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## INTRODUCTION

Nitroimidazoles are not authorised for the treatment of honey bees in the European Union (Regulation (EC) No 470/2009 of the European Parliament and of the Council). However, they can be found in honey and other honeybee products such as beeswax largely because they are illegally used in apiculture for the treatment of Nosema disease. Any toxic substances dissolved or incorporated in beeswax could be released much later when the beeswax is given to bees in the form of wax foundations sheets. Residues of these substances in the wax of the comb could contaminate honey during the next honey season. The aim of the study was to investigate whether nitroimidazole-contaminated beeswax could lead to contamination of the honey.

## MATERIALS AND METHODS

The wax foundation fortified with a mixture of 4 nitroimidazoles: metronidazole (MNZ), ronidazole (RNZ), dimetridazole (DMZ) and ipronidazole (IPZ) at the concentration level of 10 mg kg<sup>-1</sup> (Fig. 1) was placed in a beehive to let the honeybees (*Apis mellifera* L.) draw out the contaminated wax foundation to a honeycomb. At 1 month from the start, the frame filled with capped honey was removed from the hive for a first sampling of honey. Next, the honeycomb was further incubated for 5 months in the laboratory at 35 °C and sampled monthly (Fig. 2). In the sampled honey, the concentrations of nitroimidazoles and their main metabolites: hydroxymetronidazole (MNZOH), 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI), hydroxyipronidazole (IPZO) were determined by LC-MS/MS and compared to those determined in the nitroimidazole-containing wax foundation.

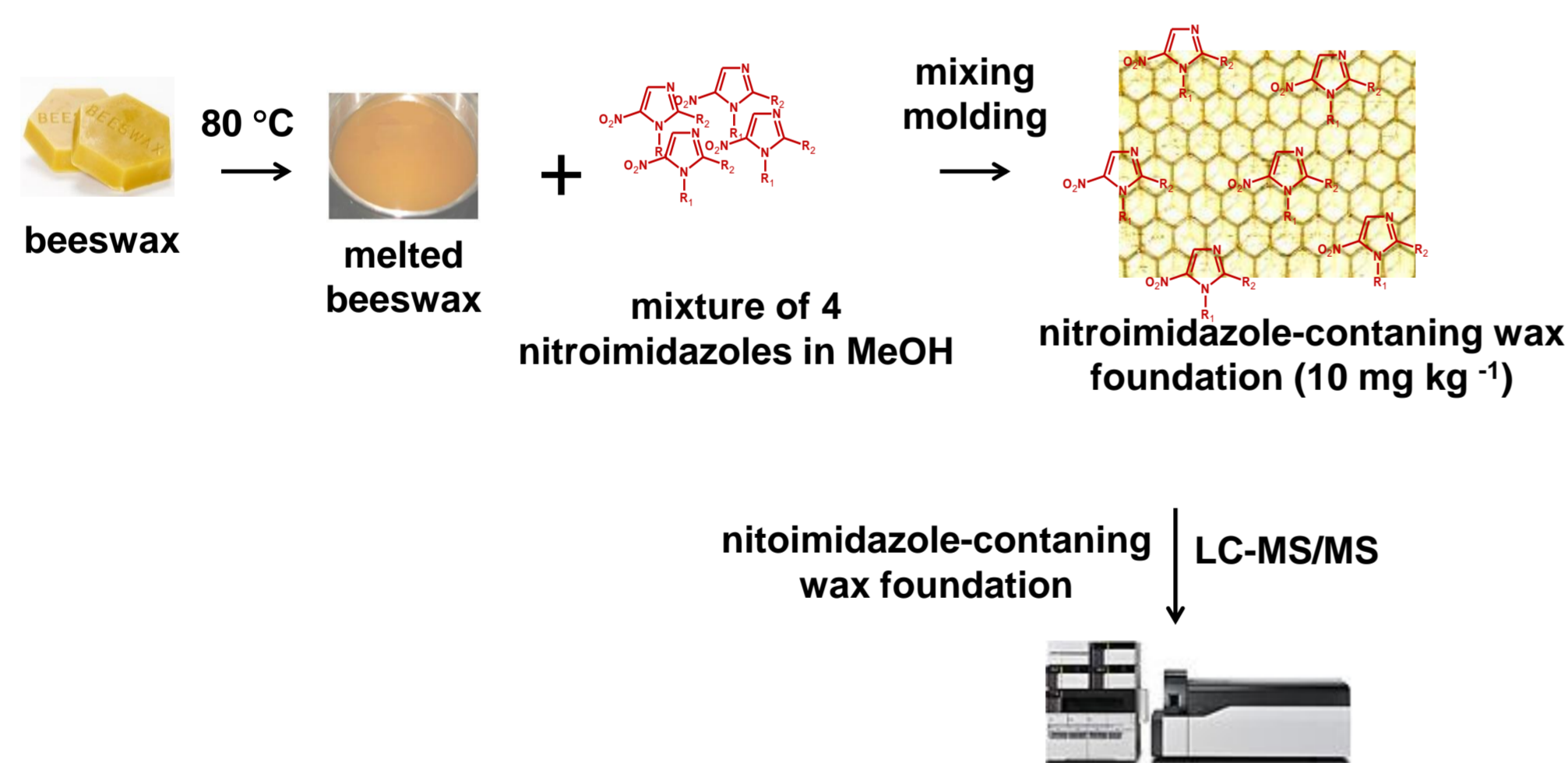


Fig. 1. The preparation of nitroimidazole-containing wax foundation.

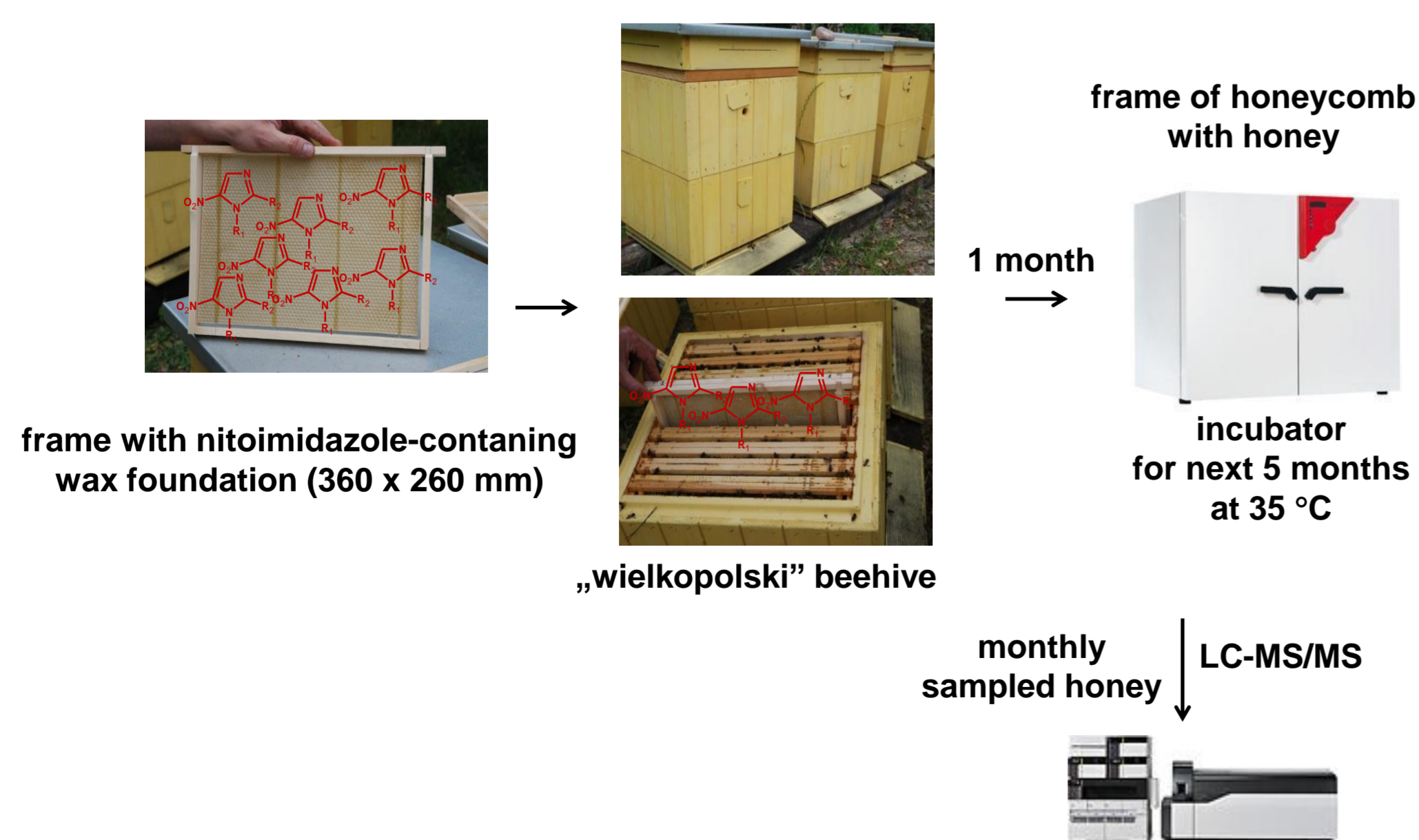


Fig. 2. Beehive treatment and incubation of nitroimidazole-containing wax foundation

## RESULTS

The highest concentration for MNZ (368 µg kg<sup>-1</sup>) and RNZ (233 µg kg<sup>-1</sup>) was observed at the fourth month (Fig. 3) whereas for DMZ (8 µg kg<sup>-1</sup>) and IPZ (8 µg kg<sup>-1</sup>) the highest content was found in the second month from the start of the experiment (Fig. 4). When we took into account that a frame completely filled with honey on both sides of the comb contained 110 g of beeswax and 2 488 g of honey and this ratio was constant, then the maximum amount of initial MNZ, RNZ, DMZ and IPZ that migrated from contaminated wax foundation to honey was 89.38%, 54.62%, 2.65% and 2.02%, respectively.

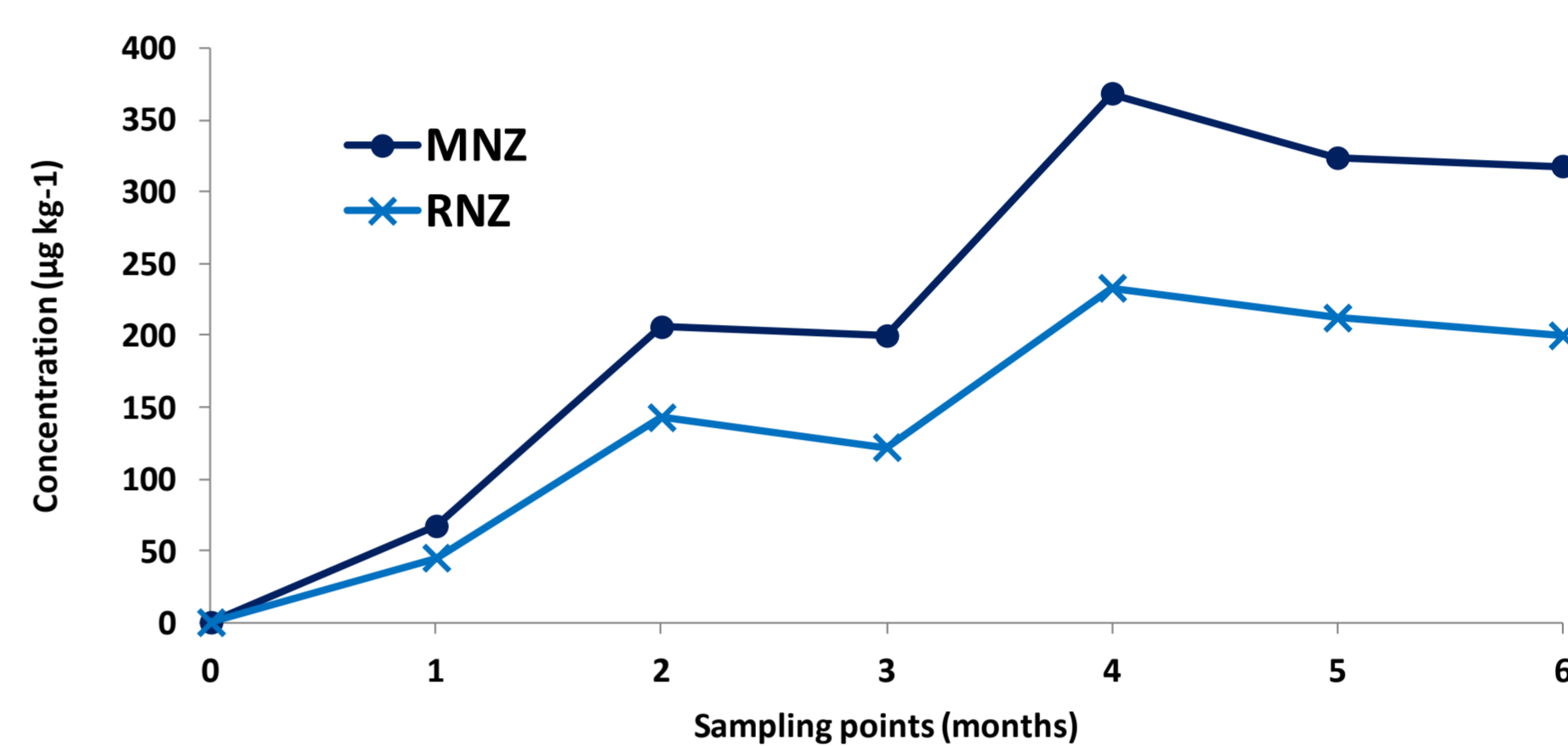


Fig. 3. Concentrations of MNZ and RNZ in honey sampled from a honeycomb drawn out on a wax foundation contaminated with MNZ, RNZ at the level of 9324 µg kg<sup>-1</sup> and 9646 µg kg<sup>-1</sup>, respectively.

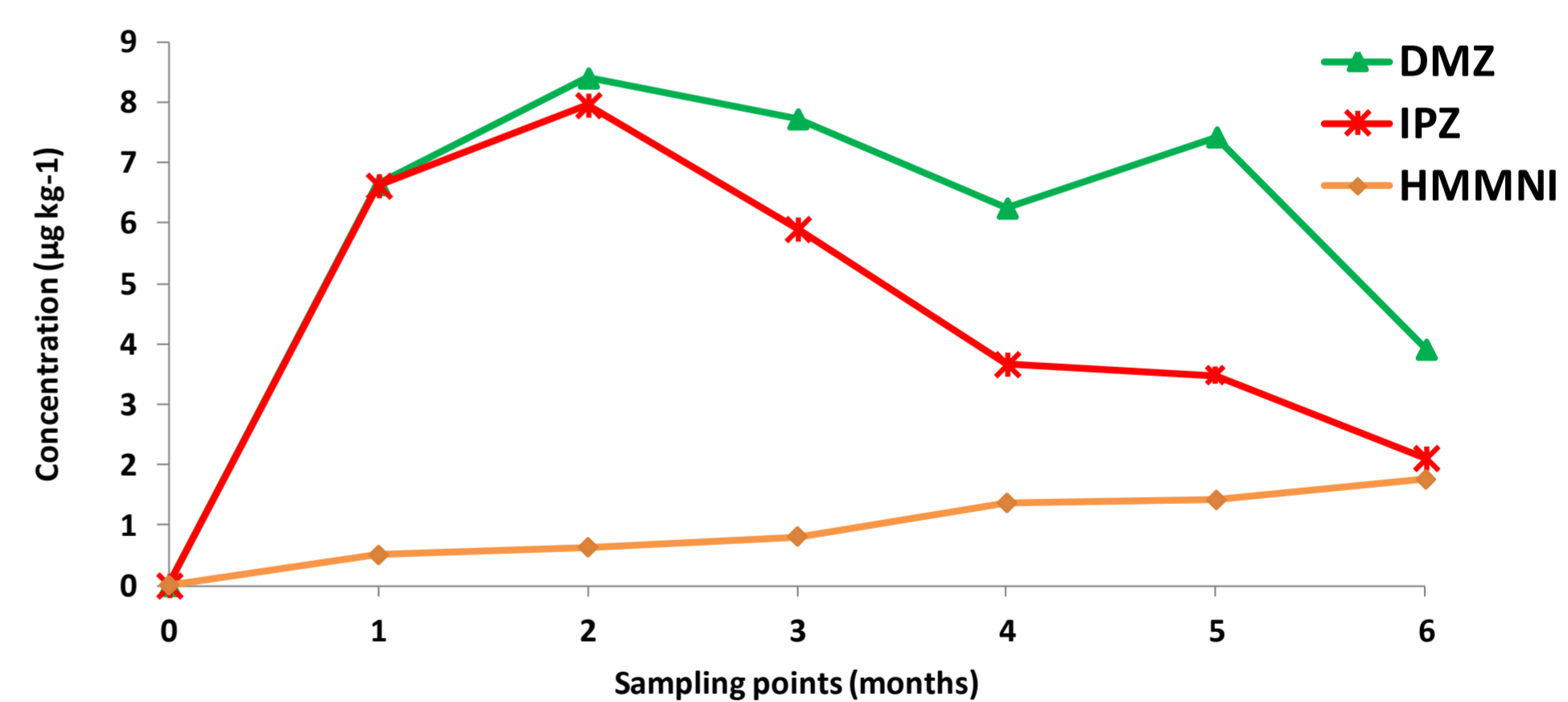


Fig. 4. Concentrations of DMZ, IPZ and HMMNI in honey sampled from a honeycomb drawn out on a wax foundation contaminated with DMZ, IPZ and HMMNI at the level of 7175 µg kg<sup>-1</sup>, 8926 µg kg<sup>-1</sup> and 22 µg kg<sup>-1</sup>, respectively.

## CONCLUSIONS

- Each of the tested nitroimidazoles could migrate from beeswax to honey kept in the contaminated comb.
- Metronidazole has the most and ipronidazole has the least migration potential from contaminated beeswax to honey.
- The control of nitroimidazoles in beeswax should be carried out to ensure high quality and safety of beeswax itself, beeswax foundation and honey.

## FUNDING

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