



The bioavailability and toxicity of ZnO and Ni nanoparticles and their bulk counterparts in different sediments

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Abstract

Purpose The intensive development of nanotechnology raises a question of the potential consequences of the presence of nanoparticles (NPs) in the different components of the environment, including sediments. The aim of this study was to evaluate the toxicity of nanoparticles of ZnO and Ni and their bulk counterparts in bottom sediments (SD1, SD2) with different properties collected from the Vistula River in Poland.

Materials and methods Sediment samples with NPs at a concentration of 100 mg kg⁻¹ were incubated for 17 months in the dark or under a photoperiod of 12 h light/12 h dark. The Microtox[®] (bacteria, *Vibrio fischeri*) and OSTRACODTOXKIT F[™] (ostracods, *Heterocypris incongruens*) tests were used to evaluate toxicity. In addition, the contents of Zn and Ni were determined in extracts (H₂O and CaCl₂) of the bottom sediments.

Results and discussion The Zn concentration was much lower in the SD1 sediment with the addition of NPs/bulk particles (30–230 µg kg⁻¹) compared to the SD2 sediment (280–1140 µg kg⁻¹). The toxicity of ZnO and Ni was determined by the type of bottom sediment and the parameter studied.

Both nano- and bulk-ZnO and Ni caused the mortality of *H. incongruens* at a level of 13.3–53.3 %. The influence of ZnO and Ni on the growth of *H. incongruens* was observed to be the opposite. ZnO resulted in growth stimulation, while Ni resulted in growth inhibition of *H. incongruens*. Both ZnO and Ni stimulated *V. fischeri* luminescence. In most cases, the incubation of ZnO and Ni under the photoperiod increased the toxicity or decreased the stimulation of *V. fischeri* bioluminescence and *H. incongruens* growth compared to the dark-incubated sediments.

Conclusions The study provides new and important information on the ecotoxicological effects of ZnO and Ni nanoparticles in different sediments and under various environmental conditions that may be useful for the risk assessment of this new group of contaminants.

Keywords Bioavailability · Ecotoxicity · *Heterocypris incongruens* · Nanoparticles · Sediments · *Vibrio fischeri*

1 Introduction

Due to the intensive development of nanotechnology, more and more nanoproducts are available to consumers. As a result of the increasing use of products containing nanoparticles (NPs), they are released into and spread in the environment (Batley et al. 2013). The nanosize of particles (1–100 nm) determines a number of beneficial properties (i.e., higher specific surface area, reactivity, and solubility) compared to their bulk counterparts. Nevertheless, the properties that are useful from the point of view of industry can pose a threat to living organisms. Nanoparticles can penetrate into the inside of an organism more easily than their bulk counterparts, where they can cause various types of dysfunction (Klaine et al. 2008). Because the nanosize results in a higher specific surface area,

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it intensifies the toxic effects caused by the same contaminants with a macro particle size (Nowack and Bucheli 2007; Klaine et al. 2008).

Aquatic ecosystems are particularly vulnerable to the presence of NPs, the effects of which (toxicity and bioaccumulation) have been investigated extensively (e.g., Franklin et al. 2007; Heinlaan et al. 2008; Blinova et al. 2010). In spite of a real risk associated with the presence of NPs in the environment, little attention has been paid to bottom sediments. Potentially, bottom sediments will be the main sink of NPs as a consequence of the increasing presence of NPs in surface waters (Hanna et al. 2013). Nanoparticles are deposited in bottom sediments as a result of sedimentation of NPs present in the water and also as a result of the death and sinking of aquatic organisms that have accumulated NPs (Hanna et al. 2013). The presence of NPs in bottom sediments determines the contact (by digestion or dermal contact) of these contaminants with sediment-dwelling organisms. These organisms are an important food source for higher aquatic organisms (Harkey et al. 1994). By exerting a toxic effect on benthic organisms, NPs can thus indirectly disturb biological life in water bodies.

The problem of the presence of NPs in bottom sediments, including their impact on living organisms, has been a less popular topic of research compared to water environments (Franklin et al. 2007; Blinova et al. 2010) or soil environments (Unrine et al. 2010; Ge et al. 2011; Joško and Oleszczuk 2013). The existing research on NPs in bottom sediments has been conducted using various test organisms. Both the toxicity and accumulation of various NPs have been determined (Oberholster et al. 2011; Fabrega et al. 2012; Buffet et al. 2012; Hanna et al. 2013). The impacts of NPs vary substantially, which is dependent on the type of NPs and their concentration, and also on the test organism. However, there is a lack of information on the effect of the properties of bottom sediments (e.g., different sediments) and various abiotic factors on the toxicity of NPs in sediments. The influence of contact time between NPs and bottom sediment on NP-induced toxic effects is particularly important in this respect. The research shows (e.g., Coutris et al. 2012; Joško and Oleszczuk 2013) that this is an important factor in determining the toxicity of NPs in soils. Evaluation of the influence of aging on the toxicity of NPs is especially justified in benthic sediments due to a much longer retention time of NPs in these sediments compared to other environments (Liu and Cohen 2014). The existing studies have been conducted on sediments recently contaminated by NPs (e.g., Fabrega et al. 2012; Hanna et al. 2013), which may not reflect the actual conditions experienced in many natural environments. The studies on soils have revealed that NP-induced toxic effects may significantly decrease with the prolongation of contact time between NPs and the soil (Joško and Oleszczuk 2013). Furthermore, the existing studies of bottom sediments have been hitherto

conducted on one sediment type, whereas the physicochemical properties of the matrix are of key importance for the fate of NPs (among others, their bioavailability and toxicity) (Shoults-Wilson et al. 2011; El-Temsah and Joner 2012; Joško and Oleszczuk 2013). Benthic sediments are characterized by great variation in terms of grain size distribution and chemical properties within one body of water. Therefore, in ecotoxicological research on NPs, there is a need to use sediments characterized by different properties.

Light conditions are another factor that can significantly influence the toxicity of NPs. Bottom sediments, in particular, in the littoral zone, can be periodically uncovered by receding water and exposure to sunlight. Lately, the effect of solar radiation has been researched more frequently in the context of the toxicity of NPs in waters or soils (Ma et al. 2012; Joško and Oleszczuk 2013). This is particularly important in the case of photocatalysts (e.g., ZnO) which, under the influence of UV irradiation, produce more reactive oxygen species (ROS) that are responsible for a greater toxicity compared to NPs unaffected by irradiation (Ma et al. 2011).

The aim of this study was to determine the toxicity of ZnO and Ni NPs in bottom sediments with varying properties. This toxicity was studied in relation to two representative organisms; i.e., the bacteria *Vibrio fisheri* and the ostracod crustacean *Heterocypris incongruens*. The study compared the toxicity of NPs with their bulk counterparts and also evaluated the toxicity of NPs with or without access of sunlight.

2 Materials and methods

2.1 Nanoparticles

The study used two types of NPs containing heavy metals (Zn and Ni). The selection of these NPs was dictated by their common use in consumer products (i.e., cosmetics, paints, pesticides, batteries, dyes) (Nowack and Bucheli 2007). Nanoparticles ZnO (nano-ZnO50 and nano-ZnO100), Ni (nano-Ni), and their bulk counterparts (bulk-ZnO and bulk-Ni) were purchased from Sigma-Aldrich (USA). The primary particle sizes of the NPs were as follows: nano-ZnO50 <50 nm; nano-ZnO100 <100 nm; and nano-Ni1 <100 nm. The size of NPs was determined by transmission electron microscope (JEM-3010 TEM JEOL, Ltd., Japan). The complete characterization of the investigated NPs (e.g., surface area, TEM images) is presented in Joško and Oleszczuk (2013).

2.2 Sediments

Two bottom sediments with varying physicochemical properties were used: loamy fine sandy (SD1) and medium sand (SD2). The sediments were sampled from the surface layer

(0–10 cm) with a dipper in October in 2011 at two sites in the littoral zone of the Vistula River (N 51° 28' 15", E 21° 54' 39"), in its middle course near the city of Puławy, Poland. The average annual mean flow and average discharge of this part of Vistula River are 465 and 489 m³ s⁻¹, respectively.

2.3 Analytical methods

The sediment samples were air-dried and sieved through a 2-mm sieve. The chemical properties of the sediments were determined by standard methods used in soil analysis (Van Reeuwijk 1993). The particle size distribution of the sediments was determined with the areometric method. The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/sediment ratio of 2.5. The total of the exchangeable bases (TEBs) were determined in the 0.1-M HCl extract. The cation exchange capacity (CEC) and concentrations of P₂O₅, K₂O, Mg, Ca, and Na were determined according to Van Reeuwijk (1993). Total organic carbon (TOC) was determined by TOC-VCSH (Shimadzu, Japan) with Solid Sample Module SSM-5000 (Ghani et al. 2003). The total nitrogen (N_t) was determined by the Kjeldahl's method without the application of Dewarda's alloy (Cu–Al–Zn alloy-reducer of nitrites and nitrates) (Van Reeuwijk 1993). The total heavy metal content was measured with ICP-OES (Leeman Labs: PS 950 apparatus with ICP induction in argon) (Rauret 1998). The composition of organic matter was determined in accordance with Schnitzer's method (Griffith and Schnitzer 1975).

Potentiometric titration was used to determine the surface charge density of sediments and nanoparticles. The sample weights were standardized to their surface area to compare results. The surface charge density was measured for the electrolyte concentrations of 2 % NaCl (a diluting agent (diluent) used in Microtox[®]) and standard freshwater (medium used in OSTRACODTOXKIT F[™]) as a function of pH. The pH of samples (sediments and nanoparticles) was measured in the diluent (2 % NaCl) and standard freshwater (5.75 mg L⁻¹ KCl 64.75 mg L⁻¹ NaHCO₃, 123.25 mg L⁻¹ MgSO₄ × 7H₂O, 294 mg L⁻¹ CaCl₂ × 2H₂O) after 24 h in a liquid/sample ratio of 5. The potentiometric titration was measured in a special Teflon cell whose structure minimized ions adsorption on the cell walls. The Teflon cell also possessed a water thermostat and temperature sensor to assure a constant system temperature. In the cell, two electrodes were also immersed: a glass electrode REF 451 and a calomel electrode pH G 201-8. The inert gas (nitrogen) was passed through the system in the cell to remove CO₂ from the system. The measuring arrangement also included an automatic burette for titration by the NaOH solution. The set was automated and operated by a computer. The data were collected using the *titr_v3* program of W. Janusz.

2.4 Preparation of samples for incubation and extraction of Zn and Ni from sediments

The sediments with NPs were homogenized in an overhead stirrer for 24 h. The material thus prepared was subsequently incubated in the dark or under photoperiod conditions (12 h light/12 h dark) at ambient temperature (22 °C ± 4 °C). The concentration of NPs and their bulk counterparts in the soil corresponded to 100 mg kg⁻¹ of sediment. After the incubation period (that lasted 17 months), the toxicity of the investigated material was determined (Microtox[®], OSTRACODTOXKIT F[™]). The concentrations of Zn and Ni in the extracts of the bottom sediments were also determined.

The Ni and Zn from the bottom sediments were extracted with redistilled H₂O and 0.01 M CaCl₂ following the methodology described by Cao et al. (2009). It is assumed that the metal concentrations in the CaCl₂ fraction correspond to metal fractions that are bioavailable to organisms (Cao et al. 2009). After 24-h shaking, the extracts (H₂O or CaCl₂) were centrifuged (at 20,000g for 20 min) and the extract was filtered through a 0.4-μm pore-size Millipore filter. The CaCl₂ extracts were acidified (pH = 2) using HNO₃ (Cao et al. 2009).

2.5 Bioassays

The Microtox[®] Toxicity Test (tests an acute toxicity) was used to evaluate the inhibition of the luminescence in the marine bacteria *V. fischeri*. Luminescence inhibition of the extract was assessed for 15 min of exposure carrying out the Basic Solid-Phase Test (Basic SPT), which allows the testing of solid samples (soils and sediments). The extracts were prepared with 7-g sediment and 35-ml 2 % NaCl, which were placed in a 50-ml beaker. Samples were stirred on a magnetic stirrer for 10 min. Extracts were transferred to cuvettes (in incubator wells) from the region adjacent to the wall and about 2 cm above the bottom of the beaker, while the sample was stirring. The light output of the luminescent bacteria from the sediment's extract was compared with the light output of a blank control sample. The Microtox[®] test was performed using the Microtox[®] bacteria reagent (Microtox[®] Acute Toxicity Testing Reagent) and prepared according to the test protocol.

Chronic toxicity determination of sediment samples was performed in a short-term contact test using the ostracod test kit (OSTRACODTOXKIT F[™], MicroBioTests, Nazareth, Belgium). Cysts of *H. incongruens* were transferred into a Petri dish filled with 10 ml of standard freshwater (U.S. Environmental Protection Agency medium hard reconstituted water) and were incubated at 25 °C and permanent illumination (approximately 3000–4000 lux). Prefeeding was performed with algae (spirulina powder) that were contained in the test kit. Ostracods started to hatch after approximately 38 h

and were immediately used for testing. Algae (*Selenastrum capricornutum*) used as feed in the test plate were reconstituted according to the manufacturer's recommendations. Each well of a test plate was filled in the following order: 2-ml standard freshwater, 1000- μ l sediment sample, 2-ml algal suspension, and 10 ostracods. The test plate was sealed by a laboratory film (Parafilm), covered by a lid and incubated at 25 °C in the dark. After 6 days, the mortality of test organisms was determined. The length of the organisms was measured according to the user guide provided by the manufacturer (Ostracodtoxkit FTM 2004). Growth inhibition (GI) of *H. incongruens* was calculated as

$$GI = (A-B) \div \{A\} \times 100 \quad (1)$$

where A is the increment of the ostracods in sediments without NPs, and B is the increment of ostracods in the investigated sediments contaminated with NPs.

2.6 Data analysis

Mean values were taken from each triplicate data set. The differences between each treatment and the control, as well as between treatments, were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. All statistical analyses were performed using Sigma Plot 11.0 software with a level of confidence of $P < 0.05$.

3 Results

3.1 Sample characteristics

The bottom sediments were characterized by varying physicochemical properties (Table 1). Only the pH value of both sediments was at a similar level (7.2–7.4). pH has a significant impact on the mobility of heavy metals; by using two sediments with a similar level of pH, a potential reason for differences in toxicity between the sediments was thus excluded. The SD2 sediment showed much lower values of CEC and TEB than the SD1 sediment. The content of minerals (K_2O , Mg, Na, Ca) and the N_t content were also lower in the SD2 sediment relative to SD1. Among the macronutrients, only the content of P_2O_5 was at a similar level in both sediments. The TOC content, which is an important factor determining the availability of many contaminants, was four times higher in the SD1 sediment than in SD2. Higher concentrations of Cd, Zn, Pb, Cr, and Ni and of individual organic matter fractions (i.e., humic acids (HA), fulvic acids (FA), and cellulose) were determined

Table 1 Physicochemical properties of sediments used in the experiment

	SD1	SD2
Sand	78	99
Silt	15	
Clay	3	
pH	7.4	7.2
CEC	278	73.2
TEB	269.1	66.7
K_2O	53	23
P_2O_5	112	120.4
Mg	63	20
Na	185	56.6
Ca	5200	1271.9
N_t	0.2	0.001
TOC	0.5	0.1
Heavy metals		
Cd	0.9	0.8
Zn	75.1	31.3
Pb	27.5	19.9
Cr	17	12.1
Cu	-	-
Ni	12.2	4.33
Composition of organic matter		
Humic acids	10.6	4.6
Fulvic acids	23.9	9.4
Cellulose	22.8	9
Residual fraction	42.8	77

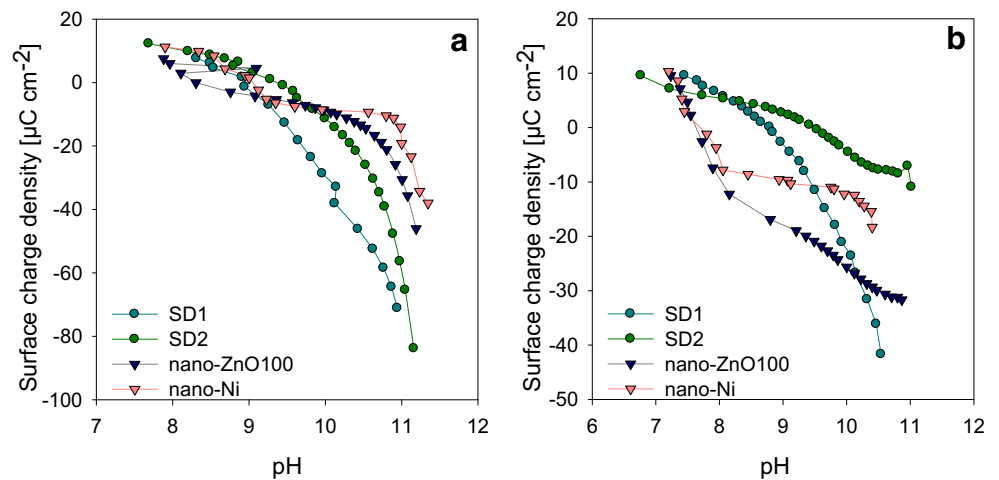
Sand, silt, clay [%]

pH reactivity in KCl, TOC total organic carbon content ($g\ kg^{-1}$), N_t total nitrogen content ($g\ kg^{-1}$), CEC cation exchange capacity ($mmol\ kg^{-1}$), TEB the total of the exchangeable bases ($mmol\ kg^{-1}$), P_2O_5 , K_2O , Mg available forms of phosphorus, potassium, and magnesium ($mg\ kg^{-1}$), Na and Ca content ($mg\ kg^{-1}$), heavy metals content ($mg\ kg^{-1}$), the composition of organic matter—fraction (%)

in the SD1 sediment compared to SD2. The contents of native heavy metals in both sediments did not affect significantly the test organisms, since their response did not differ from the effects found in the control (reference materials for the biotests used).

Figure 1 presents the surface charge density of samples as a function of pH. Based on the pH of samples in the solutions (i.e., standard freshwater and diluent), the sediments and nanoparticles were characterized by positive surface charges. In the diluent (Fig. 1a), the surface charge density was $6.97\ \mu C\ cm^{-2}$ (SD1), $11.31\ \mu C\ cm^{-2}$ (SD2), $1.42\ \mu C\ cm^{-2}$ (nano-ZnO100), and $2.97\ \mu C\ cm^{-2}$ (nano-Ni), whereas the surface charge of the samples in standard freshwater was (Fig. 1b) $3.8\ \mu C\ cm^{-2}$ (SD1), $5.38\ \mu C\ cm^{-2}$ (SD2), $0.5\ \mu C\ cm^{-2}$ (nano-ZnO100), and $2.93\ \mu C\ cm^{-2}$ (nano-Ni).

Fig. 1 Surface charge density of nano-ZnO100 and nano-Ni and sediments (SD1, SD2) in **a** diluent used in Microtox[®] and **b** standard freshwater used in OSTRACODTOXKIT F[™]

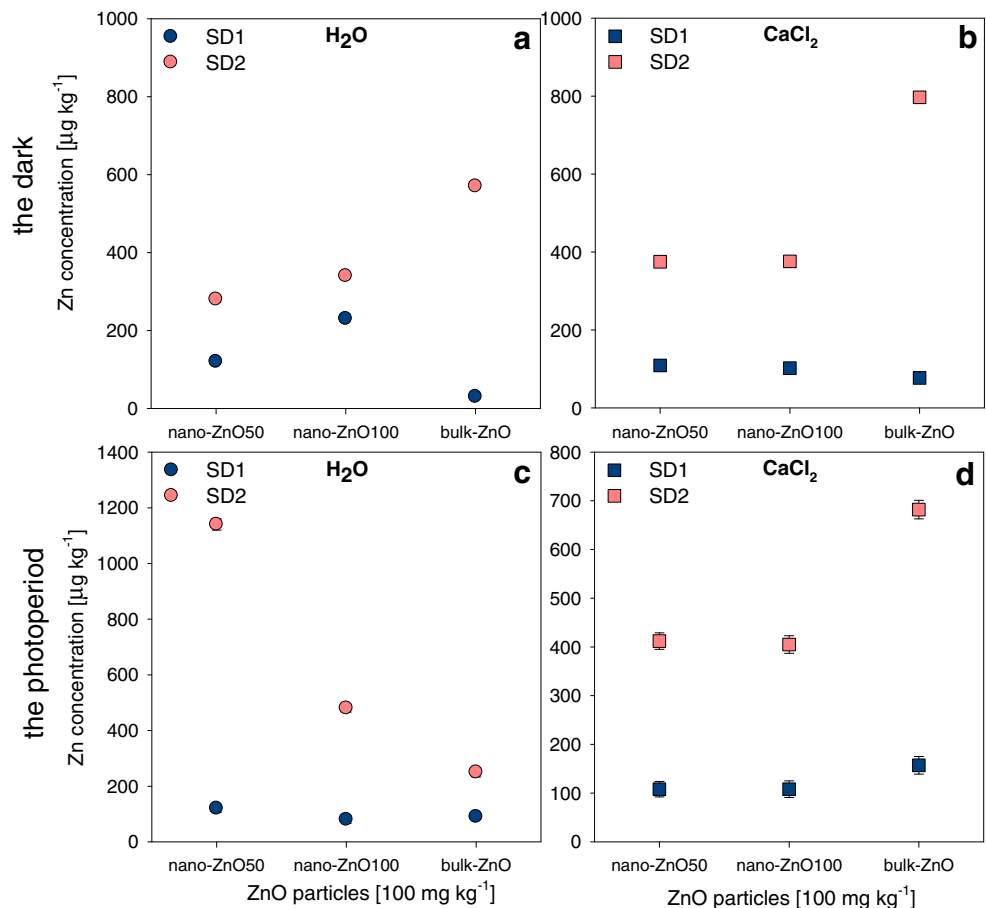


3.2 Concentrations of Zn and Ni in H₂O and CaCl₂ extracts

In the extracts examined (H₂O and CaCl₂), the Ni concentrations were too low to be detected. The Zn content in the extracts varied, which was dependent on sediment type, size of ZnO particles, extraction solvent used, and light conditions (Fig. 2).

The dark-incubated SD1 sediment contained less Zn in the H₂O and CaCl₂ extracts than the SD2 sediment also incubated in the dark (Fig. 2a, b). In the H₂O extracts of the SD1 sediment, the highest Zn concentration was found in the sediment with the addition of nano-ZnO100 (230 µg kg⁻¹), followed by the sediment with nano-ZnO50 (120 µg kg⁻¹), while the sediment with bulk-ZnO (20 µg kg⁻¹) had the lowest Zn content (Fig. 2a). Unlike SD1, in the H₂O extracts of the SD2

Fig. 2 Zinc concentration in sediment extracts of H₂O (a, c) and CaCl₂ (b, d). Sediments were treated with nano-ZnO and bulk-ZnO and incubated under different light conditions



sediment, the Zn concentration was highest in the sediment with the addition of bulk-ZnO ($570 \mu\text{g kg}^{-1}$), followed by the sediments with nano-ZnO100 ($340 \mu\text{g kg}^{-1}$) and nano-ZnO50 ($280 \mu\text{g kg}^{-1}$) (Fig. 2a).

The Zn concentration in the CaCl_2 extract did not differ significantly for the SD1 sediment with the addition of nano-ZnO50 and nano-ZnO100 ($102\text{--}109 \mu\text{g kg}^{-1}$) (Fig. 2b). On the other hand, a lower value was recorded for the sediment with the addition of bulk-ZnO ($70 \mu\text{g kg}^{-1}$). The CaCl_2 extracts of the SD2 sediment containing nano-ZnO50 and nano-ZnO100 did not differ from each other either (371 and $376 \mu\text{g kg}^{-1}$), but the Zn content was much higher in the sediment with bulk-ZnO ($797 \mu\text{g kg}^{-1}$) than in the case of NPs.

3.3 Effect of sunlight on Zn concentrations in H_2O and CaCl_2 extracts

Incubation under variable light conditions affected the Zn concentration in the extracts studied (Fig. 2c, d). The exceptions were the H_2O and CaCl_2 extracts of the SD1 sediment with the addition of nano-ZnO50 and the CaCl_2 extract of the SD1 sediment with the addition of nano-ZnO100. Regardless of light conditions, in the abovementioned cases, the same Zn concentration was found in the respective extracts.

The H_2O extract of the light-incubated SD1 sediment with nano-ZnO100 was characterized by a threefold lower Zn concentration than the same dark-incubated sediment (Fig. 2c). But, an opposite trend was observed for the bulk-ZnO-containing sediment. In this case, the Zn concentration was three times higher in the sediment incubated in light compared to that incubated in the dark. With regard to the CaCl_2 extract of the SD1 sediment, differences depending on the presence of light were only observed after adding bulk-ZnO. The CaCl_2 extract of the sediment incubated under photoperiod conditions was characterized by higher Zn concentration ($157 \mu\text{g kg}^{-1}$) compared to incubation in the dark ($77 \mu\text{g kg}^{-1}$) (Fig. 2d).

In the SD2 sediment, the Zn concentrations in the H_2O extracts were dependent on the ZnO particle diameter (Fig. 2c). The H_2O extract of the dark-incubated SD2 sediment to which nano-ZnO50 or nano-ZnO100 had been added had a lower concentration of Zn than the extract obtained from the same sediment incubated in the presence of light. However, the differences in the concentration levels were much higher in the case of the extracts with nano-ZnO50 ($860 \mu\text{g kg}^{-1}$) than with nano-ZnO100 ($100 \mu\text{g kg}^{-1}$). In the case of the H_2O extract of the sediment with bulk-ZnO, a reverse trend was observed, as the Zn concentration after incubation in light was lower than that under no light conditions. The CaCl_2 extracts of the SD2 sediment were characterized by a similar trend as the H_2O extracts (Fig. 2d), since the CaCl_2 extracts of the SD2 sediment with nano-ZnO50 and nano-

ZnO100 incubated under photoperiod conditions showed a lower (by about $30 \mu\text{g kg}^{-1}$) concentration of Zn compared to no light conditions. But, the extracts of the sediment with bulk-ZnO kept in the dark showed a higher Zn concentration (by $115 \mu\text{g kg}^{-1}$) than in the presence of light.

3.4 Toxicity of NPs and their bulk counterparts to the bacteria *V. fischeri*

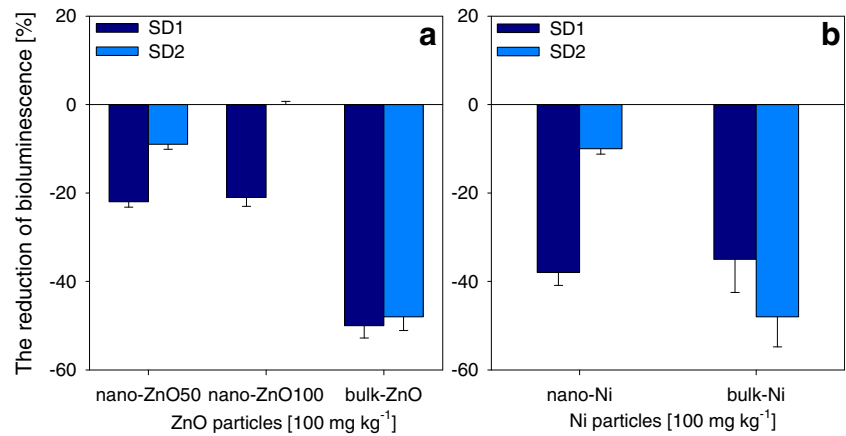
The effect of the particle size of ZnO and Ni on the toxicity of the sediments varied depending on the type of bottom sediment. In both sediments, nano-ZnO and nano-Ni as well as their bulk counterparts resulted in a strong stimulation of *V. fischeri* bioluminescence. The only exception was the SD2 sediment to which nano-ZnO100 had been added (Fig. 3a, b).

When comparing these two sediments to each other, it was observed that after adding nano-ZnO, the SD1 sediment showed a greater stimulation of *V. fischeri* bioluminescence (by 13–21 %) than SD2 (Fig. 3a). In both sediments, bulk-ZnO stimulated *V. fischeri* bioluminescence at a similar level (50 %). Nano-ZnO resulted in a much lower stimulation than bulk-ZnO in both sediments. The presence of nano-Ni in the sediments also had a stimulating effect on *V. fischeri*; similar to the case of nano-ZnO, a greater stimulation was observed in the SD1 sediment than in SD2 (Fig. 3b). No significant differences were found in the stimulation of *V. fischeri* between the SD1 and SD2 sediments containing bulk-Ni. Comparing nano-Ni and bulk-Ni in the SD1 sediment, no difference was found between them, while in the SD2 sediment, bulk-Ni stimulated *V. fischeri* bioluminescence to a much greater extent than nano-Ni.

3.5 Effect of nanoparticles and their bulk counterparts on the mortality and growth of *H. incongruens*

Adding ZnO or Ni, regardless of their form, caused the mortality of *H. incongruens*. The mortality level was primarily dependent on sediment type (Fig. 4a, b). Irrespective of the size of ZnO particles added, the mortality of *H. incongruens* was higher in the SD2 sediment than in SD1 (Fig. 4a). In the SD1 sediment, the ZnO-induced mortality was at a level of 13.3–20 %, whereas in the SD2 sediment, it was at a level 43.3–53.3 % depending on the type of ZnO. In the case of Ni, differences between the sediments were observed only for bulk-Ni; similar to the case of ZnO, it was higher in the SD2 sediment than SD1 (Fig. 4b). In both sediments, nano-Ni resulted in the mortality at a level of 36.7–40 %. In the case of Ni, differences were observed in the mortality rate depending on the particle size; nano-Ni in the SD1 sediment showed much higher toxicity than bulk-Ni, while in the SD2 sediment, an opposite trend was found.

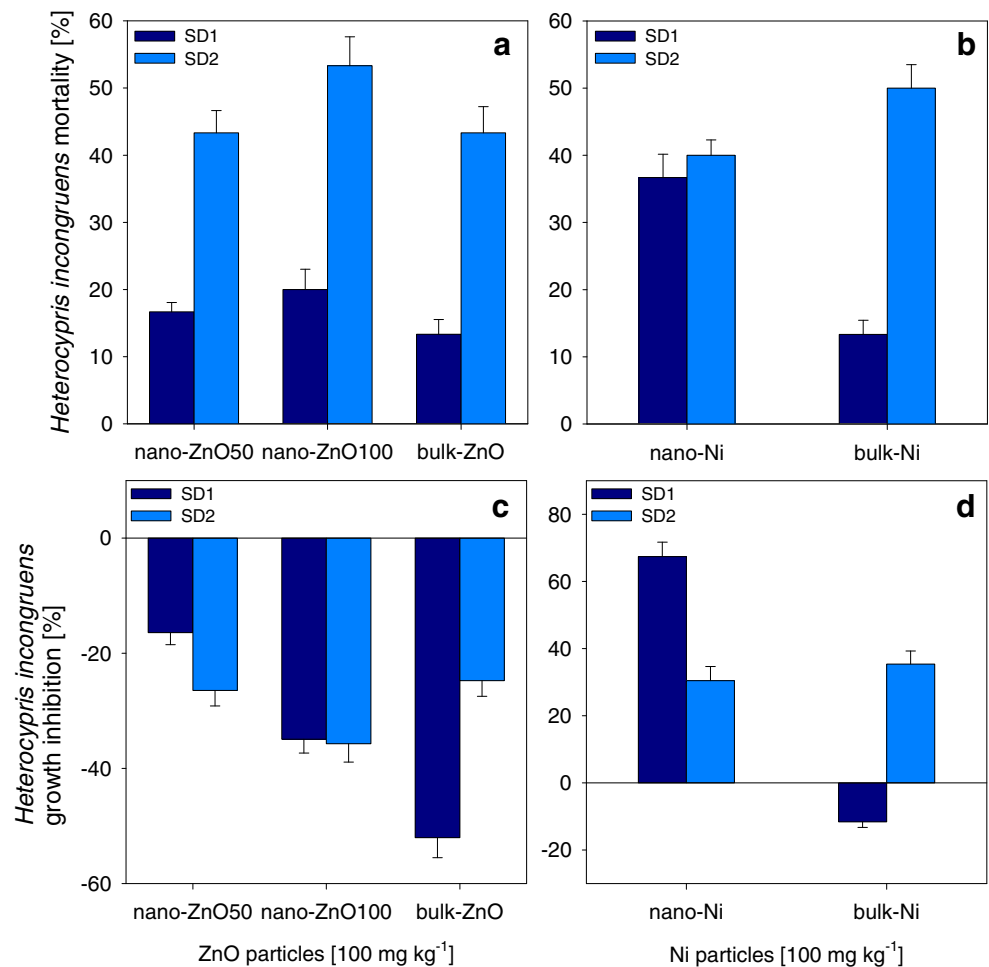
Fig. 3 Influence of ZnO (a) and Ni (b) on the bioluminescence of bacteria, *Vibrio fischeri* in different sediments



The largest differences between the toxicity parameters investigated were found during the evaluation of the effect of ZnO and Ni on the growth of *H. incongruens* (Fig. 4c, d). Both growth inhibition and stimulation of *H. incongruens* were observed under the influence of the contaminants studied. In both sediments, nano- and bulk-ZnO stimulated the growth of *H. incongruens*

(Fig. 4a). In the SD1 sediment, however, the range of stimulation depended on the ZnO particle size. In the SD1 sediment, increased stimulation was observed with increasing ZnO particle size (from 16.4 to 52 %), whereas in the SD2 sediment, the growth stimulation of *H. incongruens* was at a similar level regardless of the ZnO particle size (25–35 %).

Fig. 4 Effect of ZnO (a, c) and Ni (b, d) nanoparticles and their bulk counterparts on the mortality and the growth inhibition of *Heterocypris incongruens* in different sediments



In the case of Ni, both stimulating and inhibitory effects were observed (Fig. 4d). The level of effects was dependent on sediment type. Nano-Ni inhibited the growth of *H. incongruens* in the sediments SD1 (67.4 %) and SD2 (30.5 %). But, bulk-Ni stimulated the growth of *H. incongruens* in the SD1 sediment, whereas in the SD2 sediment, it inhibited their growth. Similar to the case of ZnO, differences in *H. incongruens* growth inhibition between nano-Ni and bulk-Ni were only observed in the SD1 sediment where nano-Ni caused a greater toxic effect than bulk-Ni. In the SD2 sediment, no significant differences were observed between nano-Ni and bulk-Ni.

3.6 Toxicity of nanoparticles to *V. fischeri* and *H. incongruens* under photoperiod conditions

The presence of light had a significant influence on the toxicity of ZnO and Ni to the test organisms (Fig. 5). An exception was the SD1 sediment (Fig. 5a), where no significant differences were found in the level of *V. fischeri* bioluminescence between various incubation conditions. On the other hand, light was observed to affect *V. fischeri* in the SD2 sediment, as manifested in the significant differences in toxicity between the sediments incubated in the dark and under photoperiod conditions (Fig. 5b). The SD2 sediment contaminated with ZnO and Ni after incubation in light caused the inhibition of *V. fischeri* bioluminescence, whereas incubation of the SD2

sediment in the dark had a stimulating effect on *V. fischeri*. The largest differences between the incubation of the SD2 sediment in the light and dark were observed for bulk-ZnO and bulk-Ni (Fig. 5b).

As far as the mortality of *H. incongruens* is concerned, the effect of the presence of light was dependent on the type of compounds investigated. In the case of incubation of the SD1 sediment containing nano-ZnO50 in the presence of light, the mortality of *H. incongruens* was found to decrease in relation to the dark-incubated sediment (Fig. 5c). In the SD2 sediment, on the other hand, a decrease in toxicity after incubation in light was found for nano-Zn100 (Fig. 5d). In the other cases (both sediments with bulk-Ni, the SD1 sediment with nano-ZnO100, and the SD2 sediment with nano-ZnO50), a reverse trend was observed (Fig. 5c, d). In both bulk-ZnO-containing sediments, light was not found to affect the mortality of *H. incongruens*.

Light conditions also played an important role in determining *H. incongruens* growth inhibition/stimulation in both sediments (Fig. 5e, f). Only in single cases (i.e., the SD1 sediment with nano-ZnO50 and nano-ZnO100 as well as the SD2 sediment with nano-Ni) were no differences found in the effect on the growth of these organisms depending on sediment incubation with/without light. Keeping the SD1 sediment with bulk-ZnO in the presence of light resulted in a lower stimulation of *H. incongruens* growth compared to incubation in the dark (Fig. 5e). But when nano-Ni and bulk-Ni were added to

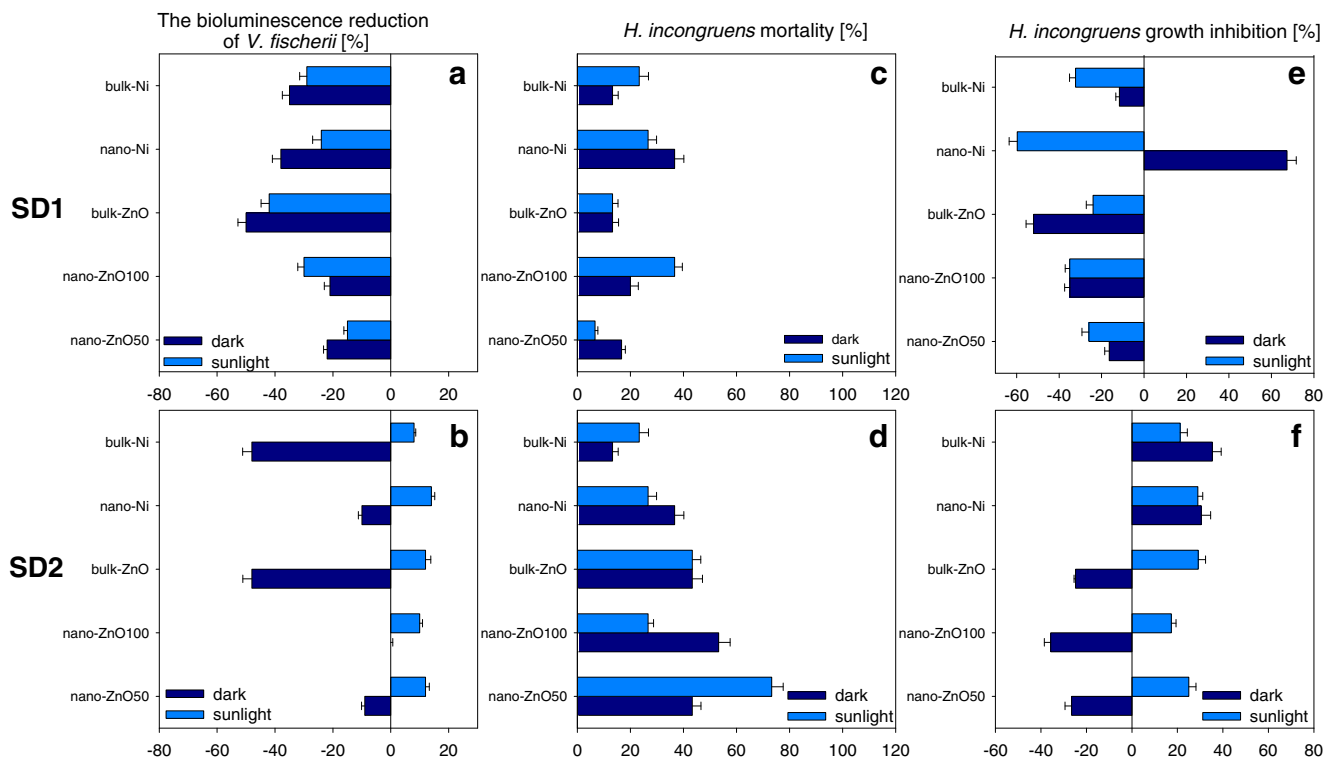


Fig. 5 Effects of nanoparticles and their counterparts (100 mg kg^{-1}) on *V. fischerii* (a, b) and *H. incongruens* (c, d, e, f) depending on different sediments and light conditions

the SD1 sediment, higher toxicity or lower stimulation of *H. incongruens* growth was observed in the absence of exposure to light. The SD2 sediment with ZnO (nano- and bulk-) incubated under photoperiod conditions was responsible for the toxicity at a level of 17.4–29.2 %, whereas after incubation in the dark, this sediment significantly stimulated (24.8–35.7 %) the growth of *H. incongruens* (Fig. 5f). In the case of the SD2 sediment with bulk-Ni, an opposite trend was found. Incubation without light caused greater *H. incongruens* growth inhibition compared to photoperiod conditions.

4 Discussion

Bottom sediments are one of fluvial compartments most exposed to the presence of NPs (Hanna et al. 2013). This represents a risk for the functioning of the benthos which is an important compartment in aquatic environments (Dale et al. 2013). Only a few studies have shown the toxicity of various NPs present in bottom sediments in relation to sediment-dwelling organisms (Oberholster et al. 2011; Coleman et al. 2013; Hanna et al. 2013). These studies have dealt with nano-ZnO (Buffet et al. 2012; Fabrega et al. 2012; Hanna et al. 2013), with a lack of data on the toxicity of nano-Ni. Our research revealed significant mortality of *H. incongruens* in the sediments containing both nano-ZnO and nano-Ni. The high toxicity of nano-ZnO was also confirmed by Hanna et al. (2013) in relation to other aquatic organisms. Hanna et al. (2013) observed high mortality of the amphipod *Leptocheirus plumulosus* in the presence of nano-ZnO occurring in the bottom sediments. Substantial toxicity of nano-ZnO has also been demonstrated in relation to *Hyalella azteca* (Poynton et al. 2013). In the latter case, however, the study was conducted without bottom sediments. Fabrega et al. (2012) also observed its negative influence on the growth and reproduction of the amphipod *Corophium volutator*. The activity of dissolved ions from NPs is reported as one of the main reasons responsible for the toxic effects of NPs (Adams et al. 2006; Beer et al. 2012; Yang et al. 2012). Our study revealed a general trend showing that the mortality of *H. incongruens* is associated with the concentration of Zn in the aqueous solution of both ZnO-containing sediments (Figs. 1a and 3a). The mortality of *H. incongruens* was lower in the SD1 sediment than in SD2, where a higher content of Zn was found at the same time. The differences in the levels of Zn concentration in the extracts originating from the different sediments are likely associated with the physicochemical properties of these sediments. The organic matter content is particularly important in this respect. The SD1 sediment was characterized by a lower TOC content as well as lower percentages of HA and FA in organic matter than the SD2 sediment. The higher content of organic matter and its

composition (a higher percentage of HA and FA) result in greater immobilization of contaminants (Ma et al. 2011), which was also confirmed in our study. Furthermore, HA may adsorb on the surface of NPs (heteroaggregation), which may settle to the bottom of the channel faster and become less bioavailable and toxic (Deonaraine et al. 2011; Nur et al. 2015). Additionally, the immobilization of nanoparticles by sediments (mainly, clays) may consist of electrostatic interaction between NPs and sediments. However, based on the surface charge density analysis, the NPs as well as the sediments were characterized by positive charge at the range of measured pH, what prevents the attachment of NPs to the surface of sediment particles. Apart from ions dissolved in the aqueous solution, in the case of *H. incongruens*, the toxicity of NPs can also be determined by the nutritional preferences of these organisms. When taking up organic and mineral nutrients, these organisms can also absorb NPs that are adsorbed in the sediment components. Such a phenomenon has already been observed earlier (e.g., Campos et al. 2013) in the case of *Daphnia magna* exposed to nano-TiO₂.

It is a surprising fact that in spite of the observed mortality of *H. incongruens* in the ZnO-containing sediment, at the same time, ZnO was found to have a stimulating effect on the growth of *H. incongruens*. The growth stimulation of *H. incongruens* could have been due to the action of Zn²⁺ ions released from NPs and their bulk counterparts. Zinc is a micronutrient required for the functioning of organisms (Sikorski 1990). As shown in earlier studies, in small amounts, Zn can stimulate the growth and development of organisms (Joško and Oleszczuk 2013). This confirms the increasing growth stimulation of *H. incongruens* with the increasing concentration of bioavailable Zn (in CaCl₂) in the SD1 sediment (Figs. 1b and 3c), while a similar relationship was not observed in the SD2 sediment. In the SD2 sediment, *H. incongruens* growth stimulation did not differ significantly between the individual types of ZnO, whereas the Zn concentration in CaCl₂ underwent significant changes depending on the particle size (e.g., the smaller diameter of NPs, the lower Zn concentration). The nanoparticles may aggregate much faster than bulk particles, what might result in the decrease of their solubility because of the smaller surface area of these aggregates (Hotze et al. 2010). The stimulating effect of Zn might have been cancelled by the type of sediment, since the SD2 sediment was characterized by a higher content of organic matter and nutrients, and therefore, the nutritional conditions were more favorable than in the SD1 sediment.

V. fisheri bacteria was the other test organism. The bioluminescence of *V. fisheri* was stimulated under the influence of both NPs (ZnO and Ni) and their bulk counterparts. The existing studies using *V. fisheri* have shown the toxic effect of various NPs (including ZnO) (Heinlaan et al. 2008; Strigul et al. 2009). It should however be stressed that these studies have been conducted only for aqueous solutions (Heinlaan

et al. 2008; Mortimer et al. 2008). Our preliminary analysis of the toxicity also revealed a significant reduction in bioluminescence in the aqueous solution; at a concentration of 1 mg L^{-1} , the reduction in bioluminescence was 65 % for nano-ZnO50, 45 % for nano-ZnO100, and 60 % for bulk-ZnO. In the present study (with sediments), a substantial stimulation of bioluminescence was observed despite the fact that the Zn concentration determined in the CaCl_2 fraction was close to 1 mg L^{-1} . However, in our study, the sample preparation method (stirring for 10 min with 2 % NaCl) could have determined the lower exposition of bacteria to the Zn^{2+} and Ni^+ ions, which proved to be stimulating for the metabolism of the bacteria. The solubility of NPs increases with time (Franklin et al. 2007) and it should therefore be presumed that after 10-min stirring, the Zn and Ni concentration was lower than in the case of the analysis of the extracts (24-h stirring).

The effect of light conditions on the toxicity of NPs has been hitherto studied mainly in water (e.g., Fenoglio et al. 2009; Ma et al. 2012) and in soil (e.g., Joško and Oleszczuk 2013). Sunlight is an important factor in the evaluation of toxicity due to the fact that it supports the process of generation of ROS (Ma et al. 2012). This is essential in the case of photocatalysts, which ZnO is. ROS may lead to cell dysfunction, including death (Ahamed 2011; Campos et al. 2013). Moreover, under light conditions, the aggregation of NPs is slower and thereby a higher concentration of metals may be released (Dalai et al. 2013; Lee and An 2013). This is also confirmed by our study in which higher Zn concentrations were observed in the extracts of the sediments incubated in light compared to the sediments kept in the dark. Nevertheless, the Zn concentration and the toxicity of nano-ZnO50 and nano-ZnO100 were found to be correlated only in some cases. The level of inhibition/stimulation by NPs may perhaps be driven not only by both ROS generation and metal ions but also by the effect on behavior (gut clogging or adhesion) additionally determined by sediment type. The existing few studies on the toxicity of NPs under the influence of solar radiation do not give unambiguous answers concerning the NP toxicity mechanisms either. The study by Lee and An (2013) on TiO_2 toxicity to green algae, *Pseudokirchneriella subcapitata*, proved that ions released from NPs were responsible for their toxicity. Ma et al. (2011), on the other hand, claim that ROS production is the decisive reason for the toxicity of nano-ZnO to nematodes (*Ceanorhabditis elegans*). Furthermore, apart from the type of test organism, the properties of the matrices can also play a key role in determining toxicity. As revealed by the study of Dasari and Hwang (2013), the toxicity of NPs under light exposure conditions was determined by the type of humic acids.

5 Conclusions

The present study showed that the effect of NPs on the toxicity of bottom sediments significantly varied depending on the size and type of NPs, sediment type, type of organism type, parameter evaluated, and also light conditions. Regardless of the sediment, ZnO and Ni increased the mortality of *H. incongruens* and the stimulation of *V. fischeri* bioluminescence. On the other hand, the growth of ostracods was stimulated by ZnO and inhibited by Ni (except for one sediment with bulk-Ni). However, the level of the effects frequently varied depending on the size of contaminant particles and sediment type. In most cases, lower toxicity or greater stimulation was observed in the SD1 sediment than in SD2. The properties of the sediments, in particular TOC, were responsible for this trend. The present study confirms the varying effects of ZnO and Ni NPs on organisms in different sediments. It demonstrates the need to investigate the various kinds of nanoparticles individually.

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