

Effects of Metal and Metal Oxide Nanoparticles on Honey Bees (*Apis mellifera* L.)

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Introduction

Environmental pollution is the release of chemical waste into water, land, or air that may cause acute or chronic detrimental effects to the Earth's ecological balance or lowers the quality of life. Pollutants can cause primary damage, with direct adverse effects on the environment, or secondary damage that can be detected over long time periods in the form of minor perturbations in the balance of the biological food webs. In global industry, the steady pursuance of miniaturization has led to the creation of minute particles, the nanoparticles (NPs; 0.1–100 nm).

The industrial division that deals with NPs is defined as nanotechnology, which is still young and promising and has been developing quickly for the last 20 years [1, 2]. Unluckily, very limited information is available about the risks of using NPs [3]. Nano toxicology is the branch that analyzes the adverse effects of NPs and their influence on the environment [4]. Serious ecological consequences and effects on human and animal health have been found to be related to the presence of NPs in bio systems [5]. The most risky implications are associated with the chronic consumption and inhalation of NPs [6].

Many of the currently available nanoparticles (NP)-containing consumer products contain ZnO or TiO₂ NPs and the likelihood that ZnO NPs are released into the environment is constantly increasing [7, 8]. Zinc oxide NPs can be found in many different products such as optoelectronic devices, sunscreens, paint pigments, rubber components, cosmetics, food additives, and medicines [9, 10]. Such abundance of products in global use which are containing ZnO NPs raises sensitivity concerning their toxic potential [11].

Previous studies concerning the toxicity of manufactured ZnO NPs to different taxa including algae, bacteria, and plants, terrestrial and aquatic invertebrates and vertebrates suggests a relatively high measured acute toxicity of ZnO NPs (in the low mg L⁻¹ levels), dependent upon test species, physicochemical properties of the material and the test methods [12]. It has been reported that generation of reactive molecules and free radicals from molecular oxygen by dissolved ionic zinc from NPs, or by

NPs themselves, is a common mode of the action of ZnO NPs in all species tested. There are a lot of studies conducted on the toxicity of nanoparticles in recent years. Once focused on the aquatic environments, while others are now being initiated and conducted on terrestrial environment and organisms. The appearance of nanotechnology products and their inevitable release into the environment can also result in adverse effects on honey bees [13].

Honey bees plays an important ecological and economical roles as pollinators of crops and produce honey that can be harvested for consumption. The western honey bee, *Apis mellifera*, is an essential pollinator for agricultural crops in many countries, pollinating \$15 to \$20 billion worth of crops in the United States alone and more than \$200 billion globally [14]. Bees also provide important pollination services to wild plants, of which in Europe 80% need insects for pollination, so confirming their ecological importance [15].

In many parts of the world attempts were made to use bees to assay the degree of environmental pollution. Moreover, many investigators commend upon the possibility to use bees and its products to monitor the purity of the environment [16-18]. Among the most important pollinators that faced many threats by environmental pollution, pest control and potentially, by products of nanotechnologies is the honey bees. Therefore, there is a crucial need for investigating the potential toxic effects of heavy metals NPs on honey bees.

Cronn and Richard investigated the toxicity of two cadmium salts to honey bees (Hymenoptera: *Apis mellifera* L.) and investigated the association between oral dose with the bioaccumulation of least molecular weight cadmium-binding proteins [19]. Honey bees were fed syrup containing cadmium as either cadmium chloride or cadmium sulfate. Bees were also injected with a saline solution containing cadmium chloride. The results suggested that cadmium is moderately to highly toxic to honey bees, depending upon the form used and length of exposure. The relationship between the actual dose administered and the level of whole body low molecular weight cadmium-binding protein was positively correlated. The level of tissue protein increase dramatically during days one and two, remaining elevated from days three through seven.

Taste perception and proboscis extension reflex (PER) have been used to determine the adverse effect of selenium (SE) on

the gustatory response of honey bee (*Apis mellifera* L.) foragers [20]. Antennae and proboscises were induced with both organic (selenomethionine) and inorganic (selenate) forms of Se that usually present in Se-accumulating plants. Methionine was also tested. Each compound was prepared in 1 M sucrose at 5 concentrations, with sucrose alone as a control. Bees fed selenate showed the least responsiveness to sucrose, which may lead to a shortage in incoming floral resources required to support nest mates and larvae in the field. Accordingly, foraging on nectar containing Se (particularly selenate), may cause reduction in population numbers due to direct harmful effects. Given that bee workers are willing to feed on food resources containing Se and may not avert Se compounds in the plant tissues on which they are foraging, they may suffer similar adverse effects as reported in other insect guilds.

The acute toxic effects of Ag-TiO₂, ZnO-TiO₂ and TiO₂ nanoparticles on *Apis mellifera* have been studied [21]. The adverse effect of the ZnO-TiO₂ composite was found to be caused by the zinc oxide nanoparticle loaded into TiO₂ nanoparticle. The concentration group that made the greatest difference in all the other concentration groups on the lethality rates in nanoparticles was 100, 1, 10 mg/l for TiO₂, ZnO-TiO₂, Ag-TiO₂ respectively. It has been concluded that, the mortality rate increased with the extension of the time of exposure of honey bees to nanoparticles and with the increase in the concentration rate.

The potential neurotoxicity of ingested zinc oxide nanomaterials (ZnO NMs) or zinc ions (Zn²⁺) have been investigated on honey bees [22]. A variety of biomarkers, including the neurotoxicity biomarker acetyl cholinesterase (AChE), metabolic impairment, feeding rate, and survival, as well as the activities of a stress-related enzyme glutathione S-transferase have been evaluated. The activity of AChE was found to be elevated in bee workers exposed to either of the tested substances. The feeding rate only increased in the group treated with Zn²⁺ ions. Most of the reported effects are due to Zn²⁺ ions. It has been concluded that zinc ions either originating from Zn salt or Zn-based NPs have a neurotoxic potential and thus can contribute in the colony failure.

The toxicity of boron particles on *Apis mellifera* have been evaluated [23]. Bee workers have been exposed for 96 h to nano and non-nano boron particles at concentrations 0.001, 0.01, 0.1 and 1 ppm. The LC50 values of nano and non-nano boron particles after 48 and 96 h exposure were 229.09 ppm, 0.33 ppm and 62.33 ppm, 4.69 ppm, respectively. Obtained results indicate that nano boron is very toxic at 96 h. Generally, the toxic effects of boron particles on honey bees increased a long with the extension of time of exposure.

The effects of environmentally realistic concentrations of metals Al, Pb and Cd have been examined on honey bees [24]. Bee workers have been exposed for 10 days to these metals (dissolved in syrup). The quantification of metals combined with syrup consumption in bees revealed the following order for metal bioconcentration ratios: Cd>Pb>Al. Metallothionein-like proteins (MTLPs), Alphatocopherol and lipid peroxidation were quantified. Bee workers exposed to increasing amounts of Cd, showed a marked augmentation in the levels of MTLPs. An

increase in a-tocopherol content have been noticed in bee groups exposed to lead (Pb) and Cd, while alteration of lipid peroxidation was observed only in bee workers exposed to Al. Obtained results raise concerns about the bioavailability and the additional threats posed by heavy metals for pollinators in agricultural lands.

Although, there is a plenty of studies elucidating the toxicity of metals and its oxide nanoparticles on aquatic and terrestrial environments and their organisms, a scarcity of studies for their toxicity on honey bees have been observed. So, more comprehensive studies are required to address the histopathological and ultra-structurally effects of heavy metals NPs on tissues of the alimentary canal of honey bee workers and to investigate and elucidate their potential toxicity on brood, queen, drones vitality, colony vigor and its potential role in colony collapse disorders.

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