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INTRODUCTION

The application of nanoparticles (NPs) is increasing drastically, especially in crop production. The repeated inputs of metal-based NPs in agri-field could increase their concentration in soil, and cause a threat to sustainable crop production.

The present study was designed to determine the role of spore-forming metal tolerant bacteria (MTB) and biochar (B) to alleviate the toxic effects of high dose of ZnO NPs (2000 mg kg⁻¹) on plants (*Hordeum sativum* L.) spiked to the soil.

METHODS

The five treatments were used such as 1) clean soil, 2) soil+NPs, 3) soil+NPs+MTB, 4) soil+NPs+B and 5) soil+NPs+B+MTB in plastic vessels in triplicate.

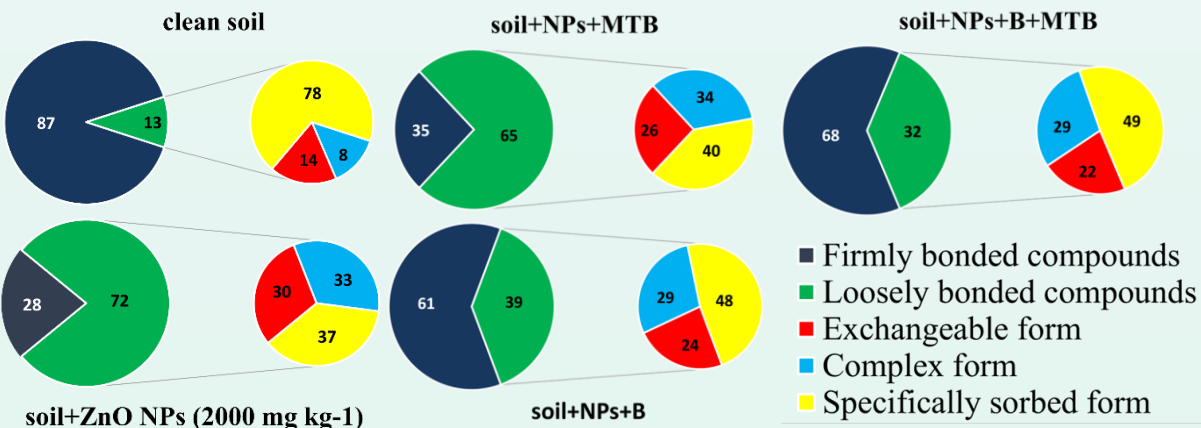
The ecological conditions of the soils were assessed by the value of the total content of HM (X-ray fluorescence analysis) and their mobile compounds (extraction with ammonium acetate buffer (AAB), 1% solution of EDTA in AAB using atomic absorption spectroscopy) in soils.

HMs were extracted from plant ash by dissolution in 20% HCl solution followed by AAS analysis. Anatomical and morphological features in the tissues of the plants affected by HM were analyzed using light-optical microscopic methods.



RESULTS

The application of MTB to the contaminated soil reduced the mobility of Zn by 7%, mainly due to exchangeable compounds, and B reduced mobility up to 33%, because of a decrease in equally exchangeable, complex, and specifically sorbed forms. The combined introduction of MTB and B reduced most effectively the actual and potential content of Zn compounds in soil.



Content of Zn in *Hordeum sativum*, mg/kg

Treatment	root	stem
clean soil	20±1	18±1
soil+NPs	1982±119	1267±84
soil+NPs+MTB	1548±77	1125±56
soil+NPs+B	737±52	406±24
soil+NPs+B+MTB	522±26	285±20

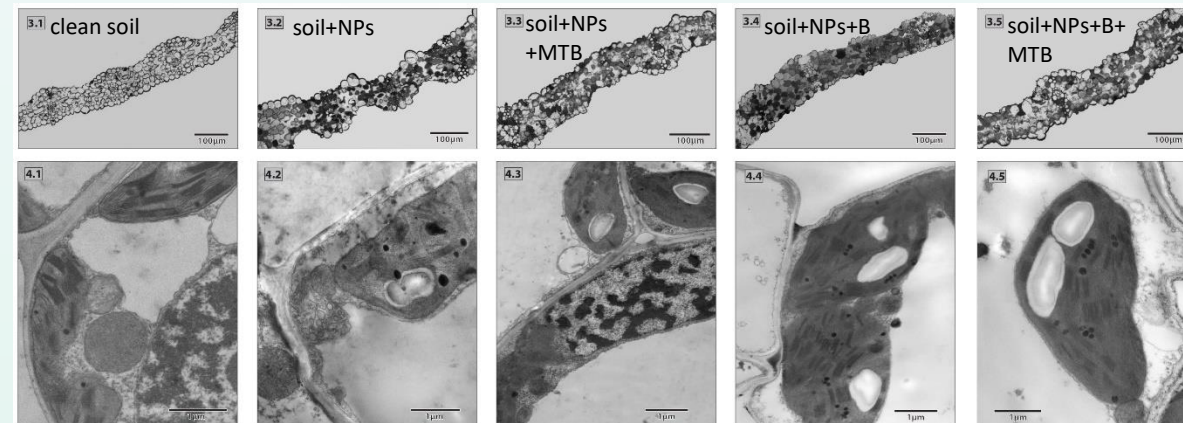
The content of Zn in *H. sativum* tissues was increased drastically, especially in ZnO NPs contaminated soil. MTB and B in the contaminated soil reduced Zn accumulation in *H. sativum* roots by 20% and 63%, and in the aboveground tissues by 11% and 68%, respectively, compared to ZnO NPs polluted soil without amendments. The combined application of MTB and B showed the greatest decrease in Zn accumulation in *H. sativum* tissues.

Morphological parameters of *Hordeum sativum*, cm

Treatment	root length	height of plants	leaf length
clean soil	25.5±2.3	29.8±3.0	18.4±3.6
soil+NPs	12.3±2.8	17.9±3.4	12.5±4.0
soil+NPs+MTB	16.5±3.0	23.6±3.5	15.2±3.7
soil+NPs+B	18.2±2.0	27.4±3.2	17.7±3.0
soil+NPs+B+MTB	22.7±2.5	25.1±2.7	18.0±4.0

The root length and *H. sativum* height was decreased by 52% and 40% in contaminated soil. The addition of B, both separately and in combination with MTB reduced root length by 48% and 85%, and plant height by 53% and 40%, respectively, compared to polluted control.

The anatomical results also showed an improvement in cellular- sub-cellular organelles, especially in chloroplast by B and in combination with MTB.



CONCLUSIONS

The results indicate that metal-tolerant bacteria and biochar could be an effective soil amendment to decrease metal toxicity enhance crop growth, and improve soil health.

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