ECOTOXICITY OF ORGANOMETAL HALIDE PEROVSKITES TESTED ON *PSEUDOMONAS PUTIDA*

ROSICKÁ Petra[1,2], NGUYEN H. A. Nhung[1,2], ŠEVCŮ Alena[1,2], LEDERER Tomáš[1], RYSOVÁ Miroslava[1,2]

[1]*Technical University of Liberec, Institute for Nanomaterials, Advanced Technologies and Innovations, Liberec, Czech Republic, EU; petra.rosicka@tul.cz*

[2]*Technical University of Liberec, Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Liberec , Czech Republic, EU*

Abstract

Organometal lead halide perovskite is relatively new material, which has proved to be a promising material for construction of solar cells for photovoltaic technology. However, little is known about the toxicity of perovskite nanomaterials. Therefore, we performed several experiments to evaluate potential ecotoxicity of perovskites on soil bacteria *Pseudomonas putida*. Three types of perovskites were tested ($CH_3NH_3PbI_3$, $CHNHNH_3PbBr_3$, $CH_3NH_3PbBr_3$), each of them containing toxic lead, which leaked from the material. Therefore we determined the concentration of leaked lead using inductively coupled plasma mass spectrometry (ICP-MS). The effect of perovskites and lead on bacterial metabolism and viability was evaluated using respirometry and fluorescence analysis. Three concentrations of perovskites were tested - 50, 100 and 500 mg/L and four concentrations of lead in form of $Pb(NO_3)_2$ (100, 200, 500 and 1000 mg/L) in diluted Soya nutrient broth medium. The bacterial respiration was negatively affected by 500 and 1000 mg/L of lead; however it was not affected when similar concentration that leaked from the perovskite (100 mg/L and 200 mg/L) was added. Moreover, none of the three perovskite materials caused significant toxic effect towards bacteria.

Keywords: Perovskite, ecotoxicity, *Pseudomonas putida*, respirometry

1. INTRODUCTION

Organometal lead halide perovskites have become very popular in solar cell technology over the last few years. Perovskite materials offer several advantages which allow widespread use and application in photovoltaic cells. The material exhibits special electrical, structural and optical properties which allow great efficiency of photovoltaic energy conversion while it is made of quite cheap components [1, 2].

The mineral $CaTiO_3$ is commonly called perovskite but in this case the term perovskite is collective designation for ABX_3 structures. Where A is an organic cation coordinated generally methyl ammonium, B is a metal ion for example Pb or Sn, which is octahedrally coordinated by an anion X representing halogen ion - Cl, I or Br [1, 3].

There are many different factors which can cause degradation of the perovskite materials and thus also corresponding devices. These factors include impact of water, crystalline structure, temperature effect or influence of UV light; perovskites are also sensitive to oxygen [4 - 6]. Moreover, perovskite materials contain a hygroscopic amine group which is decomposed in the presence of moisture and oxygen [6].

Concerns raise about the potential health hazard of the perovskites due to toxic lead content and the material being insufficiently stable. The perovskites could be released into natural environments, e.g. soil and waterways during the device production and handling. The investigation of potential toxicity of the perovskite materials and especially the role of potentially leaked lead on microorganisms and then on higher organisms and humans is therefore highly important [2].

This work is focused on the assessment of potential toxicity of perovskite materials using soil bacteria *Pseudomonas putida*. All three types of perovskites contain toxic lead, which can leak from the material. We tested three concentrations of each type of the perovskite - 50, 100 and 500 mg/L and four concentrations of lead in the form of $Pb(NO_3)_2$ - 100, 200, 500 and 1000 mg/L. The experiment was performed in diluted Soya nutrient broth medium which served as a substrate and nutrients source for the testing microorganism.

2. MATERIALS AND METHODS

2.1. Perovskites

Three types of perovskites $CH_3NH_3PbI_3$ hereafter MALI, $CHNHNH_3PbBr_3$ hereafter FALB and $CH_3NH_3PbBr_3$ hereafter MALB were obtained in powder form from EPFL, Switzerland.

2.2. Leakage of lead from perovskites

Each perovskite was suspended and mixed in sterilized deionized water (DI) to achieve a stock solution of 10 g/L concentration. The stock solution was used to prepare a sample for determining the leakage of lead. The final concentration of perovskites - 0.5 g/L was tested in diluted Soya nutrient broth medium (the same medium which was used for respiration tests and live/dead cells analysis). Lead concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS) after 24 hours incubation.

2.3. Respiration of *P. putida*

Bacterial respiration of *P. putida* was monitored for 24 h at 27 °C when exposed to three types of perovskite nanomaterials (MALI, FALB and MALB) and $Pb(NO_3)_2$. Oxygen consumption and carbon dioxide production were monitored using Micro-Oxymax respirometer (Columbus Instruments International, USA). The respiration was measured according to the standard methodology of EN ISO 9408, using perovskites nanoparticles instead of organic compound. Soya nutrient broth was used as a readily degradable substrate providing increased substrate (exogenous) respiration.

Based on our previous experiments, diluted Soya nutrient broth with 2 g/L of total organic carbon (11 ml DI water + 4 ml Soya nutrient broth) was used as a medium in all tests. The initial absorbance (i.e. cell density) of *P. putida* in the samples was adjusted to be 0.01 at 600 nm. We tested MALI, FALB and MALB in three final concentrations - 50, 100 and 500 mg/L, along with $Pb(NO_3)_2$ and cells only in diluted Soya nutrient broth medium as a control sample. Because the lead leaked from the perovskite nanoparticles, sample with bacteria and $Pb(NO_3)_2$ were prepared in parallel. The concentration $Pb(NO_3)_2$ was used according to the concentration of leaked lead showed in **Table 1**, it was determined using ICP-MS. Following experiment on determination of respiration activity in $Pb(NO_3)_2$ was performed in more concentrations - 100, 200, 500 and 1000 mg/L. Each sample in all experiments was prepared in duplicates.

2.4. Determination of dead cells

Cell viability was evaluated using the LIVE/DEAD® BacLight™ Bacterial Viability Kit, which allows observation and comparison of both living and dead cells (cells with damaged membranes [i.e. dead or dying] turn red while cells with intact membranes turn green). Fluorescence analysis was performed using Synergy HTX Multi mode Reader (BioTek) with filter set with excitation 485/20 nm and emission 645/40 nm for dead cells and excitation 485/20 nm and emission 528/20 nm for live cells. The analysis was performed after 6 and 24 hours of incubation at 27 °C. The same medium was used for the fluorescence analysis and respiration tests.

Each perovskites suspension (MALI, FALB, MALB) was added to the bacterial culture in a range of final concentrations: 0, 50, 100 and 500 mg/l and $Pb(NO_3)_2$ as determined in the leakage experiment (**Table 1**). 1 ml aliquots were transferred from each sample to a 24-well plate. Samples were incubated at 27 °C for 6 and 24 h. Thereafter, 100 µl of each sample was transferred to a 96-well plate and 100 µl of live/dead staining was added to each sample and incubated in dark for 15 minutes. After incubation, fluorescence was measured using multi-plate reader. Each sample was prepared in triplicates.

2.5. Scanning electron microscopy (SEM) analysis of perovskites

Size and morphology of the perovskite nanoparticles were characterized using SEM. Prior to analysis, 10 µl of each type of perovskite suspended in DI water (10 g/L) and small amount of powder of perovskite was transferred onto a conductive, adhesive tape coated specimen mount and dried. Dried samples were Au/Pt sputtered (5 nm) and visualized using Vega3 SEM (Tescan Ltd.).

3. RESULTS AND DISCUSSION

All tested perovskite nanomaterials were not stable in terms of lead leakage. Concentrations of the leaked lead in samples with MALI and FALB perovskite were very similar. The highest amount of the leaked lead was from the perovskite MALB - 161 mg/L (**Table 1**).

Table 1 Leakage of lead from perovskite nanomaterials

Perovskite type	Concentration of leaked Pb (mg/L)
MALI	126
FALB	133
MALB	161

There was no significant effect of perovskites MALI, FALB and MALB on the respiration activity of *P. putida*. Although the concentrations of leaked lead were rather high, no negative effect of $Pb(NO_3)_2$ against bacteria was observed. The difference in maximal respiration rate of all samples ranged up to 25 % of the control sample within 24 hours (**Figure 1a, 1b, 1c**). When wider range of concentrations of lead ($Pb(NO_3)_2$) were tested, 500 mg/L and 1000 mg/L significantly reduced the respiration rate of *P. putida*. Respiration activity was reduced by 90 % in both cases while the difference in maximal respiration rate for lower concentrations of lead - 100 mg/L and 200 mg/L was not significantly different from the control samples (**Figure 1d**).

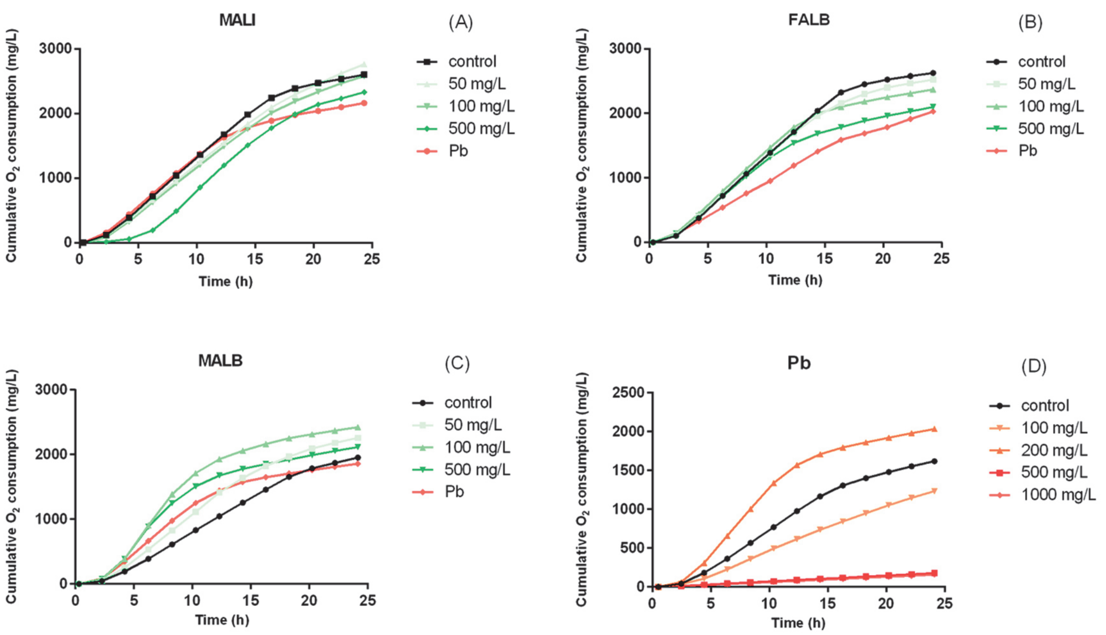


Figure 1 Cumulative oxygen consumption in bacterial samples with added perovskites (A, B, C) and in bacterial samples with lead added in higher concentrations (D)

There was no significant effect of perovskites (MALI, FALB and MALB) and $Pb(NO_3)_2$ on *P. putida* viability (**Figure 2**) determined using LIVE/DEAD kit and fluorescence analysis. No difference was observed between samples exposed for shorter and longer time (6 and 24 h).

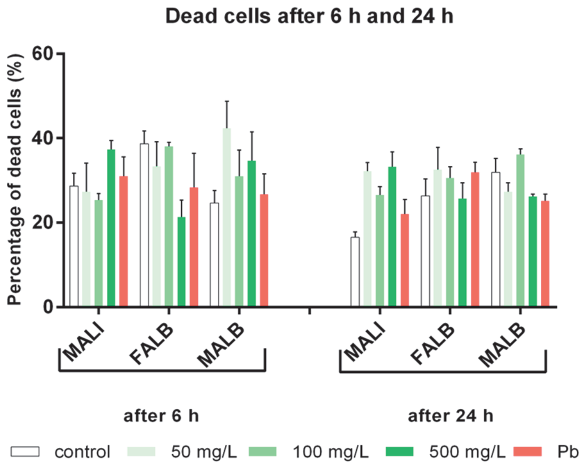


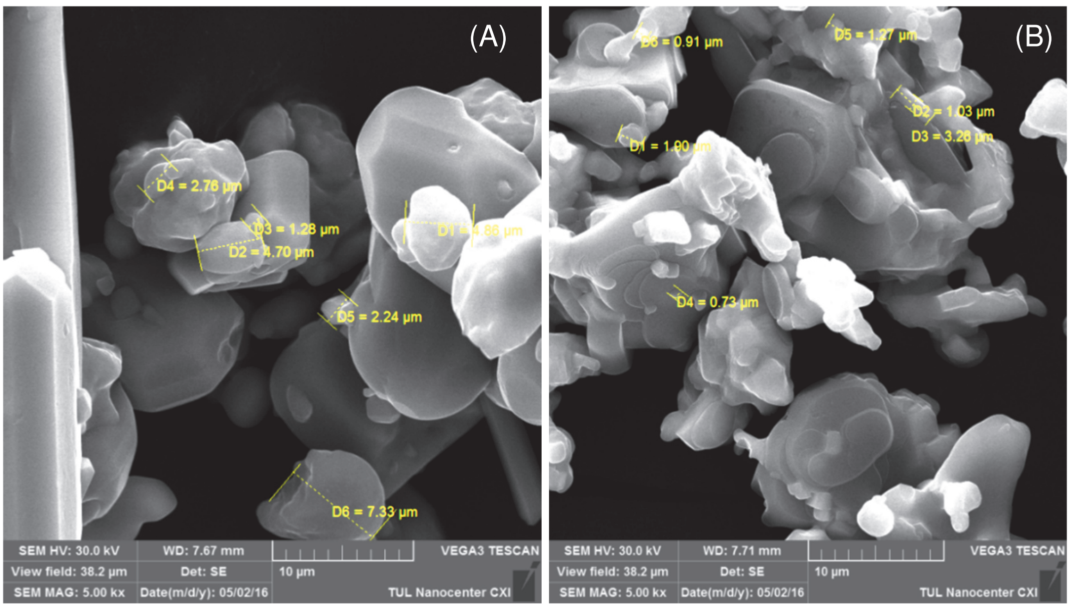
Figure 2 Percentage of dead cells of *P. putida* exposed to perovskite nanoparticles (MALI, FALB and MALB) and the lead

3.1. SEM images of perovskites

The morphology and size of the perovskite FALB and MALB particles in powder form were similar (**Figure 3a and 3b);** the size of the smallest particles was around 1 µm. On the other hand, FALB and MALB crystallized in a thin needle in DI water. Both perovskite types contain Br (bromine) (**Figure 3c**). Perovskite MALI contains I (iodine) and in this sample any needle shaped crystals were not observed in DI water (**Figure 3d**). The following reaction shows the effect of water molecule on the chemical stability of MALI [6]:

$CH_3NH_3PbI_3$ (s) → PbI_2 (s) + CH_3NH_3I (aq).

Lead iodide forms single crystals with regular hexagonal microstructures [7]. If we expect similar reaction for perovskite with bromine (FALB, MALB), $PbBr_2$ very likely rises from its decomposition. The formation of crystals in the form of needles is probably caused with $PbBr_2$.



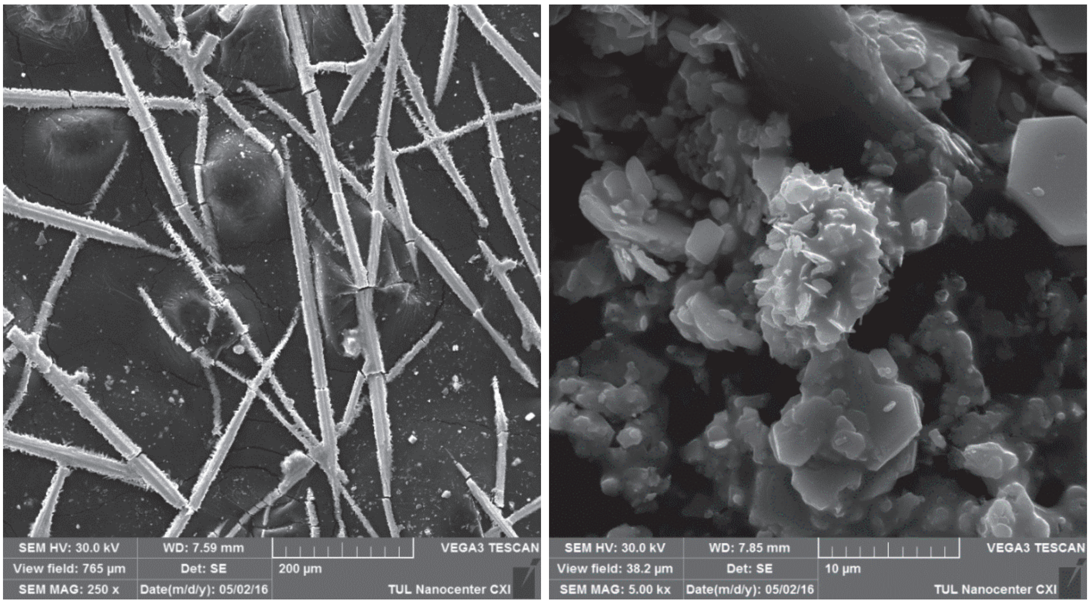


Figure 3 SEM images of perovskite particles in powder form (A - FALB, B - MALB) and in deionized water (C - FALB, D - MALI)

4. CONCLUSION

Generally, none of the three tested perovskite materials (MALI, FALB, and MALB) showed significant toxic effect against *P. putida*. Respiration of the bacterial culture was not affected even in high concentration (500 mg/L) of the nanomaterials. The same conclusion was attained from the live/dead fluorescence analysis, no significant toxicity, and no significant differences within perovskites were observed. The potentially toxic factor - leakage of lead from the perovskites was tested in parallel. The highest concentration of leaked lead (161 mg/L) was detected in MALB. Even such a high concentration of lead did not cause toxic effect against *P. putida*. Respiration of bacterial culture was reduced by 90 % only in samples with high concentrations of lead (500 and 1000 mg/L). The highest tested concentration of lead without any toxic effect was 200 mg/L. SEM images revealed that the size of particles was in the order of micrometers rather than nanometers. Perovskites containing Br (FALB and MALB) crystallized in a thin needle showing that the materials were not stable in the water environment.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°604602. The work of P. Rosická was supported by the Ministry of Education of the Czech Republic within the SGS project no. 21176/115 of the Technical University of Liberec. The authors acknowledge the assistance provided by the Research Infrastructure NanoEnviCz, supported by the Ministry of Education, Youth and Sports of the Czech Republic under Project No. LM2015073. The results of project LO1201 were obtained through financial support of the Ministry of Education, Youth and Sports under the framework of targeted support within the "National Programme for Sustainability I" and the OPR&DI project Centre for Nanomaterials, Advanced Technologies and Innovation (CZ.1.05/2.1.00/01.0005).

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