Cosmetic nanomaterials in wastewater: Titanium dioxide and Fullerenes

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Abstract:

The rapid growth in cosmetic industry across the world is mainly due to the application of nano-ingredients in cosmetics to enhance their properties and invention of new nano-molecules. And also the weaker regulations on application of nanoparticles as cosmetic ingredients and no safety assessment of cosmetics before release into market has led to uncontrolled production and usage. Unavoidable release of considerable amount of cosmetic nano-particles into wastewater introduces them into the environment via treated wastewater effluent and sludge. This review briefly gives the information about behavior of cosmetic-nanomaterials, mainly titanium dioxide (TiO_2) and fullerenes (C60) within the wastewater treatment plant and current research on their characterization and toxicity. Considering the current analytical methods for evaluating the behavior of these nanomaterials in the wastewater, there is still a need to advance these technologies. Furthermore, a better understanding and modeling of nanomaterials

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fate in wastewater treatment plants is essential for effectively predicting their impacts on the receiving environment.

Key words

Cosmetics nanomaterials, titanium dioxide, fullerenes, fate in wastewater, toxicity, characterization

Contents

Introduction

Over 10 thousand years of experience and use of cosmetics materials makes them one of the most prevalent goods in community with the annual revenue of 255 billion dollars. The annual growth of cosmetic economy was 3.2% from 2009 to 2014 (statista.com), which increased its global economy to around one trillion dollars in the following year (Sánchez et al. 2011). Nanotechnology, these days at annual global growth rate of 18 %, is being employed in various regular products, for example, in cosmetic materials. According to their properties, extent, and diversity of cosmetic nanomaterials, a uniform definition will be necessary (Batley et al. 2012). High level of consumer protection, free movement of goods and legal certainty for manufacturers, international level acceptance as well as scientific research on toxicity and environmental fate will be pivotal. According to European Commission, "Cosmetic nanomaterial" (CNM) means an insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale of 1 to 100 nm in the final formulation ((EC), 2013). The main interest of nanotechnology applications in cosmetics is to improve dispersibility and stability of ingredients, enhance their antimicrobial, antioxidant properties and transparency (reducing whiteness) (Wiechers and Musee, 2010).

Diverse titanium dioxide (TiO_2) and fullerene nanomaterials (NMs) are being manufactured by many companies to change their properties for specific cosmetic applications. Their global production statistics and direct release through washing out without any biodegradation confirms that these CNMs are one of the main sources in wastewater treatment plants (WWTPs). TiO_2 and fullerene NMs have antimicrobial properties and the scientific research in this field has increased (Delina Y. Lyon etal. 2006; Roy et al. 2010; Besinis et al. 2014; Jesline et al. 2014). This provides data and knowledge useful in elucidating the potential risk of impairing the function of microbial communities in WWTPs.

It can be easily deduced that the most important source of recalcitrant, non-biodegradable compounds in wastewater has been introduced by cosmetic products. It may also be the main source of nanomaterials in the municipal wastewater due to higher consumption of cosmetics per capita in USA and Europe. This is the right time to address the effect of CNMs on WWTPs efficiency (biological treatment) and also the WWTP efficiency on the removal of NPs because of exponential usage of nanomaterials. Our general focus in this review will be on insoluble cosmetic nanoparticles (CNPs) in WWTPs. This paper mainly discusses the gaps in regulations on usage of nanomaterials in cosmetics, their fate in WWTPs and finally their toxicity after being released into the environment.

Cosmetics nanomaterials (CNMs)

It is important to have a technology which can reach the target areas in a stable form and sustain the local effect for a long time in a biological system. By using nanotechnology, achievement of the target effects in cosmetic world can be obtained. Conventional cosmetics integrating “nanotechnology” have been considerably developed a placed on the consumer market over the past few years. Cosmetic nano-ingredients are divided into two groups: i) soluble and/or biodegradable nanoparticles which disintegrate upon application to skin into their molecular components (e.g. liposomes, microemulsions, nanoemulsions), and ii) insoluble particles (e.g. TiO_2, fullerenes, quantum dots). Figure 1 shows the nano-cosmetics categorization based on formulation and ingredients.

Nano-titanium dioxide

Titanium dioxide (titania, TiO_2) is a naturally occurring white pigment which has very high refractive index, brightness and resistance to discoloration under ultraviolet (UV) light in exposure applications. Hence, it has disinfectant, self-cleaning qualities and strong oxidative potential. It finds extensive applications ranging from paints (60% of the world’s consumption) to plastics (25%) and paper manufacturing, printing inks, fibers, rubber, cosmetic products and foodstuffs (pigment applications), as well as catalysts, electric conductors and chemical intermediates (Ceresana, 2013; Johnson et al. 2011). It is also accounted as a most produced nanomaterial due to its production level of 90,000 ton Ti/year (Kiser et al. 2009). Around 65 % of 2000 tons of nano-TiO_2 production in 2005 was used in personal care products (PCPs), such as topical sunscreens, white-colored shampoos, deodorants, and shaving creams which was worth around $ 70 million. In US, this number was estimated to increase to 2.5 million tons every year in the next two decades (Zhu et al. 2011). Recently, its production was around ten kilo tons which comprises 10% of all nanomaterials (Yang et al. 2013). Survey on the concentration of nano-titanium in cosmetic products showed a fluctuation between 90 μg Ti/mg in sunscreen to <0.01 μg Ti/mg in shaving creams. In toothpastes, for instance, the concentration varied between 0.7 to 5.6 μg/mg (0.1-0.5% of weight), while for sunscreen, it was 14 to 90 μg/mg (Weir et al. 2012).

The FDA has approved the safety of nanosized TiO_2 (0.2–100 nm) for use as a colorant and UV blocker and color enhancer (Singh and Nanda, 2014). However, in some pharmaceutical compounds, like safety coating of pills which are directly swallowed by humans, TiO_2 concentration can reach 0.014 μg/mg. Therefore, the controversy exists as to the safety of TiO_2 nanoparticles used in the cosmetics and food industry (Weir et al. 2012). The fundamental problem in the safety of cosmetics nanoparticles (CNPs) usage is a lack of information. There have not been enough studies conducted to assess how they interact with other chemicals in the formulation, with biological systems and also in the environment, once they are released.

Fullerene

The fullerene molecule (buckyballs, C_{60}) structure is formed by five-membered and six-membered carbon rings in the form of closed sphere or tube (Fowler and Ceulemans, 1995) which makes it consequently hydrophobic and decreases its solubility (10^{-18} mol/L or 1.3×10^{-17} g/L) (Batley et al. 2012). High density electron on the surface of fullerene is related to it extreme small size (0.7nm diameter). As a result, fullerene possesses strong anti-oxidant properties similar to Vitamins C and E; in addition to long-acting antioxidant action (Markovic and Trajkovic, 2008; Water, 2011; Yin et al. 2009). Furthermore, its unique porous structure, in association with high rate of derivatization makes it the best carrier of different molecules and facilitate its delivery inside the human body (Benn et al. 2011; Farré et al. 2010). Different kinds of fullerene used in cosmetics range from C60, C70, higher-order fullerenes, fullerene-based molecules (i.e., C60O, $C60O_2$, etc.), and fullerene derivatives (i.e., C60-PVP) which are soluble in water (Bakry et al. 2007). Modified fullerene promotes dispersion in water with consequent integration of water and cosmetic products (Benn et al. 2011; Nowack and Bucheli, 2007). Therefore, in the recent years, it is widely used as a cosmetic ingredient and in skin care products due to its anti-oxidant action, increases protein denaturation temperature, normalization of cellular metabolism, anti-inflammatory and antihistamine effect (Lens, 2009). In some cosmetic products, the concentration of fullerene can reach 1.1 mg-C60/g-cosmetic (Benn et al. 2011). Table 2 gives the information about current cosmetic nanomaterial fullerene products in market and its concentration

Legislation for nanomaterials usage in cosmetics

Recent regulations that have been introduced to the industries cannot catch up their safety and environmental assessment. Because of their unique properties and unpredictable change in the behavior depending on the surrounding environment; it has been not possible to extrapolate existing safety data on conventional chemicals to predict the safety of nanomaterials. The OECD (Organization for Economic Co-operation and Development) guidelines for testing of chemicals or (EC) No 440/2008, which underline the test methods for the European Community Regulation on chemicals (REACH), was not specifically designed for testing nano-scale materials. Nonethless, in2007, safety analysis programs of Manufactured Nanomaterials were established (Gartiser et al. 2014). Recently, Federal Drugs and Cosmetics Act (FD&C) and the Fair Packaging and Labeling Act (FPLA) set some regulation on their use, such as addition of color additives. Although FDA has advised manufacturers to ensure the safety of their products and ingredients, still it is not necessary for companies to share their safety information with FDA, and performing specific tests to demonstrate the safety of individual products or ingredients. FDA, and U.S. Customs and Border Protection can also inspect manufacturing facilities, encouraging cosmetic firms to report product formulations (not legally required). They are trying to increase consumer awareness about the importance of cosmetic-related problems. Finally, in June 2011, FDA has issued a draft "Guidance for Industry - Safety of Nanomaterials in Cosmetic Products" in which they have proposed certain points that the industry should consider when including nanotechnology in FDA-regulated products.

The 4th annual meeting of International Cooperation on Cosmetics Regulations (ICCR) on cosmetics held in Canada in July 2010 led to the formation of a Joint Industry/Regulator Working Group (WG) for nanomaterial safety to review the existing safety approaches in use of nanomaterials in cosmetics (ICCR, 2011). Except for their identification, the Acts and Regulations administered by Health Canada have no special information reference to nanomaterials. Similarly, under the Japanese Cosmetics Regulation, except for the categorization of negative and positive effects of cosmetic ingredients, there is no specific regulation for safety evaluation of nanomaterials.

At present, the EU's Cosmetics Regulation (Regulation (EC) No 1223/2009) is the only one specifically covering the use of nanomaterials in cosmetics. The Regulation requires cosmetic products containing nanomaterials to be notified to the Commission six months prior to being placed on the market, and nanoscale ingredients to be labelled (name of nano ingredient, followed by 'nano' in brackets).

To the best of our knowledge, these regulations only consider the safety and toxicity (local and systematic) of NMs in human beings from the biological point of view. They do not take into account the fate and toxic effects of these cosmetic NMs on the ecosystems after being released into the environment. As these NMs are directly released into the environment and do not undergo any biodegradation, they would keep cycling in the environment and are subject to dilution, oxidation or long term retention in the sediments or soils. Therefore, many efforts are required on their ecotoxicological evaluation. The current legislations have the stringent rules only on the safety dosage of nanomaterials in the cosmetics. Limits on concentrations of NMs present in the wastewater effluent and sludge for safe release into the environment are not included. This gap is mainly due to the lack of sufficient knowledge concerning toxicity of NMs in the environment. Hence, the development of new authority and regulatory structure might be the only way to effectively address these challenges.

Cosmetic nanomaterials in wastewater

Lack of information on environmental risk assessment of nanomaterials has mostly restricted their fate assessment in the municipal sewage treatment plants as the main source of nanomaterial in environment (Gartiser et al. 2014). CNMs, for example, TiO_2 have been used by some researchers as an indicator of predicting the environmental concentration (Musee, 2010). Wang et al. (2012) predicted 90% of TiO_2 in municipal wastewater originating from the cosmetic product (Wang et al. 2012). About 95% of Nano-TiO_2 used in cosmetic products was released to wastewater, as it does not penetrate the skin (Muller and Nowak, 2008; Johnson et al. 2011). As WWTPs have not been optimized for efficient nanomaterial removal, they translocate to the effluent and sludge in the environment (Bystrzejewska-Piotrowska et al. 2009; Batley et al. 2012), potentially disrupting numerous biological ecosystems (Wiechers and Musee, 2010). Kiser et al. (2009) estimated 45-50 (mg/person)/day for the daily load of titanium in the wastewater, which raises the total concentration of titanium including particles larger than 700 nm in the range of 0.1 to 3 ppm (Kiser et al. 2009). The maximum titanium concentration reported in WWTP influent was 2.8 mg/L. Consequently, the predicted environmental concentration for nano-TiO_2 in water increased in the range of 0.7-16 µg/L (Muller and Nowak, 2008). Considerable amount of fullerenes (Kiser et al. 2010b) also get directly released into the environment due to their use and application (Brar et al. 2010; Batley et al. 2012). As regard to accumulation of CNPs in aquatic life, TiO_2 concentration, for example, increase to 100 μg/L in fish, 980 μg/L for an algae and 1000 μg/L no observed effect concentration (NOEC) for river bacteria (Johnson et al. 2011). Land application of concentrated sewage sludge with nanomaterials increases their further release potential to agricultural field. Therefore, the highest fluctuation of concentration for these nanoparticles was observed in soil. In the developing countries and some parts of Europe, the concentration was less than 0.1 ng/kg; while, in agricultural soil exposed to biosolids, the concentration could rise to 2 mg/kg (Johnston et al. 2010; Gottschalk et al. 2013). By all these routes, humans could be exposed to high dosage of CNPs, especially in future. Table 3 presents the concentration of CNPs in different environmental media.

Fate of Cosmetic NMs in WWTPs

Although NMs are being used for around a decade, focus on environmental assessment of nanomaterial just dates back to 2007 as per Boxall et al. (2007). Due to their high consistency (Gottschalk et al. 2013), CNPs could be appreciably introduced to wastewater treatment through indoor usage or by dumping of cosmetic products in landfill (Nowack and Bucheli, 2007). An examination of 10 WWTPs showed 96% removal of titanium in influent sewage; still the effluents contain around 25 μg/L of TiO_2 from 4 to 30 nm size (Westerhoff et al. 2009; Benn et al. 2011). In Arizona WWTP, the removal efficiency for nano-TiO_2 in the influent at 185 μg/L, were 59, 84 and 91% for primary, secondary and tertiary stages, respectively (Kiser et al. 2010b). Researchers inferred that the fate of cosmetic NPs in WWTPs are so complicated and difficult to control due to: (1) undetermined source of pollution (Benn et al. 2011; Sánchez et al. 2011); (2) analysis problem of free NPs; and (3) functionalization of NPs with abundant organic matter present in wastewater (Brar et al. 2010; Urban et al. 2010).

Effect of aggregation

Understanding of the aggregation of CNPs is pivotal in predicting their fate in WWTPs, as well as its toxicity, since it changes CNPs chemical and physical properties with consequent effect on mobility, persistence, bioavailability and reactivity of nanoparticles (Thio et al. 2011; Shih et al. 2012). High surface charge, lower particle size, and increased ionic strength make nanoparticles susceptible to aggregation (Batley et al. 2012). It also dramatically changes the stability of CNPs in water (Huang et al. 2008).

Different factors affect the aggregation of CNPs in aquatic phase. When TiO_2 dosage was varied from 5 to 120 ppm, for example, aggregate size increased from 826 to 2368 nm (Long et al. 2006). It can also happen in the presence of divalent anions (e.g. sulfate ions), due to the increase in ionic strength (Shih et al. 2012). Aggregation of nano-TiO_2 is closely related to its zeta potential. Zeta potential of nano-TiO_2 increased at neutral pH when Fe(III) concentration rise due to the formation of Fe(III)-hydroxyl colloids. Furthermore, by introduction of 500 mmol/L NaCl, the zeta potential reduced from 45 mV to 20 mV (Shih et al. 2012). Therefore, the extra usage of coagulant may lead to adsorption or agglomeration or conjugation of NPs (Brar et al. 2010).

Factors, such as surface charge of NPs, pH, ionic strength, and natural organic matter (NOM) (e.g. organic acids, sugars, cellulosic materials, alginate, proteins, lipids) control aggregation, deposition, bioavailability and adsorption of NPs (Chang et al. 2009; Thio et al. 2011). Extracellular polymeric substances reduce the aggregation propensity, by increasing the stability of CNPs, especially in the case of TiO_2 (Kiser et al. 2010a) and fullerene (Batley et al. 2012). These materials consist of diverse aliphatic, aromatic, carboxylic, phenolic, alcoholic and quinoid groups, which interact with the active site of hydrophobic NPs. Therefore, in their presence, attachment of TiO_2 on these functional groups decreases the zeta potential and stabilizes the solution due to surface charge neutralization (Zhang and Guiraud, 2013). The combined effect of electrostatic repulsion and steric repulsion could stabilize the TiO_2 particles leading to decreased sedimentation rate (Li and Sun, 2011), and the bioavailability in biological processes (Thio et al. 2011). Since adsorption of hydrophobic nanoparticles to humic acid is more easier than metal NPs and performed only by surface modifications, colloidal aggregates of fullerenes were effectively removed up to 97% in laboratory jar tests (Batley et al. 2012). On the contrary, other researchers claimed that the presence of cations in solution of humic acid, compensate their negative effect on stabilizing aquatic NPs, due to the formation of COO–Fe(III) complex (Li and Sun, 2011).

Pre-treatment

Since the nanoparticles are smaller than suspended solids (SS) and colloides, preliminary treatment processes (sieving, bar screens, centrifuges) are not likely to remove a significant amount of ENMs (Neale et al. 2013). Nonetheless, two mentioned CNPs (TiO_2 and fullerene) are highly hydrophobic (Batley et al. 2012); sthus, the probability of their absorption to large particles such as, toilet paper and large fat globules is higher. Even though their removal by screening and sieving has not been investigated, appreciable amount of adsorbed TiO_2 and fullerene have been apparently removed by elimination of large particles. Unfunctionalized CNPs are not normally removed by this process, however, around 10% of TiO_2 was removed by primary settling stage (Johnson et al. 2011). One study on the raw sewage illustrated that with 843 µg/L of initial Ti concentration, 96% was attached to TSS= 336 mg/L. Hence,with removal of TSS from 366 to 97 mg/L, total TiO_2 removal reached 46% in the primary clarifier. It also reported that derivatization of fullerene in pre-ozonation process negatively affects its removal efficiency which further decreased during coagulation process (Hyung and Kim, 2009).

Primary treatment

Primary treatment involves sedimentation, flotation, coagulation/ flocculation and filtration; hence, the stability of CNPs (their tendency to resist agglomeration and aggregation) will determine the effectiveness of removal processes at this stage of the treatment process (Neale et al. 2013). According to Stoke's law, intact NPs are impossible to settle. Therefore, the mobility and fate of hydrophobic nanomaterials in primary clarifier, largely depends on aggregation and deposition behavior (Chen and Elimelech, 2008). Bridging or electrical double layer compression, as the main cause of aggregation, is highly dependent on the different factors, such as pH, ionic strength, electrolyte species and concentrations (Liu et al. 2013). It has been reported that in real WWTPs, the removal efficiency of primary treatment could rise to 60% for the influent concentration of 3300 - 8100 μg/L Ti (Kiser et al. 2010b).

Investigation of different factors in removal of fullerene by flocculation in wastewater showed that the complexity of wastewater in comparison with water helped the removal by adsorption to hetero-precipitation of alum flocs (Wang et al. 2013). It seemed that all of the NPs below the size of 5 nm are effectively removed by coagulation. On the contrary, large size NPs with more than 300 nm are just removed by self-agglomeration after longer retention time (24 h) (Chang et al. 2007). In laboratory scale jar test, 97% of fullerene was removed by means of alum coagulation, flocculation, sedimentation, and filtration sequence processes (Batley et al. 2012).With no alum added, still 15% of fullerene was removed by adsorption on the wastewater suspended solids (Wang et al. 2013b). Meanwhile, Kiser et al. (2010a) stated that by addition of 2 mM of $NaHCO_3$ in the TSS solution of 1500 mg/L , 50% of fullerene was removed (Kiser et al. 2010a). In neutral pH, the removal was associated with hydrolyzed aluminum species, while at basic pH, metal precipitates were the main factor. Alkalinity enhanced the nC60 removal efficiency (up to 71%) through the formation of sodium aluminum hydroxycarbonate precipitates, especially at higher pH (Wang et al. 2013). At 100 mg $CaCO_3$/L alkalinity and pH 6-8, and 10 ppm $AlCl_3$, 60% of fullerene was removed. However, the removal sharply decreased when the pH deviated from this value (Hyung and Kim, 2009). It has also been reported that highest deposition rate of fullerene onto silica surface occurred at 30 mM of NaCl, and 1 mM of Ca^{2+}. In the presence of minerals, the charge screening between fullerene nanoparticles and silica surfaces increased, leading to enhanced deposition rate (bridging effect of metallic cations). On the contrary, the presence of humic acid drastically decreased this rate (three times) due to faster adsorption on the humic compounds. Fullerene coated with humic acids repel each other (Chen and Elimelech, 2008). In the presence of 1% sodium dodecyl sulfate (SDS), removal efficiency of nano-TiO_2 decreased from 50 to 33% (Kiser et al. 2010a).

Suspended solids also enhance the production of flocs by acting as the formation core leading to increase in the removal efficiency to 46% at 25 mg/L of alum. Sewage organic matter, on the other hand, decreased the removal efficiency (by 17% or less) since they cause disaggregation of fullerene nanoparticles by means of the steric hindrance effect, and increase in the surface hydrophobicity (Wang et al. 2013b). They also occupied the active sites of aluminum hydroxide flocs (Wang et al. 2013; Zhang et al. 2008). Wang et al. (2013) showed that increasing the concentration of sewage organic matter from 40 to 75 mg/L, nC60 removal efficiency decreased by around 17%. On the other hand, humic substances could improve the flotation performance, since they can be easily separated as a foam (Shen, 2008). Under optimum conditions, dissolved air flotation with the dosage of 7.8–9.15 mg/L DOC, colloidal nanoparticle can be removed up to 91.4% of agglomerated TiO_2 (Zhang and Guiraud, 2013).

It is obvious that because of their size, free CNPs readily escaped from the sand filtration; yet, granular activated carbon may show higher removal efficiency for fullerene and TiO_2 in water treatment and tertiary treatment processes. The study on filtration showed an average of 82% of removal efficiency by biosolids leaving around 10 to 50 μg/L of TiO_2 in the effluent (Kiser et al. 2009). Furthermore, filtration was so important for removal of residual flocs, that it was decisive for overall removal efficiency of NPs in water and wastewater treatment (Hyung and Kim, 2009; Brar et al. 2010). One study showed that by means of 0.45 μm filtration, only 1–8% of nano-TiO_2 remained in the water (Zhang et al. 2008).

Biological Treatment

Adsorption to activated sludge seems to be the major removal mechanism for cosmetic nanoparticles in all the studies, since the biodegradation (Chang et al. 2007), and volatilization do not occur in their case (Liu et al. 2013). Adsorption onto sludge takes place in two stages: adsorption on surface driven by electrostatic force, followed by uptake through sludge mass due to three possible mechanisms: 1) passive diffusion, (2) facilitated transport across an intact membrane; or (3) diffusion across a disrupted membrane (Kiser et al. 2010a). The capacity of adsorption is so high that exposure to even low amount of sludge (50 mg TSS/L) could remove 10% of fullerene (Kiser et al. 2010a). With 400 mg/L TSS of biomass, about 88% and 23% of fullerene and TiO_2, respectively were sorbed to the biomass (Kiser et al. 2010b).

Till date, research has been mostly limited to the activated sludge and sequence batch reactor processes for nanoparticles. Table 2 shows the removal conditions of CNPs by these processes. It seems that WWTPs are quite efficient in CNPs removal since they can reach more than 95% for nano-TiO_2 and fullerenes (Batley et al. 2012; Wang et al. 2012; Gartiser et al. 2014). Because of high hydrophobicity of CNPs, they are easily adsorbed to the bulk of sludge, leading to increase in their concentration in the sludge (Kiser et al. 2009; Gartiser et al. 2014). The comparison between TiO_2 concentration in the sludge and the effluent showed that at least 95% of nano-TiO_2 was adsorbed onto sludge, while the rest of the particles more than 600 nm size were identified (Gartiser et al. 2014). Consequently, the TiO_2 concentration in sludge was estimated to be 23 times to that of the concentration in the effluent (Gartiser et al. 2014). Biomass concentration also affects the removal, since an increase of biomass from 0 to 2.2 g/L in sequencing batch reactor (SBR) led to, the removal of fullerene from 65% to 96%. By further decrease of biomass to only 0.6 g/L, the removal efficiency was still high (around 92%) (Thio et al. 2011). By considering the nano-TiO_2 with the size lesser than 700 nm, its removal efficiency in biological treatment decreased to only 42% on average (Gartiser et al. 2014). In real WWTPs, however, around 90% of nano-TiO_2 with the size less than

450 nm were removed by adsorption on to sludge in which the concentration in the influent, effluent and sludge was 30, 3.2 µg/L and 305 mg/kg, respectively (Johnson et al. 2011).

The effluent concentration detection was strongly related to the suspended solids, which agglomerated TiO_2 with the size lesser than 0.7 µm (Gartiser et al. 2014). It could also be the result of coating, functionalization, or other surface modification that decreased their removal (Kiser et al. 2010b). Ruled by diffusion, functionalization seems to have hindered the NPs' interaction with biomass surfaces (Kiser et al. 2010a). Zeta potential of cosmetic nanomaterials is a critical factor for their removal by bioadsorption. In the study of Kiser et al. (2010a), fullerene (ζ= -52 mv), which has the highest zeta potential amongst different nanomaterials, showed around 80% of removal, regardless of TSS concentration, while TiO_2 adsorption was totally dependent on TSS and less than 25% (Kiser et al. 2010a). Many older or smaller WWTPs employ fixed-film biological reactors (e.g., trickling filters) rather than the suspended biomass systems thus effecting removal rates and ways. Further research into NM removal by attached microbial communities is therefore needed (Thio et al. 2011).

Effect on microorganisms

Concentration of microorganisms inside biological treatment seem to have an important role to resist better the shock of nanomaterials toxic effects (Musee et al. 2011). Besides, concentration of nanoparticles itself largely influences on its toxic potential. About 50 ppm of TiO_2 nanoparticles has almost no effect on the biodegradation or nitrification (Gartiser et al. 2014; Zheng et al. 2011). As the data showed, almost all of TiO_2 and fullerene discharged into the sludge increased their concentration and made the microorganisms in sludge digester susceptible to its toxic effect. Most of the studies so far have normally focused on the toxic effect of nano-TiO_2 on sludge digestion processes. For instance, Mu et al. (2011) examined the effect of nanoTiO_2 on anaerobic digestion by fermentation experiments using waste activated sludge as the substrate. Nano-TiO_2, in doses up to 150 g/ kg-TSS showed no inhibitory effect (Mu et al. 2011). Other studies reached same results for TiO_2 nanoparticles on ordinary heterotrophic organisms (OHO), ammonia-oxidizing bacteria (AOB), and anaerobic biomass. By using 84 g/L of TiO_2 (much higher than the concentration in a municipal WWTP) significant inhibition occurred (83% at 4 h of exposure). In thermophilic anaerobic tests, TiO_2-NPs even had positive effects on production of biogas by 10% (García et al. 2012). The presence of nano-TiO_2 did not affect methane generation at concentrations of 6 to 150 mg/ g-TSS. On the contrary, under illumination, 100 mg/L of nano-TiO_2 can enhance hydrogen gas production up to 46.1% by promoting photosynthetic bacteria, and inhibit the activity of hydrogen-uptake enzymes (Yang et al. 2013).

The antimicrobial activities of nano-TiO_2 are generally attributed to their nano size effect and reactive oxygen species (ROS) generation leading to triggering of the generation of hydroxyl radical (OH*) in the presence of sunlight. The formation of intracellular ROS can indicate the effect of adsorbed TiO_2 inside the cell (Battin et al. 2009). In the absence of oxygen, the generation of OH* is limited. Hence, the adverse effect of TiO_2 in anaerobic processes is compensated (Yang et al. 2013). Because of fragility of nitrification bacteria, the main impact of NPs toxic effect on aeration basin was on nitrification performance. It was observed that high dose nanoTiO_2 had negative effect on long term nitrification bacteria (Zheng et al. 2011), by interrupting their key enzymes. In short term exposure, 50 mg/L nano-TiO_2 had no effect on nitrogen and phosphorous removal. Higher concentrations of nano-TiO_2 decreased the nitrogen removal from 80.3% to 24.4% during long-term exposure (70 days), whereas biological phosphorus removal was unaffected (Yang et al. 2013). Another study on nitrogen removal from wastewater in a SBR showed no adverse effect of TiO_2 in the concentration range of 2-50 mg/L. However, increase in the TiO_2 NPs concentration from 0, 100, and 200 mg/L dramatically decreased the removal efficiency from 80 to 36.5 and then 20.3% for these concentrations, as well as it had significant effect inhibitory effect on dehydrogenase activity during the de-nitrification process (Li et al. 2013). Inside sludge digestion plants, the concentration of 6 mg/g TSS of TiO_2 had no significant effect on methane generation (Mu et al. 2011).

Tertiary treatment

Even though titanium oxide based nanomaterials have been developed for potential use in the photocatalytic degradation of various chlorinated compounds (Chang et al. 2009; Brar et al. 2010; Hamdy et al. 2014), until this date, there are few studies on removal of TiO_2 in tertiary treatment. A study at one plant confirmed that the filtration in tertiary treatment removed on average 82 % of TiO_2, decreasing the effluent concentration from 50 to 10 μg/L. In this study on real WWTP, the TiO_2 concentration decreased from 20 to 12 μg/L by tertiary treatment which included filtration and disinfection (Kiser et al. 2009). In fullerene case, until date, there is no study on its degradation in tertiary treatment. It seems that in near future, the research on photo degradation of fullerene by advanced oxidation process will be performed. Figure 2 shows the fate of CNMs, TiO_2 and fullerenes along the WWTP. Even the fraction of nanomaterial in effluent can be toxic to aquatic life. Nanomaterials can also find its way through the food chain and groundwater by means of land application of sludge (Gartiser et al. 2014).

Sludge treatment

In spite of the fact that large portion of the TiO_2 nanomaterial was flushed into the sewage sludge, till date, no environmental guidelines have regulated the concentration of nanomaterials in the sludge. As CNPs get removed from wastewater, they become concentrated in biosolids by either direct adsorption of NPs, or clusters of aggregated CNPs with a size of several hundred nanometers (Thio et al. 2011). The majority of total titanium in the raw sewage (around ppm) goes to sludge, raises the concentration of Ti to 1.8 to 6.4 mg Ti/g SS, (average 2.8). A survey of the USEPA on

83 WWTP biosolid samples observed a range of Ti from 0.018 to 7.02 mg Ti/g SS in the sludge (Kiser et al. 2009). The average Ti content in activated sludge was around 538 μg/g in European sewage sludge (Johnson et al. 2011). Even stabilized biosolids collected from the WWTPs had an average Ti concentration of 1.1 mg Ti/g SS (Kiser et al. 2010b). Simulated data on nano-TiO_2 also showed the concentration of 0.3 to 23.2 mg/kg in the sludge (Yang et al. 2013). Gottschalk et al. (2009) calculated 1 ng/kg and 89 μg/kg for the annual increase of fullerene and nano-TiO_2 in the sludge (Gottschalk et al. 2009). Benn et al. (2011) predicted the concentration of 0.9 μg/kg-biosolids of n-C60 or C60-polyvinylpyrrolidone (PVP) in the biosolids caused by cosmetic fullerene.

To the best of our knowledge, to this date, there is no regulation for the maximum concentration of CNPs in wastewater sludge; however, further increase in their daily use, as well as future studies in the toxicology field will make CNPs, a case to study. Considerable increase of CNPs in the biosolids has raised attention towards their proper disposal. Also, the land application seems to be the most proper and sustainable way of disposal, new strict regulations discourage using this option in comparison to incineration. Even this method results in major concerns, such as deposition of ashes into landfills, and emission of heavy metals into the atmosphere (Thio et al. 2011). Generally, presence of variety of mineral and organic matter in sludge could form strong bond with nanomaterials and later mobilize them through the soil. The life cycle, accumulation in soil or sediment, and transformation of nanomaterials during land application must be investigated in future studies (Batley et al. 2012).

Characterization of TiO_2 and fullerene NPs

Due to certain distinctive properties and behavior, detailed characterization and safety assessment of nanomaterials is crucial. In general, physic-chemical properties of NMs play a crucial part in safety assessment which is more significant for nanomaterials than conventional chemicals. For an adequate nanomaterial description and to fill the current gaps in knowledge with regard to the effects of nanomaterials in relation to different physicochemical parameters, characterization of NPs should be done efficiently (behavior, size, distribution, stability, etc.). Furthermore, the choice of characterization methods are dependent on the matrix composition in which NMs are present, chemical composition, properties (functional groups on the surface, charge) and concentration of NMs and the analytical equipment. Table 3 shows the different parameters to be considered for NMs characterization depending on the matrix in which NMs are present and also demonstrate the instrument specifics for characterization.

The existing single methods will not provide sufficient data for NMs description (size (aggregates or agglomerates), charge, area and behavior) in complex media, such as WW. Different measurement techniques analyze a given parameter in distinct ways, such as some techniques measure individual primary particles, while others measure aggregates or agglomerates. Hence, results of different measurement techniques may not be directly comparable for the same parameter. Characterization of nanomaterials is generally more difficult in WW because of the presence of complex matrices and interaction of nanomaterial with matrix may change the basic physico-chemical characteristics of NMs with time. It may be necessary to use a combination of methods for detection and characterization of a nanomaterial in WW.

TiO_2 is insoluble in water and is expected to be in the sludge phase (Kiser et al. 2009). Until date, there are only limited practical studies on the exact compositional, morphological, and structural characteristics of residual TiO_2 NPs in sewage sludge. Kim et al. (2012) identified nano-TiO_2 across the sewage sludge and they characterized (morphology and chemistry) TiO_2 behavior using a series of electron microscopic techniques, such as scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM), transmission electron microscopy (TEM) combined with energy dispersive X-ray spectroscopy. Nano beam diffraction and high-resolution TEM were used to assess crystal structure of TiO_2. They found that treatment methods in WWTP affected the surface modified TiO_2 properties, mainly size and reactivity with organic matter. Also, pH changes in the surrounding environment affected the charge on the surface of NMs (zeta potential) and led to non-selective, electrostatic interactions with trace metals (silver) in the sludge.

C60 fullerenes are sparingly soluble in many solvents; this affects their separation by reverse phase high performance liquid chromatography (HPLC). In reverse phase HPLC, both stationary and mobile phase are non-polar for C60. Some applications were found for water soluble fullerenes (derivatized) by reverse phase HPLC. In other experimental studies, the characterization and quantification of hydoxylated fullerenes (fullerol) was studied. They specifically used the amide phase hydrophilic interaction liquid chromatography (HILIC) column for this water soluble fullerol separation and also compared the separation efficiency of a C_{18} column with HILIC (Chao et al. 2011). Inefficiency in retention of fullerols by RHPLC may be due to hydroxyl groups and the negative surface charges of fullerols, which may interfere in reverse-phase separation. Many researchers explored the UV/Vis spectroscopy and mass spectrometric analyses for detection and subsequent quantification of fullerenes in complex matrices (Wang et al. 2010; Chao et al. 2011). Chao et al. (2011) studies reported decrease in UV absorbance spectra for fullerols compared to fullerenes and also lack of distinct absorption peaks between fullerene and fullerols and the potential presence of interfering substances in environmental samples may severely limit its use. Soft ionization techniques (matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization) were preferred for fullerols to preserve diagnostic hydroxyl moieties in the fullerol sample.

Wang et al. (2010) concluded that the best extraction procedure for C60 in wastewater matrices; liquid-liquid extraction was applicable to all water and wastewater matrices and SPE could be used for water and wastewater matrices with lower dissolved solids. They have used combination of different extraction and detection methods (UV-Vis spectroscopic and mass spectrometric) to quantify nC60 in engineered wastewater matrices. They reported that mass spectrometry gives the m/z of the charged molecules and provides the specificity to differentiate and quantify different order fullerenes.

The shape and surface chemistry of fullerenes are quite different from other NPs. Fullerenes undergo different derivatizations in wastewater matrices and also the functionalized NPs in wastewater behave differently with time due to continuous changes in matrix composition. This instability in structural properties and matrix of surrounding environment affects the characterization and quantification of fullerenes and the choice of analytical methods. Finding effective concentration of fullerenes in cosmetic formulations prevents the excess usage and production of them; this will decrease the high exposure of fullerene NMs in the environment.

Toxicity of engineered TiO_2 and fullerene NPs

Toxicology will play an important role in assessing the safety of new materials and nanotechnology application. Presence and toxicity evolution of NPs in living systems is a unique novel challenge because such materials were not generally present in the environment during the course of biological evolution. Conventional risk assessment methodologies based on mass metrics may be adequate for soluble and/or biodegradable nanoparticles, but for the insoluble particles, their number, surface area and size distribution constitutes important additional information for safety assessment. From toxicity point of view, TiO_2 has been classified as biologically inert. LC_{50} of TiO_2 was reported around 80 mg/L (Peralta-Videa et al. 2011), and because of low level of toxicity, EC_{50} could not be measured (Sánchez et al. 2011). However, the physical, optical and chemical properties change dramatically at nano-scale (Wang et al. 2007).

Intact bacterial cell membranes have effective pore size typically ranging from 2 to 3 nm (Demchick and Koch, 1996) and are not permeable to NPs of approximately 10 nm. However, these particles might pass through substantially damaged membranes. Fullerene and its derivative forms, on the other hand, easily crossed the external cellular membrane and migrated into the mitochondria finally altering the protein structure (Long et al. 2006). Due to this reason, cellular damage includes cell dysfunction and cell lysis after 21 days exposure of fullerene to *Daphnia magna* will be inevitable (Liu et al.

2013).The exposure time of aggregate CNPs determined their penetration in skin. Penetration of 4 nm TiO_2 from innermost corneocyte layer can occur after 48 hours (Wu et al. 2009). Since these nanomaterials cannot be degraded inside the bodies, they gradually accumulated in tissues. The higher level of bioaccumulation also interrupted the metabolism leading to growth and reproduction malfunctioning (Zhu et al. 2010). Chronic exposure of nano-TiO_2 during long exposure time (21 days) displayed reproduction deficiency, growth retardation, paralysis, and even death.

Cosmetics nanomaterials combine two contrast electron-donating and -accepting properties, which can generate oxy-radicals in the body, or simultaneously act as an oxy-radical scavenger (Zhu et al. 2006). Strong electrostatic interaction between membrane cells and high surface charge nanomaterials disrupted the membrane integrity, finally causing cellular metabolic disturbance, especially for bacteria which have singular layer cell or higher interaction with nanoparticles. While they enter into the cells, generation of ROS causes peroxidation of various organelle constituents leading to oxidative stress (Liu et al. 2013; Musee et al. 2011). The intracellular oxidants produced by these radicals diffuse into the microglial plasma membrane where they can potentially damage the proteins, lipids, and DNA of neighboring cells, especially neurons and mitochondria (Long et al. 2006). In skin, the pace of ROS production increased under lamination after dermal exposure. These free radicals reduced the skin collagen content, increased wrinkles and caused skin ageing (Wu et al. 2009; Li and Sun, 2011).

Although attachment of CNPs onto soil particles decreased its toxic effects on soil microorganisms (Chen and Elimelech, 2008), still massive release and hydrophobic surfaces of NPs and their strong interactions with highly toxic inorganic and organic chemicals compounds in the environment are found to act as contaminant carriers (Bernhardt et al. 2010; Zhu et al. 2011). For TiO_2, the adsorption of highly toxic tri-butyl tin (TBT) onto nTiO_2 was faster, reaching a steady state within 120-240 min. The combination of even lower concentration of TBT and TiO_2 increased the toxicity by 20 and 70 times for the aquatic fauna (Zhu et al. 2011). These interactions between NPs and other emerging pollutants must be deeply investigated in future.

Toxicity in aquatic organisms

Many researchers concluded that in near future, the concentration of nanomaterials in water may pose a risk to aquatic life (Muller and Nowak, 2008). Furthermore, the gradual sedimentation of CNP may result in accumulation in sediments, which expose benthic animals (Zhu et al. 2011). Evaluation of cytotoxic potential of TiO_2 nanoparticles on fish cells showed the important role of agglomeration or aggregation than the concentration (Vevers and Jha, 2008).

Several studies of the toxicity of uncoated, water-soluble, colloidal fullerenes (C60) have been carried out. LC_{50} (median lethal concentration) at 48h in *D. magna* was determined to be about 800 ppb for fullerene (Sánchez et al. 2011). $nTiO_2$ showed lower toxic effect as at 2 mg/L $nTiO_2$, no inhibition of hatching was detected; yet, it was affecting 29% of abalone (*Haliotis diversicolor supertexta*) embryos by malfunctioning in hatching at 10 mg/L $nTiO_2$(Zhu et al. 2011).

Several laboratory scale experiments were carried out to find the bioaccumulation potential and acute and chronic effects of nano-scale TiO_2 in freshwater invertebrate, *D. magna* (Baun et al. 2008; Wiench et al. 2009; Zhu et al. 2010; Das et al. 2013). These studies explained that acute toxicity was independent of particle size of NPs and the size and functionalization of NPs are important in determining the chronic effects on growth and reproductive systems. Chronic effects on *D. magna*, exposed for 21 days to 5 mg/L of C60 showed a mortality rate of 40% for the highest concentration (Sánchez et al. 2011). Since fullerene can be easily partitioned into lipids, its concentration is considerably higher than the subsurface; therefore, it can be easily reached by planktons feeding on this layer.

Another important factor in toxicity of fullerene was dependent on the preparation of fullerene and its aggregation forms (Musee et al. 2011). For instance, highly toxic tetrahydrofuran nC60 killed all the Daphnia between 6 and 18 h, while no physical effects of the water-stirred-nC60 was detected after 48 h exposure time (Zhu et al. 2006). For the aquatic species, such as largemouth bass, at 0.5 ppm of C60, and exposure time of 48 hours, lipid peroxidation in the brain and glutathione depletion in the gill were reported (Sánchez et al. 2011). Oberdorster et al. (2006) studied the sub-lethal effects of fullerene NPs by evaluating the population levels in invertebrate and vertebrates. This was carried out by estimating the mRNA and protein levels in liver which indicated the cytochrome P450 isozymes. These enzymes are helpful in the cell signaling of lipid metabolism involved in either detoxication or tissue repair. They found that enzymes levels decreased after 96-h at 0.5 ppm nC60 exposure.

As mentioned earlier, CNPs can also interact with other toxic matter and facilitate their entry into organisms. Accordingly, investigation on tetrahydrofuran (THF)-solubilized nC_{60} on aquatic life showed that, in 6 hour exposure time caused the death of almost all fish (Zhu et al. 2006). For TiO_2, the adherence of aggregated TiO_2 to the surface of marine fauna, accelerated the uptake of highly toxic TBT (cumulative toxic effect) (Zhu et al. 2011). All in all, because of recent introduction to environment, literature is still bereft of long-term studies at environmentally realistic concentrations of nano-TiO_2. Figure 3 depicts the life cycle of NPs in the environment and also different ways for NMs entry into living systems.

Toxicity in humans

Apart from all applications of NPs on humans, some authors are determined that nano-cosmetic applications will be a major portal of entry for nanomaterials into body through skin (Bystrzejewska-Piotrowska et al. 2009). Around 60 days will be enough for penetration of all nano-TiO_2 to penetrate under the hairless mice skin. This time for the pig is less than 30 days for penetration of 4 to 60 nm of nano-TiO_2 (Wu et al. 2009).

Derivatization also affected the toxicity in human body as LC_{50} of fullerene varied by a factor of 10^7 according to derivatization. The nano-C_{60} aggregates are toxic to human skin cells at a LC_{50} of 20 ppb, while these values for C_3, Na+ $[C_{60}O_7\text{-}9(OH)_{12\text{-}15}]^{(2\text{-}3),}$ and $C_{60}(OH)_{24}$ were around 10, 40, and >5000 ppm (Sayes et al. 2004). It was also proved that the penetration pace may be different in derivatized NPs. Sayes et al. (2005) studied the nano-C60 cytotoxicity by monitoring the peroxy-radicals on the lipid bilayer of human cells. For instance, carboxylated (CdSe/ZnS, core/shell) quantum dots (QDs) penetration rate was faster than the simple ones (Mortensen et al. 2008). They indicated that these findings can be correlated to NP of similar size and surface chemistry, such as metal oxide NP found in sunscreens, which may also penetrate into the skin depending on its inflammatory status. After introduction into the human body by skin, nanoparticles with lower size (4 to 60 nm) accumulated generally in the organ with higher fatty acids, such as brain cells (Peralta-Videa et al. 2011). However, uptake by liver and kidneys cells is more prevalent. Around 69% of 250 mg/kg TiO_2 injected into human liver accumulated in just 5 min (Wang et al. 2007). Continuous uptake of these nanomaterials may result in a significant problem for human system.

Conclusions

The accumulation of CNMs in the environment would be exacerbated for the next decade if the concentration of fullerene increases to ppb. The current insufficient level of scientific understanding of the possible changes in properties, behavior, and effects of nanomaterials compared to conventional equivalents, will be overcome by intense research into the development and validation of methods for nanomaterials characterization and toxicological evaluations at all stages.

Sorption to sludge is the only removal pathway of cosmetic nanomaterials. Current regulations dealing with cosmetics are lax; they do not require any toxicity studies on humans before marketing and also do not consider the fate and toxic effects of these NMs once they are released into the environment. Investigation on cosmetic nanoparticles fate, characterization and toxicity in humans and in the environment is at its natal stage. Even though a lot of research is ongoing across the world on the behavior and toxic effects of cosmetic NMs on humans, still there is lack of solid information to fill the gap of health and environmental impacts of NPs to build stringent regulations for their usage.

One of the potential exposures of nanoparticles in the environment is their use cosmetics. The study, exploration and the behavior of cosmetic NPs (nano-TiO_2 and Fullerene) when applied on skin is different from when they are released into wastewater and also from the organic micro-pollutants in the environment. Apart from the use of these nano-TiO_2 and fullerene materials in cosmetics technology, their usage in other fields (water treatment, electronics, solar applications) also ultimately ends up into wastewater; hence it is difficult to detect the point source of these nanomaterials in the wastewater. It is always better to target them at point sources to remove before they enter into the complex media which ultimately affects the fate of NMs. The unique properties of NMs play an important role as critical parameters while detecting and characterizing them especially in complex media, such as wastewater is challenging. Detection and behavior of NPs in the environment is the starting point for further development and applications of nanotechnology. Surface modified (functionalized) TiO_2 NMs (doping) and fullerenes have different chemical and biological properties; this may affect the future nanotechnology applications. Apart from this, these NMs after entering into the environment naturally undergo surface modifications with different matrices based on their environmental conditions. Hence, it is also important to understand the functionalization of NPs in the environment, which is an unexplored area. Changes in functionalization of many ENPs by environmental factors or the coating of the surface by natural compounds are clearly an important process in the environment which significantly affects their behavior. European market does not contain fullerene products because the current scientific studies available on possible undesirable effects of this fullerene as a cosmetic ingredient are incomplete; therefore cosmetic regulation bans its marketability. For any new emerging technology advancement, such as nanotechnology, complete life cycle should be studied for its applications to avoid future problems. Applications of cosmetic NMs should be prohibited until appropriate research can show the potential benefits to be more important than the risks.

Abbreviations

CNMs, cosmetic nanomaterials; EC, European Commission; FDA, Food and drug administration; FD&C, Federal Food, Drug, and Cosmetic; ICCR, International Cooperation on Cosmetics Regulations; NMs, nanomaterials; NPs, nanoparticle; QDs, quantum dots; TiO_2, Titanium dioxide; WW, Wastewater; WWTP, wastewater treatment plant.

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Figures

Figure 1: Nano-cosmetics categorization based on formulation and ingredients

Figure 2: Fate of titanium dioxide and fullerene nanomaterials along the wastewater treatment plant

Figure 3: Life cycle of nanoparticles in the environment and different ways of entry of nanoparticles in to the living systems

Tables

Table 1: The mean concentration of CNPs in different environment media.

Table 2: Investigation of CNPs removal by SBR and activated sludge processes.

Table 3: Different parameters to be considered for nanomaterials (NMs) characterization depending on matrix and instruments for specific applications

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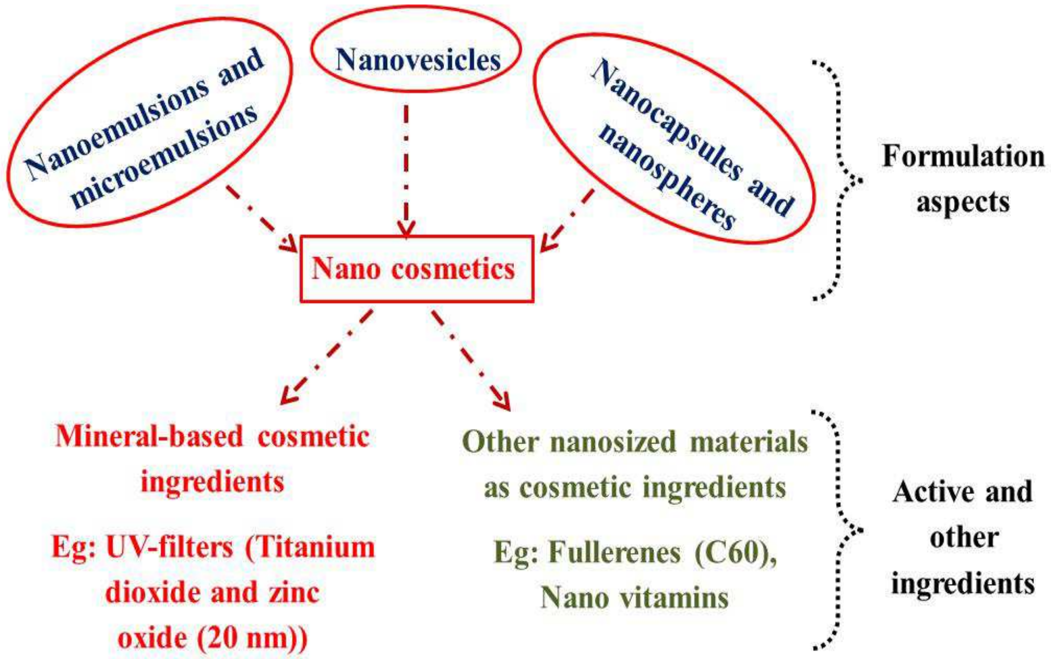

Figure 1: Nano-cosmetics categorization based on formulation and ingredients

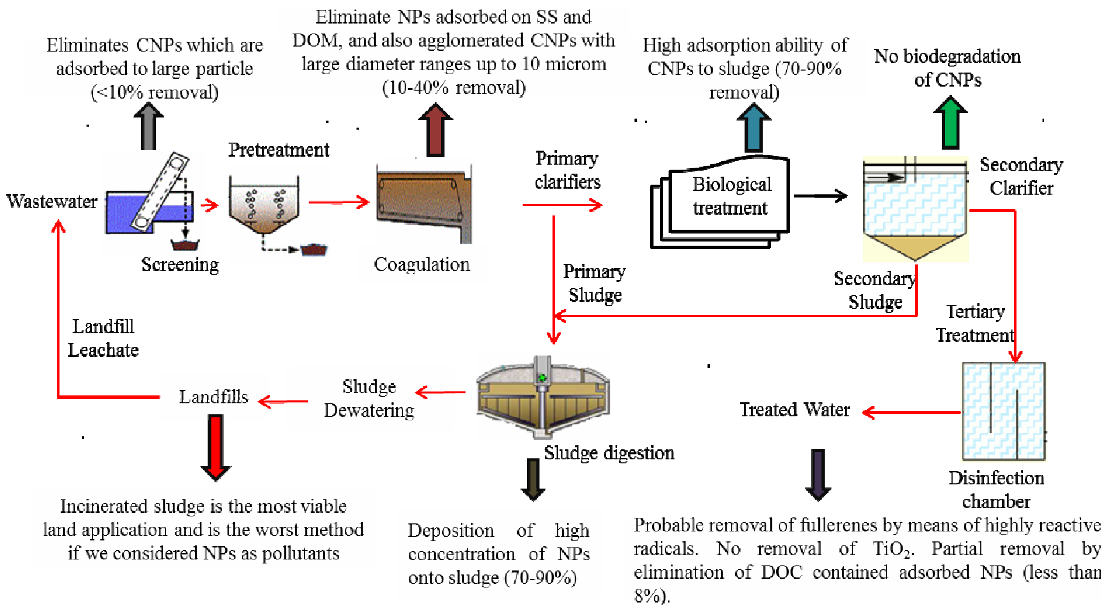

Figure 2: Fate of titanium dioxide and fullerene nanomaterials along the wastewater treatment plant

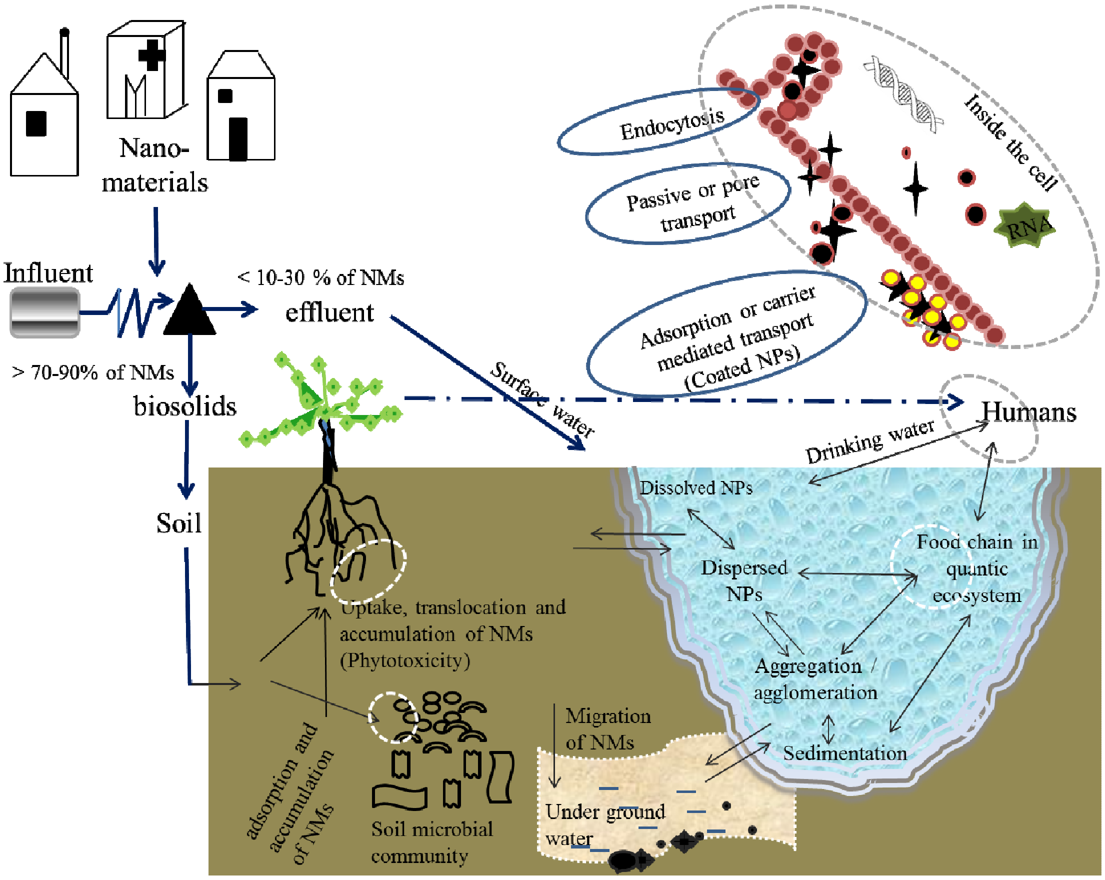

Figure 3: Life cycle of nanoparticles in the environment and different ways of entry of nanoparticles in to the living systems

Table 1: The concentration of CNPs in different environmental media

Nanoparticle type	Media	Location	Concentration	Reference
TiO_2	Surface water	predicted	0.03-1.6 µg/L	(Gottschalk et al. 2013)
			1.62 µg/L	(Batley et al. 2012)
		Thames region seaside, UK	8.8 µg/L (by sunscreen during a heatwave)	(Johnson et al. 2011)
		Europe survey	3.5 µg/L	(Gottschalk et al. 2009)
	Influent of sewage	predicted	0.7-16 µg/L	(Batley et al. 2012)
		Arizona	influent 185 µg/L	(Kiser et al. 2010b)
		Johannesburg	47.73 µg/L	(Musee, 2010)
	Effluent	prediction	4-40 µg/L	(Gottschalk et al. 2013)
		Spain	25 µg/L	(Benn et al. 2011
		China	1.75 µg/L	Yang et al. (2013)
		Arizona	17 µg/L	(Kiser et al. 2010b)
		Johannesburg	9.36 µg/L	(Musee, 2010)
	Sludge	predicted	1-2500 µg/g	(Gottschalk et al. 2013)
	river sediment	UK	5.6-91 ng/g	(Johnson et al. 2011)
		Predicted	3 µg/g	(Gottschalk et al. 2013)
Fullerene	surface water	Predicted	0.01-0.1 ng/L	(Gottschalk et al. 2013)
	Sewage	Predicted	4 and 33 ng/L	(Benn et al. 2011)
			100 ng/L 10-400 ng/L	(Gottschalk et al. 2013)

	WWTPs effluent	Spanish	0.5 ng/L-67 µg/L	(Farré et al. 2010; Farre et al. 2011)
	sewage sludge	Predicted	4 ng/L-20 ng/g,	(Gottschalk et al. 2009; Gottschalk et al. 2013)
	Sediment	Predicted	0.01-0.8 ng/kg	(Gottschalk et al. 2013)

Table 2: Investigation of CNPs removal by SBR and activated sludge processes

Type of reactor	Initial conditions	Operation Condition	Removal and concentration	Reference
activated sludge process	30 µg/L (TiO_2)		90% Sludge concentration= 305 mg/kg	Johnson et al. (2011)
	COD= 200 mg/L, TiO_2NPs= 10 mg/L	HRT=24 h, MLSS= 3000 mg/L	95%	Park et al. (2013)
		HRT= 6 hr Settling tank= 2 hr	79% of TiO_2 particle larger than 700 nm	Kiser et al. (2009)
SBR	0.5–2.5 mg/L (n-TiO_2)	TSS=1.3 g/L	70%	Park et al. (2013)
	5–2.5 mg/L(n-TiO_2 and fullerene)		97 and 95% for TiO_2 and fullerene	Wang et al. (2012)
	synthetic sewage containing, 2.9 mg/l (TiO_2)	8 h aeration, 2h settling time	88%	Kiser et al. (2009)

COD: chemical oxygen demand; HRT: hydraulic retention time; MLSS: mixed liquor suspended solids; SBR: Sequencing Batch Reactor-Membrane; TSS: Total suspended solids.

Table 3: Different parameters to be considered for nanomaterials (NMs) characterization depending on matrix and instruments for specific applications

Nanomaterial matrix	Properties to be	Characterization parameters	Instrument for specific applications

	considered		
Formulation	Physical parameters of NMs	Size, shape, surface area, surface charge, surface morphology, rheology, porosity, crystallinity and amorphicity, primary nanoparticles, agglomerates and/or aggregates.	**Size and size distribution:** TEM, XRD, DLS, SEM. **Shape and surface charge:** SEM, TEM, AFM, zeta-potentiometry **Crystallinity:** TEM, XRD. **Chemical composition:** ICP-MS/ICP-OES, XRD.
	Chemical parameters of NMs	Chemical composition, surface chemistry, oxidative capacity, catalytic activity, stoichiometry, dissolution kinetics and solubility, hydrophilicity or hydrophobicity, surface coating, impurities, intentional or unintentional surface adsorbents.	
Wastewater	Physical parameters of NMs	Size, shape surface area, surface charge, surface morphology, agglomerates and/or aggregates, concentration of NM.	**Aggregation:** Fl-FFF, SEM, Polarisation Intensity Differential Scattering (PIDS). **Surface characterization:** MALDI, EDX, BET, TG, FTIR, near-infrared, and Raman’s spectroscopy
	Chemical parameters of NMs	Chemical composition, surface chemistry, oxidative capacity, catalytic activity, hydrophilicity or hydrophobicity, surface coating	
	Wastewater characterization	COD, BOD, pH, total solids, composition of organic matter and pollutants	