Environmental Toxicity of Nanoparticles Environmental Toxicity of Nanoparticles

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***ABSTRACT**: In recent years, nanoparticles that have size of 1-100 nm is widely used for textile, pharmacy, cosmetic and treatment of industrial wastewater. Producing and using of nanoparticles widely, causes important accumulation in nature and toxicity on ecosystem. Knowledge of potential toxicity of nanoparticles is limited. In this study, six different nanoparticles nano-zinc oxide, nano-silicon dioxide, nano-cerium oxide, nano-aluminum oxide, nano-hafnium oxide, and nano-tantalum oxide which used commonly, were studied to investigate toxic impacts on organisms. We studied nine different acute toxicity test (bacteria – Escherichia coli (gram negative bacteria) ; bacteria – Bacillus cereus (gram positive bacteria) ; bacteria – Vibrio fischeri (bioluminescences bacteria) ; methane Archae Bacteria ; yeast – Candida albicans ; mold – Aspergillus niger ; algae – Chlorella sp. ; Crustacea – Daphnia magna ; lepistes - Poecillia reticula) for the effect of nanoparticles to different trophic levels. In general, the most toxic nanoparticle is nano-zinc oxide and the least toxic nanoparticle is nano-hafnium oxide. Among the used organisms in acute toxicity test; the most sensitive organism is algae - Chlorella sp ; the most resistant organism is fish- Poecillia reticula.*

Keywords: nano-zinc oxide, nano-silicon dioxide, nano-cerium oxide, nano-aluminum oxide, nano-hafnium oxide, nano-tantalum oxide, acute toxicity, trophic levels, organisms.

I. Introduction

Nanoparticles (NP) are structures with dimensions generally between 1 to 100 nm. There are widespread in nature. Different sizes, different structures, one-element or multi-element structure can be formed in different shapes and formats, or desired. NPs have wide potential : in the short term in the textile, cosmetics and dye , in the long-term medications are used in drug delivery systems to send the requested body (Kahru et al., 2010). Also NMO is widely used in the treatment of industrial wastewater (Chen et al., 2012 ; Zhou et al., 2012). This widespread production and use of nanoparticles in nature means intense accumulation. This accumulation of knowledge about the toxicity of the environment is very limited. The purpose of this work ; increasing concentrations (0,01 mg/l – 1000 mg/l) of NMO which there is limited acute toxicity studies used 9 different trophic levels starting from at the bottom of the trophic level (bacteria - *Escherichia coli* (gram negative bacteria), bacteria - *Bacillus cereus* (gram-positive bacteria), bacteria - *Vibrio fischeri* (bioluminescence bacteria), methane Archae bacteria , yeast - *Candida albicans* , mold - *Aspergillus niger* ; algae - *Chlorella sp.* ; Crustacean - *Daphnia magna* ; fish - *Poecillia reticula*). Inhibitions, death , on the basis of biodegradibility and bioaccumulation is calculated simultaneously.

NMOs because of can easily be synthesized chemically and can easily be modified consumer products ; industrial products , machinery industry , military applications, in wastewater treatment and medicine widely used (Atlı-Şekeroğlu, 2013). In particular, the development of wastewater treatment technology that uses NP is seen as an alternative solution to the growing worldwide water pollution problems. Examples of this work in the treatment of heavy metals 2 mg/l anaerobic conditions using nano -TiO_2 15.3 mg/g Zn and 7.9 mg/g have provided treatment of Cd. But the dose of nano-TiO_2 found that at a later stage of anareobic they inhibited on activated sludge microorganisms (Liang et al., 2004).

Studies about the environmental toxicity of the NMOs is very limited. The ecotoxicity of nano-TiO_2 (NMOs) is the most studied NP (Cattaneo et al., 2009; Kahru & Dubourguier, 2010). When the toxicity of nano-TiO_2 to *Daphnia magna* ; at the end of 48 hours EC_{50} values 5.5 mg/l to 20000 mg/l seem to change (Lovern & Klaper, 2006; Heinlaan et al., 2008). According to Sadiq et al. (2011) ; two different microalgae (*Scenedesmus sp.* and *Chlorella sp.)* isolated from the fresh water ecosystems were investigated and found that after 72 hours EC_{50} value is 16,12 mg/l for *Chlorella sp.* ; EC_{50} value is 21.2 mg/l for *Scenedesmus sp.*

Information about the mechanisms of toxicity of NPs on organisms are quite limited. Recent years researhers specializing on potential mechanisms of toxicity aganist multiple organisms. Nano-ZnO the most commanly used creates high toxicity on

bacteria (LC_{50} value for *E.coli* : 0,048 mg/l ; Dasari et al. 2013). Zinc ions (Zn^{+2}) connect to bacterial cells and reported that damage to physilogical function of the defeated cell to osmotic shock. Mannier et al. (2013) studied that the ecotoxicity of nano-CeO_2 to fresh water algae. Close adhesion of nano-CeO_2 on the algal cells may lead to direct physical effects, such as cell membrane disruption, or indirect effect such as the reduction of the available light necessary to the algal growth (shading effect) or the limitation of the nutrient intake by the algal cells. Bing et al. (2014) reported that small particles of less than 50 microns in diameter are filtered out of the water by fine setae located on the thoracic legs and are moved to the mouth. As a result of that, nano-SiO_2 may be bioaccumulated and bioconcentrated in filter-feeding aquatic organisms such as *Daphnia magna* and transfer to the higher levels of food chain.

II. Material and Methods

2.1 Nanoparticles Used in Acute Toxicity Tests

The environmental toxicity of nanoparticles has been studied in six different nanoparticles. These nanoparticles are nano-CeO_2 (abcr, AB249225, Lot:1266155) ; nano-ZnO (abcr, AB249229, Lot:1235097) ; nano-Al_2O_3 (abcr, AB249221, Lot:1235095) ; nano-SiO_2 (Sigma-Aldrich, 637238, Lot:MKBL8542V) ; nano-HfO_2 (Sigma-Aldrich, 202118, Lot:MKBH3310V) ; nano-Ta_2O_5 (Sigma-Aldrich, 303518, Lot:MKBJ3486V). Considering the studies in the literature on acute toxicity test nanoparticles concentration ranges were determined. Stock solutions of all NP used (100 mg/l) prepared with distilled deionized water, stirred at sonicator at 30°C for 1 hour were used in acute toxicity test.

2.2 Organisms Used in Acute Toxicity Tests

In this study, representing 6 different nutrition level 4 bacteria, 1 yeast, 1 mold, 1 algae, 1 crustacean, 1 fish was used. These are bacteria (*Escherichia coli, Bacillus cereus, Vibrio fischeri,* ve Metan Archae Bacteria), algae (*Chlorella sp.*), yeast (*Candida sp.*), mold (*Aspergillus sp.*), Crustacean (*Daphnia magna*) and fish (lepistes - *Poecillia reticula*). The culture of bacteria [*Escherichia coli* - ATCC 3509 (RSHM NO: 5010) and *Bacillus cereus* - RSKK 11015 (NTC 9946)] , yeast *(Candida albicans* ATCC 628) and mold (*Aspergillus niger)* were purchased from Turkey Public Health Institutions. Algae – *Chlorella sp.* was isolated from Gölcük Lake (Ödemiş) and was cultivated. Bioluminescent bacteria – *Vibrio fischeri* were purchased from Hach-Lange Company as a lyphilized culture. Water flea - *Daphnia magna* and fish - *Poecillia reticule* was purchased from an aquarium maker.

2.3 Methods of Acute Toxicity Tests

Stock solutions of NPs was stirred at sonicator for bacteria (*Escherichia coli* and *Bacillus cereus*) ; yeast (*Candida albicans*) and mold (*Aspergillus niger*) acute toxicity tests. 5 ml was added to sterile tubes from each NPs. In log phase bacteria/yeast/mold culture was added to an equal volume of the tube. Bacteria/yeast/mold was exposed to NPs for 24 and 48 hours. After incubation period 1 ml was taken from each tube and made serial dilutions. Then inoculations was done on nutrient agar plate/PDA plate. Increased concentration of remaining exposed to NP bacteria/yeasts/mold colonies were calculated percent inhibition compared to the control group. According to bioluminescent bacteria acute toxicity tests ; pre-test reactivation solution was added 1 ml in the 15ºC incubation block and wait 30 minutes. While waiting reactivation ; dilution of NP solutions [direct(1/1) ; ¼ ; 1/8 ; 1/16 ; 1/32] was made in a bathtub in the A chamber and 1.5 ml of NaCl solution was added on. After 30 minute incubation period ; 0.5 ml of bacteria was added to the tube at the B and C divison. After LU values measured in t=0, addition of a bathtub in a diluted sample in each compartment and after 5^{th} , 15^{th} and 30^{th} minute LU values was measured. EC_{50} values were calculated after incubation LUMISsoft IV program.

Anaerobic toxicity assasy (ATA) was held at 35ºC in the volume of 150 ml amber bottle (Owen et al., 1979). Vanderbilt Mineral Medium, 3000 mg/l glucose-COD, sodium thiogylcollate (to maintain the anaerobic environment), $NaHCO_3$ (to keep neutral pH) was added into sterile 5-Liter flask. NP solutions (1 ; 5 ; 10; 25; 50 and 100 mg/l) was added into the amber bottle. 5 liters of the mixture were distributed into each vial 75 ml and stirred sonicator for 1 hour. 40 mg/l of the anaerobic sludge was added and the mouths of the bottles were sealed with rubber stoppers. 24 and 48 hours after incubation period was measured methane. 3 % NaOH solution was used to removed CO_2 from the methane gas (Razo-Flores et al., 1997). Compared to the control group it was calculated by decreasing the amount of methane inhibition footnote. For the Algae (*Chlorella sp.*) acute toxicity tests ; NPs 0.01-0.25-0.5-1 and 5 mg/l stock solutions prepared sonicator was stirred for 1 hour. 3 series of 50 ml solutions of the NPs were placed in sterile flasks for 24, 48 and 72 hours. Each concentration and the control group of flasks containing equal amounts of algae culture was added. After 24, 48 and 72 hours incubation time, they were centrifuged. Counting of sedimented algae cells was performed in three series. It was used for calculating the percentage of inhibition of formula [(1- N/N_0) X 100] (N: The number of algal cells exposed NPs/10 µl ; N_0: The number of algal cells in the group/10 µl) (Gong et al., 2011).

According to *Daphnia magna* acute toxicity tests ; before the dilution NPs of 100 mg/l stock solutions are stirred at 30ºC for 1 hour in the sonicator. 70 ml mineral medium and 30 ml NPs solution was added into the 100 ml baeker for the test. 10 *Daphnia*

magna were added and then recorded live and mortality of *Daphnia magna* during 24 and 48 hours to calculate the acute toxicity (OECD 202, 2004). According to fish (lepistes – *Poecllia reticula*) acute toxicity test, acclimated fish was used this toxicity study. Synthetic dilution water as the volume of distilled water and 25 ml of each stock solution is made up to 2 liters and 5 fish were added. The pH of the water 7 ± 0.2, dissolved oxygen 4-6 mg/l will be adjusted. After 24 and 48 hour incubation fish deaths recorded (OECD 203, 1992). Bio-degradability test was conducted, where a 2-liter glass beaker. 10 and 100 mg/l initial COD was adjust using glucose (For 10 mg/l-COD: 0,1 g ; for 100 mg/l-COD: 0,5 g) and 10 and 100 mg/l NPs solutions were added (Before added ; NPs was stirred with sonicator). The pH of the water 7 ± 0.2, dissolved oxygen 4-6 mg/l (1N NaOH and 1 N HCl) will be adjusted. 30 mg/l-anaerobic sludge was added into the 2 liter baeker and test was started. During 28 days decreasing COD values were noted (OECD 301, 1992). Bioaccumulation test is performed in two stages. The first stage of contaminant uptake by fish are monitored in a 28-day incubation period. Aquarium 10 liter fill with water dilution in the pH 6.8 to 8.5, dissolved oxygen value of 2-3 mg/l so that ventilation is performed between. Nanoparticles concentration of 10-100 mg/l is set to be 2 different aquariums. 30 mg/l activated sludge and 10 fish was added aquariums. During this time, COD concentrations measured 0-5-10-15th days (OECD 305, 2012).

The toxicity of NPs to organisms with increasing doses were investigated in vivo inhibition aganist time and statistical analysis whether the dose-dependent. Using the ANOVA program (JMP10) inhibition of time and the relationship between variables were evaluated by multiple regression analysis. r^2 and p (<0.05) were used to describe the statistical significance between dependent and independent variables.

III. Results and Discussion

3.1 Acute Toxicity Studies with nano-CeO_2

Acute toxicity studies were performed six different trophic levels [bacteria (*Escherichia coli* ; *Bacillus cereus* ; *Vibrio fischeri* ; Metan Archae Bakterisi) , yeast - (*Candida albicans)* ; mold - *Aspergillus niger* , algae (*Chlorella sp.*) , Crustacea (*Daphnia magna*) and fish - lepistes - *Poecillia reticula*]. Table 1 shows that EC_{50} values of nano-CeO_2 different trophic levels. Dose that kills 50% of the *E. coli* bacteria for 24 and 48 hours EC_{50} value of 42.2 and 28.9 mg/l was calculated from the graphic [(R=0.996) (P=0.0004 < 0.05)]. EC_{50} values of nano-CeO_2 are 18.55 and 15.1 mg/l after 24 and 48 hours for *B.cereus* [(R=0.999)(P=0.0001 < 0.05)]. For biolumiscent bacteria - *V. fischeri* ; EC_{50} values of nano-CeO_2 after 30 minutes 39.973 mg/l [(R=0.972) (P=0.0408 < 0.05)]. Another bacteria is Methane Archae bacteria group and EC_{50} values are 58.5 and 43.2 mg/l for 24 and 48 hours [(R=0.994) (P=0.0001 < 0.05)]. EC_{50} values of nano-CeO_2 are 91.4 – 87.6 mg/l and 43.8 – 31.2 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (For *C.albicans* : [(R=0.986) (P=0.0040 < 0.05)] ; for *A.niger* : [(R=0.994) (P=0.0010 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 12.07 ; 8.1 and 7.35 mg/l) [(R=0.976) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 70.9 and 20.8 mg/l [(R=0.951) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-CeO_2 because of the lowest EC_{50} value (72 hours results EC_{50} = 7.35 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-CeO_2 because of the highest EC_{50} value (24 hours results EC_{50} = 505.1 mg/l) (P=0.0001 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-CeO_2 concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 25,1% and 18,57 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 0.33 and 38.13 for 10 and 100 mg/l nano-CeO_2. According to OECD Guideline, if BCF is less than 10 fish samples did not accumulate in the body containing NPs, if the NPs in the body of the fish BCF values greater than 10 indicate that accumulate. According to OECD Guideline, 10 mg/l nano-CeO_2 concentration is not bioaccumulative, but 100 mg/l nano-CeO_2 concentration is bioaccumulative.

Tablo 1 EC_{50} values of nano-CeO_2 different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	42.2	28.9	-
Bacteria - *B.cereus*	-	18.5	15.1	-
Bacteria - *V.fischeri*	39.973	-	-	-
Bacteria – Methane Archae	-	58.5	43.2	-
Yeast – *C.albicans*	-	91.4 **(EC_{40})**	87.6	-
Mold – *A.niger*	-	43.8	31.2	-
Algae – *Chlorella sp.*	-	12.07	8.1	7.35
Crustacea – *D.magna*	-	70.9	20.8	-
Fish – lepistes - *Poecillia reticula*	-	505.1	334.7	-

3.2 Acute Toxicity Studies with nano-ZnO

Table 2 shows that EC_{50} values of nano-ZnO different trophic levels. EC_{50} value (dose that kills 50% of the *E. coli* bacteria) of 15.6 and 12.9 mg/l was calculated from the graph for 24 and 48 hours [(R=0.990) (P=0.0022 < 0.05)]. After 24 and 48 hours EC_{50} values of nano-ZnO are 31.8 and 22.8 mg/l for *B.cereus* [(R=0.986) (P=0.0041 < 0.05)]. Another organism is *V. fischeri* and EC_{50} values of nano-ZnO after 30 minutes 16.097 mg/l [(R=0.985) (P=0.0215 < 0.05)]. EC_{50} values of Methane Archae are 50.01 and 34.99 mg/l for 24 and 48 hours [(R=0.995) (P=0.0001 < 0.05)]. EC_{50} values of nano-ZnO are 60.2 – 27.5 mg/l and 25.9 – 13.9 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (*C.albicans* : [(R=0.996) (P=0.0004 < 0.05)] ; *A.niger* : [(R=0.971) (P=0.0118 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 3.03 ; 1.6 and 0.93 mg/l) [(R=0.97) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 41.4 and 21.8 mg/l [(R=0.942) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-ZnO because of the lowest EC_{50} value (72 hours results EC_{50} = 0.93 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-ZnO because of the highest EC_{50} value (24 hours results EC_{50} = 352.2 mg/l) (P=0.0001 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-ZnO concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 16.6 % and 6.43 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 0.66 and 3.26 for 10 and 100 mg/l nano-ZnO. According to OECD Guideline, 10 and 100 mg/l nano-ZnO concentrations are not bioaccumulative.

Tablo 2 EC_{50} values of nano-ZnO different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	15.6	12.9	-
Bacteria - *B.cereus*	-	31.8	22.8	-
Bacteria - *V.fischeri*	16.097	-	-	-
Bacteria – Methane Archae	-	50.01 **(EC_{70})**	34.99 **(EC_{70})**	-
Yeast – *C.albicans*	-	60.2	27.5	-
Mold – *A.niger*	-	25.9	13.9	-
Algae – *Chlorella sp.*	-	3.03 **(EC_{80})**	1.6 **(EC_{85})**	0.93 **(EC_{80})**
Crustacea – *D.magna*	-	41.4	21.8	-
Fish – lepistes - *Poecillia reticula*	-	352.2	324.3	-

3.3 Acute Toxicity Studies with nano-Al_2O_3

Table 3 shows that EC_{50} values of nano-Al_2O_3 different trophic levels. EC_{50} values was calculated from the % inhibition graphic and calculated dose that kills 50%. Firstly, gram negative bacteria - *E. coli* tested and calculated EC_{50} values are 39.9 and 26.9 mg/l for 24 and 48 hours [(R=0.994) (P=0.0011 < 0.05)]. Other bacteria is gram positive bacteria – *B.cereus* and EC_{50} values of nano-Al_2O_3 are 40.9 and 15.22 mg/l [(R=0.956) (P=0.0221 < 0.05)]. Another organism is *V. fischeri* and EC_{50} values of nano-Al_2O_3 after 30 minutes 29.282 mg/l [(R=0.998) (P=0.0020 < 0.05)]. EC_{50} values of Methane Archae are 96,83 and 43.2 mg/l for 24 and 48 hours [(R=0.967) (P=0.0014 < 0.05)]. Other organisms are fungus [yeast and mold] and EC_{50} values are 93.1 – 72.7 mg/l and 59.2 – 26.9 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (*C.albicans* : [(R=0.994) (P=0.0010 < 0.05)] ; *A.niger* : [(R=0.978) (P=0.0077 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 4.07 ; 3.5 and 2.9 mg/l) [(R=0.943) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 36.13 and 23.37 mg/l [(R=0.969) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-Al_2O_3 because of the lowest EC_{50} value (72 hours results EC_{50} = 2.9 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-Al_2O_3 because of the highest EC_{50} value (24 hours results EC_{50} = 849.9 mg/l) (P=0.0001 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-Al_2O_3concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 43.5 % and 24.9 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 3.9 and 7.29 for 10 and 100 mg/l nano-Al_2O_3. According to OECD Guideline, 10 and 100 mg/l nano-Al_2O_3 concentrations are not bioaccumulative.

Tablo 3 EC_{50} values of nano-Al_2O_3 different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	39.9	26.9	-
Bacteria - *B.cereus*	-	40.9	15.22	-
Bacteria - *V.fischeri*	29.282	-	-	-
Bacteria – Methane Archae	-	96.83	43.2	-
Yeast – *C.albicans*	-	93.1	72.7	-
Mold – *A.niger*	-	59.2	26.9	-
Algae – *Chlorella sp.*	-	4.07 **(EC_{30})**	3.5 **(EC_{40})**	2.9
Crustacea – *D.magna*	-	36.13	23.37	-
Fish – lepistes - *Poecillia reticula*	-	849.9	771.3	-

3.4 Acute Toxicity Studies with nano-HfO_2

Table 4 shows that EC_{50} values of nano-HfO_2 different trophic levels. EC_{50} value (dose that kills 50% of the *E. coli* bacteria) of 59.4 and 30.9 mg/l was calculated from the graph for 24 and 48 hours [(R=0.9993) (P=0.0014 < 0.05)]. After 24 and 48 hours EC_{50} values of nano-HfO_2 are 29.6 and 16.7 mg/l for *B.cereus* [(R=0.943) (P=0.0325 < 0.05)]. Another organism is *V. fischeri* and EC_{50} values of nano-HfO_2 after 30 minutes 46.35 mg/l [(R=0.991) (P=0.0121 < 0.05)]. EC_{50} values of Methane Archae are 34.97 and 13.55 mg/l for 24 and 48 hours [(R=0.981) (P=0.0004 < 0.05)]. EC_{50} values of nano-HfO_2 are 41.1 – 19.7 mg/l and 27.8 – 18.4 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (*C.albicans* : [(R=0.982) (P=0.0059 < 0.05)] ; *A.niger* : [(R=0.992) (P=0.0015 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 4.57 ; 3.8 and 2.94 mg/l) [(R=0.991) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 63.49 and 50.18 mg/l [(R=0.960) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-HfO_2 because of the lowest EC_{50} value (72 hours results EC_{50} = 2.94 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-HfO_2 because of the highest EC_{50} value (24 hours results EC_{50} = 849.8 mg/l) (P=0.0178 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-HfO_2 concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 54.4 % and 19.01 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different

concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 0.941 and 38.1 for 10 and 100 mg/l nano-HfO_2. According to OECD Guideline, 10 mg/l nano-HfO_2 concentration is not bioaccumulative, but 100 mg/l nano-HfO_2 concentration is bioaccumulative.

Tablo 4 EC_{50} values of nano-HfO_2 different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	59.4	30.9	-
Bacteria - *B.cereus*	-	29.6	16.7	-
Bacteria - *V.fischeri*	46.35	-	-	-
Bacteria – Methane Archae	-	34.97	13.55	-
Yeast – *C.albicans*	-	41.1	19.7	-
Mold – *A.niger*	-	27.8	18.4	-
Algae – *Chlorella sp.*	-	4.57	3.8	2.94
Crustacea – *D.magna*	-	63.49	50.18	-
Fish – lepistes - *Poecillia reticula*	-	849.8	790.3	-

3.5 Acute Toxicity Studies with nano-Ta_2O_5

Table 5 shows that EC_{50} values of nano-Ta_2O_5 different trophic levels. EC_{50} values was calculated from the % inhibition graphic and calculated dose that kills 50%. Firstly, gram negative bacteria - *E. coli* tested and calculated EC_{50} values are 26.1 and 18.6 mg/l for 24 and 48 hours [(R=0.960) (P=0.0192 < 0.05)]. Other bacteria is gram positive bacteria – *B.cereus* and EC_{50} values of nano-Ta_2O_5 are 26.9 and 14.8 mg/l [(R=0.932) (P=0.0425 < 0.05)]. Another organism is *V. fischeri* and EC_{50} values of nano-Ta_2O_5 after 30 minutes 31.457 mg/l [(R=0.9948) (P=0.0060 < 0.05)]. EC_{50} values of Methane Archae are 29.6 (EC_{60}) and 93.1 (EC_{70}) mg/l for 24 and 48 hours [(R=0.918) (P=0.0133 < 0.05)]. Other organisms are fungus [yeast and mold] and EC_{50} values are 71.5 – 53.2 mg/l and 29.3 – 21.5 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (*C.albicans* : [(R=0.976) (P=0.0088 < 0.05)] ; *A.niger* : [(R=0.989) (P=0.0028 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 3.9 ; 3.2 and 2.4 mg/l) [(R=0.983) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 36.13 and 23.37 mg/l [(R=0.977) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-Ta_2O_5 because of the lowest EC_{50} value (72 hours results EC_{50} = 2.4 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-Ta_2O_5 because of the highest EC_{50} value (24 hours results EC_{50} = 849.9 mg/l) (P=0.0030 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-Ta_2O_5 concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 78 % and 31.5 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 7.17 and 17.9 for 10 and 100 mg/l nano-Ta_2O_5. According to OECD Guideline, 10 mg/l nano-Ta_2O_5 concentration is not bioaccumulative, but 100 mg/l nano-Ta_2O_5 concentration is bioaccumulative.

Tablo 5 EC_{50} values of nano-Ta_2O_5 different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	26.1	18.6	-
Bacteria - *B.cereus*	-	26.9	14.8	-
Bacteria - *V.fischeri*	31.457	-	-	-
Bacteria – Methane Archae	-	29.6 **(EC_{60})**	93.1**(EC_{70})**	-
Yeast – *C.albicans*	-	71.5	53.2	-
Mold – *A.niger*	-	29.3	21.5	-
Algae – *Chlorella sp.*	-	3.9	3.2	2.4

Crustacea – *D.magna*	-	29.7	36.7**(EC_{70})**	-
Fish – lepistes - *Poecillia reticula*	-	790.3**(EC_{30})**	505.8**(EC_{30})**	-

4.6 Acute Toxicity Studies with nano-SiO_2

All acute toxicity studies were performed six different trophic levels [bacteria (*Escherichia coli* ; *Bacillus cereus* ; *Vibrio fischeri* ; Metan Archae Bakterisi) , yeast - (*Candida albicans)* ; mold - *Aspergillus niger* , algae (*Chlorella sp.*) , Crustacea (*Daphnia magna*) and fish - lepistes - *Poecillia reticula*]. Table 6 shows that EC_{50} values of nano-SiO_2 different trophic levels. Dose that kills 50% of the *E. coli* bacteria for 24 and 48 hours EC_{50} value of 27.2 and 16.7 mg/l was calculated from the graph [(R=0.944) (P=0.0313 < 0.05)]. EC_{50} values of nano-SiO_2 are 39.9 and 20.9 mg/l after 24 and 48 hours for *B.cereus* [(R=0.991) (P=0.0018 < 0.05)]. Another organism is *V. fischeri* and EC_{50} values of nano-SiO_2 after 30 minutes 60.4 mg/l [(R=0.999) (P=0.0002 < 0.05)]. According to ATA (Anaeroic Toxicity Assay) EC_{50} values are 84.6 and 48.11 mg/l for 24 and 48 hours [(R=0.920) (P=0.0127 < 0.05)]. EC_{50} values of nano-SiO_2 are 92.7 – 67.2 mg/l and 18.03 – 14.6 mg/l after 24 and 48 hours for *C. albicans* and *A. niger*, respectively (*C.albicans* : [(R=0.991) (P=0.0018 < 0.05)] ; *A.niger* : [(R=0.993) (P=0.0013 < 0.05)]. The exposure time has increased with the increased growth inhibition (Algae – *Chlorella sp.* ; 24, 48 and 72 hours results EC_{50} = 2.5 ; 1.9 and 1.4 mg/l) [(R=0.976) (P=0.0001 < 0.05)]. EC_{50} values for *Daphnia magna* after 24 and 48 hours are 70.9 and 20.8 mg/l [(R=0.997) (P=0.0001 < 0.05)]. Algae - *Chlorella sp.* is the most sensitive organisms to nano-SiO_2 because of the lowest EC_{50} value (72 hours results EC_{50} = 1.4 mg/l) (P=0.0001 < 0.05). Fish – lepistes – *Poecillia reticula* is the most resistant organisms to nano-SiO_2 because of the highest EC_{50} value (24 hours results EC_{50} = 716.4 mg/l) (P=0.0008 < 0.05). According to biodegrabilitity test : 10 and 100 mg/l of nano-SiO_2 concentration of 28 daily % biodegradability values are at the end of the incubation time ; % decomposition percantages is 79 % and 34.34 % for 10 and 100 mg/l, respectively. Other toxicity test is bioaccumulation and this test aim different concentrations of the NPs was conducted to determine the release and accumulation in living organisms. BCF (Bioaccumulation Factor) values are 18.01 and 32.05 for 10 and 100 mg/l nano-SiO_2. According to OECD Guideline, 10 and 100 mg/l nano-SiO_2 concentrations are bioaccumulative.

Tablo 6 EC_{50} values of nano-SiO_2 different trophic levels

	EC_{50} Values (mg/l)			
	30 minutes	**24 hours**	**48 hours**	**72 hours**
Bacteria - *E.coli*	-	27.2	16.7	-
Bacteria - *B.cereus*	-	39.9	20.9	-
Bacteria - *V.fischeri*	60.4	-	-	-
Bacteria – Methane Archae	-	84.6 **(EC_{80})**	48.11**(EC_{80})**	-
Yeast – *C.albicans*	-	92.7	67.2	-
Mold – *A.niger*	-	18.03	14.6	-
Algae – *Chlorella sp.*	-	2.5**(EC_{60})**	1.9**(EC_{60})**	1.04**(EC_{60})**
Crustacea – *D.magna*	-	36.7	40.76**(EC_{80})**	-
Fish – lepistes - *Poecillia reticula*	-	716.4	433.1	-

IV. Conclusion

Six different NMO (nano- Ta_2O_5, nano-HfO_2, nano-CeO_2, nano-ZnO, nano-SiO_2, nano-Al_2O_3) using six different trophic level with effects were studied (bacteria - *Escherichia coli* (gram negative bacteria), bacteria - *Bacillus cereus* (gram-positive bacteria), bacteria - *Vibrio fischeri* (bioluminescence bacteria), methane Archae bacteria , yeast - *Candida albicans* , mold - *Aspergillus niger* ; algae - *Chlorella sp.* ; Crustacean - *Daphnia magna* ; fish - *Poecillia reticula*). The acute toxicity test results is made to all of the sensitivity of the used organisms and resistant nanoparticles were studied in terms of % inhibition and EC_{50} values. Nano-ZnO nanoparticles, studied was determined that the most toxic among other nanoparticles. The subsequent toxic nanoparticles are respectively the nano-CeO_2, nano-SiO_2, nano-Ta_2O_5, nano-Al_2O_3 and nano-HfO_2. We detected that for nano-ZnO acute toxicity test the most sensitive organism is algae – *Chlorella sp.* (72 hour EC_{80}=0,93 mg/l) and the most resistant organism is lepistes - *Poecillia reticula* (48 hour EC_{50}=324,3 mg/l). After *Chlorella sp.* the most

sensitive organisms are *E.coli* , Metan Archae and after lepistes – *Poecillia reticula* the most resistant organisms are yeast - *C.albicans* and mold - *A.niger*. Six different trophic levels of the toxic effects of nanoparticles that are at least nano-HfO_2. The result of lepistes - *Poecillia reticula* for the EC_{50} value of 849.8 mg/l is as determined ; this EC_{50} value is the highest value found among other nanoparticles. After lepistes – *Poecillia reticula* ; the most resistant organisms are *Daphnia magna* (24 hours EC_{50} =63,49 mg/l) ; *E.coli* ; *C.albicans* ; methane Archae ; *B.cereus* and *A.niger*.

In general, when we look at the tolerance of *Chlorella sp.* – algae to NPs nano-CeO_2 nanoparticles of sensitivity among other algal cells are exposed to the nanoparticles, is the lowest (24 hours EC_{50}=12,07 mg/l). Among the all organisms after lepistes – *Poecillia reticula* the most resistant organisms are *Daphnia magna* ; *Candida albicans* ; and methane Archae which detected highest EC_{50} values (*D.magna* : for nano-CeO_2 24 hours EC_{50}=70,9 mg/l ; *C.albicans* : for nano-Al_2O_3 24 hours EC_{50}=93,1 mg/l ; for Metan Archae bacteria : nano-Al_2O_3 24 hours EC_{50}=96,83 mg/l). Overall, the most toxic NP is nano-ZnO and the minimum toxic NP is HfO_2. Among the acute toxicity test organisms the most sensitive organism is *Chlorella sp.* – algae and the most resistant organism is lepistes – *Poecillia reticula*.

Today NPs are used in the treatment of industrial wastewater. This NPs effect six different trophic levels and all of them show different toxic effects. Although using NPs in water treatment industries that is recovered for reuse, is very economical ; toxic effects that occur with the discharge of ecosystems. We studied nine different species and observed acute and high toxicity for some NPs. Most toxic effects showing the nano-ZnO is discharged to the receiving environment should be very careful because even at low concentrations nano-ZnO is high toxic to *Chlorella sp.* (EC_{80}=0,93 mg/l). In trials with nano-ZnO 0.93 mg/l to 850 mg/l for changing EC_{50} values ; when working with the nanoparticles should be careful to this concentration range. Other most toxic of the nano-CeO_2 nanoparticles and nano-SiO_2, respectively, in the studies ; 7 mg/l to 500 mg/l and 1.04 mg/l to 720 mg/l for changing EC_{50} values; should be careful when working with nanoparticles in this concentartion range.

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