

## **Ecotoxicity of, and remediation with, engineered inorganic nanoparticles in the environment.**

Antoni Sánchez<sup>a</sup>, Sonia Recillas<sup>a</sup>, Xavier Font<sup>a</sup>, Eudald Casals<sup>b</sup>, Edgar Gonzalez<sup>b</sup> and Víctor Puentes<sup>b,c,\*</sup>

<sup>a</sup> Department of Chemical Engineering, Escola Tècnica Superior d'Enginyeria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>b</sup> Institut Català de Nanotecnologia, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>c</sup> Insitut Català de Recerca i Estudis Avançats, Passeig Lluís Companys, 23, 08010 Barcelona, Spain

\* Corresponding author: Víctor Puentes

Phone: 34-935868013

Fax: 34-935868020

E-mail address: victor.puentes@uab.es

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**Abstract**

This study presents the recent developments on the use of inorganic nanoparticles for environmental remediation in soil, water and gas polluted streams. The number of publications on these topics has grown exponentially in recent years, especially those focused on the wastewater treatment. Among them, the removal of heavy metals has become the most popular, although some works are related to the use of nanomaterials for the elimination of nutrients such as nitrogen and some persistent organic pollutants. However, this growth has not been accompanied by the knowledge about the behavior of nanoparticles once used and released to the environment. The current situation of nanoparticles toxicology (nanotoxicology) is also commented in this study. It is remarkable the high number of different toxicology tests that have been applied to nanoparticles, which often makes the results very difficult to interpret or generalize. Bioluminescence test, *Daphnia magna* and other published tests are analyzed in detail, jointly with some preliminary results obtained in our works.

**Keywords:** Nanoparticles, Toxicity, Environmental applications, Nanowaste, Nanotoxicology.

## 1. Introduction

As a result of advances in methods of producing nanoparticles with controlled composition and properties, as well as the incipient development of protocols for large-scale synthesis, in recent years the use and production of engineered inorganic nanoparticles has increased exponentially. These nanostructures are the base for manufacturing new materials that project and materialize a variety of applications: health sector and life sciences 18%, chemicals 12%, energy, communication and information technologies, transportation, and environmental applications around 8-9 % each one and 1-6 % for construction, household products, defense and security, aerospace industry, personal care, food industry and textiles [1]. Some of them have been inserted as raw matter in the productive sector and support the research tasks. The National Science Foundation estimates \$1 trillion impact on the global economy and employ 2 million workers in the field of nanotechnology by 2015 [2]. In this context it is necessary to assess the environmental risk factors of exposure to engineered nanoparticles. Apart from the exposure through intended use, unwanted dispersion (spill) or (nano)waste management have also to be considered as critical pathways to introduce nanoparticles in the environment.

Nanoparticles are sensitive to the nature and evolution of the entities that conform the interaction environment. The response of the nanoparticle environment can be extremely complex and diverse, depending on a variety of parameters involved, which makes difficult to know precisely their environmental fate. They may be either aggregated into microscopic particles or embedded in the exposed materials; they may corrode and dissolve or suffer morphological modifications. The surface of the nanoparticles experiments constant modifications and it is indeed through its surface that it interacts with its environment. Recently, a model to predict nanoparticle toxicity

was proposed where the available electronic energy levels in the nanoparticle structure are matched up with the oxidation potentials of reactions that would either remove antioxidants from cells or generate reactive oxygen species (ROS) like hydrogen peroxide or superoxide ions ( $O_2^{\cdot-}$ ) [3]. Where these two values overlap, it means that the nanoparticles can accept electrons in these processes and cause oxidative stress in cells - either by eliminating antioxidants or producing ROS. Thus, in biological environments, the toxic effects of nanoparticles will depend not only on the initial morphological properties, composition, size, additives and synthesis method employed, but also on the physico-chemical evolution in the surroundings [4-6]. Auffan et al. [7] pointed out that the chemical stability at physiological redox conditions appears to be a conditions for non-toxicity in metallic nanoparticles. Nevertheless, metallic nanoparticles with strong oxidant or reductive properties can be cytotoxic and genotoxic [7]. Therefore, it is necessary to evaluate risk factors considering the type of morphology, size, method of producing nanoparticles and the type of target and conditions of interaction [8,9].

In addition, nanoparticles have found applications directly in environmental remediation [10-12] which is reflected in the increasing number of publication and amount of funding for remediation projects. For instance, nearly one billion dollars have been allocated to the U.S. EPA in 2009 to this purpose [13]. The use of nanoparticles for environmental remediation could help to reduce this cost but the obtained benefit has to be balanced with their potential risks.

The objectives of this study are, on one hand, to present the most recent developments on the use of nanoparticles for environmental remediation and, on the other hand, to present the first attempts to assess nanoparticles toxicology and the tests available in the scientific literature.

## **2. Inorganic nanoparticles: environmental remediation applications**

While industrial sectors involving semiconductors, memory and storage technologies, display, optical and photonic technologies, energy, biomedical and health sectors produce the most nanomaterial-containing products, nanotechnology is also used as an environmental technology to protect the environment through pollution prevention, treatment and cleanup. Nanoremediation has the potential not only to reduce the overall costs of cleaning up large scale contaminated sites, but it also can reduce cleanup time, eliminate the need for treatment and disposal of contaminated soil, reduce some contaminant concentrations to near zero - all in situ [13].

In general, nanoparticles present some natural skills to be applied to environmental remediation. Due to its reduced size, the surface grows exponentially for the same gravimetric concentration as the diameter shrinks. Due also to its small size, their mobility in solution is high and the whole volume can be quickly scanned with small amounts of nanoparticles. For example, in a rough estimation, a 10 nm gold nanoparticle in water at room temperature will experience Brownian relaxation on the order of the nanosecond, and each Brownian step in solution will move it about 10 to 20 nm. Therefore, a typical nanoparticle concentration of few nanomolar will explore the total volume in the order of the centiseconds (assuming a 10% efficiency, i.e., the nanoparticle visits a new position before repeating 10 times one previous). In addition, due to its reduced size and their high curvature radii, the surface is specially reactive (mainly due to high density of low coordinated atoms at the surface, edges and vortex) [14].

These unique properties can be employed to degrade and scavenge pollutants (Figure 1). The species absorbed onto the nanoparticle can be removed by applying mild (and

affordable) gravitational (centrifugation) or magnetic (in the case of magnetic nanoparticles) gradients (Figure 2). The ability of the inorganic nanoparticles trapping pollutants is well known for cations like As, Cr, Ni, Cd, etc [15]. In the case of organic molecules, the nanoparticles can be functionalized to specifically trap determined molecules. Finally, the use of the nanoparticles as photocatalyst to promote the complete degradation of the organic matter is also being explored.

### *2.1. Heavy metals removal in water streams*

Heavy metals (or simply metals) removal with nanoparticles is the field where today literature is most abundant. Typically, the papers present an engineered nanoparticle to remove a specific metal, which is present in low amounts in drinking water. The paradigmatic case is the use of magnetic iron oxide nanoparticles to remove As ions from drinking water. The As ions spontaneously adsorb onto the iron oxide nanoparticles and the complex is removed with weak magnetic fields [15]. The drawbacks in these papers are typically the use of synthetic water and the lack of knowledge about the detailed mechanisms of interaction between nanoparticles and metals. When using synthetic water, it is clear that the results may not be translated into the real field, and some problems such as scale-up, cost evaluation and down-stream and recuperation of nanoparticles are often omitted. In the case of nanoparticles and metal interactions, the scientific knowledge is most developed, and typically adsorption is presented as the main mechanism. For instance, Langmuir and Freundlich isotherms have been proposed for arsenic removal [16] and in some cases organic modifications of the nanoparticles have been synthesized to enhance the metal removal efficiency [17].

Table 1 summarizes some of the main results recently published in this field. Although interesting, it can be observed that some of them lack of the specific characteristics or proposed model mechanisms and most of them are conducted with synthetic water.

## *2.2. Nutrients removal in water streams*

Phosphorous and nitrogen in its different chemical forms are normally appointed under the term nutrients. There are many factors that influence nutrient behavior in the environment and their interrelationships may be complex. Nitrogen and phosphorus are the main plant nutrients that warrant concern with respect to surface water systems. The nanoparticles may either absorb them or transform their oxidation state into friendly species. Some works have been published on the elimination of nitrogen with nanoparticles, although literature is scarce on this topic. For instance, Choe et al. [18] reported the reductive denitrification by nanoscale zero-valent iron. Apart from this simple method of reduction of nitrate with iron, recent studies have been focused on more complex mechanisms for nitrate removal such as the photocatalytic reduction reaction with pure TiO<sub>2</sub> nanoparticles [19] or doped with Bi<sup>3+</sup> [20]. The use of iron is appealing, however, the pyrophoricity of the iron nanoparticles makes very difficult to preserve them in the zero valent state before their real application in the environment.

To our acknowledgment, only Martin et al. [21] has reported the use of nanoparticles to remove and recover phosphate, in this case, from municipal waste waters using ferric oxide nanoparticles. In the same way, our research group has promising results with cerium oxide nanoparticles and phosphate in synthetic water. These results regarding phosphate elimination are very encouraging since, nowadays, phosphate elimination must be performed chemically or through a complex biological process.

### *2.3. Organic pollutants removal in water streams*

Although still in an incipient moment, the research on the removal of organic compounds with several types of nanoparticles has been initiated in several studies (table 2). Special attention is focused on Persistent Organic Pollutants such as DDT. Typically, the mechanisms are not well understood but the interest and the difficulty of eliminating traces of these compounds are so high that the first publications deserve attention. For instance, Ghauch et al. [22] have reported interesting results on the antibiotic elimination from water using iron nanoparticles and Wang et al. [23] have presented bismuth-compounded TiO<sub>2</sub> nanoparticles for photocatalytic degradation of rhodamine B, a commonly used dye, among others. In general, results obtained in the degradation of organic pollutants with nanoparticles are very promising and represent a significant step forward in the removal of Persistent Organic Pollutants. Table 2 summarizes some of the main results recently published in this field. As it can be observed photocatalysis and/or the use of other reactants (H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>, etc.) are the common mechanisms used to degrade the pollutants. However, it is remarkable the combination of a biological process with nanoparticles to degrade trichloroethylene [24], which represents a new view for the biological treatment of wastewaters.

### *2.4. Other polluted media: soil and gases*

#### *2.4.1. Nanoparticles for soil remediation*

This is a topic with less research when compared to water or wastewater pollutants removal. In this situation, the advantages of the Brownian dispersion are lost. Typically, the physical phenomena involved in the interactions soil-nanoparticle are not understood, which makes the results obtained in this field as only preliminary.



Moreover, nanoparticles are not in soil but they are spiked on it, which is far from the real case, as the introduction of nanoparticles in soil is one of the most difficult aspects to consider in *in situ* soil bioremediation. For *ex situ* remediation, soil columns are typically set up, and a liquid suspension of nanoparticles is added to extract or immobilize the contaminant (typically heavy metals or persistent organic compounds). Accordingly, some interesting papers have been published. Xu and Zhao [25] reported the reductive immobilization of chromate in water and soil using stabilized iron nanoparticles using laboratory batch and column experiments, whereas other studies have reported the immobilization of some organic pollutants in soils and sediments [26,27].

#### 2.4.2. Gas treatment with nanoparticles

This topic can be considered the most emerging field in the use of nanoparticles. Although the investigation of nanomaterials specifically designed to clean polluted gases is just started and the results must often be seen as preliminary, some studies have been recently published on gas treatment using nanoparticles, apart from the large number of published studies on nanomaterials used as a sensors for gas detection and measurement [28], which conforms another research area.

For instance, toxic gases such as carbon monoxide [29], toluene [30] and gases containing chlorinated compounds [31] have been successfully removed with platinum, titanium oxide and Fe-Pd bimetallic nanoparticles, respectively. Although it is probable that new applications will appear in the following years, it seems to be a common consensus about the potential of using titanium oxide nanoparticles for the reduction of polluted gases [32] since, due to their photocatalytic properties, titanium oxide nanoparticles can remove volatile organic compounds and nitrogen oxides from the air

and turn them into less harmful molecules. Thus, some preliminary attempts to preserve buildings stainless in dense traffic areas applying TiO<sub>2</sub> nanoparticles paints have been carried out.

### **3. Standardized tests for inorganic nanoparticles toxicity**

The question here is if the potential benefits of using nanoparticles do not present additional risks. What may be key is how nanoparticles may interact with toxic ionic and molecular species capturing and destroying them without presenting any hazard to microorganisms since these have defense mechanisms that molecular species have not. Otherwise, if the cleaning product is toxic itself, the interest in nanoparticles for environmental remediation will be very limited. For example, several studies of the toxicity of uncoated, water-soluble, colloidal fullerenes (C<sub>60</sub>) have been done. In one of them, the 48-h LC<sub>50</sub> (median lethal concentration) in *Daphnia magna* was determined of about 800 ppb [33]. In other, using largemouth bass (*Micropterus salmoides*), lipid peroxidation in the brain and glutathione depletion in the gill were observed after exposure to 0.5 ppm of C<sub>60</sub> for 48 h [34]. Toxicity of some metal oxide nanoparticles has been also studied. TiO<sub>2</sub> absorbs substantial UV radiation yielding, in aqueous media, hydroxyl species which may cause substantial damage to DNA [35,36]. Other studies found that Al<sub>2</sub>O<sub>3</sub> nanoparticles could reduce root growth due to the perturbation of the microbial composition of soil [37], raising concerns since the basis of many food chains depends on the benthic and soil flora and fauna, which could be affected by such nanoparticles. On the other hand, Yang and Watts [37] observed that alumina nanoparticles do not induce any detectable effects on the seed root growth using root elongation tests on *C. sativus*. Besides, Zn and ZnO nanoparticles caused significant inhibition of seed germination and root growth [38]. In this area, Warheit et al. proposed

a base set of toxicity tests to determine TiO<sub>2</sub> risk management [39]. Since available information on nanotoxicology is still in early stages, any scientific contribution on environmental risks of nanoparticles should help to regulate the use and production of nanoengineered materials.

### 3.1. Wastewater-based tests

Although not specially standardized for nanoparticles suspensions, several tests commonly applied to wastewater or even drinking water have been used for determining the toxicity of nanoparticles. These tests are easy to carry out in the laboratory, but often the results interpretation with nanoparticles is not straightforward, since the ultimate cause of toxicity is rarely known. Some of these tests are detailed below.

#### 3.1.1. Bioluminescence test

A standardized test called Microtox® system from Microbics Corporation has been used with nanoparticles. This method is based on the percentage of decrease in the amount of light emitted by the bioluminescent marine bacterium *Vibrio fischeri* upon contact with a filtered sample at pH 7 containing a known concentration of nanoparticles. Then, toxicity is inversely proportional to the intensity of light emitted after the contact with the toxic substances [40]. The effective concentration, EC<sub>50</sub>, is defined as the concentration that produces 50% of light reduction. EC<sub>50</sub> can be measured after 5 and 15 min contact time. Bioluminescent tests must be performed under a sodium chloride concentration of 22% according to the manufacturer's instructions.

Our previous results [41] did not show toxicity using this test with ferrous oxide, silver and gold nanoparticles, but other works have presented other results. For instance, in the

case of titanium oxide nanoparticles, low levels of toxicity have been found with the bioluminescence test and  $EC_{50}$  could not be measured [42], whereas boron nanoparticles showed high levels of toxicity and a value of  $EC_{50}$  ranging from 56 to 66 mg/L, depending upon the age of the solution. Differences could be attributed to different NP preparations. For instance, it should be remembered that some ions are a remnant in every NP synthesis. A paradigmatic case are commercial samples of colloidal silver intended for water purification, where the amount of ionic silver may be as high as the 90% with respect to the total silver content. This fact is mostly neglected and may have a large effect on the results.

### 3.1.2. *Daphnia magna*

This is probably the most extended test to evaluate nanoparticles toxicity [43]. *D. magna* is a cladoceran freshwater water flea which is native to northern and western North America. Domesticated, it is widely used as a laboratory animal for testing ecotoxicity. In this test, acute 48 h toxicity assays with *D. magna* are conducted following the Organisation for Economic Co-operation and Development (OECD) protocols [44]. Groups of 10 neonates (< 24 h old) are exposed to 20 ml of test concentrations in 50 ml flasks with no food present. At the end of exposures, immobile animals are recorded and median lethal concentration levels ( $LC_{50}$ ) are estimated.

*D. magna* is often very sensitive to the presence of nanoparticles, existing a large number of publications on this topic, in which sometimes it is distinguish between death and immobilization of *D. magna* [45]. In their review, Farré et al. [43] report the main data observed when exposing *D. magna* to several types of nanoparticles. In this and other works [45], ZnO and TiO<sub>2</sub> and Fullerenes C<sub>60</sub> appear to be very toxic to *D. magna*, whereas Fe<sub>3</sub>O<sub>4</sub> nanoparticles exhibit a low effect. However, our experience in

this test it is that the results are somehow erratic and a lot of replications must be carried out to obtain a reliable value of LC<sub>50</sub>.

### 3.1.3. Other aquatic microorganisms

Although not so extensively used, other superior organisms have been used for testing nanoparticles toxicity, being the most typical *Danio rerio* (Zebra fish) [43] and rainbow trout (*Oncorhynchus mykiss*) [46]. Nevertheless, it should be mentioned that in these tests the overall toxicity can be assessed, but the objective is often to find damage on specific organs after nanoparticles exposure or the accumulation of them.

Acute toxicity tests are significant to determine the effect of a sudden nanoparticles load dumped to a waste water treatment plant or to a natural water stream. However, it is also important to investigate the effect of chronic tests and bioaccumulation of nanoparticles to establish the effect of a continuous spread of nanoparticles to the environment. Bioaccumulation assays has been done with *D. magna* finding that TiO<sub>2</sub> toxicity effects are much higher at 21 days than at 72 h and higher at 72 h than at 48 h [47] or investigating the effect of TiO<sub>2</sub> nanoparticles size on chronic toxicity [48]. Also mice and marine polychaete has been used to test bioaccumulation of gold and TiO<sub>2</sub> nanoparticles respectively [49,50] showing low rates of nanoparticle in-body accumulation.

### 3.3. Germination, earthworms and other tests

These tests have been used to evaluate different nanoparticles toxicity; in a minor extent germination [37,41] and earthworms [51]. Therefore, the amount of available data is too scarce to extract reliable conclusions about the validity of these tests except for the specific situation where they have been carried out. It is evident that practically any

microorganism in which a feasible method to determine its biological activity exists is suitable to determine its toxicity when exposed to nanoparticles. This is the case of liquid respirometry for aerobic microorganisms, in which Oxygen Uptake Rate (OUR) is compared in the presence and in absence of nanoparticles and the case of anaerobic populations, in which the specific biogas/methane production is compared. Several studies have been published on this topic. For instance, Nyberg et al. [52] and Barrena et al [41] tested the effect of several nanoparticles in the production of biogas by selected anaerobic consortia. The results showed that, at the assayed concentrations, nanoparticles were not toxic in most cases, although the study was only focused on some specific types of nanoparticles. Interestingly, an accumulation of nanoparticles in or on the microorganisms was detected by Transmission Electron Microscopy (Figure 3). Also, several reviews have been published on the toxicity of nanoparticles in aerobic environments [8]. Some of these studies alert on the fact that the *in vitro* toxicity value obtained with a selected specie cannot be applied to other microorganisms [53] because some important facts are often forgotten, for instance, nanoparticles size, the specific surface characteristics of the nanoparticles [37] and their state of agglomeration [54].

#### **4. Toxicology data**

Clearly, it would be necessary to have a database combining all microorganism and nanoparticles toxicity effect for each specie, but the amount of work necessary makes the costs of such work almost impossible to face [55]. In their interesting article, Choi et al. [55], comment that it is very important to gather information about the toxicity of nanoparticles in order to have a proper regulation, but they estimated for the United States that costs for testing existing nanoparticles ranges from \$249 million for optimistic assumptions about nanoparticle hazards. Also, the time taken to complete

testing is likely to be very high (34-53 years) if all existing nanomaterials are to be thoroughly tested. Off course, maturation of the field and understanding of the involved mechanism, and common sense, will significantly shorten the time, cost and effort to properly regulate nanomaterials.

A first attempt to present toxicological effects for inorganic nanoparticles is shown in Table 3 (acute toxicity). This table is based in our previous published [41] and non-published data. Although incomplete, it can serve as a basis to develop new studies on nanoparticles toxicity, including new methodologies and types of nanoparticles. As observed in Table 3, some trends can be deduced, as the high toxicity of cerium oxide nanoparticles, the innocuous character of gold nanoparticles and the surprisingly low toxicity of silver nanoparticles, although other authors have found different results with nitrifying bacteria [56].

Finally, we want to remark that it is important to report data on the characterization of the nanoparticles used in toxicity tests. Size, synthesis methodology, specific surface or solvent and formulation used are factors that definitively affects the toxicity of a nanoparticle [41,56].

## **5. Nanoparticles as a waste**

Another important issue to consider when dealing with nanoparticles is what to do when they are to be treated or disposed as a waste. At present, it is too early to have abundant literature on this topic, but some reviews have been also published. For instance, Bystrzejewska-Piotrowska et al. [57] point out that it for any approach proposed to the treatment of nanowaste requires understanding of all its properties (chemical, but also physical and biological). Among the techniques to recycle used nanoparticles, it is clear that noble nanoparticles will be the object of recovery because of their high price. In the

case of other nanoparticles, the bioaccumulation using some species of plants and fungi can be a way to remove nanoparticles for water, air and soil, although the mechanisms of these processes are not currently known. Finally, they propose not to underestimate the effect in the long run for the handling of waste containing nanomaterials. In our lab, we have chosen to destroy the nanoparticles, either by irreversible aggregation or dissolution, and then to process the resulting material conventionally.

More recently, another study has been focused on the presence of engineered nanoparticles in wastewater and wastewater sludge, and their role when being landfilled or applied to soil [58]. They conclude that the number of unanswered questions regarding nanoparticle fate and impact is too big to make any prediction about it.

## **6. Conclusions**

This work presents a summary of the current situation of nanoparticles and their application to environmental remediation and toxicity effects. In conclusion, it can be stated that the high number of publications involving nanoparticles for the remediation of selected environments (especially wastewater) is not accompanied by a deep knowledge about the long and sometimes even the short time effects on environment that handling of nanoparticles can provoke. It is our opinion that more efforts in research are necessary on this topic to help stakeholders to promote scientifically-based regulations and for nanotechnology to be on close terms with society.

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- [14]  $10^{12}$  NP in one ml  
 Each NP is responsible to scan a volume which is  $1/10^{12}$  ml, which in cubic nm is:  
 (1 ml = 1 cm<sup>3</sup>, 1 cm<sup>3</sup> =  $10^{-21}$  nm<sup>3</sup>)  
 $10^9$  nm<sup>3</sup>  
 The volume of one 10 nm Au particle is: 523 nm<sup>3</sup>  
 Therefore, each particle has to give  $10^9/523$  steps to visit its whole corresponding volume, what is about  $10^6$  (1.91  $10^6$ ) steps, with a 10% efficiency makes a need of  $10^7$  steps, each taking 1 ns to happen means that every  $10^{-2}$  seconds the whole volume is explored.
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## Tables

**Table 1.** Some of the results obtained with nanoparticles to remove metals from water.

Removed metal(s)	Nanoparticle	Mechanism	Water	Reference
As (III), As(V)	Iron(III)–titanium(IV) binary	Adsorption	Synthetic	[16]
Mo(VI)	Maghemite	Adsorption	Synthetic	[59]
Cr (VI)	Chitosan-Fe <sup>0</sup>	Reduction	Synthetic	[60]
Pb (II)	Titanium phosphate	Adsorption	Synthetic	[61]
Pb (II)	Silica-alumina	Adsorption	Synthetic	[62]
Cr (VI)	Zero-valent iron	Reduction	Synthetic	[25]
Cu (II)	Fe <sub>3</sub> O <sub>4</sub> chitosan bound	Adsorption	Synthetic	[63]
U (IV), U (VI)	Zero-valent iron	Reduction	Real	[64]
Hg (II)	FeS	Adsorption	Synthetic	[27]
Cd (II)	TiO <sub>2</sub>	Adsorption /	Synthetic	[65]
Co (II)	Zero-valent iron	Adsorption	Synthetic	[66]
Pb(II), Ni(II)	Fe <sub>3</sub> O <sub>4</sub> chitosan bound	Adsorption	Synthetic	[67]
Au, Ag, Pt, Pd	Several	Adsorption	Real	[68]
Fe (III)	Fe <sub>3</sub> O <sub>4</sub> chitosan bound	Adsorption	Synthetic	[69]

**Table 2.** Some of the results obtained with nanoparticles to remove organic pollutants from water.

Organic Pollutant	Nanoparticle	Comment	Reference
Trichloroethene and chlorobenzene	Palladium/magnetite	Photocatalytic degradation	[70]
Trichloroethylene	Powder Activated Carbon and Fe <sub>2</sub> O <sub>3</sub> ,TiO <sub>2</sub> , and SiO <sub>2</sub>	Nanoparticles enhances the adsorption on Powder Activated Carbon	[71]
Rhodamine B	Fe <sub>3</sub> O <sub>4</sub>	Chemical reaction with H <sub>2</sub> O <sub>2</sub> as oxidant	[72]
Acetone, benzene, and toluene	In(OH) <sub>3</sub>	Photocatalytic degradation (UV)	[73]
Rhodamine 13	BiFeO <sub>3</sub>	Fenton reaction	[74]
Trichloroethylene	Bimetallic particles of nickel on iron, supported on functionalized carbon nanotubes	Catalytic breaking of C-Cl bond	[75]
Alachlor and phenanthrene	Copper oxide	Chemical reaction with H <sub>2</sub> O <sub>2</sub> as oxidant	[76]
Trichloroethylene	Bio-Pd	Dechlorination with bio-Pd, hydrogen gas and formic acid	[24]
Acid Black 24	Fe <sup>0</sup>	Photocatalytic degradation (UV) and H <sub>2</sub> O <sub>2</sub> as oxidant	[77]
Hexachlorocyclohexanes	Fe <sup>0</sup>	Reaction rather than sorption was the operative mechanism for the pollutant removal	[78]
Acid Black-24	TiO <sub>2</sub> and Fe <sup>0</sup>	Photocatalytic degradation (UV)	[79]
Anthracene-9-carboxylic acid	CdSe	Photocatalytic degradation (Green Monochromatic Light)	[80]
Lindane	Fe <sup>0</sup>	Catalytic degradation	[81]

**Table 3.** Acute toxicological data on inorganic nanoparticles. Concentration of nanoparticles was the maximum reached in the laboratory without agglomeration. Based on Barrena et al. [41], Garcia et al. [42] and other unpublished studies of the authors. Since nanoparticles concentration was different in each toxicity test, the file concentration range corresponds to the range of concentrations used in the different tests. Bioluminescence, *Daphnia magna*, aerobic and nitrification consortia tests results are expressed as EC50 values. The Germination tests values are expressed as Germination Index. Anaerobic toxicity test is expressed as percentage of biogas reduction.

		Nanoparticle				
		CeO <sub>2</sub>	TiO <sub>2</sub>	Fe <sub>3</sub> O <sub>4</sub>	Au	Ag
Solvent		HMT <sup>‡</sup>	TMAOH <sup>¥</sup>	TMAOH <sup>¥</sup>	Trisodium citrate	Sodium borohydrate
Concentration (µg/mL)	range	0.02-0.57	0.48-1.01	18-116	oct-62	16-100
Mean size (nm)		6.5	7.5	6	10	29
Bioluminescence test (Microtox)		0.021 mg/mL	EC50>45% of the tested concentration	EC50>45% of the tested concentration	EC50>45% of the tested concentration	EC50>45% of the tested concentration
<i>Daphnia magna</i> (Standard test)		0.012 mg/mL	0.016 mg/mL	2.3·10 <sup>-4</sup> mg/mL	No data	No data
Germination test (several seeds tested)		0%	40-60%	70-75%	100-120%	75-95%

Anaerobic consortium (biogas production)	87%	No effect	No effect	18%	No effect
Aerobic consortium (oxygen uptake rate)	0.18 mg/mL	No effect	No effect	No effect	33% inhibition at 0.13 mg Ag-NP/mL
Nitrification consortium (oxygen uptake rate)	0.21 mg/mL	No data	No effect	No effect	No effect

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<sup>¥</sup> Tetramethylammonium hydroxide

<sup>£</sup> Hexamethyl tetramine

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## Legends to Figures

### **Figure 1: Schema of different methods of cleanup using nanoparticles. 1)**

Nanoparticles that degrade contaminants *in-situ*. For instance, photocatalysis of organic matter using  $\text{TiO}_2$  nanoparticles. 2) Nanoparticles that adsorb contaminants. For instance,  $\text{CaCO}_3$  adsorb Ni,  $\text{Fe}_3\text{O}_4$  adsorb As and Cr. Adsorbed heavy metals lose their toxicity. Moreover, nanoparticles can be separated from media using magnetic or gravitatory fields. 3) Nanoparticles conjugated to molecules that adsorb contaminants. For instance, nanoparticles conjugated to cyclodextrins that adsorb persistent organic pollutants. Moreover, nanoparticles can be separated from media using magnetic or gravitatory fields.

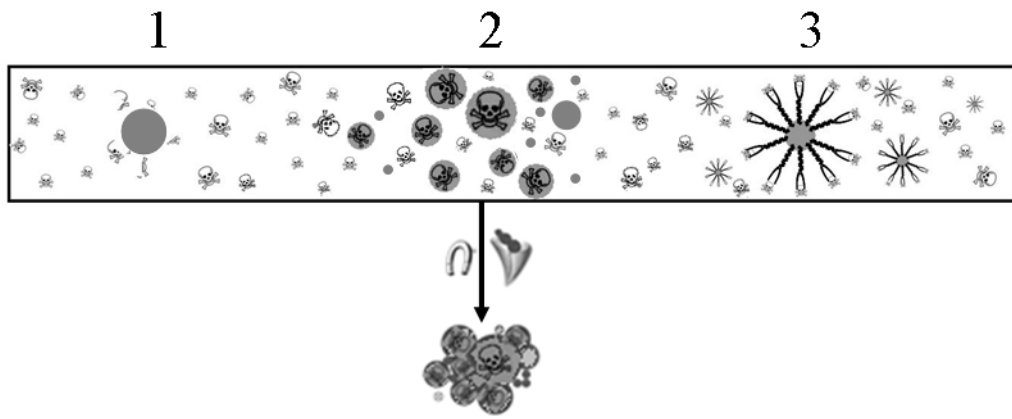
### **Figure 2: Magnetic removal of contaminated water using $\text{Fe}_3\text{O}_4$ nanoparticles.**

**Left:**  $\text{Fe}_3\text{O}_4$  nanoparticles mixed with sludge in 1:1 proportion. **Right:** nanoparticles trap pollutants and due to the magnetic character of the particles the composite is separated from liquid driven by a magnetic field. Image was taken 1 minute after the magnet was placed.

**Figure 3: Up:** TEM images of model nanoparticles produced in our lab and used for the experiments mentioned in the text. Scale bars are 20 nm **Down:** Image of anaerobic bacteria after a toxicology test with silver nanoparticles. Scale bar is 200 nm.

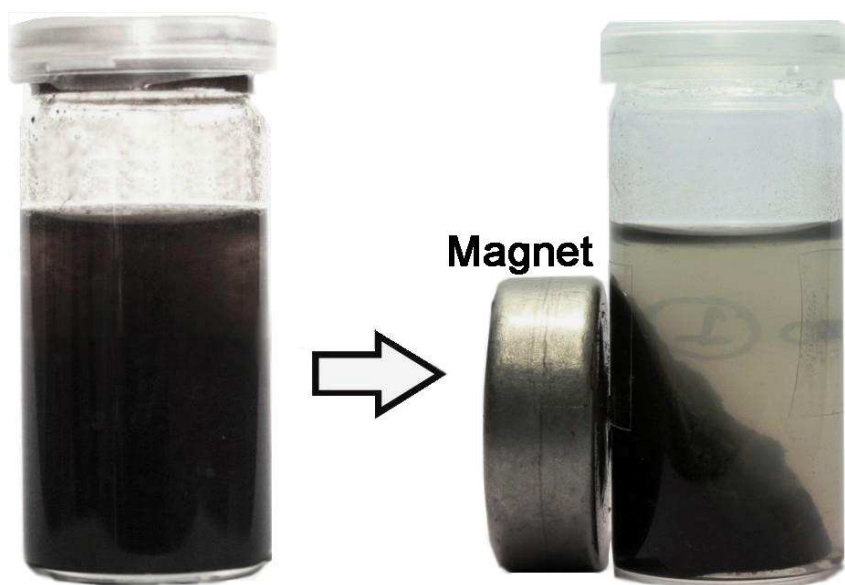


**Figure 1.** Sánchez et al.



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**Figure 2.** Sánchez et al.



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Figure 3. Sánchez et al.

