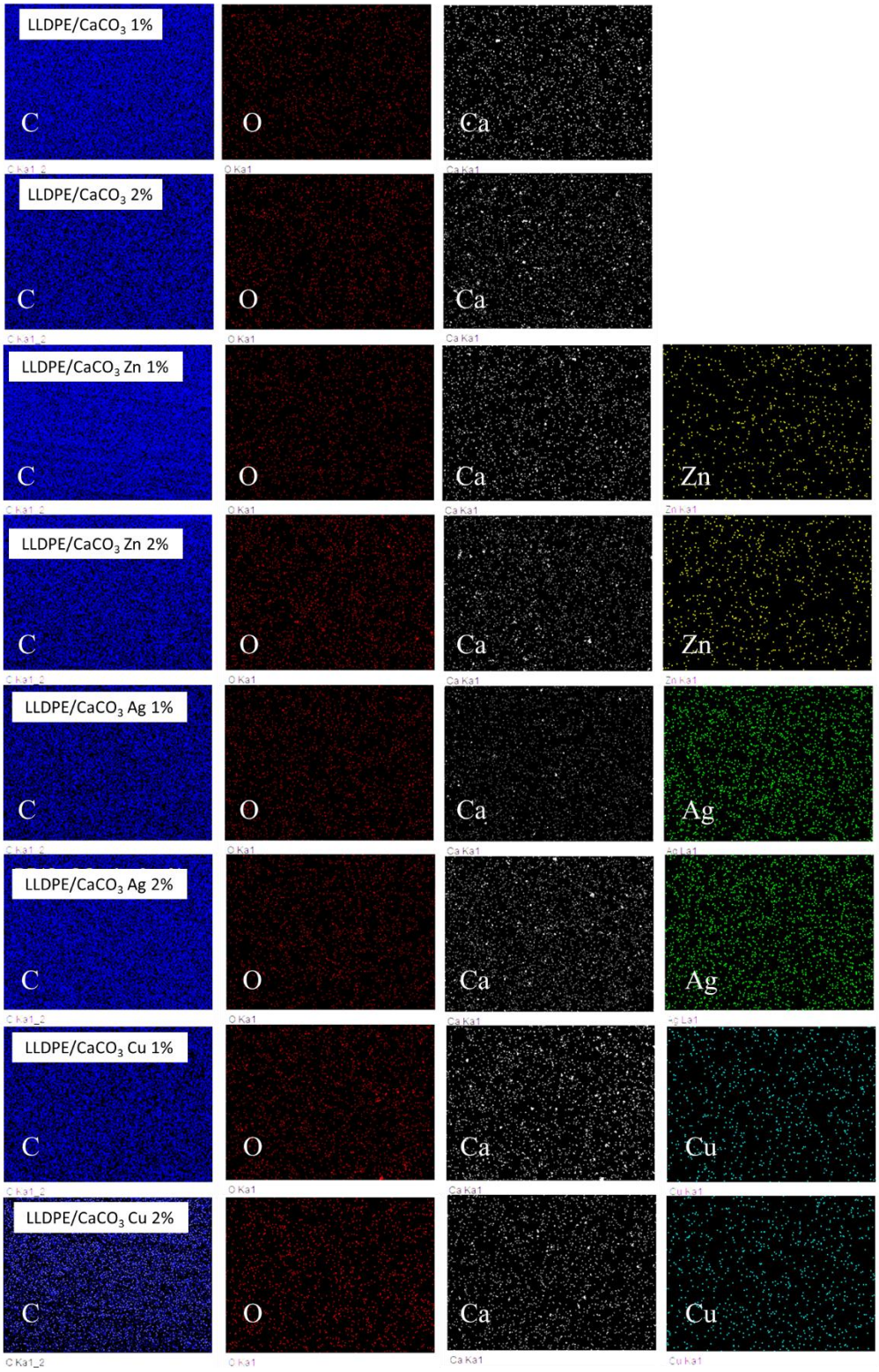


**Figure S4** EDX spectra of LLDPE/$CaCO_3$ and LLDPE/ $CaCO_3$ containing metal ions (Ag, Zn, Cu).

**Table S2**. SEM-EDX mapping elemental analysis of LLDPE added with different concentrations of $CaCO_3$ series modified metal ions

| Sample | Atom (%) | | | | Total |
|---|---|---|---|---|---|
| | C | O | Ca | Zn/Ag/Cu | |
| LLDPE/$CaCO_3$ 1% | 98.14 | 1.30 | 0.57 | 0 | 100 % |
| LLDPE/$CaCO_3$ 2% | 97.49 | 2.09 | 0.42 | 0 | 100 % |
| LLDPE/$CaCO_3$ Zn 1% | 98.57 | 1.18 | 0.16 | 0.10 | 100 % |
| LLDPE/$CaCO_3$ Zn 2% | 97.95 | 1.47 | 0.36 | 0.23 | 100 % |
| LLDPE/$CaCO_3$ Cu 1% | 98.38 | 1.21 | 0.29 | 0.11 | 100 % |
| LLDPE/$CaCO_3$ Cu 2% | 97.83 | 1.46 | 0.52 | 0.25 | 100 % |
| LLDPE/$CaCO_3$ Ag 1% | 98.38 | 1.41 | 0.14 | 0.12 | 100 % |
| LLDPE/$CaCO_3$ Ag 2% | 97.86 | 1.59 | 0.36 | 0.24 | 100 % |

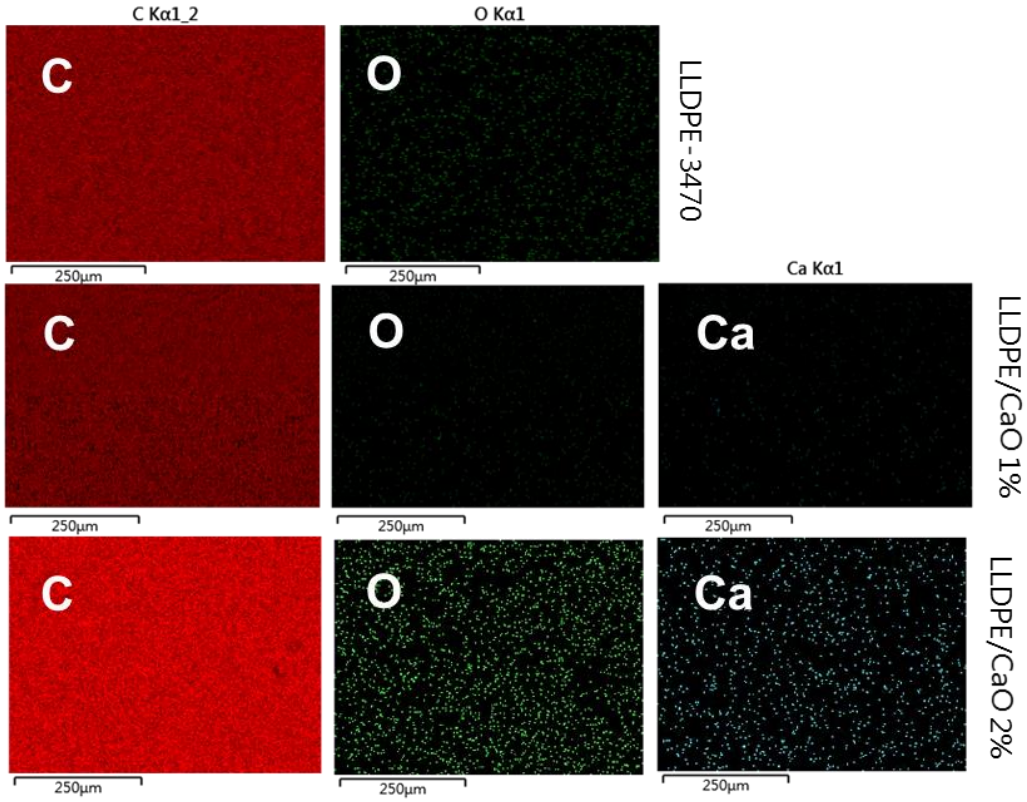


**Figure S5** EDX spectra of LLDPE-3470 and LLDPE/CaO with different concentrations (1% and 2%).

**Table S3.** The atomic percentage of LLDPE/CaO data from EDX measurement

| Sample | Atom (%) | | |
|---|---|---|---|
| | C | O | Ca |
| LLDPE-3470 | 99.4 | 0.6 | 0 |
| LLDPE/CaO 1% | 98.4 | 1.1 | 0.5 |
| LLDPE/CaO 2% | 97.6 | 1.6 | 0.8 |

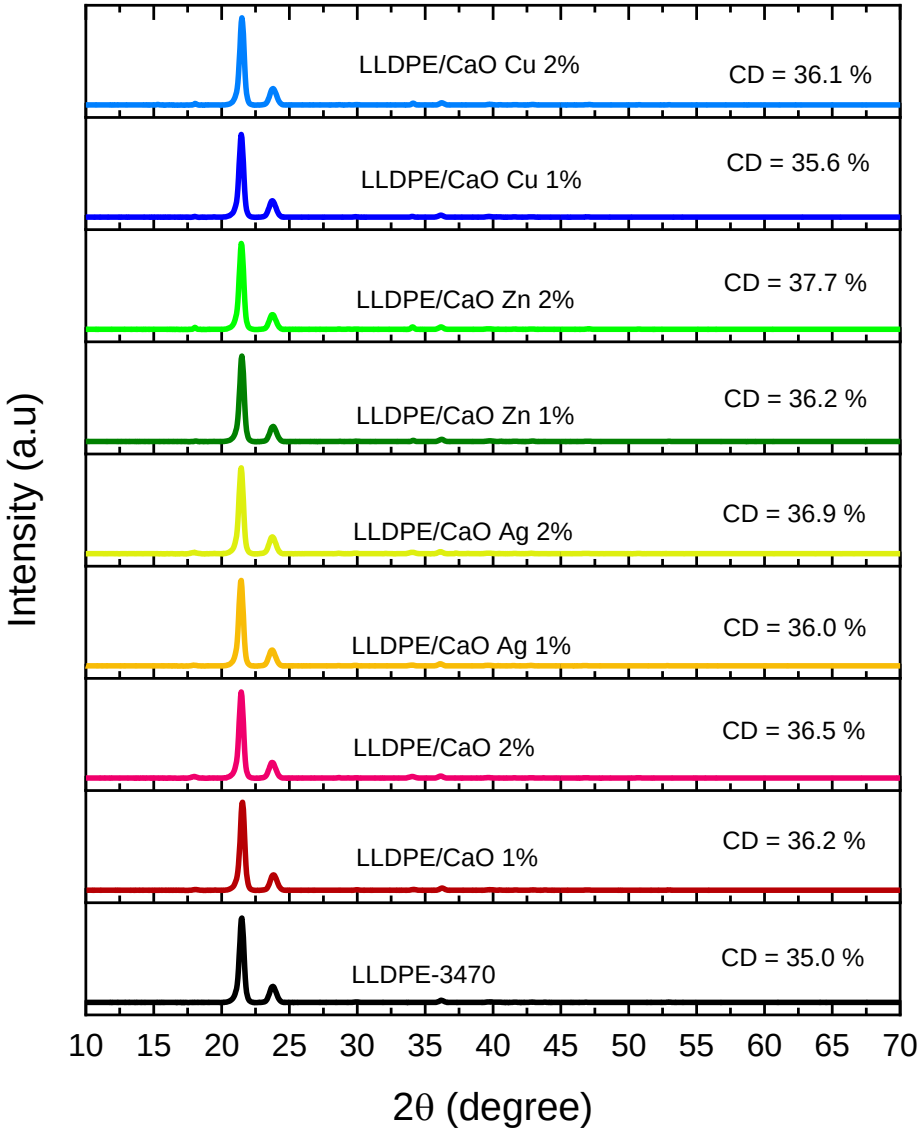


**Figure S6** XRD pattern of samples and degree of crystallinity (CD)

**Table S4.** Position of crystalline peaks and crystal indices for LLDPE and LLDPE nanocomposites with different ratios of CaO/Ag, Zn, Cu nanoparticles

| Sample | Peak 2θ (°) | | FWHM β | | Crystallite Size (nm) | | d-spacing (nm) | | Degree of Crystallinity X (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 110 | 220 | 110 | 220 | 110 | 220 | 110 | 220 | |
| LLDPE | 21.48 | 23.78 | 0.42 | 0.58 | 20.00 | 14.66 | 4.13 | 3.74 | 35 |
| LLDPE/CaO 1% | 21.53 | 23.83 | 0.42 | 0.58 | 19.93 | 14.71 | 4.12 | 3.73 | 36.2 |
| LLDPE/CaO 2% | 21.45 | 23.74 | 0.42 | 0.57 | 20.26 | 14.96 | 4.14 | 3.74 | 36.5 |
| LLDPE/CaO Ag 1% | 21.43 | 23.73 | 0.42 | 0.57 | 20.21 | 14.84 | 4.14 | 3.75 | 36 |
| LLDPE/CaO Ag 2% | 21.44 | 23.75 | 0.42 | 0.57 | 20.12 | 14.90 | 4.14 | 3.74 | 36.9 |
| LLDPE/CaO Zn 1% | 21.49 | 23.80 | 0.42 | 0.57 | 19.99 | 14.78 | 4.13 | 3.74 | 35.6 |
| LLDPE/CaO Zn 2% | 21.46 | 23.76 | 0.42 | 0.57 | 20.15 | 14.89 | 4.14 | 3.74 | 36.1 |
| LLDPE/CaO Cu 1% | 21.45 | 23.75 | 0.42 | 0.58 | 20.15 | 14.64 | 4.14 | 3.74 | 36.2 |
| LLDPE/CaO Cu 2% | 21.49 | 23.80 | 0.42 | 0.42 | 20.24 | 20.32 | 4.13 | 3.74 | 37.7 |

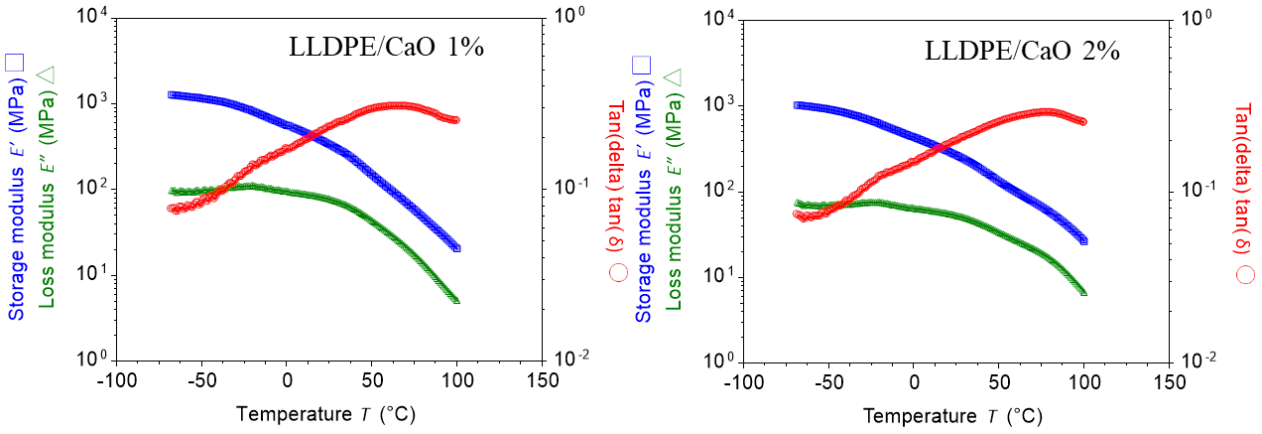


**Figure S7**. Typical DMA thermogram of LLDPE with different (%) of CaO. Storage Modulus (E') and Loss Modulus (E''), and Loss Factor tan(delta) are plotted as a function of temperature.

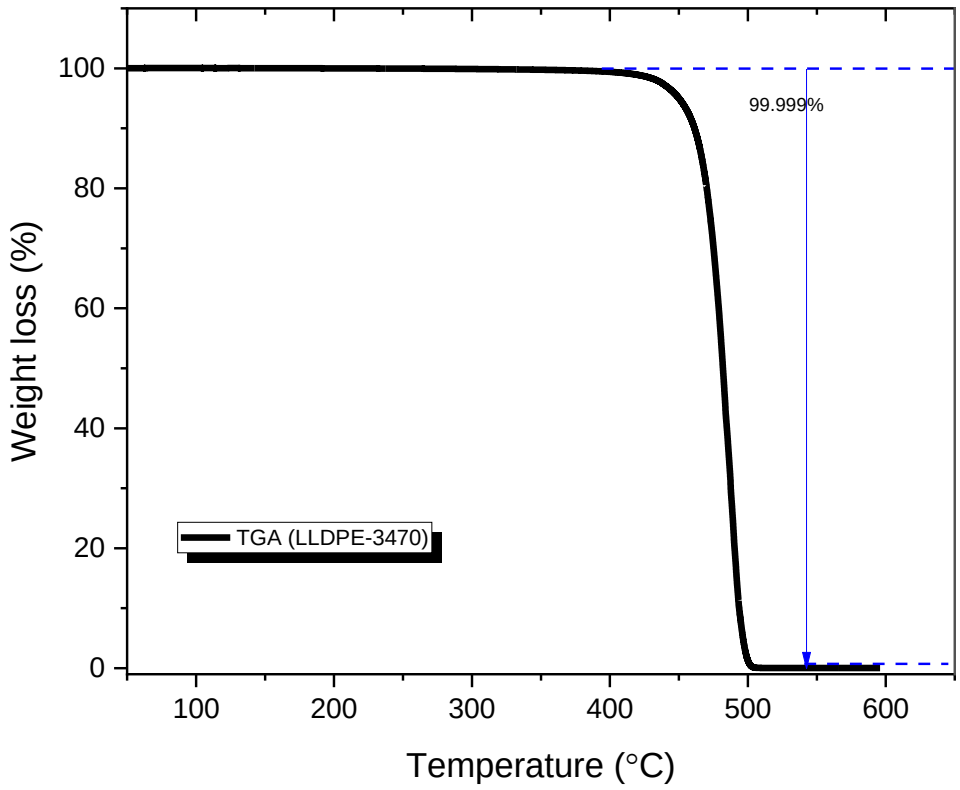


**Figure S8**. Thermogravimetric analysis (TGA) of LLDPE-3470.

**Table S5** Thermogravimetric analysis (TGA) analysis data of LLDPE and LLDPE biocomposites with different ratios of CaO/Ag, Zn, Cu nanoparticles

| | Sample | Ash testing residue (%) | Mass loss (%) |
|---|---|---|---|
| **Thermogravimetric analysis (TGA)** | LLDPE-3470 | 0.00 | 99.999 |
| | LLDPE/CaO 1% | 0.85 | 99.008 |
| | LLDPE/CaO 2% | 1.78 | 98.077 |
| | LLDPE/CaO Ag 1% | 0.66 | 99.307 |
| | LLDPE/CaO Ag 2% | 1.39 | 98.211 |
| | LLDPE/CaO Zn 1% | 0.72 | 99.214 |
| | LLDPE/CaO Zn 2% | 1.83 | 98.487 |
| | LLDPE/CaO Cu 1% | 0.53 | 99.320 |
| | LLDPE/CaO Cu 2% | 1.31 | 98.440 |

**Table S6** antibacterial activity (*Escherichia coli* and *Staphylococcus aureus*) of powder CaO-Metal ions

| **Materials** | **Antibacterial ring, Inhibition zone diameter (mm)** | |
|---|---|---|
| | ***Escherichia coli*** | ***Staphylococcus aureus*** |
| Control | 32.00 ± 1.53 | 32.00 ± 2.08 |
| CaO-Ag | 22.00 ± 2.65 | 21.00 ± 1.53 |
| CaO-Zn | 29.00 ± 1.00 | 24.00 ± 2.08 |
| CaO-Cu | 23.00 ± 1.00 | 22.00 ± 2.65 |

**Table S7** Antibacterial efficacy (%) *E.Coli* of LLDPE/CaO 1% and LLDPE/CaO 2% with a deferent metal ion.

| | **Materials** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LLDPE/CaO | | LLDPE/CaO Ag | | LLDPE/CaO Zn | | LLDPE/CaO Cu | |
| | 1% | 2% | 1% | 2% | 1% | 2% | 1% | 2% |
| **Antibacterial efficacy (%)** ***E.Coli*** | 90.60 ± 1.15 | 99.5 ± 0.46 | 99.9 ± 0.49 | 99.9 ± 0.15 | 81 ± 1.15 | 98.5 ± 1.15 | 82.7 ± 1.53 | 99.9 ± 0.10 |

**Table S8**. Classification antimicrobial sensitivity[1, 2]

| **Diameter Zone of inhibition (mm)** | **Antimicrobial activity** |
|---|---|
| ≤ 10 | Not active |
| 11-15 | Weak |
| 16-20 | Middle |
| ≥ 20 | Strong |

## References


1. Indriani, V., et al., *Antibacterial Effect of Curcuma zedoaria Extract on Bacillus cereus and Staphylococcus epidermidis.* Althea Medical Journal, 2020. **7**(1): p. 6-10.
2. Zainuddin, A. and A.H. Saputro. *Antimicrobial effectiveness measurement system based on circle detection algorithm*. in *2017 International Seminar on Sensors, Instrumentation, Measurement and Metrology (ISSIMM)*. 2017. IEEE.

# Parametric and Non-parametric tests

**Analysis of variance (ANOVA) of LLDPE/CaO-Metal ion biocomposites.**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| LLDPE/CaO 1% | 3 | 270.3 | 90.1 | 1.33 |
| LLDPE/CaO 2% | 3 | 298.5 | 99.5 | 0.21 |
| LLDPE/CaO Ag 1% | 3 | 299.7 | 99.9 | 0.01 |
| LLDPE/CaO Ag 2% | 3 | 299.7 | 99.9 | 0.01 |
| LLDPE/CaO Zn 1% | 3 | 243 | 81 | 0.25 |
| LLDPE/CaO Zn 2% | 3 | 295.5 | 98.5 | 0.25 |
| LLDPE/CaO Cu 1% | 3 | 248.2 | 82.73333 | 2.413333 |
| LLDPE/CaO Cu 2% | 3 | 299.7 | 99.9 | 0.01 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 1398.152 | 7 | 199.736 | 356.4062 | 2.38E-16 | 2.657197 |
| Within Groups | 8.966667 | 16 | 0.560417 | | | |
| Total | 1407.118 | 23 | | | | |

**Nonparametric Kruskal-Wallis Test of LLDPE/CaO-Metal ion biocomposites.**

| LLDPE/CaO 1% | LLDPE/CaO 2% | LLDPE/CaO Ag 1% | LLDPE/CaO Ag 2% | LLDPE/CaO Zn 1% | LLDPE/CaO Zn 2% | LLDPE/CaO Cu 1% | LLDPE/CaO Cu 2% |
|---|---|---|---|---|---|---|---|
| 89 | 99 | 99.8 | 99.9 | 81 | 99 | 84 | 99.8 |
| 91.3 | 99.9 | 99.9 | 99.8 | 80.5 | 98 | 81 | 99.9 |
| 90 | 99.6 | 100 | 100 | 81.5 | 98.5 | 83.2 | 100 |

$$H = \left[\frac{12}{n_T(n_T+1)}\sum_{i=1}^{k}\frac{R_i^2}{n_i}\right] - 3(n_T+1)$$

$k$ = number of populations

$n_i$ = the number of observations in sample $i$

$n_T = \sum_{i=1}^{k} n_i$ = the total number of observations in all samples

$R_i$ = the sum of the ranks for sample $i$

| | |
|---|---|
| **H** | 20.46 |
| **chi-square** | 5.99 |
| **p-value** | 3.60E-05 |

**Nonparametric Methods, Kruskal-Wallis Test**

**Analysis of variance (ANOVA) of CaO-Metal ion powder for *E.Coli* bacterial.**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| CaO Ag (E.Coli) | 3 | 66 | 22 | 7 |
| CaO Zn (E.Coli) | 3 | 87 | 29 | 1 |
| CaO Cu (E.Coli) | 3 | 69 | 23 | 4 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 86 | 2 | 43 | 10.75 | 0.010386 | 5.143253 |
| Within Groups | 24 | 6 | 4 | | | |
| Total | 110 | 8 | | | | |

**Nonparametric Kruskal-Wallis Test of CaO-Metal ion powder for *E.Coli* bacterial.**

| CaO Ag (E.Coli) | CaO Zn (E.Coli) | CaO Cu (E.Coli) |
|---|---|---|
| 20 | 29 | 21 |
| 21 | 30 | 23 |
| 25 | 28 | 25 |

$$H = \left[\frac{12}{n_T(n_T+1)}\sum_{i=1}^{k}\frac{R_i^2}{n_i}\right] - 3(n_T+1)$$

$k$ = number of populations

$n_i$ = the number of observations in sample $i$

$n_T = \sum_{i=1}^{k} n_i$ = the total number of observations in all samples

$R_i$ = the sum of the ranks for sample $i$

| | |
|---|---|
| **H** | 5.60 |
| **chi-square** | 5.99 |
| **p-value** | 0.06 |

**Analysis of variance (ANOVA) of CaO-Metal ion powder for *S. Aureus* bacterial.**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| CaO Ag (S. Aureus) | 3 | 64 | 21.33333 | 2.333333 |
| CaO Zn (S. Aureus) | 3 | 73 | 24.33333 | 2.333333 |
| CaO Cu (S. Aureus) | 3 | 66 | 22 | 1 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 14.88889 | 2 | 7.444444 | 3.941176 | 0.080735 | 5.143253 |
| Within Groups | 11.33333 | 6 | 1.888889 | | | |
| Total | 26.22222 | 8 | | | | |

**Nonparametric Kruskal-Wallis Test of CaO-Metal ion powder for *S. Aureus* bacterial.**

| CaO Ag (S.Aureus) | CaO Zn (S. Aureus) | CaO Cu (S. Aureus) |
|---|---|---|
| 20 | 26 | 21 |
| 23 | 23 | 23 |

$$H = \left[\frac{12}{n_T(n_T+1)}\sum_{i=1}^{k}\frac{R_i^2}{n_i}\right] - 3(n_T+1)$$

$k$ = number of populations

$n_i$ = the number of observations in sample $i$

$n_T = \sum_{i=1}^{k} n_i$ = the total number of observations in all samples

$R_i$ = the sum of the ranks for sample $i$

| | |
|---|---|
| **H** | 4.47 |
| **chi-square** | 5.99 |
| **p-value** | 0.11 |