The release of ZnO nanoparticles from Portland cement nanocomposites and its effects on model microorganisms

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Introduction

Nanoparticles (NPs) are widely used in different industry sectors, e.g. cosmetics, electronics, agriculture and civil engineering. It has been proven that the incorporation of nanomaterials, such as metal oxides, to cement-based composites, can improve their mechanical properties, electrical resistivity and durability. One of NPs - zinc oxide, is well-known due to its photocatalytic and semi-conductive character. However, there is still not enough information about the release of NPs incorporated in cementitious materials to the environment and their effect on organisms.

Objective

The study aimed to assess the release of ZnO nanoparticles from cement blocks (7- and 28-days-old blocks, abraded and crushed blocks and blocks treated with 600 °C and 800 °C) after 72-hours-long leaching procedure. The effects of the incubation with the leachates were tested on model microorganisms.

Materials and Methods

Materials and mix design

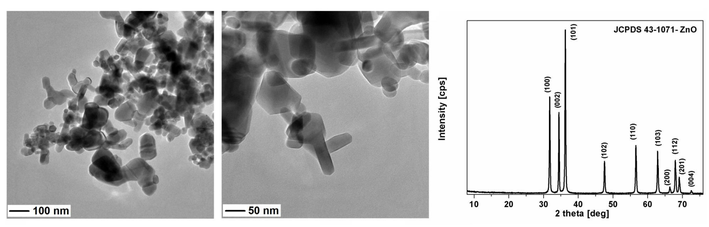

Figure 1. TEM images and XRD pattern of ZnO NPs.

The ZnO NPs structure was analysed using TEM and XRD (Fig. 1). Mortars with water-cement ratio (w/c) equal to 0.5 were produced conforming the EN 196-1. The ratio of cement to sand to water was set as 1:3:0.5. Overall, three types of cement mortars were produced. Control (plain) mortar was designated as C, while mortars containing addition of 1 % and 3 % (by weight of cement) of ZnO nanoparticles were designated as 1Z and 3Z, respectively.

Methods

Samples tested included leachates of 7- and 28-days-old blocks (7 and 28), abraded (A) and crushed (C) blocks and blocks treated with 600 °C (600) and 800 °C (800). The release of the nanoparticles was assessed with AAS and ICP-MS. Growth kinetics assay was performed by optical density (OD600) measurements of the cultures and the growth rate was calculated according to Tsuchiya *et al.* (2018) and compared to the control growth rate. 24-hour toxicity assay was carried out in optical density (OD600) measurements of the cultures (t0 and t24) and the viability of the cells was assessed by AlamarBlue® assay. Biofilm formation experiment was carried out with AlamarBlue® to assess cells viability and later treated with 1% *w/v* crystal violet in order to measure the biomass production (OD595).

References

Jajda, H. M. *et al.* (2013) 'Comparative efficacy of two standard methods for determination of iron and zinc in fruits, pulses and cereals', *Journal of Food Science and Technology*, 52(2), pp. 1096–1102. doi: 10.1007/s13197-013-1088-6.

Tsuchiya, K. *et al.* (2018) 'A decay effect of the growth rate associated with genome reduction in Escherichia coli', *BMC Microbiology*. BMC Microbiology, 18(1), pp. 1–10. doi: 10.1186/s12866-018-1242-4.

Contact

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Results

AAS and ICP-OES analyses

Tab. 1 Concentrations of Zn in tested leachates measured with ICP and AAS (ND – not detected)

Sample	C7	1Z7	3Z7	C28	1Z28	3Z28	CA	1ZA	3ZA	CC	1ZC	3ZC	C600	1Z600	3Z600	C800	1Z800	3Z800
ICP (mg/L)	0,002	0,003	0,0811	0,0079	0,028	0,0269	0,003	0,0223	0,0426	0,0048	0,0796	0,372	0,0041	0,014	0,1555	0,0032	0,027	0,2557
AAS (mg/L)	ND	ND	0,044	ND	ND	ND	ND	ND	0,078	ND	0,008	0,273	ND	ND	0,102	ND	ND	0,162

24-hour toxicity assay

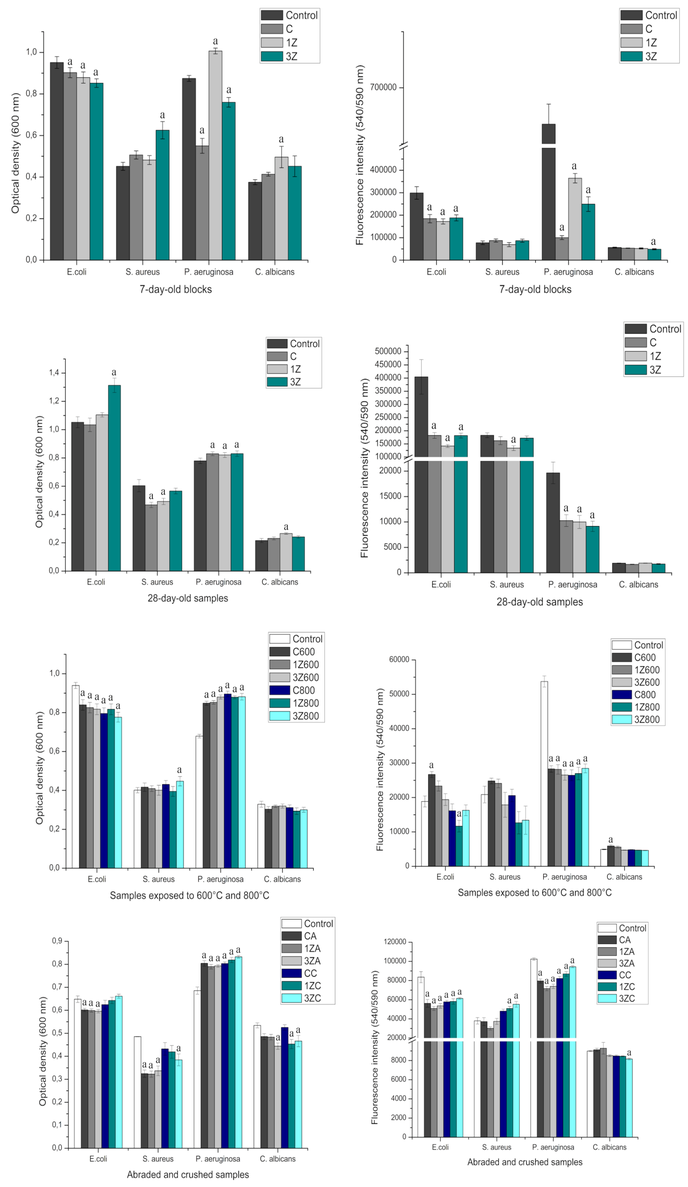

Fig. 2 Optical density and fluorescence measurements of the microbe cultures exposed to the tested leachates for 24 hours

Growth kinetics

Tab. 2 Growth rates (μ) of the cultures incubated with the tested leachates in comparison to the control growth rates

Sample	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*
C7	103%	94%	111%	117%
1Z7	119%	99%	101%	105%
3Z7	107%	96%	126%	96%
C28	102%	94%	110%	99%
1Z28	112%	107%	95%	102%
3Z28	115%	91%	91%	107%
CA	112%	110%	95%	107%
1ZA	104%	95%	94%	105%
3ZA	114%	101%	80%	84%
CC	103%	105%	99%	98%
1ZC	107%	96%	98%	96%
3ZC	114%	104%	105%	96%
C600	108%	102%	102%	90%
1Z600	116%	91%	100%	107%
3Z600	112%	95%	100%	87%
C800	106%	106%	104%	93%
1Z800	109%	106%	103%	87%
3Z800	109%	103%	91%	96%

Biofilm formation assay

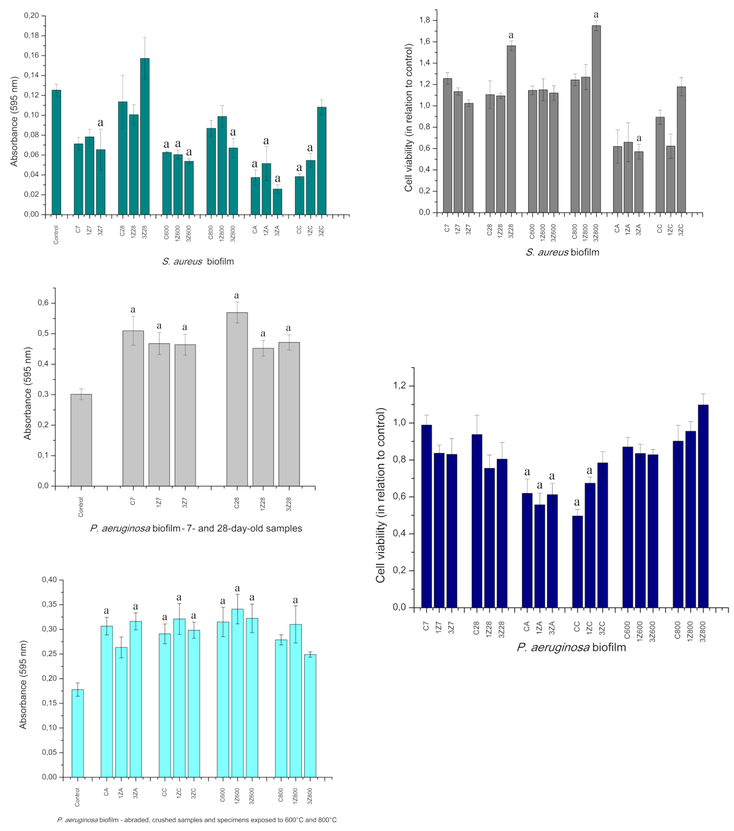

Fig. 3 Biofilm biomass and fluorescence measurements of the cultures incubated with the tested leachates

Conclusions

1. Both spectroscopic methods confirmed the presence of Zn in filtered leachates with the addition of 3% Zn (all samples besides 28-day-old blocks) and in abraded 1% sample. It indicates that syringe filtering of the samples did not cause the gathering of all the nanoparticles on the filter surface. However, filtering might lead to the reduction of the nanomaterial concentration, e.g. abraded samples clogged the filter pores relatively quickly. On the other hand, filtering process allowed the separation of the unwanted residues and agglomerates. Moreover, the differences in the concentrations detected using ICP and AAS were noted. Nevertheless, the concentrations are relatively low and may be caused by the standard deviation characteristic for these spectroscopic methods as shown by Jajda *et al.* (2013).
2. In most cases the cell viability was decreased or equal to the control values. The only exceptions were samples that were crushed (1ZC and 3ZC) with *P. aeruginosa*, control samples treated with 600 °C (C600) with *E. coli* and *C. albicans* and 3% Zn samples (3Z28 and 3Z800 in biofilm assay).
3. The choice of the microbial model was affecting the observations. For example, the differences between two Gram-negative bacteria were noted – the OD of *P. aeruginosa* increased in the experiments with temperature-treated and crushed samples while the OD of *E. coli* decreased or was equal to the control value.
4. *Pseudomonas aeruginosa* biofilm biomass was greater than the control biomass in most tested samples while the cell viability decreases. Perhaps it is a defence mechanism of the bacterium incubated in unfavourable conditions. On the other hand, the experiment with 3Z800 sample showed the cell viability on the control level and no significant biomass increase. Moreover, *S. aureus* incubated with the same sample showed the decreased biofilm biomass but higher cell viability. It may indicate the lower toxicity of the above-mentioned sample.
5. Presented research shows rather the effect of cement mortar blocks than ZnO NPs on model microorganisms. Obtained results do not indicate the pronounced toxicity of tested nanomaterial to microbes, which may indicate that the release of the nanoparticles from cement mortars is lower than concentration toxic to test microorganisms.

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