The aim of this research is to estimate the toxicity and toxicokinetics of zinc nanoparticles (nano-ZnO) in the soil invertebrates, represented here by a model organism - the earthworm *Eisenia andrei*. The production and consumption of metal nanoparticles has grown rapidly over the past approximately 10 years. Yet, their toxicity to terrestrial invertebrates is still poorly understood compared to the more common environmental pollutants, such as metals in the ionic form. Toxicokinetics of metal nanoparticles, i.e., their uptake from the environment, assimilation and elimination by an organism, is almost completely unknown for invertebrates, and such studies do not exist at all for soil invertebrates. It is also not known whether and to what extent metal nanoparticles affect energy budgets of organisms – do they elevate metabolic rate due to increased costs of detoxification, or maybe slow down the metabolic processes via affecting the redox processes? The specific properties of nanoparticles and their fate in the environment suggest that all these processes may differ for metal nanoparticles in comparison with much better known metal ions.

In the proposed study toxicity, toxicokinetics and impact of nano-ZnO on the energetic processes of the earthworm will be compared with the effect of the ionic form of zinc ($ZnCl_2$). The concentration of zinc in the body and the energy reserves (sugars, fats and proteins) as well as energy consumption at cellular level will be measured in animals exposed to two different forms of zinc in the soil. The whole body respiration rate will be also measured periodically to determine the impact of each of the forms of zinc on the overall metabolic rate.

The project fits perfectly into the current trends in ecotoxicology, understood as one of the disciplines of the basic science. It aims at clarification of some general mechanisms, widely discussed in the scientific community for many years. At the same time it focuses on the very new and important problem of increasing exposure of organisms to nanoparticles. The fate of toxic substances in the body, their accumulation and elimination (i.e., toxicokinetics) and the mechanisms of utilizing the energy resources in the presence of stress are extensively studied problems in the context of new stressors continuously emerging in the environment. Such a new stress factor present in virtually all environments are metal nanoparticles widely used in various industries. It has been estimated that the production of the most popular nanomaterials is approx. 300,000 tons per year, and there are over 1,300 products containing nanoparticles. Once released to the environment, nanoparticles may interact with its different compartments, and this interaction is determined by both the properties of nanoparticles (e.g., solubility in water, colloidal stability, reactivity) and the properties of the environment (e.g., temperature, pH, soil organic matter content). Previous studies showed that nano-ZnO can be toxic to organisms under some circumstances, and their toxicity is determined by such characteristics as size, shape, solubility and a tendency for aggregation. If metal nanoparticles are not dissolved, they may be accumulated in the body, and the process of accumulation may be completely different than in the case of ionic forms of the same metal. If the nanoparticles dissolve, either in the soil solution or in the digestive tract of an animal, their toxicokinetics should be comparable to the toxicokinetics of ionic forms. Therefore, in this study the fate of nano-ZnO both in the soil and in the body will be compared with those processes for the ionic forms of zinc.

Two main experiments will be conducted, in which earthworms will be exposed to soil contaminated with either nano-ZnO or $ZnCl_2$ for 42 days (contamination phase) followed by the subsequent decontamination phase, in which the earthworms previously exposed to the contaminated soil will be transferred to clean soil. In the first experiment, the earthworms will be sampled for analysis of internal Zn concentrations to capture the dynamic changes of Zn concentration in their bodies, possibly different for different Zn forms present in the soil (nano-ZnO versus Zn). Some individuals will be additionally sampled to identify the nanoparticles in their body by using electron microscopy. The individuals sampled in the second experiment will be used to measure respiration rate at the level of the organism and for *Cellular Energy Allocation* (CEA) measurement. The latter will be done by biochemical analyses of energy reserves (Er) measured as the amount of proteins, lipids and carbohydrates, and cellular-level respiration rate (Ec). The difference between Er and Ec represents the net energy budget, i.e., CEA of the test organism.

Ecotoxicology and Stress Ecology Team at the Institute of Environmental Sciences, Jagiellonian University, where the project will be allocated, has an extensive experience in research on metal toxicokinetics in terrestrial invertebrates. Also, costs of living of organisms in contaminated environments and detoxification mechanisms related to this issue have been studied by this group for many years, and the PhD supervisor has significant achievements in this research area. The Faculty of Biology and Earth Sciences and Faculty of Chemistry have all necessary equipment to carry out the proposed research. The applicant is already experienced in experimental work and statistical analysis of toxicokinetic data thanks to the earlier work on the MSc project, which was a part of the bigger project entitled "Toxicokinetics metals in invertebrates: models and physiology" (NCN 0384/B/P01/2011/40, project leader: prof. Ryszard Laskowski). Moreover, the applicant is currently involved in the study of "Effect of metal bioavailability on the toxicokinetics and compartmentalization of metals in carabids" (HOMING PLUS/2013-8/1; project leader: dr. Agnieszka Bednarska).

The novelty of this project comes from a comparison of Zn toxicokinetics after exposure to two different forms of Zn in soil and linking the changes in toxicokinetics with changes in the cellular energy budget of earthworms. This will be a significant step towards understanding the mechanisms responsible for the toxicokinetics of metal nanoparticles in invertebrates. Such data for nanoparticles do not currently exist, but they are crucial for predicting effects of prolonged exposures to their sublethal concentrations. Although the project does not aim at solving practical problems, in the long perspective it can be a significant contribution to *Ecological Risk Assessment.* Especially, that the experiments will be conducted on the standard species for ecotoxicological tests. The proposed research, due to the novelty of the proposed topic, will have a good chance to be published in the best international journals and high citation rates in the future. The results will be, of course, presented at major international conferences in the field of ecotoxicology.