BIOMONITORING OF ENGINEERED NANOPARTICLES IN THE ENVIRONMENT

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Abstract

Continuous increase in nanoparticle production raises concerns about their fate in the environment leading to the need of monitoring of their presence, concentration and effects on the living systems. In the studies performed so far, attention was paid mainly to the toxicity/ecotoxicity of the nanomaterials to the range of taxonomic groups – from bacteria to animals and plants – with varying results. Biomonitoring is a technique utilising organisms or their tissues for the assessment of the pollution of the environment and it has been successfully applied to a vast array of pollutants and as such, it is a promising way of monitoring engineered NPs in the environment as well. In this paper, concept of biomonitoring is introduced and general overview of known interactions of NPs and organisms is presented along with the evaluation of possible applications of biomonitoring techniques in the nanoparticle pollution assessment.

Klíčová slova: biomonitoring, nanoparticles, environment, pollution

1. INTRODUCTION

Although atmosphere has a natural background level of nanoparticles, it is rather insignificant in comparison with those NPs originating from the human activities [1]. However, these are, by no means, only NPs manufactured on purpose – according to Remédios at al. [2], substantial portion of anthropogenic airborne NPs are unwanted or incidental NPs from by-products of various types of combustion, food cooking, and chemical manufacturing; welding or refining and smelting. NPs originating in combustion are becoming of higher concern, especially due to the ever-growing production of waste that is being taken care of in incineration plants [3]. In big cities, traffic was identified as a major source of nanoparticle air pollution [4]. Anyhow, with growing NP production, the need for monitoring of their presence in the environment grows as well. Biomonitoring techniques present a suitable and affordable way of meeting this need [5]. In this paper, the definitions of biomonitoring are based on those stated in Markert et al. [6]: biomonitoring is defined as a technique utilising living organisms to obtain quantitative information on the state of the environment (as opposed to bioindication defined as providing qualitative information). Biomonitoring methods can be further divided to the passive methods – those utilising organisms naturally occurring in the monitored area - and the active methods – those utilising organisms introduced (transplanted) to the site artificially. [7]

For the successful application of a certain taxon in biomonitoring study, knowledge of its autecology and interaction with the pollutant under scrutiny is essential. There are a number of taxa routinely used in biomonitoring the ecological characteristics and demands are well known, however, there is poor understanding of the interaction between organisms and NPs so far. Although there have been some studies carried out from the ecotoxicological point of view, little is known from the perspective of bioaccumulation and hence possible application in biomonitoring of nanoparticle pollution. Though the interaction and uptake mechanisms of NPs in organisms have been generally not well studied, it is already apparent that there are two major ways of how NPs permeate the organisms. In aquatic environment, the uptake is generally – depending on the particular species and its metabolism – most direct: through gills and/or whole body surface in animals and through the body surface in plants [8]. In terrestrial environment, the uptake in animals takes place mainly by means of ingestion or inhalation [9] specific uptake mechanisms of terrestrial plants were far less studied and are, therefore, rather obscure. In this paper, contemporary knowledge of the interaction between organisms traditionally thought as suitable for biomonitoring and/or bioindication and metal or metal oxides NPs is summarised and possibilities of utilisation of organisms in monitoring of nanoparticle pollution are discussed.

2. NANOPARTICLES AND HETEROTROPHIC ORGANISMS

Various heterotrophic organisms were surveyed to assess the interaction between nanoparticles and living systems ranging from bacteria to mammals, though almost exclusively in aquatic environments. When the influence of nanosized TiO_2, SiO_2 and ZnO on bacterial flora was tested [10], all of the applied oxides had harmful effects on aquatic bacteria, significantly enhanced under light conditions (induced photocatalysis). This is, however, hardly surprising since the antibacterial properties of nanosized metal oxides are known and widely utilised. Moreover, research carried out by Miller et al. [11] focused on assessing the toxicity of TiO_2 and found out that originally is insignificant toxicity of TiO_2 rises rapidly when the UV light is present.

The response of complex organisms to exposure to NPs is much more intricate. Whilst environmentally reasonable concentrations of TiO_2 NPs applied to model organism *Caenorhabditis elegans* [12] have an adverse effect on locomotion and enhanced ROS production, no change in behaviour of *Daphnia* (well-known and abundant small crustacean) was observed [13]. TiO_2 was confirmed to have neither genotoxic nor ecotoxic effect on *Daphnia magna* in study of Lee, Kim and Choi [14] though, according to the same study, both CeO_2 and SiO_2 had ecotoxic effect. Larva of aquatic midge *Chironomus riparius* was exposed to the same NPs with similar results. Long term exposition of *Daphnia magna* to nanosized TiO_2 lead to food intake, growth and reproduction alteration showing that chronic toxicity is the main risk factor of TiO_2 presence in aquatic environment [15]. The same was reported by Dabrunz et al. [16], in their study, they also observed higher toxicity of nanosized TiO_2 compared to the non-nanosized TiO_2. Noss et al. [17] then observed the swimming behaviour of *D. magna* and found a similar negative impact.

Bivalve molluscs are a taxon used commonly for biomonitoring – their good performance as biomonitors is attributed to their ecological niche of filtrators [18,19]. *Mytilus hemocytes* was used to determine ecotoxical potential of nanosized C60 fullerene, TiO_2 and SiO_2, however no major effect was found, though there was some stress indicated on a biomolecular level [20]. On the other hand, other species of the same genus – *Mytilus galloprovincialis* – was exposed to TiO_2 NPs and there was distinct negative effect of it on the digestive gland and haemocytes of the mollusc [21]. Marine bivalve mollusc *Scrobularia plana* and polychaete *Hediste diversicolor* were exposed to CuO NPs [22]. Biochemical biomarkers showed enhanced stress in both species and behaviour disruption in *Scrobularia plana*. Same species showed similar effects when exposed to gold NPs although in environmentally rather improbable concentrations [23].

Fish are a group of organisms routinely utilised in assessment of aquatic environment for they are known bioaccumulators. *Piaractus mesopotamicus* were exposed to TiO_2 NPs solution in various concentrations (0.1, 10, and 100 mg/L) and light conditions [24]. There was no lethal effect observed, nevertheless biomarkers in the fish liver, brain and gills shown altered physiological activity.

Interesting results were obtained by Blinova et al. [25] who discovered that harmful effects of silver NPs on two aquatic crustaceans was under natural circumstances somewhat mitigated – this phenomenon was attributed to the presence of dissolved organic carbon. Negligible attention was paid to the interaction of terrestrial vertebrates and nanoparticles per se – the studies performed were focused mainly on assessing the medical applicability of certain NPs and used animals merely as a surrogate for human tissues. Generally speaking, mammals can be considered not vulnerable to the effects of NPs, even in *in vitro* cultures, especially in contrast with algae and crustaceans [26].

3. NANOPARTICLES AND AUTOTROPHIC ORGANISMS

Not much attention was focused on the interaction of plants and NPs and most of the studies carried out in this field were, again, bound to the aquatic environment – either it was using water algae and/or embryophytes or hydroponic cultivation of terrestrial plants. Observed effects of NPs on particular plant species vary significantly – both positive and negative effects of their introduction to the plant environment were reported. Nevertheless, when assessing possible NP pollution, plants may be much more suitable biomonitors since they are immobile and cannot escape the exposition to the pollutant and hence are forced either to succumb to the pollutant and die or immobilize the pollutant and bioaccumulate it in their tissues.

Algae are have been used to assess impact of NPs on aquatic environment. Nevertheless, they have been utilized only as test organisms for ecotoxicological surveys and not as biomonitors. Standard ecotoxicological indicators as EC50 were determined in *Desmodesmus subspicatus* colonies exposed to TiO_2 NPs by Hund-Rinke and Simon [27]. Nevertheless, the authors pointed out that since there is no knowledge on the possible concentration of NPs in the environment, these values are yet senseless. Sadiq et al. [28] later associated the toxic effect of TiO_2 NPs on algae with the adhesion to the cellular wall and thus shading of the chloroplasts. Cytotoxicity of TiO_2 NPs toward algae *Scenedesmus obliquus* and the possible application of microalgae for purification of the aquatic environment from nanoparticle pollution by their bioaccumulation were studied [29]. Photo-induced toxicity was, once again, indicated but TiO_2 NP ability to penetrate the cells of algae and was observed as well; the concentration of TiO_2 in the supernatant was significantly lower when in presence of algal cells.

Important question whether the NPs affect plants the same way as an ion of the metal they are composed of was dealt with by Qian et al. [30]. In experiment involving introducing the silver NPs and ions to the model organism *Arabidopsis thaliana*, the nano-form of silver was found to be more toxic than its ion form. Nano-silver particles were more easily accumulated in leaves, induced root elongation inhibitory effect and disruptive effect on thyllacoid structure and chlorophyll itself. The same negative effect on root elongation – caused by ZnO NPs – was reported by Lin and Xing [31]. Uptake of CuO NPs and cadmium sulphide/zinc sulphide (CdS/ZnS) quantum dots in aquatic plant *Schoenoplectus tabernaemontani* and its potential in bioaccumulation was studied recently [32]: NPs have the ability to penetrate the roots whilst quantum dots were found to have stronger toxic effect. NPs tended to accumulate in roots and not to translocate to shoots. When the same CdS/ZnS quantum dots were introduced to the environment of *Arabidopsis thaliana*, only adsorption was observed, no internalization whatsoever, regardless if in stems, leaves or roots [33]. Despite that, oxidative stress was observed in the plants exposed, once again with no indication of the particular mechanism.

In wheat and rapeseed, nonaquatic plants, however, the translocation of accumulated carbon nanotubes from roots to stem was observed – the plants being cultivated hydroponically as well [34]. In environmentally reasonable concentrations, there was no observed phytotoxic effect on neither of the plants. When the uptake and possible toxic effects of NPs (Ag) on terrestrial plants growing not hydroponically but rooted in soil or agar were explored, it was found out that the soil considerably reduces NPs bioavailability in soil hence there was much lower phytotoxicity and uptake observed in *Phaseolus radiatus* and *Sorghum bicolor* [35] When the plants are exposed to TiO_2 NPs via leaves, the particles gets internalized in the tissues of the plants as well [36]. On the leaves of lettuce, the accumulation of NPs in leaves was observed without the change of their chemical form. Though the NPs were incorporated to the leaves, no significant phytotoxic effect was observed.

Increasing size and concentration of silver NPs have detrimental effect on rice (*Oryza sativa*) seeds and seedlings [37]. However, the greatest NP accumulation in roots occurred when the smaller (20 nm) particles were introduced though, once again, the translocation of the particles to the stem and leaves was considerably limited. Translocation to the stem took place in pumpkin plants but not in wheat when both plants were exposed to CeO_2 NPs [38]. No phytotoxic effect was observed. Rate of translocation of the same NPs from underground to aerial parts of the plant *Phaseolus vulgaris* was a found to be a function of time of the exposure in a recent study [39]. In some species – in this case pumpkin *Cucurbita pepo* – the translocation of carbon-coated iron NPs throughout the body of plant was observed to take place even for a long range distances [40]. This indicates that accumulation and translocation are not only concentration and time dependent but are also strongly dependent on the plant species and properties of the particular NP.

No cellular uptake and no phytotoxic effect whatsoever did occur when individuals of *Lemna minor* were exposed to TiO_2 NPs. The NPs nevertheless adhered to the cell walls. [41]. When Al_2O_3 NPs were introduced to the *Lemna minor* environment, not only there was no observable toxic effect, the plant actually benefited from the exposition resulting in its greater production of biomass [42].TiO_2 NPs were also subjected to the standard toxicity Allium test – though some mitotic effect was observed, overall the TiO_2 NPs were found to have rather low phytotoxic properties [43]. This is in a good accordance with recent findings of Song et al. [44] who conclude that TiO_2 NPs indeed do accumulate in plants but, at least in the species observed, its phytotoxic potential is low to none. In the hydroponic culture of *Arabidopsis thaliana*, the effect of exposure to the TiO_2 NPs was even positive in terms of chlorophyll production [45]. Moreover, exposure to nano-sized TiO_2 was found to improve the light-harvesting mechanism in spinach [46].

4. CONCLUSION

Interactions of organisms and NPs are not yet thoroughly known, nevertheless, the extent of knowledge is sufficient to perform at least preliminary biomonitoring studies. Studies clearly indicate that whilst the NPs (mainly metal oxides) are indeed quite often harmful to animals, their effects on plants are mainly mechanic and limited. Therefore, animals seem to be more suitable for qualitative bioindication – through study of the dynamics of populations, behaviour changes etc., plants are promising in the field of quantitative biomonitoring, particularly in bioaccumulation studies. For the biomonitoring of NPs in terrestrial ecosystems, species that are not vulnerable to the NPs and have easily penetrable tissues are recommended.

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