Review article

Zinc and nano-ZnO – influence on living organisms

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Abstract :*The use of zinc is very common in many industries. The multitude of advantages, and capabilities make use of different forms of penetration of zinc into the environment. Due to its ability to migrate, zinc is also accumulated in living organisms. Zn is an essential trace element but both the deficiency or excess are extremely dangerous for living organisms. Nowadays nanozinc has been increasingly used. Nanozinc is a component of daily used products, like plastics, ceramics, glass, cement, rubber, foods and many others. This leads to spreading nanozinc the environment with the possibility of its penetration into living organisms. This article is a review of the impact of zinc and zinc nanoparticles on a variety of organisms: ranging from microorganisms through plants and animals, to people.*

Keywords: *zinc, nano-ZnO influence on organisms, nano-zinc, zinc NPs ecotoxicity.*

Introduction

Zinc is an essential micronutrient for many living organisms. It is necessary for the proper functioning of enzymes and many organs. Unfortunately, its excess is extremely harmful, and can lead to malfunctioning of proteins, changes in DNA structure, changes in the metabolism of toxic reactive oxygen species, and inhibition of growth and germination in plants.

Due to increasing use of this element in the form of nanoparticles, the impact of zinc on living organisms can be strengthen when compared to the molecular one. However, the exact impact and mechanisms of its action are not yet fully understood.

The aim of presented review is to discuss the influence of zinc and its nanoparticles on living organisms with particular emphasis of its toxicity.

Properties of zinc and its uses

Zinc is the metal of Group IIB of the Periodic Table. It is shiny and blue-white. In the normal temperature zinc is crystalline and brittle, but at temperatures ranging from 110°C to 150°C becomes plastic. Zinc is a reactive element that reacts with non-metals and diluted acids [1].

Zinc is present in the soil mainly in the form of ores. This element can be obtained from the primary ore or recycled materials. There are two methods used for zinc obtaining: pyrometallurgical and hydrometallurgical [2].

Zinc is used as a white pigment in paints, activator in the rubber industry, in the production of plastics, cosmetics, wallpaper, printing inks. In the pharmaceutical it is used, among others, as an antioxidant in the formulations of anti-aging skin. The most important use of zinc is plating, or coating highly corrosive metals [3, 4].

Zinc is also used for the production of alloys: bronze (copper, tin and zinc) and brass (copper and zinc). Zinc alloys are used in a wide variety of products, including auto parts, roofing, gutters, faucets, pipes, electric fuses, metal type, everyday items and building materials [2, 3].

A widely used zinc compound is ZnO, often in the form of nanoparticles. In this form ZnO it is currently used in the production of plastics, ceramics, glass, cement, rubber, foods (source of Zn nutrient), as ingredients of personal care products, including cosmetics, also with a UV filter that absorb UV light [3, 4].

Zinc in the environment

Zinc is an element that occurs naturally in the environment as mineral (in bound form) e.g. $ZnCO_3$ or ZnS. It is a component of the earth's crust and its amount in the rocks is 10-120 ppm. Areas where there are scattered zinc deposits are located at depths of 50-400 m underground. Zn usually occurs in the divalent form of Zn^{2+}. However, it can also exist in soil in the form of complex compounds, like $ZnOH^+$, $ZnHCO_3^+$, $Zn(OH)_3^-$ and ZnO_2^- [1].

Zinc is one of the most active metals in the soil. The content of zinc in soils worldwide varies within a broad range from 35 mg to 12400 mg kg^{-1} of soil [1]. In the Polish soils, the range of zinc concentrations is from 10.27 mg do 5 805 mg kg^{-1} of soil, and its average content is 79.81 mg kg^{-1} [5]. In accordance with Kabata-Pendias and Pendias (1993) the concentration of zinc in polluted soils are dependant on both source of pollution and soil location [1]. For example in soils of Greece in the area of mining and metallurgical industry the concentration of zinc can reach the level of 16 000 mg kg^{-1} of soil and in Great Britain in the area of inactive mine the zinc amount in soil ranges from 220 to even 66 400 mg kg^{-1} of soil [1].

Zinc content in the waters is varied and depends mostly on the surrounding geological rocks and environment contamination. The natural content of zinc in unpolluted waters of rivers is about 10 μg/l, while in sea water it is lower due to its absorption by the clay and organic deposits [1, 6, 7]. In spite of high susceptibility to migration, zinc penetrates underground waters in relatively small quantities [1]. The average concentration of zinc in drinking groundwater is equal 15 μg/l [1].

Zinc in the environment gets mainly from the combustion of fossil fuels: coal and crude oil, as well as from municipal wastewater. Additionally, it is a component of plant health products and fertilizers. It is easily soluble therefore migrates directly into groundwater. The concentration of zinc in living organisms tissues is proportional to the quantity of food (in plants - the content in the soil). It is absorbed into the organisms by the same route as aluminum and iron, as a result of metabolic disorders [8].

Due to the increasing use of nanoparticles of zinc, their level in the environment is likely to increase. Recent studies have shown a potential for the use of nanoparticles of zinc in biomedicine and therapeutics, as carriers of genes and drugs. At the same time, more and more research suggests high toxicity of ZnO nanoparticles to living organisms (e.g. Algae, bacteria, zebrafish) as well as cell lines (at the level of DNA damage) [9].

Bioavailability of zinc

Bioavailability is the amount of mineral intake that the body is capable of absorbing and using to obtain a certain metabolic function. Zinc bioavailability is defined on three basic steps: absorption, penetration into systemic circulation and use in the cells [10].

Higher plants take zinc from soil in various forms: Zn^{2+}, hydrated ions and organic chelates, proportionally to its content in the soil. However, the zinc bioavailability is the function of soil conditions. It can be generalized that in well oxidizing acid soils zinc is easily mobile and available to plants, while in poorly reducing neutral or alkaline soils Zn is substantially less available [11]. The type of soil also influence the zinc bioavailability for plants. Low zinc assimilation is characteristic of spodic soils, calcaric and salty soils. In addition, the limitation of the bioavailability of zinc for plants may be due to the fertilization of soils with phosphorus and calcium compounds [1].

The zinc bioavailability for animals is dependent on its form. The most organic zinc sources are more than twice as bioavailable as inorganic forms (eg zinc sulphate) and three times as much as zinc oxide [10]. Inorganic zinc is broken down in the digestive tract, which causes it to interact with other components of the diet, such as calcium. This can lead to the formation of insoluble compounds that the animal can not digest or absorb (diet antagonism when one component of the diet reduces the availability of another component). On the other hand, trace minerals of organic origin are strongly bound to the organic compound and do not decompose to the small intestine where they can be readily absorbed [12].

In dietary studies in humans, bioavailability is determined by determining the absorption capacity. Delivery of zinc by food depends on its quantity and bioavailability. It is estimated that the bioavailability is about 20-30% of total zinc contained. Various agents can reduce the absorption of zinc, such as other metals such as copper, and to a lesser extent calcium and iron. Based on the mean bioavailability, the recommended daily intake of zinc is in the range of 10 to 15 mg in adults but may be higher under certain circumstances such as pregnancy and lactation, with an additional 5-10 mg [13].

Effect of zinc on living organisms

There are three mechanisms of toxic action of ZnO and ZnO nanoparticles: a) the release of toxic substances, b) interaction with a medium that can result in the production of free radicals, c) direct interaction with the tissues, and protein-DNA. Nanoparticles of zinc operate in accordance with all three mechanisms. This makes it necessary to carry out numerous studies on the effects nano-ZnO on the environment and living organisms [4].

Microorganisms

ZnO has an antibacterial properties. However, the influence of ZnO on both microorganisms – Gram-positive or Gram-negative – is ambiguous. According to Tayel et al. [14] and Reddy et al. [15] zinc oxide more potent Gram-positive bacteria (*S. aureus*), and the results Pasquet et al. [16] and Applerot et al. [17] show a potent antibacterial activity against Gram-negative bacteria (*E. coli*) [14-17].

Similarly, scientists do not agree on the bacteria defense mechanisms that is activated during the exposition to ZnO. Some of them believe that zinc oxide is adhered to the peptidoglycan in Gram-positive cells, and some consider that the lipid bilayer of the bacteria are more sensitive to ROS produced by the action of ZnO [16].

Pasquet et al. [16] also studied the effect of ZnO on the growth of microscopic fungi. It has been shown that zinc oxide resulted in only a slowdown of growth, but did not affect the size of the yeast *C. albicans*. Differences in the influence of ZnO on fungi and bacteria growth may resulted from the fungal ability to produce spores. ZnO inhibits the growth of mold mycelium of *A. brasiliensis* at low concentrations, and stops it completely in case of high concentrations. However, in the case of *Aspergillus*, it was shown that high concentrations have a positive effect on the growth of this microorganism. This suggests that this compound has antibacterial properties and does not fungicidal (affects only the morphology of the cells) [16].

Plants

Zinc is an essential element for plants, but in high concentrations has a toxic effect. Phytotoxic effect of zinc depends on soil pH, the quantity of organic substance, and in case of plants its development phase. Zinc deficiency occurs if its content in plants is less than 20 mg/kg, and excess – 300-400 mg/kg. Zinc deficiency impairs the metabolism, the synthesis of RNA and DNA, and the functioning of the proteins, leading to inhibition of plant growth. Excess causes chlorotic and necrotic changes. Studies show that the phytotoxicity of zinc is mainly based on incomplete and delayed germination and deformations in the root zone. Dicotyledonous plants are more sensitive to zinc excess than monocot plants. It is believed that it is caused by movement of contaminants from roots to the aerial parts at an early stage of plant development. Another explanation is the capacity of the root system and the ratio of the mass of the aerial parts to the underground [18-21].

Research conducted by Baran et al. [18] on vetch (*vicia*), peas (*Pisum sativum*), mustard (*Sinapis alba*) and flax (*Linum usitatissimum*) with increased zinc content in soil showed that the more zinc in the soil, the lower the yield received. The research showed that even at 50 mg/kg of soil dry weight there was a decrease of 3-18% (flax – 3% peas – 17%, mustard – 18%, vetch – 13%) in growth when compared to control plants and was between 14-65% (flax – 25%, peas – 65%, mustard – 33%, vetch – 14%) for 250 mg/kg of soil dry weight also compared to control plants. At a dose of 750 mg/kg dry weight soil flax, mustard and vetch did not grow; peas yield was about 83% lower than in control plants. The most sensitive to zinc pollution was flax – at 50 mg/kg, dry weight in soil 50% inhibition of root growth and germination was observed. the most resistant were beans and mustard – inhibition of root growth and germination of about 10-15% [18].

Baran et al. [19] also studied the accumulation of zinc in plant tissues. It has been shown that depending on the plants and its parts it varied. Larger amounts were found in the underground part of the plant. The comparison analysis of some plant species show that roots of flax accumulated about 2, 3 times more zinc than its aerial tissues. Similarly, the concentrations of zinc in roots of mustard and peas were 2 and 1, 2 times higher than in plant shoots, respectively. Simultaneously, flax contained more zinc in the aerial parts, in contrast to mustard seeds and peas. The research shown that the ability of bioaccumulation of zinc depends largely on the species [19].

Nanoparticles of zinc can be used in pesticides, herbicides and fertilizers as trace elements. This applies to stimulate growth and concomitant germination. Test carried out by Suresh et al. [22] in peanuts (*Arachis hypogaea*) have shown that soaking seeds in nano-ZnO during growth have a significant effect on the content of protein, chlorophyll, carbohydrates, and other cellular components [22]. Zinc oxide nanoparticles exhibit phytotoxicity at concentrations above 400 mg/kg. Increased surface activity of nano-ZnO makes it easier to adhere to the surface of the root, which causes weakening of its functions. ZnO NPs may have different mechanisms of toxicity due to their properties: a very large surface area and high surface energy. At the same time it has been shown that the addition of ZnO NPs to the soil increase the bioavailability of zinc particles [23].

Research also showed that ZnO nanoparticles cause a reduction in wheat plant growth (*Triticum aestivum L.*) and increased production of ROS [24]. The impact consisted mainly on a root elongation perennial ryegrass (*Lolium perenne*), radish (*Raphanus sativus*) and rapeseed (*Brassica napus*). The tests carried out showed the presence of nano-ZnO in the cytoplasm, nuclei and other cellular structures. It was also demonstrated that the nanoparticles have a negative impact on the growth of soybeans (*Glycine willd.)* [25].

Cakmak et al. [26] showed that zinc deficiency also is harmful to plants. The study was conducted in cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* L.) and apple (*Malus domestica*).

Root permeability and root secretion were checked. In all examined species, Zn deficiency caused increased root secretion, amino acids, sugars and phenols. Zinc deficiency caused various effects depending on the species. In most plants (except for tomato) deficiency caused depress shoot growth more than root growth. The leaves of the examined plants had symptoms of chlorosis [26].

Animals

Zinc is one of the most important trace elements found in the animal body. It has many features in it. It is an activator of over 200 enzymes, responsible for DNA replication and digestion. Moreover, it affects reproduction and lactation. As a constituent of insulin, it participates in the metabolism of sugar. Optimal zinc levels also have a beneficial effect on the immune system of animals.

Animals accumulate zinc in kidneys, gonads and in the liver mainly in form of its complexes with proteins. The inhalable form, the concentration of 15 mg/m^2, zinc causes the flu-like illness. Zinc poisoning manifest itself via vomiting, impaired immune response, fatigue and abdominal pain. Studies in animals have shown toxicity of 20 and 120 nm nano-ZnO. The harmful effect was observed within the organs such as liver, heart, spleen, pancreas and bones [7, 20, 28].

The study of Bai et al. [27] on zebrafish (*Danio* rerio) embryos after 96 hours of exposure to 50 and 100 mg/l of nano-ZnO showed mortality of zebrafish at a level of about 30 and 65%. No increased mortality was observed at concentrations 0,5-6,3 mg/l of nano-ZnO. It is believed that it is caused by creation of nano-aggregates that block the access of oxygen through channels in the pores of choriocarcinoma or by the formation of ROS. Additionally, it showed a higher toxicity of ZnO nanoparticles than ZnO [27].

In contrast, Kuang et al.[29] showed that even 1 mg/l of 35 nm ZnO NPs affect reproduction and survival of the species [29].

Choi et al. [30] conducted a study comparing the toxicity of nano-ZnO and $ZnSO_4$ on the embryonic development of zebrafish larvae. Researchers have shown that with the same concentration nano-ZnO is more toxic then Zn^{2+}. In both cases malformations could be observed, but in the case of nano-ZnO more of them were present [30].

Exposure of mice to 30 nm zinc oxide nanoparticles present in food demonstrated an increased activity of alanine aminotransferase and alkaline phosphatase in the mice tissues and caused damage and apoptosis of liver DNA [29].

A similar effect was observed in rats was cause by 20 nm ZnO NPs. In addition it impaired their metabolism [29]. Studies in rats have shown that inadequate amounts of zinc in the body have teratogenic effects on the fetus. In young, juveniles causes lethargy, irritability, impaired learning and memory. These symptoms persist even after reaching adulthood by these individuals [31].

Concentration of zinc in feed for fatteners and sows should be 50-60 mg/kg, and for piglets – 100 mg/kg. The most common effect of zinc deficiency for animals (less than 30 mg/kg) is parakeratosis, which manifests itself on the skin surface. Long-term zinc deficiency, leads to cracking and keratosis of the skin, also causes keratosis of the tongue and mucous membranes of the mouth. In piglets, zinc deficiency is manifested by growth inhibition. Female pigs suffering from deficiency of the element are exposed to difficulties during labor and lactation disorders. Male pigs can suffer from infertility. In addition, zinc has anti-diarrheal effects and is used in piglets when discontinued from mothers.

Excess zinc is also harmful. Its content in feed should not exceed 250 mg/kg. Higher doses cause movement disorders. Zinc content above 2000 mg/kg is toxic to pigs [32].

The recommended dose of zinc for dairy cows is 50 mg/kg. However, for greater certainty, it is better to use a double level. Phytin contained in plants such as in soy or in silage causes zinc in the body is not absorbed. Zinc deficiency is manifested in the form of hair loss, growth retardation, abnormalities in the treatment of abrasions, reproductive problems. In young cow with impaired absorption of zinc the symptoms are diarrhea, skin damage, alopecia, immune system dysfunction and inhibited growth occur. Much zinc accumulates in the fetal and placental tissues as it is needed for proper fetal growth and development. The concentration of zinc in the liver of the calf decreases after childbirth. After the first year of life, it reaches a value similar to that of adults. Excess zinc in cows is very rare and results in lack of appetite, stomach ulcers, liver damage [28, 33].

Humans

Daily requirement of zinc for adults is 10-15 mg/day, 10 mg/day for children and 3-5 mg/day for infants. Natural sources of zinc are eggs, meat, fish, onion, pumpkin or garlic. The high content of zinc is mainly found in the muscles, bones, brain, liver, pancreas and spleen [21].

In the human body zinc regulates work of skeletal, reproductive, and circulatory systems; it is relevant during creation of enzymes that regulate the metabolism of carbohydrates and proteins; determines the proper growth and development; regulates the immune system, and helps in processes of learning and memorizing. Zinc ions are involved in the regulation of apoptosis, DNA repair, or inhibition of activation of enzymes (due to the formation of so-called zinc fingers – the characteristic motifs in the amino acid sequence). Deficiency causes hair loss, allergies and skin diseases. On contrary the excess deposits in kidneys and liver, and blocks the absorption of elements such as phosphorus and calcium; it may also be carcinogenic. It is believed that an excess of zinc ions is responsible for the deposition of β-amyloid depositions in Alzheimer disease [21, 31].

Zinc balance in the body is particularly important for the operation of the pancreas. The zinc ions are involved in regulating the signals of insulin, glucagon, and activation of digestive exoenzymes. Another organ, which needs zinc for proper functioning is brain. This element regulates the transmission of nerve signals to the synapses. It is necessary for the proper reception of auditory, taste, odor, pain, visual, in managing emotions, learning and memory [31, 34, 35].

The test results obtained by Condello et al. [9] demonstrated the possibility of using ZnO NPs in treatment of colon cancer in humans. Authors found that the compound enters the tumor cell in two ways: by diffusion (isolated nanoparticles and the agglomerates of 100 nm) and endocytosis [9]. In contrast, Wang et al. (2016) showed that 30 nm zinc nanoparticles caused apoptosis of lung cancer cells and reduction of their viability. Numerous publications suggest that the toxicity of ZnO nanoparticles is mainly due to the formation of ROS [29, 36].

Conclusions

Zinc is widely used in the industry, cosmetics, pharmaceutical, paints and plastics, for coating metals to protect against corrosion and many others. It is common in the environment and it is also introduced to the environment by human, both in particulate form, and, more and more often as nanoparticles. Many studies show that it is necessary for living organisms as a trace element. However its excess and deficiency are very dangerous. Recent research has focused mainly on the effects of excess of zinc in a variety of organisms. The main threats are: RNA and DNA damage, damage to organs (including liver, lung, spleen, brain), emergence of Alzheimer disease, and disturbances in plants growth and germination.

The impact of zinc on living organisms is quite well studied and described. However, despite increasingly wider use of nano-zinc, its impact on organisms is not yet sufficiently understood.

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