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Fate and behaviour of TiO_2 nanomaterials in the environment, influenced by their shape, size and surface area

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Fate and behaviour of TiO_2 nanomaterials in the environment, influenced by their shape, size and surface area

by

Thomas A.J. Kuhlbusch, Carmen Nickel, Bryan Hellack
IUTA e.V., Air Quality & Sustainable Nanotechnology, Duisburg, Germany

Stefan Gartiser, Felicitas Flach
Hydrotox GmbH, Freiburg, Germany

Andreas Schiwy, Hanna Maes, Andreas Schäffer
RWTH Aachen, Biology V, Aachen, Germany

Lothar Erdinger
Universitätsklinikum Heidelberg, Department of Infectiology, Hygiene and Medical Microbiology, Heidelberg, Germany

Stephan Gabsch, Michael Stintz
TU Dresden - Mechanical Process Engineering, Dresden, Germany

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IUTA e.V.
Air Quality & Sustainable Nanotechnology
Bliersheimer Straße 60
47229 Duisburg

Hydrox GmbH
Bötzinger Str. 29
79111 Freiburg

RWTH Aachen
Biology V
Worringerweg 1
52074 Aachen

TU Dresden
Mechanical Process Engineering
01062 Dresden

Universitätsklinikum Heidelberg
Department of Infectiology,
Hygiene and Medical Microbiology
Im Neuenheimer Feld 324
69120 Heidelberg

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Wörlitzer Platz 1
06844 Dessau-Roßlau
Germany
Phone: +49-340-2103-0
Fax: +49-340-2103 2285
Email: info@umweltbundesamt.de
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5. Autor(s), Family Name(s), First Name(s)

[1] Nickel, Carmen
[1] Hellack, Bryan
[2] Gartiser, Stefan
[2] Flach, Felicitas
[3] Schiwy, Andreas
[3] Maes, Hanna
[3] Schaeffer, Andreas
[4] Gabsch, Stephan
[4] Stintz, Michael
[5] Erdinger, Lothar
[1,6] Kuhlbusch, Thomas A.J.

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[1] IUTA e.V., Air Quality & Sustainable Nanotechnology, Bliersheimer Straße 60, 47229 Duisburg, Germany
[2] Hydrotox GmbH, Bötzinger Str. 29
79111 Freiburg, Germany
[3] RWTH Aachen, Biology V, Worringerweg 1
52074 Aachen, Germany
[4] TU Dresden - Mechanical Process Engineering, 01062 Dresden, Germany
[5] Universitätsklinikum Heidelberg, Department of Infectiology, Hygiene and Medical Microbiology, Im Neuenheimer Feld 324
69120 Heidelberg, Germany
[6] Universität Duisburg-Essen, Center for Nanointegration Duisburg-Essen (CeNIDE), Duisburg, Germany

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16.Abstract

The *OECD Guidelines for the Testing of Chemicals* are internationally accepted standards and used for risk assessments, but their applicability to nanomaterials is not tested. Therefore the applicability of the guidelines - here especially those related to soil and sewage plant tests - was investigated in this study. The results obtained for the three TiO_2 nanomaterials are also important for closing the gap of information about environmental behaviour. The specific nanomaterials studied were UV Titan M262, which is coated with alumina and dimethicone, and the uncoated P25 and PC105.

The fate and behaviour of P25 was studied in a laboratory sewage treatment plant – based on the OECD Guideline 303A. The experiments in the laboratory sewage plant were performed over a period of three weeks. Altogether 1344 mg P25 in a suspension were added in three different concentrations each for one week; first week 1 mg/L, second week 5 mg/L and third week 10 mg/L. The chemical analysis showed that only 3 – 4% of the added TiO_2 was found in the effluent. The main part of the nanomaterial was adsorbed to the sludge.

The mobility of the three TiO_2 nanomaterials was tested in columns with three different soil types – based on the OECD Guideline 312. The tests were carried out in duplicate. 500 mg of the nanomaterials was applied as suspension of 5 g/L to the top of each soil column. After termination of

the experiments, segments of the soil and the eluate were chemically and microscopically analysed. The bulk chemical analyses of the soil segments indicate transport only for UV Titan M262. Microscopic analysis, however, showed transport of isolated TiO_2 agglomerates for all three nanomaterials. In the eluate no Titanium concentration > 5 µg/L (LOD) was detected.
The adsorption behaviour of P25 and UV Titan M262 in different soil types was tested based on the OECD Guideline 106. 2.5 mg TiO_2 were applied as suspension of 1 g/L to a soil/solution mixture consisting of 5 g soil and 22.5 mL 0.01M $CaCl_2$ solution. The mixture was homogenised and afterwards centrifuged. The supernatant was analysed for its Titanium content to derive the non adsorbed fraction. The Titanium content in the supernatant was in all cases < 5% of that added to the system.
It can be concluded, that the OECD Guidelines 303A and 312 are applicable to nanomaterials, here specifically TiO_2. Still specific recommendations should be taken into account as given in the corresponding section of the report. OECD Guideline 106 was found to be not useful for nanomaterial testing due to lack of possibilities for differentiating adsorbed from non adsorbed (agglomerated) TiO_2 nanomaterial.

17. Keywords
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5. Autor(en), Name(n), Vorname(n)
[1] Nickel, Carmen
[1] Hellack, Bryan
[2] Gartiser, Stefan
[2] Flach, Felicitas
[3] Schiwy, Andreas
[3] Maes, Hanna
[3] Schaeffer, Andreas
[4] Gabsch, Stephan
[4] Stintz, Michael
[5] Erdinger, Lothar
[1,6] Kuhlbusch, Thomas A.J.

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6. Durchführende Institution (Name, Anschrift)
[1] IUTA e.V., Luftreinhaltung & Nachhaltige Nanotechnologie,
Bliersheimer Straße 60, 47229 Duisburg, Deutschland
[2] Hydrotox GmbH, Bötzinger Str. 29
79111 Freiburg, Deutschland
[3] RWTH Aachen, Biology V, Worringerweg 1
52074 Aachen, Deutschland
[4] TU Dresden - Institut für Verfahrenstechnik und Umwelttechnik, Arbeitsgruppe Mechanische Verfahrenstechnik, 01062 Dresden, Deutschland
[5] Universitätsklinikum Heidelberg, Department für Infektiologie, Hygiene und Med. Mikrobiologie, Im Neuenheimer Feld 324, 69120 Heidelberg, Deutschland
[6] Universität Duisburg-Essen, Center für Nanointegration Duisburg-Essen (CeNIDE), Duisburg, Deutschland.

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15. Zusätzliche Angaben

16. Kurzfassung

Die OECD Richtlinien für die Testung von Chemikalien sind international akzeptierte Standards und werden für Risikoanalysen genutzt: Allerdings ist ihre Anwendbarkeit auf Nanomaterialien bisher kaum untersuch worden. Daher wurde die Anwendbarkeit der Richtlinien – speziell diejenigen für Böden und Laborkläranlagen – in dieser Studie untersucht. Die Ergebnisse welche für die drei TiO_2 Nanomaterialien erlangt wurden sind zudem von Bedeutung um Informationslücken zum Umweltverhalten teilweise zu schließen. Die in dieser Studie untersuchten Nanomaterialien waren das mit Aluminiumoxid und Dimeticon beschichtete UV Titan M262, sowie die unbeschichteten Materialien P25 und PC105.

Das Verhalten und der Verbleib von P25 wurde in einer Laborkläranlage, basierend auf die OECD Richtlinie 303A, untersucht. Die Untersuchungen in der Laborkläranlage wurden in einem Zeitraum von drei Wochen durchgeführt. Insgesamt wurden 1344 mg P25 in Suspension in drei verschiedenen Konzentrationen für jeweils eine Woche appliziert; In der ersten Woche 1 mg/L, in der zweiten Woche 5 mg/L und in der dritten Woche 10 mg/L. Die chemische Analyse zeigte, dass nur 3 – 4% des zugegebenen TiO_2 im Ablauf der Laborkläranlage wiedergefunden wurden. Der Hauptteil der Nanomaterialien wurde am Schlamm adsorbiert.

Die Mobilität der drei TiO_2 Nanomaterialien wurde in Bodensäulenversuchen mit drei verschiedenen Bodentypen basierend auf der OECD Richtlinie 312 untersucht. Die Versuche wurden als Duplikate durchgeführt. Insgesamt wurden 500 mg des Nanomaterials in Form einer 5 g/L konzentrierten Suspension auf jede Bodensäulen von oben aufgegeben. Nach Beendigung der Versuche wurden sowohl Segmente des Bodens als auch das Eluat chemisch und mikroskopisch analysiert. Die chemischen Untersuchungen zeigen lediglich für UV Titan M262 einen möglichen Transport in den Säulen. Mikroskopisch konnte ein Transport isolierter TiO_2 Agglomerate für alle drei Nanomaterialien nachgewiesen werden. Im Eluat wurde kein Titan in Konzentrationen > 5 µg/L (LOD) detektiert.

Das Adsorptionsverhalten von P25 und UV Titan M262 wurde an verschiedenen Bodentypen basierend auf die OECD Richtlinie 106 untersucht. 2,5 mg TiO_2 wurden als Suspension mit einer Konzentration von 1 g/L zu einer Boden/Lösung Mischung, bestehend aus 5 g Boden und 22,5 mL 0,01 M $CaCl_2$ Lösung, gegeben. Die Boden/Suspension Mischung wurde anschließend geschüttelt, zentrifugiert und der Überstand chemisch auf Ti untersucht um die nicht adsorbierte Fraktion zu bestimmen. In allen Fällen war der Titananteil im Überstand < 5% von dem, was zugegeben worden war.

Die durchgeführten Untersuchungen zeigen, dass die OECD Richtlinien 303A und 312 für Nanomaterialien, speziell TiO_2, angewandt werden können. Allerdings sollten spezielle Empfehlungen in Betracht gezogen werden, welche im entsprechenden Abschnitt dieses Berichtes dargelegt sind. Die OECD Richtlinie 106 ist aufgrund der fehlenden Möglichkeit zwischen „adsorbierten“ und „nicht adsorbierten“ (agglomerierten) Nanomaterialien (hier TiO_2) zu unterscheiden für die Testung von Nanomaterialien nicht geeignet.

17. Schlagwörter
Titaniumdioxid, Nanomaterial, Kläranlage, Boden, Adsorption, Verhalten, Mobilität

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List of abbreviation

BV:	blank value
CV:	coefficient of variation
DI:	deionised water
DLS:	dynamic light scattering
DOC:	dissolved organic carbon
EDX:	energy dispersive X-ray spectroscopy
FET:	fish embryo toxicity
HMM:	Heidelberg University Hospital, Department of Infectiology - Hygiene and Medical Microbiology
ICP/OES:	inductively coupled plasma optical emission spectrometry
ICP/MS:	inductively coupled plasma mass spectrometry
IEP:	isoelectric point
IGEOS:	Institute for Geosciences, University of Heidelberg
IME:	Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg
IUTA:	Institute of Energy and Environmental Technology, Duisburg
JRC:	Joint Research Centre
LOD:	limit of detection
LSTP:	laboratory sewage treatment plant
OSW :	organic synthetic wastewater
Rpm:	runs per minute
SD:	standard deviation
SDS:	sodiumdodecylsulphate
SDW:	synthetic drinking water
SEM:	scanning electron microscopy
SHP:	sodium hexametaphosphate
SRM:	standard reference material
STP:	sewage treatment plants

1. Introduction

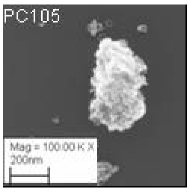
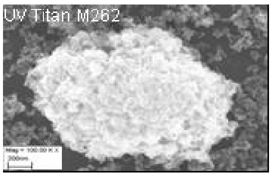
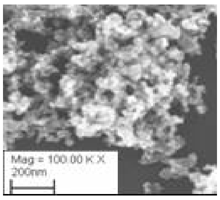
Engineered nanomaterials are used in many everyday life products. Since 2006 the use of nanomaterials in products showed an increase of 521% (Woodrow Wilson database – 2011) and further growth of applications is expected. Hence environmental and human exposure to these nanomaterials during their life cycle is anticipated (Kaegi et al., 2008, 2010). One pathway into environmental compartments is the release into the aquatic, sediment or soil ecosystems via direct or indirect emission e.g. by sewage treatment plants.
Information about the fate and behaviour in the environment after release are still scarce but important for risk assessments, as recent studies demonstrated potential toxicity of some nanomaterials (Oberdörster et al., 2004, 2006, Poland et al., 2008, Zhang et al., 2007, Hund-Rinke et al., 2006).

For the effect analysis of chemicals, a multiplicity of standardised test methods exist and the results are used in risk assessments. The *OECD Guidelines for the Testing of Chemicals* for examples are international harmonised and accepted standards. Nanomaterials can show different behaviour compared to the bulk material or chemicals (Tiede et al., 2008, Nel et al., 2006, Burleson et al., 2004). Therefore careful tests and evaluations of these guidelines in view of their applicability for testing nanomaterials have to be conducted. If needed specific modifications of the guideline have to be agreed upon.

Titanium dioxide nanomaterials are widely used in many different products and its release from products to the environment has been shown by e.g. Kaegi et al., (2008) or Hsu and Chein (2007). Titanium dioxide (in bulk and nanoscale form) is used in huge quantities as white pigment or in the nanoscale form as UV-filter in consumer products such as paints, paper coatings, plastics, and sunscreens. Industrial applications include its use in self-cleaning coatings as well as a photo catalyst in advanced wastewater treatment or NOx-removal in ambient air. Some of the above applications may lead to a release into the aquatic environments and due to this into sewage treatment plants and possibly soil systems.

In this study three different functionalised and non-functionalised TiO_2 nanomaterials (P25, PC105, and UV Titan M262) were therefore tested – P25 in a laboratory sewage treatment plant and P25, PC105 and UV Titan M262 in soil compartments (Table 1)

Table 1: Titanium dioxide nanomaterials: physical properties based on the information provided by the OECD Sponsorship program.

	PC105 – NM102	**UV Titan M262 – NM103**	**P25**
Crystalline form	anatase	rutile	anatase 86 % rutile 14 %
Shape	essentially spherical	essentially spherical	spherical
Density	4.13 g/cm^3 [#]	3.79 g/cm^3 [#]	4.13 g/cm^3 [#]
Coating	none	Al_2O_3 + dimethicone (hydrophobic)	none
Primary particle size (distribution)	15 – 25 nm (560 nm – 1090 nm)	20 nm (180 nm – 720 nm)	21 nm [+]
Surface area (by BET)	90 m^2/g	60 m^2/g	50 ± 15 m^2/g
Particle size in used suspensions (at pH 5)	560 nm*	180 nm*	220 nm*
Refractive index	2.59	2.75	2.7
Adsorption	0.01	0.01	0.01
Use	photo catalyst	cosmetics	photo catalyst, cosmetics
			

* Average of DLS measurements (this study) after 10 min sonication; n = 5

[#] Values for TiO_2 based on those of rutile and anatase and the given ratio (IARC, 2010)

[+] No confidence interval provided in the material data sheet

The tests methods chosen are based on the OECD guidelines for testing of chemicals:

- OECD test guideline 303A – Simulation Test - Aerobic Sewage Treatment: Activated Sludge Units
- OECD test guideline 312 – Leaching in Soil Columns
- OECD test guideline 106 – Adsorption / Desorption - Using a Batch Equilibrium Method

The nanomaterials were suspended in water for use in the environmental tests to simulate the most likely pathway into environmental compartments of these materials. This also provides the possibility to generate and use homogeneous and comparable basic conditions. In this study a realistic worst case in view of particle size distribution in water was employed which was defined here by the use of nanomaterial suspensions with an average agglomerate size aiming at < 250 nm diameter to minimise possible interference by large agglomerates. The stability of the suspensions was tested by measuring the size distribution of the agglomerates and zeta potential in the suspension as well as observation of any visual sedimentation. A suspension was defined stable if the variations of the results were < 10% within 24 h.

The subsequent sections present and discuss the results obtained in following order:

- Preparation and characterisation of suspensions for the different environmental tests,
- Development, establishment and validation of an analytic method for the Titanium analysis of the samples,
- Environmental tests including test realisation, analysis and data interpretation separately for each test guideline, for:
  sewage plant,
  soil leaching,
  and soil adsorption testing.
- Finally recommendation for the investigated OECD test guidelines.

2. Suspension preparation and chemical analysis

2.1 Suspension preparation

The titanium dioxide nanomaterials have to be suspended in different aquatic media for the environmental tests. To conduct those tests reproducibly, the suspensions have to be stable for 24 h. The stability was investigated by measuring the size distribution, the width of the size distribution expressed as polydispersity index (PdI) < 0.5 and zeta potential (< 10% variance). The different requirements for the different environmental media are summarised in Table 2.

An average agglomerate size of < 250 nm (= target agglomerate size) in suspension was decided to represent a realistic worst case scenario with regard to particle size.

Table 2: Requirements of the different suspensions for the environmental tests.

	Aerobic Laboratory Sewage Treatment Plant (LSTP) Simulation Test (OECD 303A)	**Leaching in Soil Columns (OECD 312)**	**Adsorption / Desorption: Using a Batch Equilibrium Method (OECD 106)**
Material	P25	P25, UV Titan M262, PC105	P25, UV Titan M262
Media	Synthetic drinking water (SDW - according to DIN EN ISO 6341)	Deionised water (DI water)	Deionised water (DI water)
Stability	At least 24 h	At least 24 h	At least 24 h
Stock suspension	100 mg/L	5 g/L	1 g/L
Applied suspension	Dilution of suspension to 1 mg/L, 5 mg/L and 10 mg/L	5 g/L	1 g/L

Size analysis of the suspensions was performed with dynamic light scattering (DLS Zetasizer Nano ZS 3600, Malvern; HPPS Malvern; Nanophox, Sympatec; Zetatrac, Microtrac, Beckmann Coulter CZ). The agglomerate size is detected as hydrodynamic diameter expressed as Zeta average diameter in nanometre (Z.average).

A comparison measurement with the different instruments were conducted and showed a good comparability (Annex I). Comparable results were also found in a DLS round robin test with ten different laboratories, which was conducted to test the comparability of DLS measurements in different laboratories. For this comparison measurements a polystyrene particle (182 ± 5 nm, BS Particle GmbH – HS) and TiO_2 (P25 – 100 mg/L, 1 wt% SHP) suspension was centrally prepared, than distributed to the participating laboratories and measured. Additionally suspension of P25 was prepared in the different laboratories following the final SOP defined in the context of this project (Annex I). A good comparability between the different laboratories was found (Nickel et al., in preparation).

The quality of the DLS measurements is influenced by several parameters, like stability of the suspension, temperature of the medium, refraction index and particle size. Larger particles scatter in a higher intensity than smaller, e.g. the scattered intensity of a 10 nm particle is a factor 10^6 higher than that of a 1 nm particle of the same composition (Rayleigh approximation), hence resulting in a higher signal.

2.1.1 First studies – suspension preparation

For the suspension preparation different methods for the preparation of stable P25 suspensions were tested, by varying suspension method, time, energy, concentration and pH.

The P25 suspensions were prepared in a first test series with fixed mixing times and concentrations (100 mg/L) in deionised (DI) water varying the type of mixing:

- Stirring
- Ultrasonic bath – 240 Watt (W)
- Ultrasonic homogeniser using 600 W and 200 W

A stable suspension was only achieved with an ultrasonic homogeniser. By using a 200 W homogeniser a sonication time of 10 min for a volume of 100 mL DI water with pH 5 (200 W Bandelin Sonoplus, pulse 0.2 / 0.8) was sufficient for the preparation of a stable suspension, which meets all stability criteria (Figure 1). The change in the zeta potential within the first 24 h may indicate that the particles underwent changes due to adsorption / desorption processes or that some particle with a lower zeta potential were lost to the wall or agglomeration not leading to measurable changes.

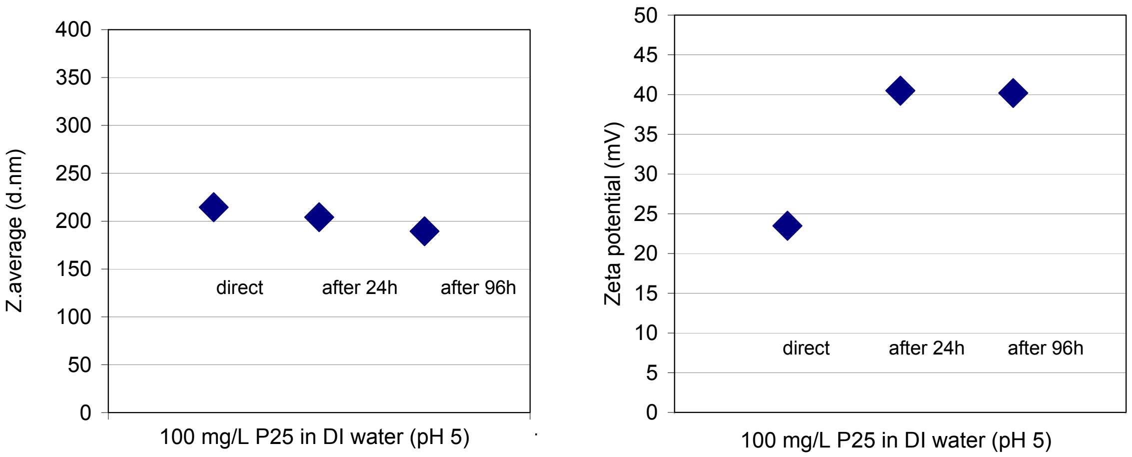


Figure 1: Z.average (left) and zeta potential (right) of P25 in DI water (pH 5) after 10 min sonication, measured directly, 24 h, 96 h thereafter; n = 5.

Increased sonication time leads to a decrease of the average agglomerate size. Ten minutes of sonication were found to be sufficient for P25 to reach the target agglomerate size (Figure **2**).

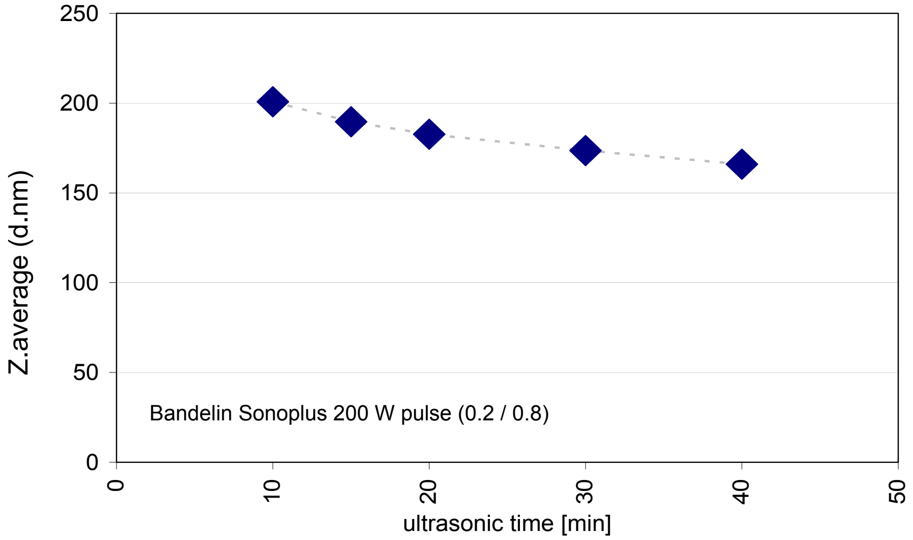


Figure 2: Z.average of P25 (100 mg/L) in 100 mL DI water (pH 5) after 10, 15, 20, 30 and 40 min sonication. Standard deviation (SD) < 1; n = 5.

A suspension of TiO_2 fulfilling the stability requirements given above was achieved for P25 in DI water with a sonication time of 10 min in 100 mL with cooling of the beaker by ice water. The latter avoids heating of the suspension caused by the sonotrode induced energy influx.

Based on these findings, suspensions in this study were prepared using an ultrasonic homogeniser. The influence of different parameters like sonication time, nanomaterial concentration, pH and ionic strength on the stability and particles size was further tested.
On a basis of an inter-laboratory comparison (three laboratories) measurement differences in the suspension characteristics were detected even following the same suspension preparation procedure. The DI water composition was found to be a significant source of error and consequently we decide to use the same DI water from one laboratory for harmonisation.

→ *Particles sizes in suspensions are closely related to the sonication time.*

Effect of nanomaterial concentration on the particle size distribution - exemplary investigation with P25

The size analysis of the suspensions with varying P25 concentrations showed stable suspension for 24 h – here DI water was used with 1 wt% sodium hexametaphosphate (SHP) as stabiliser. No significant differences of the Z.average results between the same concentrations at t = 0 h vs. t = 24 h were detected via t- test, exceptional for 1mg/L. The t-test for t = 0 h (1 mg/L) vs. t = 24 h (1 mg/L) revealed a significant difference on a significance level of p = 0.05. Results are not in detail shown in the (Figure 3).

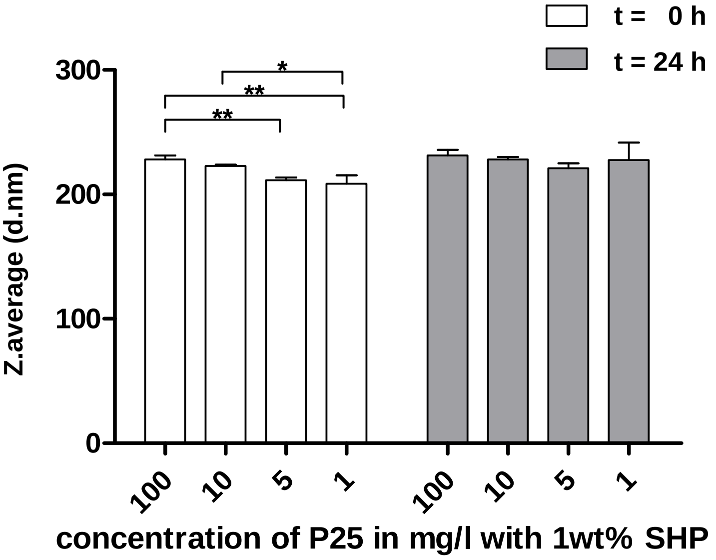


Figure 3: Z.average P25 (100, 10, 5 and 1 mg/L) in 100 mL DI water with 1 wt% SHP sonicated for 10 min. Error bars show determined SD. Statistic analysis via ANOVA and Bonferroni´s test with selected pairs were performed for both time points, * marks significant difference $p < 0.05$, ** $p < 0.01$; n = 3.

P25 particles were detected in range of 100 - 5 mg/L with good reproducibility. Particle concentration of 1 mg/L showed a high variance > 5 % between the separate DLS measurements and additionally the quality report of the Malvern Zetasizer Software was failed often due to the detection limit of the dynamic light scattering method. Consequently statistical analysis for concentrations < 5 mg/L should be handled with care due to the LOD of the DLS. However concentration dependent differences in the Z.average within the time point t = 0 was detected, showing significant lower Z.averages for 5 mg/L and 1 mg/L compared to the highest concentration of 100 mg/L. Also the Z.averages for 10 mg/L and 1 mg/L are significantly different. These differences equilibrated within the first 24 hours. No significant differences were determined anymore at this time point most likely due to agglomeration and sedimentation.

Conclusion first suspension tests:

- → *No stable suspension could be prepared with stirring or sonication with an ultrasonic water bath.*
- → *The use of an ultrasonic homogeniser was necessary (200 W Bandelin Sonoplus, pulse 0.2 / 0.8).*
- → *With a sonication time of 10 min in a volume of 100 mL a stable suspension with a target Z.average agglomerate sizes of < 250 nm could be prepared in DI water (pH 5).*
- → *With increasing sonication time agglomerate sizes decrease.*

→ *A significant change in Z.average in dependence of the concentration was determined for t = 0 h but not for t = 24 h. For both time points the lowest concentration of 1 mg/L was not measurable with appropriate reproducibility.*

→ *The suspension preparation procedure was adopted for the suspension preparations in this study.*

The results were used to prepare a standard operation procedure for preparation of suspensions (SOP – Annex I).

2.1.2 Suspension for laboratory sewage treatment plants

Tab water contains different minerals with varying concentrations between different locations. Hence the stability of the P25 suspension showed high variance in dependence on which laboratory produced the suspension. Consequently synthetic drinking water (SDW) based on DIN EN ISO 6341 was used as defined liquid matrix for the laboratory sewage treatment plant (LSTP) experiment instead of tap water (mentioned in the OECD guideline). Nevertheless, the higher ionic strength of the SDW leads to unstable P25 suspensions. Therefore the addition of a dispersing agent was found to be necessary and four different substances were tested (Table 3).

Table 3: Dispersing agents.

Name	CAS
Sodium hexametaphosphate (SHP)	10124-56-8
Sodiumdodecylsulfate (SDS)	151-21-3
Humic acid	1415-93-6
Triton X	9002-93-1

With Triton X no stabilisation effect could be detected (failed DLS quality report). With humic acids the preparation of a reproducible stock solution was found to be not feasible. Therefore it was not used for the following analysis.

Only SHP and SDS were found to be suitable additives for these tests to achieve stable particle suspensions (detailed information Annex I). SDS inhibits microbial activity and thus would influence the sludge in the tests. Hence SDS was excluded from further testing and SHP adapted with a concentration of 1 wt% for the suspension (thus, the final concentration for the stock suspension for the OECD test 303A was 1 g/L SHP). The resulting concentrations in the inflow to the laboratory sewage treatment plant (LSTP) were consequently 0.01 wt% for 1 mg/L, 0.05 wt% for 5 mg/L and 0.1 wt% for 10 mg/L, respectively.

Effect on sonication time on the agglomerate size in synthetic drinking water

For the laboratory sewage treatment plant experiments the suspension preparation was conducted in a volume of 300 mL. Therefore the needed sonication time was tested again.

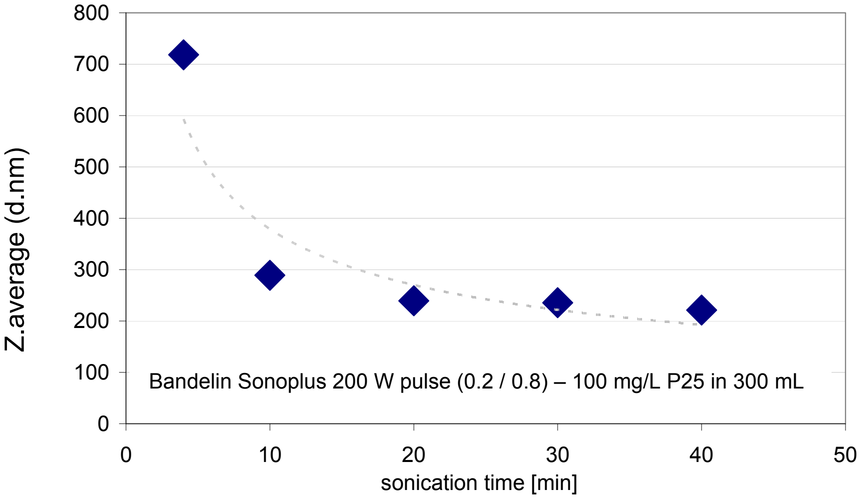


Figure 4: Z.average of 100 mg/L P25 in 300 mL SDW after 5, 10, 20, 30 and 40 min sonication. Error bars = SD covered by the symbols; n = 5.

Effect of dilution and pumping on the stability of the suspension

The stability of the suspension was also investigated during the LSTP tests. The size and zeta potential of the stock suspension (100 mg/L - which was diluted to the target concentration for the LSTP experiment) were measured directly after preparation and after 24 h, as well as the diluted suspension and the suspension after the peristaltic pump.

All determined particle size distributions of the suspensions showed no significant deviation to the stock suspension (
Figure **5**). The average agglomerate size was 230 nm with a negative zeta potential lower than -35 mV.

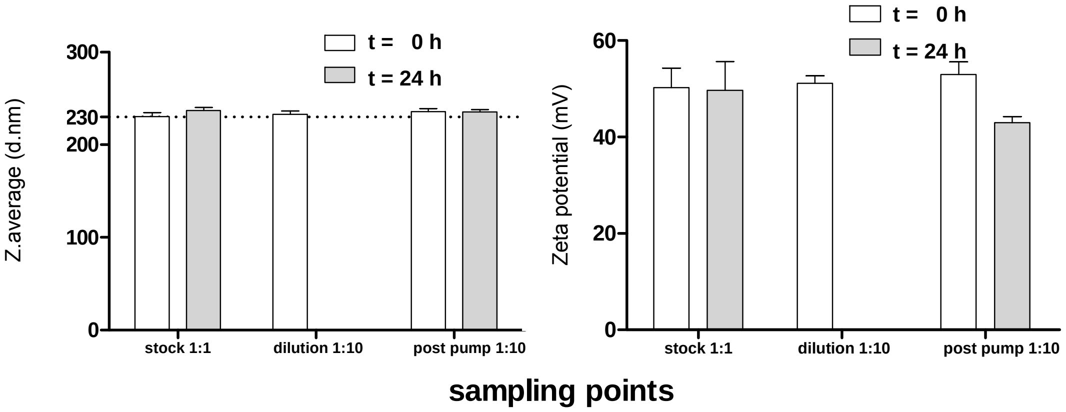


Figure 5: Size (left) and zeta potential (right) analysis of 300 mL SDW with 100 mg/L P25 and 1 wt% SHP sonicated for 30 min with 200 W with SD as error bars. Measured time points 0 h and 24 h; n = 2.

→ *No effect of the dilution and pumping on the agglomerate size in the suspensions was detected.*

Occupational safety – workplace exposure particle measurements

Measurements of a possible particle release during handling were conducted. No nanomaterial release during weight, suspension preparation or during operation (full report, only in German, can be found in Annex IV) was detected.

Conclusion suspension preparation in SDW for the LSTP experiments

→ *No stable suspension could be prepared in SDW without a dispersion additive.*
→ *Using 1 wt% sodium hexametaphosphate a stable suspension could be prepared.*
→ *Dilution and pumping did not effect the stability and size distribution of the suspension.*
→ *Occupational measurements detected no particle release during handling and operation.*

2.1.2 Suspension preparation for the soil experiments

For the leaching experiments in soils (OECD 312) the three TiO_2 materials, P25, PC105 and UV Titan M262, had to be suspended in DI water with concentrations of 1 g/L and 5 g/L. For the adsorption experiments (OECD 106) P25 and UV Titan M262 were suspended in DI water (pH 5) with a concentration of 1 g/L regarding to the experience of the first studies with P25 (chapter 2.1.1 First studies – suspension preparation).
For a first test with the ultrasonic homogeniser (10 min, pulse 0.2 / 0.8) suspensions of the three materials at a nominal concentration of 100 mg/L was set-up in a volume of 100 mL DI water (pH 5) and were measured directly after suspension and after 24 h (Table 4).

Table 4: DLS measurements of P25, PC105 and UV Titan M262 suspension with a concentration of 100 mg/L in 100 mL after 10 min sonication (200 W homogeniser); n = 5.

	P25 Z.average (d.nm) (SD)	**PC105 Z.average (d.nm) (SD)**	**UV Titan M262 Z.average (d.nm) (SD)**
Direct	199 (1.01)	500 (4.62)	188 (3.01)
After 24 hours	214 (3.32)	969 (7.66)	198 (3.21)

The suspension of UV Titan M262 showed the smallest agglomerate size, followed by P25 and than PC105, with an agglomerate size > 500 nm. PC105 was not as stable as UV Titan M262 or P25 which showed only a small size increase (< 10%). Visually no sedimentation of PC105 was detected after 24 h. It is conceivable that few larger agglomerates in the suspension covered smaller agglomerates (uncertainty of the DLS measurements).

Effect of sonication time on the agglomerate size

P25, PC105 and UV Titan M262 (100 mg/L) were sonicated in a volume of 100 mL for 10, 15, 20, 30 and 40 min. The average agglomerate size was negatively correlated with sonication time (decreasing particle size with increasing sonication time).

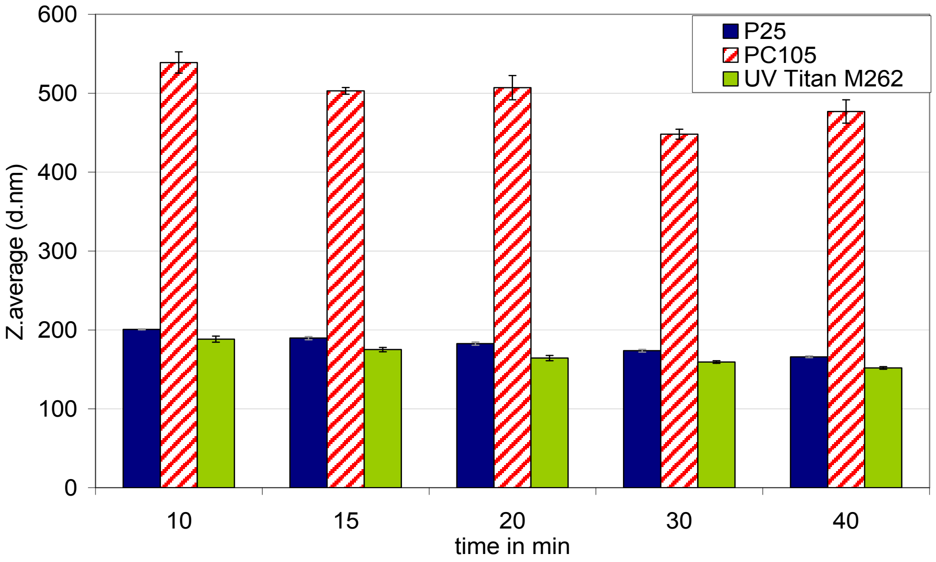


Figure 6: Z.average of 100 mg/L P25, PC105 and UV Titan M262 in 100 mL DI water, sonication for 10, 15, 20, 30 and 40 min with SD as error bars; n = 10.

Sample sonicated for 40 min had the lowest Z.average with 165 nm for P25, 150 nm for UV Titan M262 and around 480 nm for PC 105 respectively. Overall P25 and UV Titan M262 showed comparable Z.average around 180 nm ± 8% for P25 and 170 nm ± 7% for UV Titan M262, whereas the Z.average , for PC105 was a factor of three higher (500 nm ± 6%), but in agreement with the manufactures instructions (Table 1).

Effect of pH on the particle size and suspension stability

The influence of the pH (adjusted by HCl / NaOH) on the suspension stability of the three materials was tested in DI water (Figure 7).

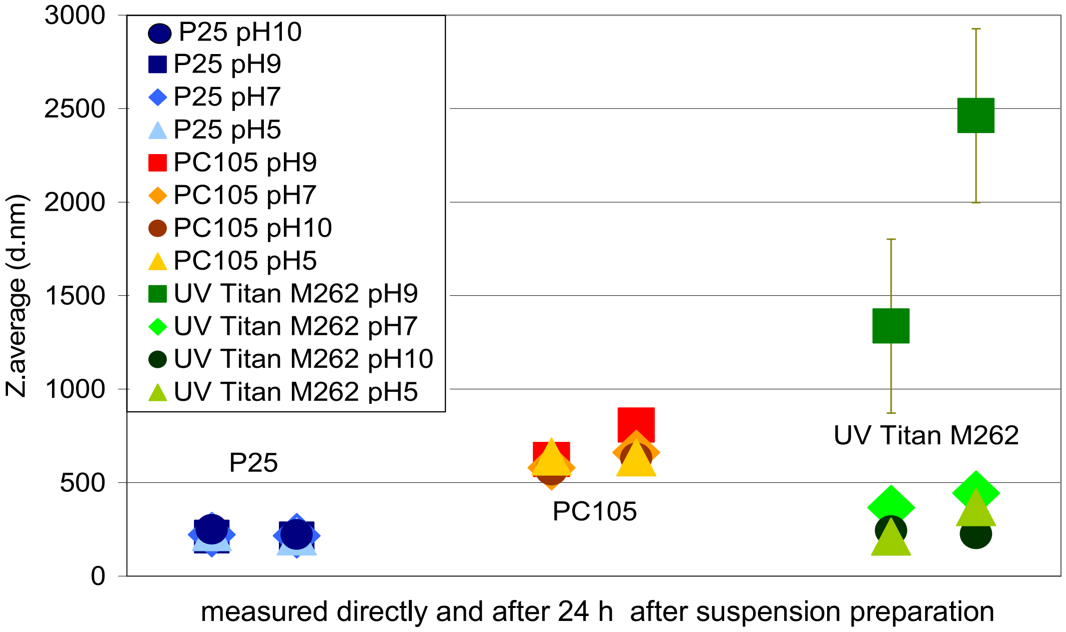


Figure 7: pH dependent DLS measurements of the Z.average of P25, PC105 and UV Titan M262 at pH 5, 7, 9 and 10 in DI water with SD as error bars; n = 3.

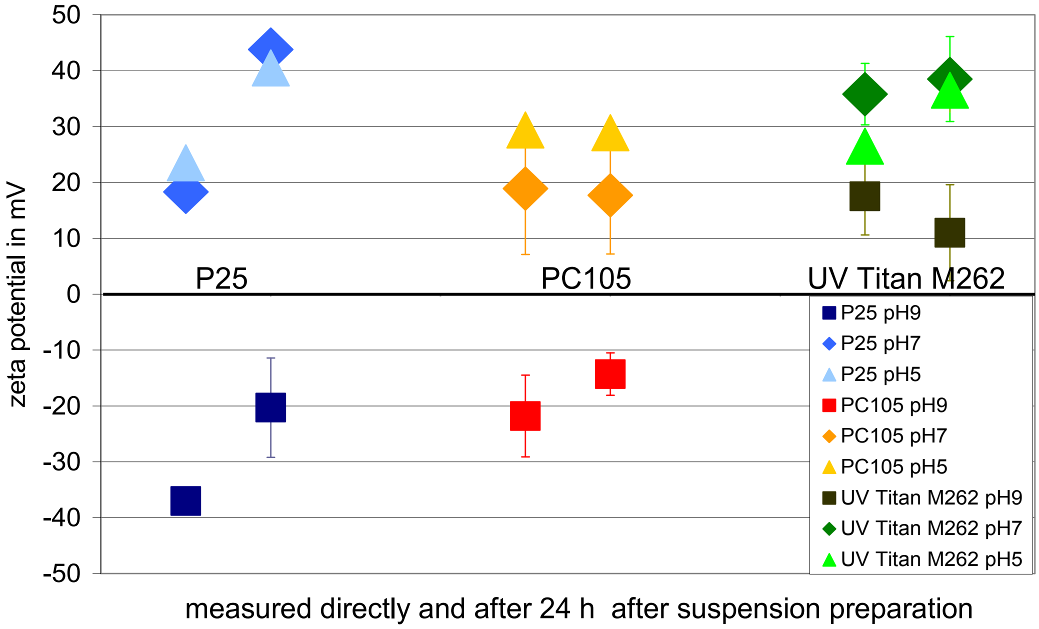


Figure 8: pH dependent measurements of the zeta potential of P25, PC105 and UV Titan M262 at pH 5, 7, and 9 in DI water; SD as error bars; n = 5.

For P25 no pH effect on Z.average at the tested pH values could be detected. For PC105 increasing agglomeration size with increasing pH was observed. At pH 9 agglomeration and sedimentation was visually detected, the same was seen for UV Titan M262 with the highest agglomeration at pH 9 the isoelectric point (IEP) of this material. The detected IEP is comparable with the IEP of alpha aluminium oxide (IEP pH 9.1) (Rubio-Hernández et al., 2006, Shin et al., 2006).

In the pH range of the tested soils (pH 5.1 – 6.7) no destabilisation effect of the pH to the nanomaterial suspensions is expected and due to this no additive was used. But the stabilisation effect of SHP was additionally tested for PC105 and UV Titan M262 at different pH values – 6, 7, 8 and 9 to derive more information about the agglomeration behaviour with and without 1 wt% SHP (Annex I).

Conclusion suspension preparation in DI Water for the soil experiments

→ *Target Z.average of < 250 nm was achieved with a sonication time of 10 min for P25 and UV Titan M262 in 100 mL DI water (pH 5). This was not the case for PC105 which showed about a factor three higher particle sizes compared to P25 and UV Titan M262.*

→ *A pH dependent effect for PC105 and UV Titan M262 for both, zeta potential and Z.average was detected.*

→ *UV Titan M262 does not remain hydrophobic after sonication. It is assumed that the hydrophobic hydrosilicon coating is removed by this procedure. The Al2O3 coating seems to be not removed as deduced from the IEP.*

→ *P25 does not show large pH dependent size effects but significant changes in the zeta potential.*

→ *In the pH range of the tested soils (pH 5.1 – 6.7) no destabilisation effect of the pH to the nanomaterial suspension is expected and due to this no additive was used.*

2.2 Chemical analysis

This chapter focuses on the chemical analysis for Titanium performed for the detection of (titanium dioxide) TiO_2 in the different matrices, soils, sewage sludge and suspensions.

TiO_2 occurs in nature as rutile, anatase and brookite. Brookite form orthorhombic crystals and is not often used for commercial applications. The most often used forms are anatase and rutile. Anatase is industrially produced in large amounts due to its technological properties. Rutile is the most stable form and the equilibrium state at all temperatures. Upon heating the metastable anatase and brookite phases both convert to rutile. The TiO_2 is a white pigment, not soluble in water having acidic and basic properties.

Being insoluble in water, TiO_2 needs to be digested to a soluble form prior to quantitative wet chemical analysis. TiO_2 can be dissolved in strong acids under formation of Titanium salts. Using sulphuric acid, Titanium dioxide can be dissolved as Titanylsulphate ($TiOSO_4$ H_2O). The subsequent analysis may be performed by diverse detection methods, depending on the individual nature of the samples. Several methods are available depending on the form of the analyte and the matrix, respectively (e.g. mass or emission spectrometry).

General remarks concerning chemical analysis

Titanium is a ubiquitous metal which is present in several chemical forms at least in trace concentrations in the environment. Therefore, all glassware used for the chemical analysis was machine cleaned and rinsed with doubly quartz distilled water. Cleanliness was monitored by analysis of (procedure) blank. Calibration was done using commercial standard solutions. Doubly quartz distilled water was used to prepare calibration solutions from traceable Titanium standards (CertiPUR 1.70243.0100) from Merck, Darmstadt, Germany. The standard solution is traceable to standard reference material (SRM) of the National Institute of Standard and Technology (NIST, USA). The standard solution contains 1004 ± 5 mg/L Ti as $(NH_4)_2TiF_6$ in H_2O. All samples were digested and analysed at least in duplicate. Blank and reference materials, as far as available, were analysed together with project samples. Limit of detection (LOD) was estimated by analysis of calibration standards of known concentration. At least five calibration points were measured. All measurements were done using external calibration. Calibration curves were completely linear with $R^2 > 0.95$. For measurements performed by ICP/MS the LOD was < 1 µg/L. For ICP/OES measurements the LOD was < 5 µg/L.

Analytical methods: Detection of Titanium in this study

The concentration of the Titanium in the suspensions and environmental samples were analysed by Inductively Coupled Plasma - Mass Spectrometry (ICP/MS) or Inductively Coupled Plasma - Optical Emission Spectrometry (ICP/OES). The operating mode of the two methods is described in the following section.

Analysis by ICP/MS

An inductively coupled plasma (ICP) contains a sufficient concentration of ions and electrons to make the gas electrically conductive. It is sustained in a so called torch placed inside an induction coil supplied with a radio-frequency electric current. A flow of argon gas (usually 14 to 18 L/min) is introduced to ignite and maintain the plasma. The temperature of the plasma is about 10,000 K.

Another flow (app. 1 L/min) of gas is introduced into the central tube of the torch. This gas flow passes through the centre of the plasma, where it forms a channel that is cooler than the surrounding plasma but still much hotter than a chemical flame. Samples to be analysed are introduced into this central channel.

As a droplet of nebulised sample enters the central channel of the ICP, it evaporates and, at least in theory, any solids that were dissolved in the liquid vaporize and break down into atoms.

For coupling to mass spectrometry, the ions from the plasma are extracted through two cones into a quadrupole mass spectrometer. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

Analysis by ICP/OES

The abbreviation ICP/OES stands for inductively coupled plasma optical emission spectrometry. Inductively coupled plasma is used to produce excited atoms. During relaxation excited atoms release electromagnetic radiation of very specific wavelength which may be employed to identify the element. The intensity of this emission is indicative of the concentration of the element within the sample. In practice, all elements emit several different wavelengths.

ICP/OES is usually less sensitive than ICP/MS. Additionally, ICP/OES is not applicable to all elements because some elements emit in areas of the electromagnetic spectrum difficult to detect. Another problem may arise by the presence of unspecific emissions of the matrix. Titanium, however, is easy to detect at several wavelengths including 337.280 nm and 336.122 nm.

ICP/OES analysis was done in cooperation with the Institute for Geosciences (IGEOS), University of Heidelberg. All analysis was done using an ICP/OES system “Vista MPX” of Varian (now Agilent; USA) equipped with a conical glass vaporizer and a cyclone vaporizer. Measurement of samples digested by aqua regia was done using an inert V-slit vaporizer and an inert Sturman-Masters cyclone vaporizer.

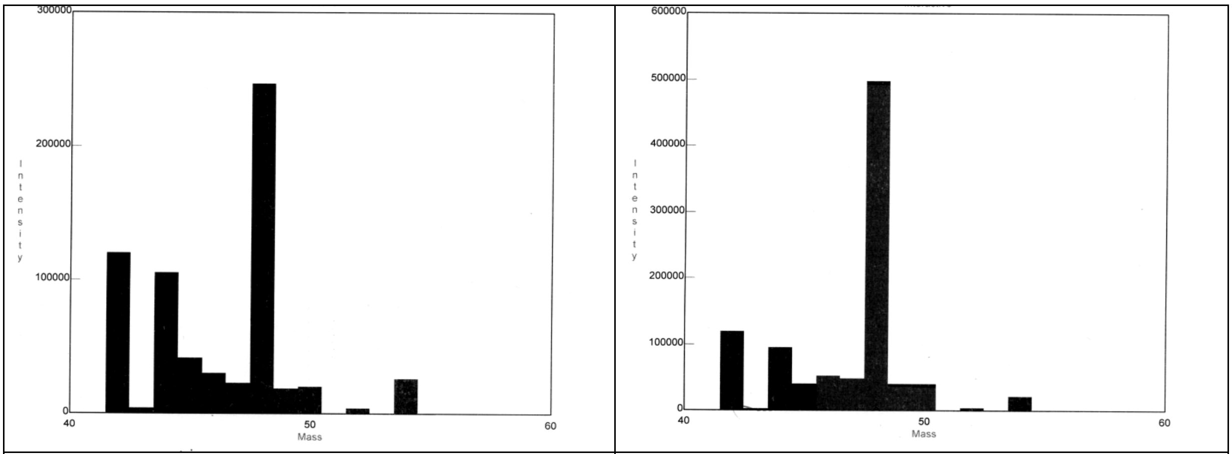
ICP/MS: Analytical problems

The analysis with ICP/MS (LOD < 1 µg/L) is more sensitive as the analysis with ICP/OES (LOD < 5 µg/L), but interferences occurred by the ICP/MS analytic.

Like any other analytical procedure, ICP/MS may produce erroneous results and in contrast to other metals, analysis of Titanium is not a standard or routine task and analytical problems resulting from extreme matrices or differences in matrices may be underestimated. In case of ICP/MS interferences may result from isotopes or molecules showing similar m/z relations like certain Ti isotopes (Figure 9).

Table 5: Ti isotopes and isobare overlap with molecular ions and isotopes of other elements.

Isotope (Ti)	Mass	Prevailance (%)	Interference
46	45.9526	8.0%	Ca, CO_2, SiO, NO_2, Zr^{+++}
47	46.9518	7.3%	NO_2, PO, SiO, CCl, Zr^{++}
48	47.948	73.8%	Ca, ArC, CCl, SO, NO_2, PO, Zr^{++}, POH
49	48.9479	5.5%	PO, CaH, CCL, HSO, SOH
50	49.9448	5.4%	Cr, V, SO, CCl, ArC, ArN, HSO



Ti calibration solution 50 µg/L

Ti calibration solution 100 µg/L

Figure 9: Counts for the 40 to 60 amu (atomic mass unit) analysing Titanium calibration solutions. A mass from 46 - 50 relate to Titanium.

Elements usually consist of stable mixtures of isotopes. Therefore, relation of different isotopes should be more or less stable. For chemical analysis the most suitable isotope is selected, depending on the LOD and low to none interferences. Figure 9 depicts mass spectra of Titanium calibration solutions of different concentrations. Whilst the above mentioned masses of Titanium double their intensity, the observable background masses close to the mass of Titanium keep their original intensity. Mass 48 clearly shows highest intensity, followed by 46 and 47, 49 and 50 are slightly smaller and of the same size.

For the chemical analysis of the Titanium the suspension and environmental samples has to be digested. For first studies the classical molten $KHSO_4$ method (Annex II) was used as digestion media, later H_2SO_4 or HCl, HNO_3, HF digestion was used (described later on in this chapter) for the sample preparation.

By using sulphur containing media interferences occurred with the mass spectrum of Titanium (Figure 10 and Figure 11). However, the background intensity increases with increasing salt concentrations. Figure 10 shows the mass spectrum of $KHSO_4$ solution having about the same concentration like samples after digestion. Obviously, background noise in the area of Titanium is strongly increased with strong peaks showing at masses 48, 49 and 50. Masses 46 and 47 show lowest interferences, however, there are measurable background signals, possibly interfering.

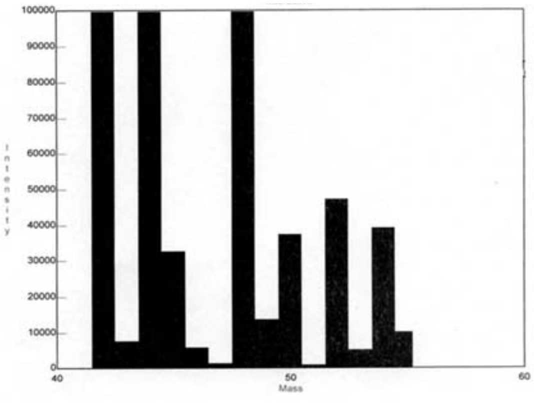
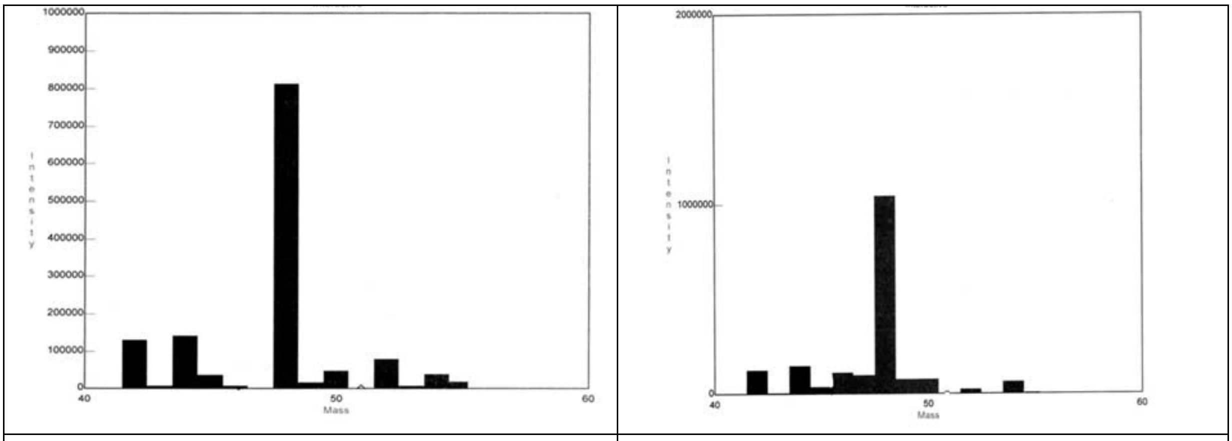


Figure 10: Mass spectrum of $KHSO_4$ solution.

Figure 11 compares mass spectra of a blank value and an activated sludge sample. As seen before there is a strong peak at mass 48 lying over the most abundant isotope of Titanium.

Therefore, sulphur containing analytical matrices like recommended in most analytical procedures seems not to be suitable for the detection of low Titanium concentrations.



blank value

activate sludge

Figure 11: Mass spectra of samples.

However, at elevated concentrations of the analyte, the error is normally small. Unfortunately, most of the samples within this project contained low Titanium concentrations. Therefore, the ICP/MS method was abandoned and analysis was done with ICP/OES.
ICP/OES shows only minor interferences with the matrix and therefore seems to be much more suitable for the analysis of small concentrations of Titanium dioxide in sulphur rich matrices.

Comparison of ICP/MS and ICP/OES Analysis

As described before, significant problems with the analysis with ICP/MS evolved if samples were digested by H_2SO_4 or $KHSO_4$ containing low concentrations of Ti. Figure 12 depicts results for Ti analysis made by ICP/OES and ICP/MS using the same samples digested with H_2SO_4/microwave. Titanium concentrations in these samples were comparably high and comparison clearly indicates equivalency of both methods.

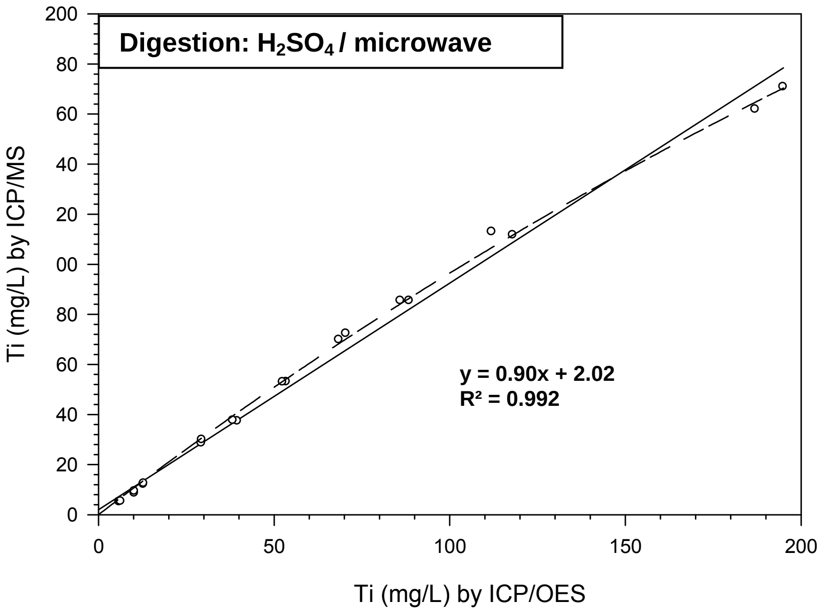


Figure 12: Comparison of Ti analysis by ICP/OES and ICP/MS. All samples were digested using the H_2SO_4 / microwave method.

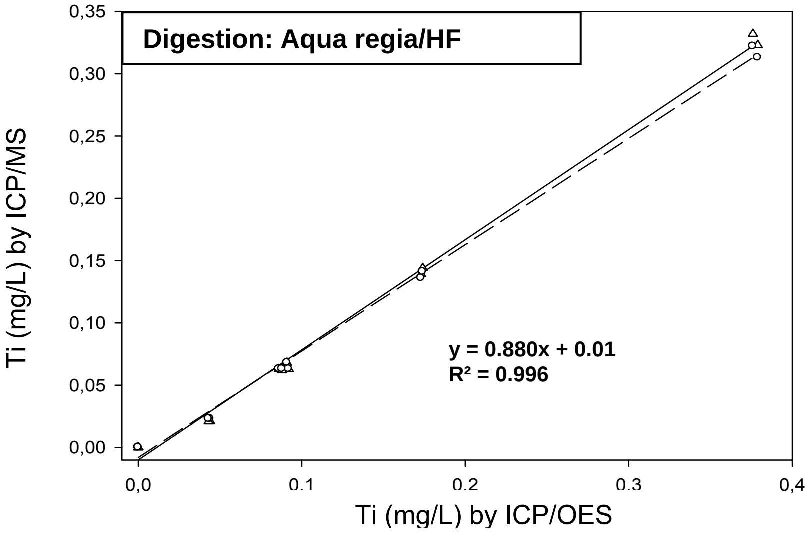


Figure 13: Comparison of Ti analysis by ICP/OES (IGEOS) and ICP/MS two different by two different laboratories. All samples were digested using aqua regia/HF. Circles: ICP/MS measurement of HMM, triangles: ICP/MS measurement of IGEOS.

Samples digested using aqua regia/HF and following analysis by ICP/MS and ICP/OES show comparable results, also at the lower end of the concentration range. Figure 13 compares series of sample analysis by ICP/OES and two independent series of analysis by ICP/MS, performed in different laboratories. Linear regression analysis shows $R^2 = 0.997$. However, all results are lower as compared to ICP/OES. No specific reason can be given since both methods were calibrated with the same standard.

Analytical methods: Digestion

For the analysis of waste water and sludge samples, the organic matrix needs to be destroyed. Additionally, Titanium dioxide needs to be transformed into a soluble form. Sulphate is the common and most suitable form for chemical analysis.

Methods for Titanium analysis tested

For analysis, standardised methods are available:

1. DIN EN ISO 11885 recommends for most analytes aqua regia. However, for TiO_2 detailed instructions are given in the Annex II. According to the method described there, TiO_2 should be dissolved using a mixture of ammonia sulphate and sulphuric acid.

2. The “classical method” for the digestion of TiO_2 minerals is digestion in molten $KHSO_4$. This method is described in textbooks whilst there is no standardised method available. Heating of $KHSO_4$ to 250°C releases water leaving potassium bisulphate or pyrosulphate behind. The latter serves as oxide ion acceptor and therefore serves as the final digestion agent. Higher temperatures will lead to the formation of SO_3 and decomposition of the digesting agent. During the first part of the project soil samples were analysed using digestion in molten $KHSO_4$. To this end, 50 mg of soil or sediment were placed in a platinum digestion vessel. Three grams of $KHSO_4$ were added and mixed with the sample. This mixture was heated up three times until white fumes are formed. The resulting cake is dissolved under heating in 4 mL concentrated sulphuric acid (24%). The white coloured solution is transferred to a volumetric flask and filled up to 1 L. Usually, samples were diluted 1:10 for the analysis.

Two further methods for the digestion of TiO_2 have been investigated by the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in Schmallenberg, Germany within the context of a research project. These methods were adopted for the purposes of this project and are detailed below.

Aqueous and sewage sludge samples: Digestion using aqua regia/HF

3. Titanium dioxide in aqueous matrices is digested using a mixture of aqua regia and HF. Using this method, the organic matrix is probably destroyed by aqua regia and Titanium is transformed into a stable fluoride salt.

Up to 10 mL of sample (depending on the type of sample, details are given in the result tables) were transferred into a plastic test tube for centrifugation. Samples were concentrated to about 100 µL using a commercial concentration apparatus (Turbova, Zymark, Germany) at 70°C under a light nitrogen stream. After addition of aqua regia (2.4 mL HCl, 36 - 38% J.T. Baker Ti < 0.2 ppb) and 0.8 mL HNO_3 (69%, Ti < 0.5 ppb) and 0.8 mL of HF (48%, Roth supra, Ti < 1 ppb) the mixture was vortexed for 1 min. The reaction vessels were placed in an ultrasonic bath (Sonorex RK 510S) and digestion was finished within 30 min. Caps of the reaction vessels must not be closed tightly because reaction gases may be formed. Build up of pressure must be avoided at any time.
For the destruction of residual HF, 1 mL of boric acid solution (4%, Merck, Germany), was added. Finally, the digestion solution still containing solids was centrifuged at 4000 rpm for 20 min and the supernatant was diluted and measured as indicated in the results tables.

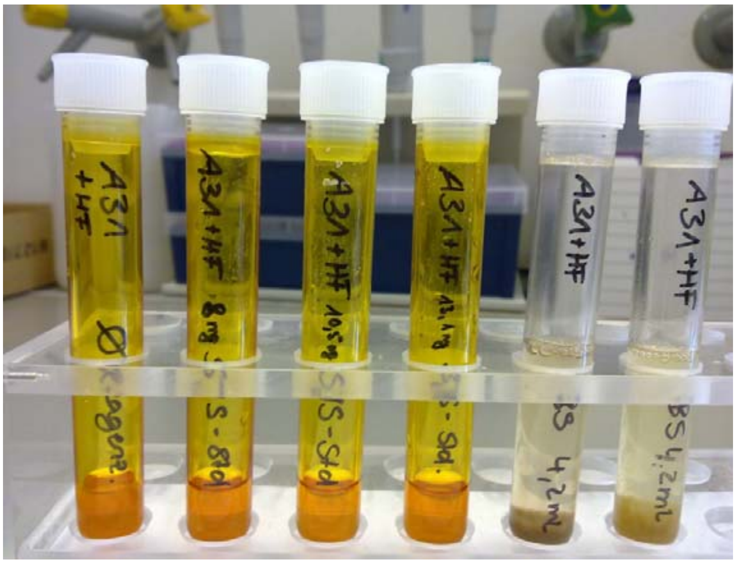


Figure 14: Digestion of samples using aqua regia/HF. Yellow coloured (first four samples of the left) samples were completely dissolved whilst the two samples on the right side contained solid residues.

As mentioned before, this method was adopted from IME. All liquid samples should be dissolved using this method. However, it turned out that liquid samples of 4 mL were not digestible using this method. The reason for this is that the digesting acid mixture is diluted by the sample in a way that digestion reactions are hindered. The sense of aqua regia is to form chlorine in statu nascendi being able to oxidize most structures. Additionally it may be speculated that HF transforms TiO_2 to its fluoride salt. However, if the sample is diluted too much, reactive species from aqua regia cannot form and digestion is incomplete. Figure 14 depicts six samples after digestion. Samples on the left side were concentrated to 100 µL like described before whilst samples on the right side were not concentrated and contain residues. Therefore, all samples digested using this method were concentrated prior to digestion.

Analysis of standardized suspension materials

With this adapted digestion method the aqueous samples were prepared. Figure 15 depicts a histogram for results of TiO_2 particles in suspension prepared by project partners. The preparation of suspensions is detailed later. However, for these data the interpretation is more difficult because less data points are available. Therefore results for the analysis of different TiO_2 particles are summarized in Figure 15. Only for suspensions containing relatively high TiO_2 concentrations (reference value: 5 mg/L) normal distribution is visible (with two outliers) whilst for concentrations of 1 mg/L, 0.1 mg/L, and 0.01 mg/L the shape of the resulting distribution is not clear.

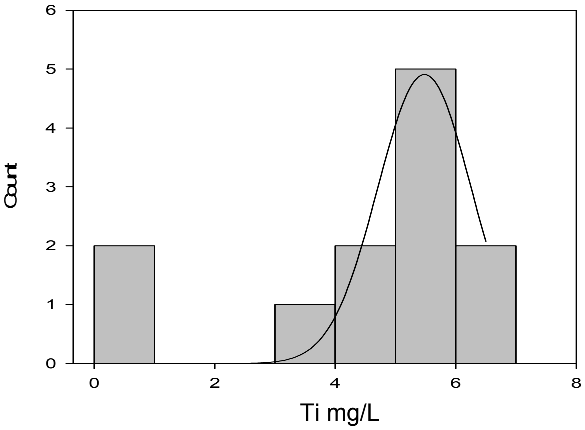


Figure 15: Histogram of TiO_2 particle suspension containing 5 mg/L TiO_2. Data are normally distributed with the exception of two outliers.

Figure 16 compares target reference and measured values for three different standard materials. Obviously, the error of the measurement (1σ) is not concentration dependent but varies over the concentration range. Therefore, an average error for the analysis of suspensions has to presume. Average error for Titanium determination of suspensions was determined to be 16%. Compared to other analysis, this increase in error may be explained by stability problems of the suspension. A noticeable deposit was formed of the bottom if the test tube was standing undisturbed for more than half an hour. Although the suspension was vigorously shaken prior to each analysis, increased errors may result from this instability.

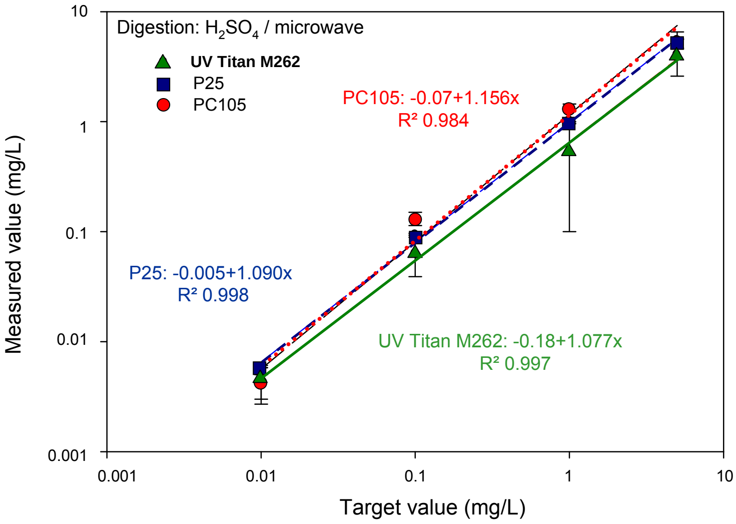


Figure 16 Target vs. measured Ti concentrations. Linear regression for each nanomaterial

Soil, samples: Microwave / H_2SO_4 digestion

4. The other method employed here is closer to “classical” methods for TiO_2 digestion. Samples are simply digested by concentrated H_2SO_4, however, using high pressure digestion vessels allowing a much higher digestion temperature.

An exact amount of soil (about 200 mg) is placed in a Teflon reaction vessel. 5 mL H_2SO_4 (95%, Roth supra, Ti < 1 ppb) is added. The reaction vessel is placed in an automated microwave digestion apparatus (MLS Ethos plus) and digested by heating up to 218 °C within 1 h and holding the temperature for 30 min. Samples are allowed to cool to room temperature and are filled up to a defined volume. Samples are centrifuged at 4000 rpm for 20 min, and supernatant is passed through a 0.45 µm membrane filter. For measurement, samples were diluted to appropriate concentrations suitable for the analytical method.
A comparison study between the Fraunhofer Institute in Schmallenberg (IME) and the Heidelberg University Hospital, Hygiene and Medical Microbiology (HMM) using the analytical method described before was performed prior to the analysis of the samples. The purpose of this part of the project was simply to show reproducibility of the method. Therefore three uncertified soil reference materials as well as the above mentioned SRM 2709a were analysed in parallel.

Figure 17: SRM 2709a after digestion. The white dispersion is centrifuged prior to analysis.

Figure 17 depicts SRM 2709a “San Joaquin Soil” after microwave digestion described above but before centrifugation. Obviously not all components of the material are digestible. However, the opaque-white dispersion may be separated by centrifugation. The identity of the white dispersing particles was not analysed.

Analysis of standardized soil materials

For the purposes of this project, SRM 2709a “San Joaquin Soil” was purchased as a reference soil. It contains a certified value for Titanium (0.342 ± 0.024%) based on neutron activation and x-ray fluorescence analysis. This reference soil was selected because it contains a certified value for Titanium (0.342 ± 0.024%). Analysis of a certified soil was done to elucidate whether digestion of the matrix is sufficient, whilst TiO_2 suspensions prepared and delivered by project partners were analysed to investigate digestion of TiO_2 particles. Unfortunately, no certified material containing a known concentration of TiO_2 particles is available.

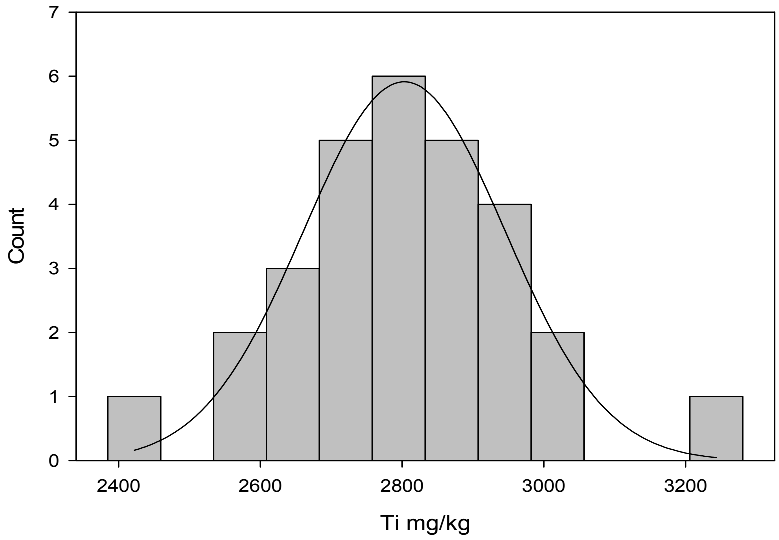


Figure 18: Histogram for San Joaquin Soil (SRM 2709a) after sulphuric acid/microwave digestion method. Mean value was 2801 ± 173 mg/kg, normally distributed.

Figure 18 displays a histogram for 29 independent analysis of SRM 2709a. All samples were digested using the “sulphuric acid/microwave method” detailed later. Mean value was 2801 ± 173 mg/kg (0.280 ± 0.017%). These results are significantly lower than concentrations indicated in the certificate of the SRM which most likely is due to the chosen digestion method. Not all Titanium bound in a mineral matrix is dissolved by the chosen method.

2.2.1 Recovery in different matrices

Based on the results of the analytic and discussion with the co-project FKZ 3709 65 416 and 3709 65 418 (both coordinated by IME) following digestion methods were used (Table **6**):

Table 6: Overview of the applied digestion methods for the different matrices.

	Stock suspensions (DI water and SDW)	**Laboratory sewage treatment plant - effluent**	**Laboratory sewage treatment plant - sludge**	**Soil - eluate**	**Soil – matrix**
HCl, HNO_3, HF	√	√	√	√	-
H_2SO_4 microwave	-	-	-		√

Further to the validation of the principal analytical method the identification and calculation of the Titanium in the different matrices are crucial information for further data interpretation and conclusion. Therefore an evaluation of the different digestion methods for different matrices was conducted to test for the recovery of TiO_2 nanomaterials.

Stock suspensions in synthetic drinking water – Laboratory sewage treatment plant

The experiments in the laboratory sewage treatment plant (LSTP) were conducted with different concentrations of P25 – 100 mg/L, 50 mg/L and 10 mg/L (for details see chapter 3.1 Laboratory sewage treatment plant – OECD 303A). For the experiment P25 suspensions were prepared in synthetic drinking water (SDW), which was added to the system every 24 h. Before the sewage treatment plant tests were performed the recovery of three different P25 concentrations in synthetic drinking water was analysed (Figure 19).

The recovery analysis revealed a concentration dependent linear correlation ($R^2 = 0.99$) with lower recovery rates for stock suspensions of higher concentration. Overall an average recovery of 92 ± 3% (arithmetic mean) and 88% for the slope of the stock suspension in synthetic drinking water was determined (Figure 19). Therefore an average recovery of 90% for Ti detection, based on arithmetic mean and slope, from particulate TiO_2 is derived.

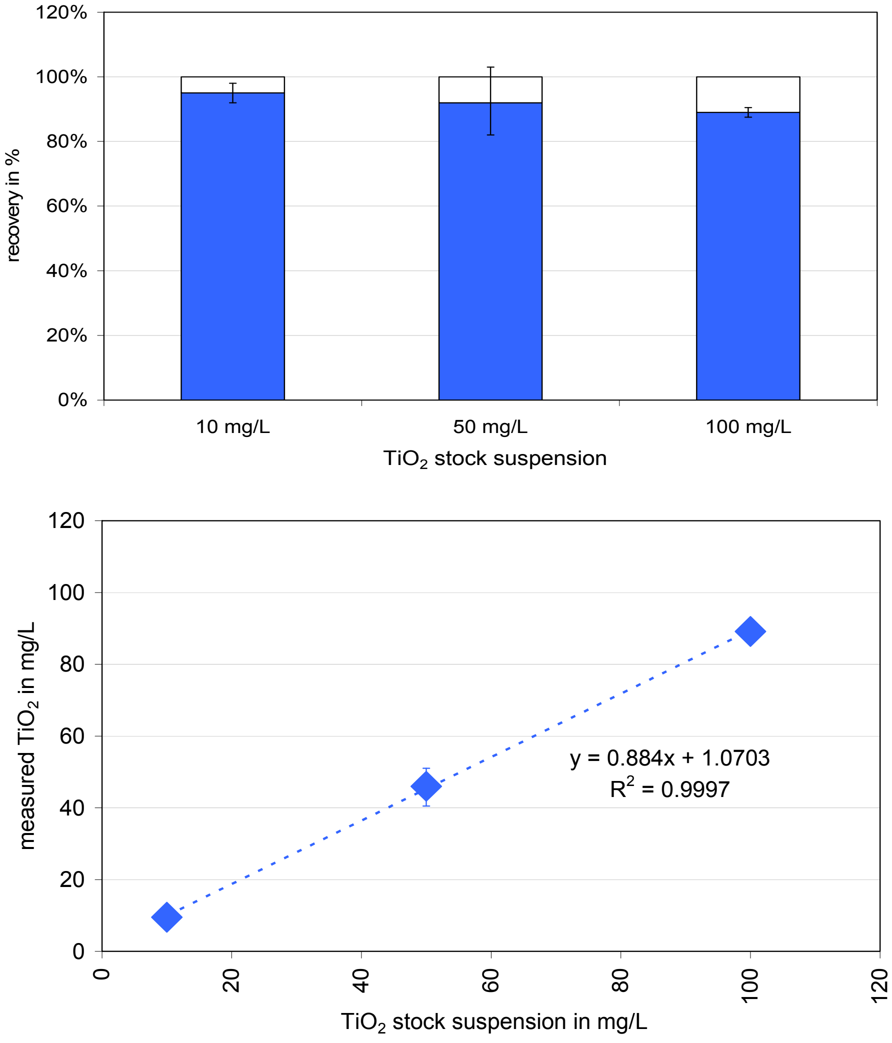


Figure 19: Recovery (upper and lower figure) of the TiO_2 content from the stock suspensions in synthetic drinking water using HCl, HNO_3, HF digestion. Error bars = min and max; n = 2.

➔ *A concentration dependent linear correlation of the recovery rate was found with an average recovery rate of 90% for SDW. The variability between the results of the different concentrations was within the range of the uncertainty of the detection method.*

Stock suspension in deionised water – soil experiments

All three different TiO_2 materials (solid) had to be suspended in deionised water (see also chapter 3.2 Leaching experiments in soil columns – OECD 312) before they could be used in the leaching experiments in soils as well as for the adsorption experiments. Therefore the recovery rate for Titanium in deionised water was tested for three different concentrations for each TiO_2 material. A linear correlation between stock suspension and measured concentration with a $R^2 = 0.99$ was detected for all materials tested (Figure 20) with slopes ranging from 1.03 to 1.23.

The lowest recovery rate (64% - 80%) was always determined for the lowest Titanium concentration of 10 mg/L while the highest recovery rates were found for the concentrations of 1000 mg/L and 5000 mg/L (103% - 149%). This indicates a concentration dependent and linear recovery rate for all materials (Figure 21).

A material dependent recovery rate is also indicated by the data, which vary between arithmetic average recovery rates of 92% and 114%.

The coating of the UV Titan M262 or the different crystal forms of the materials could be the reasons for this finding: PC105 100% anatase; P25 86% anatase, 14% rutile; UV Titan M262 100% rutile. The lowest recovery was detected for the rutile form and the maximum recovery for the anatase crystal form.

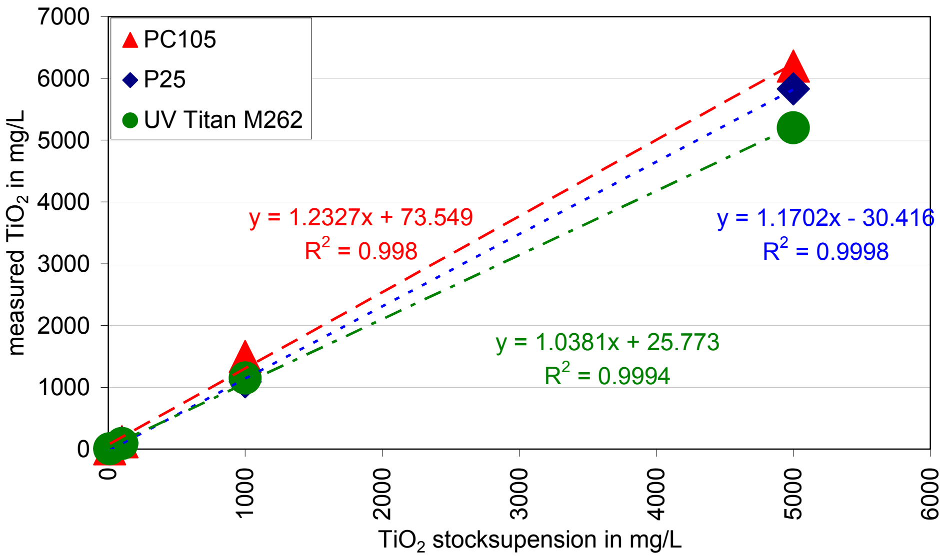


Figure 20: Linear correlation of TiO_2 concentrations in DI water as prepared and measured using HCl, HNO_3, HF digestion. Stock suspension concentrations 10 mg/L, 100 mg/L, 1000 mg/L and 5000 mg/L. Error bars covered by the symbols; n = 3.

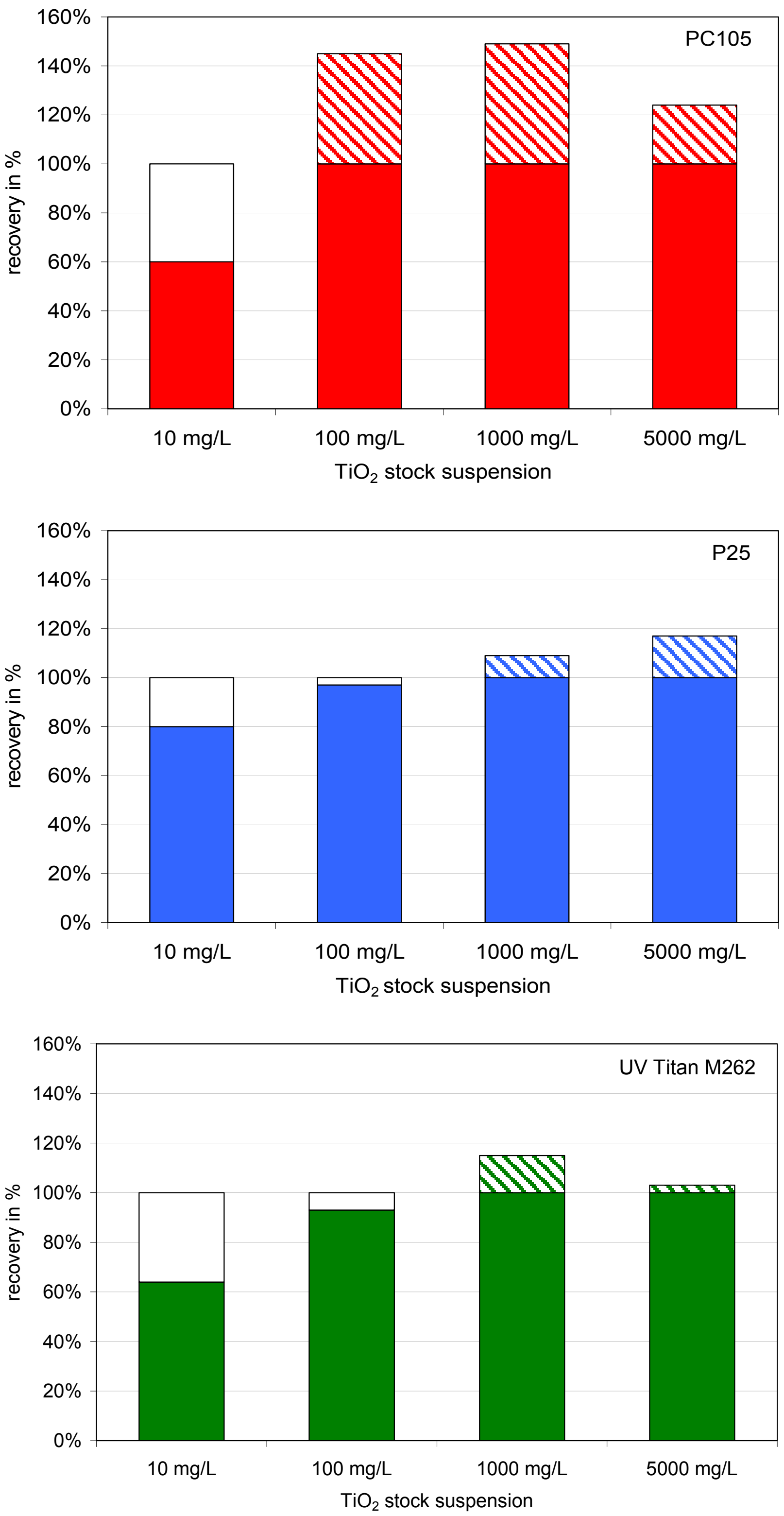


Figure 21: Ti recovery rate for the three different TiO_2 nanomaterials in DI water using HCl, HNO_3, HF digestion. Four concentrations: 10 mg/L, 100 mg/L, 1000 mg/L and 5000 mg/L. PC105 (upper figure), UV Titan M262 (central figure) and P25 (lower figure). Filled part of the bar = measured recovery value.

In summary a material, concentration dependent and linear correlated ($R^2 = 0.99$) recovery was determined. The overall arithmetic average recovery was determined to be 105% ±

42%. The slopes for all materials ranged between 103% and 123 % with negligible Y-axis values. With the concentrations measured mainly at the lower end of the concentration range tested here an average recovery of 100% in DI water is assumed.

➔ Taking into account concentrations measured in project samples, a *recovery rate (average) of 100% in DI water is assumed. The high variance with values differencing by 50% have to be noted.*

Recovery in sewage sludge

In the laboratory sewage treatment plant experiment, the sewage sludge beside the effluent was analysed for its TiO_2 content to conduct the mass balance for the P25 in this system. To derive information about the recovery rates, sewage sludge of the reference system was spiked with different TiO_2 stock suspensions. A linear recovery with an R² = 0.98 of the added P25 was identified with an average arithmetic recovery rate of 58% and 37% based on the slope was determined (Figure 22).

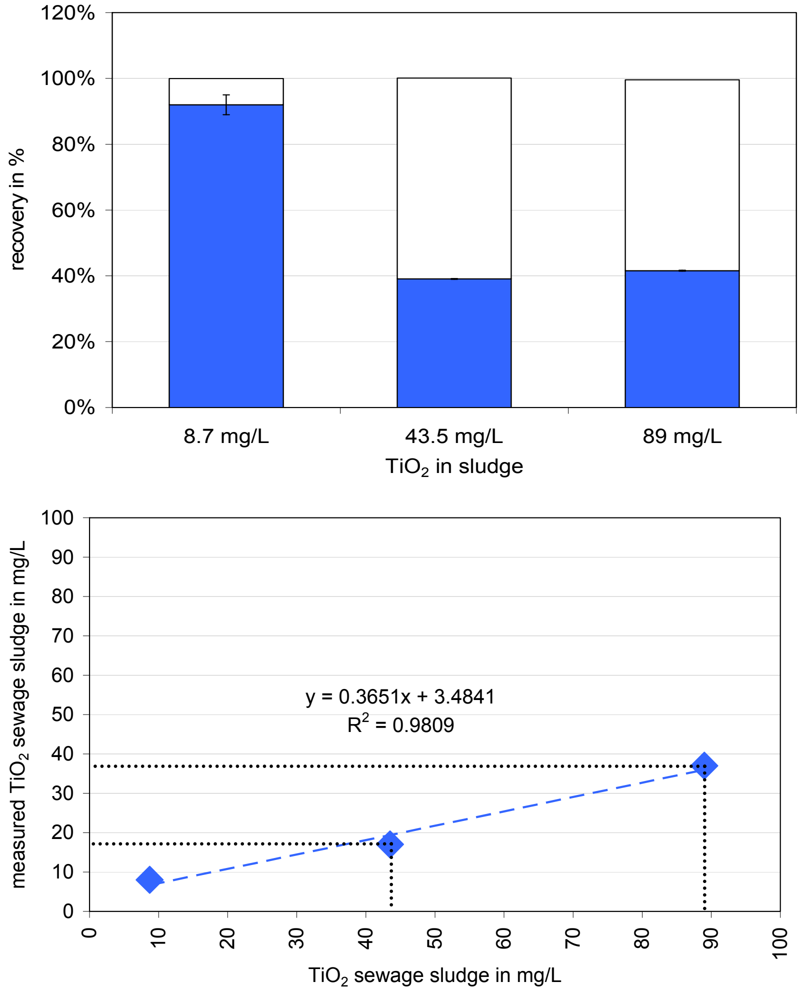


Figure 22: Recovery (upper and lower figure) of the TiO_2 content from spiked sewage sludge aliquots using HCl, HNO_3, HF digestion. Filled part of the bar = measured recovery value, error bars = min and max; n = 2.

An unsatisfying recovery rate of 39% and 42% for the two samples spiked with the highest concentration (43.5 mg/L and 89 mg/L) was detected.

The main part of the sewage sludge (about 75%) consists of organic carbon. The inorganic carbon is further reduced during the test through the removal of surplus sludge. Therefore an influence of the matrix on the recovery rate or interference with the analysis devices seems implausible (complete chemical digestion of the organic carbon).

The sample preparation could have an effect on the recovery rate, because a homogeneous spiking of the sewage sludge can be a problem, due to agglomeration of the particles in suspension by contact with the sludge, which could lead to an inhomogeneous distribution in the sample. The unsatisfying recovery rate of 39% and 42% of the samples spiked with the highest concentration (43.5 mg/L and 89 mg/L) can be an indication for this.

No clear explanation on the variance in the recovery rate can be given at this time. Based on the implausibility of this finding, the plausibility of the mass balance for the sewage plant experiments and the results for the stock suspensions we assume that the spiked sewage sludge could not be used and were treated as outliers. Based on the recovery rate of the stock suspensions in synthetic drinking water for P25 a recovery rate of 90% may be assumed for the interpretation of the mass balance in the sewage plants experiments.

➔ *An insufficient and implausible recovery was detected for samples with the two highest concentrations. Based on the results of the recovery rate for the other matrices and in the context of the chemical analysis, these results were treated as outliers and the recovery rate of the stock suspension in synthetic drinking water of 90% may be taken into account for interpretation of the mass balance of the sewage plant experiments.*

Recovery in soils

Two reference soils (reference soil materials SRM 2709a - San Joaquin Soil, NIST and the BCR 142 - light sandy soil, JRC) were analysed for their Ti content for a first method validation and further data comparison (Figure 23), as the analytical procedure was described in chapter 2.2. A comparison measurement with the co project FKZ 3709 65 416 and 3709 65 418 (lead by Fraunhofer IME) of different soil samples was conducted (results in Annex II).

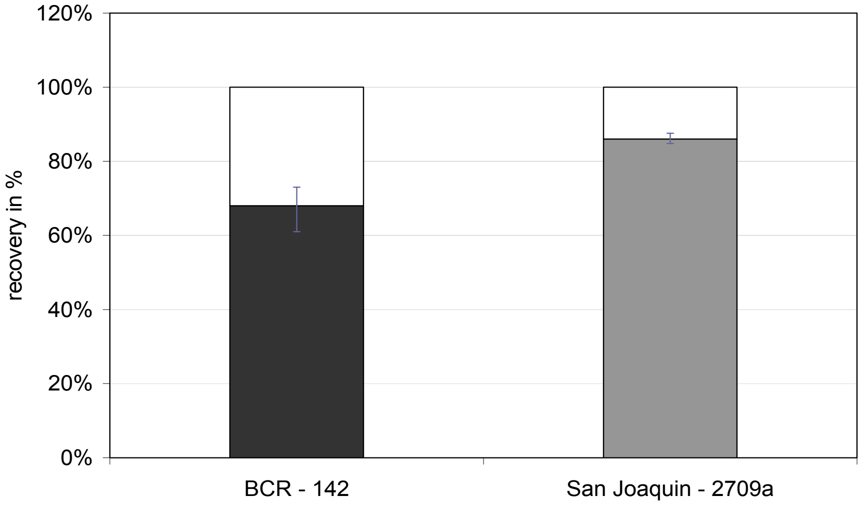


Figure 23: Recovery of Ti content of two reference soils - using H_2SO_4 microwave digestion. 100% is the value for the reference Ti content of the soils. Filled part of the bar = measured recovery value, error bars = min and max; n = 6.

It is expected that with the used H_2SO_4 microwave digestion, not all silicates and minerals of the soil were digested, therefore explaining the lower recovery rate of on average 72 ± 4%. This is not of relevance for the conducted experiments since only the recovery rate of added TiO_2, which was tested with spiked soils, is of relevance for the mobility analysis of the nanomaterial.

Uncorrected values were used for the following analysis, unless specifically indicated differently.

The recovery of the three different TiO_2 materials in the soil matrix was tested in different soils. First two soils (A01 and G03) were spiked with P25 to derive information about the recovery rate of the added P25 (Figure 24).

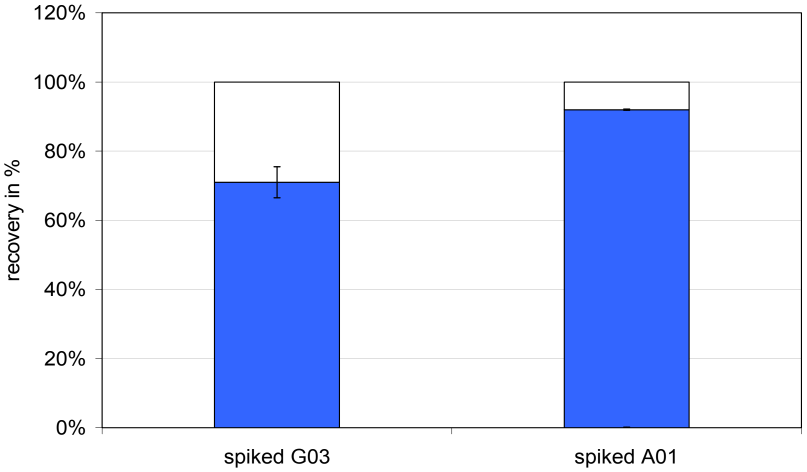
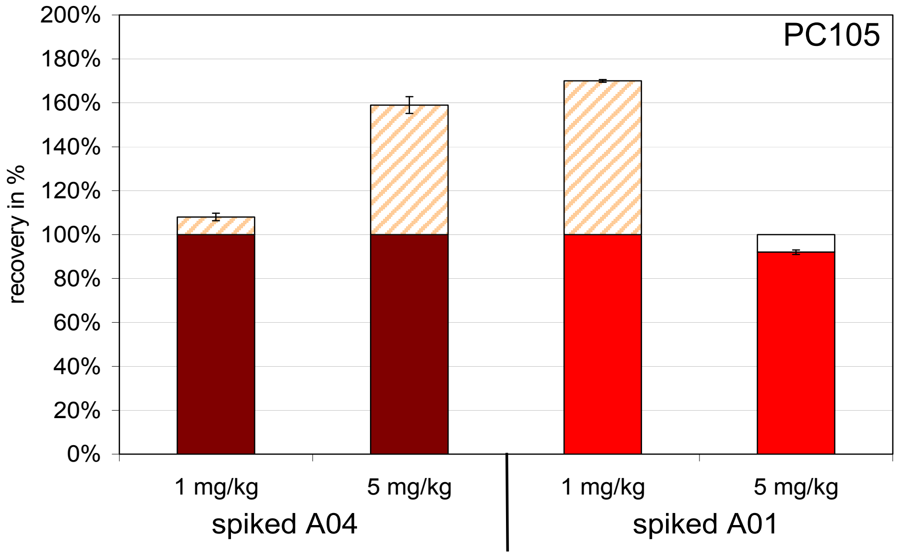


Figure 24: Recovery of P25 (40,000 mg/kg) in soil type G03 - Eutric Cambisol and A01 - Dystric Cambisol (H_2SO_4 microwave digestion. Filled part of the bar = measured recovery value, error bars = min and max; n = 2).

The recovery rate of P25 (40,000 mg/kg) was measured, to make sure that no matrix (different mineral content) or concentration dependent effect has an influence on the recovery rate. Analogue PC105 and UV Titan M262 were tested (Figure 25) by using lower concentrations (1 mg/kg and 5 mg/kg).



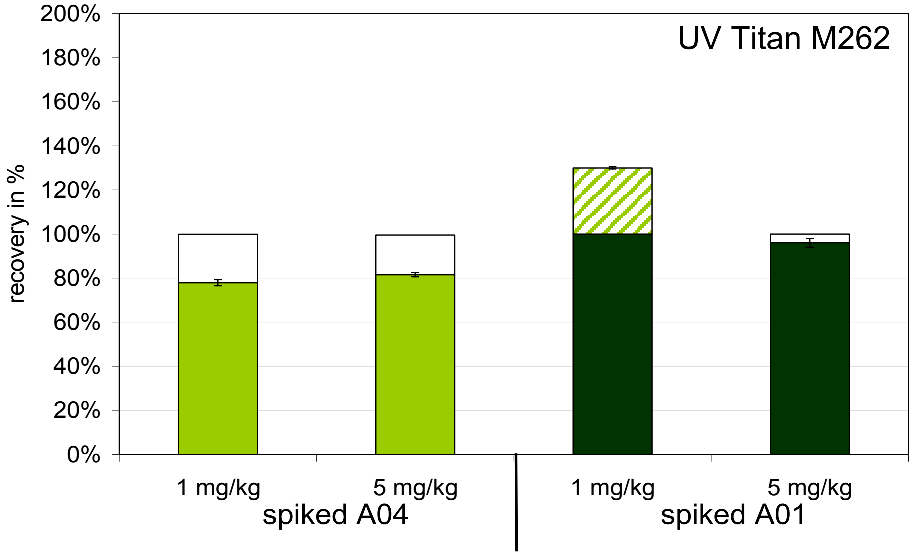


Figure 25: Recovery of PC105 (upper figure) and UV Titan M262 (lower figure) using a H_2SO_4 microwave digestion for soil type A04 – Gleyic Podsol and soil type A01 - Dystric Cambisol. Filled part of the bar = measured recovery value, error bars = min and max; n = 2.

The recovery rate of the two tested soils was 72 ± 4%. The low recovery can be due to the chemical digestion method chosen.
The recovery of P25 was 82 ± 12% for both soils with a higher recovery of 92% for soil A01. The average recovery for PC105 was 132% ± 40% and for UV Titan M262 96% ± 27% with a higher recovery in soil A01 with 113% and a lower recovery in soil A04 with 80%.
The recovery tests in the stock suspension showed a material dependent recovery with a higher recovery for PC105 and a lower recovery for UV Titan M262. Similar results were found for the spiked soils. If this was considered it could be that the type of the material could affect the recovery in soils, which we took into account for further analysis.

➔ *The recovery of the three different TiO_2 nanomaterials in a complex soil matrices showed a higher variance compared with the recovery of the stock suspension. The higher variance could be caused by matrix effects and the difficulty of the preparation of homogeneous samples of the spiked soils.*

A rough trend was shown with the highest recovery rate for PC105 (132% ± 40%), than UV Titan M262 (96% ± 27%) and than P25 (82% ± 12%).
The recovery rate of the different TiO_2 nanomaterials in the complex soil matrix is acceptable against the background of the natural high amount of Ti in natural soils and was within the range of the uncertainty of the detection method (chapter 2.2).
Overall a recovery rate of 100 % for TiO_2 in soil matrices is deduced from all values.

Conclusion

The highest variance of the analysis was detected for soils, the most complex matrix tested here. This finding may be due to the matrix itself or by the preparation of the spiked sludge or soil (inhomogeneous dispersion of the nanomaterials in the samples – local accumulation). A nanomaterial dependent recovery is indicated (Table 7):

- PC105 highest recovery for the liquid and the soil matrix,
- UV Titan M262 medium recovery the liquid matrix and lowest recovery in soil matrix,
- P25 the lowest recovery in the liquid and a medium recovery in soil matrix.

Table 7: Recovery rates in percent of the tested TiO_2 nanomaterials in different matrices.

	SDW – suspension average (min; max)	**DI – suspension average (min; max)**	**Sludge average (min; max)**	**spiked soil A01 average (min; max)**	**spiked soil A04 average (min; max)**	**spiked soil G03 average (min; max)**
P25	**92** (89; 95)	**101** (80; 117)	**58** (39; 92)	**92** (92; 93)	-	**71** (63; 79)
PC105	-	**121** (64; 149)	-	**132** (92; 170)	**133** (108; 159)	-
UV Titan M262	-	**94** (64; 115)	-	**113** (96; 130)	**80** (78; 82)	-

- ➔ *The overall recovery for the (liquid) samples for the LSTP experiment was 90%.*
- ➔ *The overall recovery for the stock suspensions for the soil experiments was 100%, with a trend of material dependent recovery: PC105 (121% ± 35%), P25 (101% ± 18%) and UV Titan M262 (94% ± 27%).*
- ➔ *The overall recovery of the three added TiO_2 materials in soils was 100%, with a trend of material dependent recovery: PC105 (132% ± 40%), UV Titan M262 (96% ± 27%) and P25 (82% ± 12%).*
- ➔ *The material dependency was used for the interpretation of the mass balance in the soil column experiments, but the values were not corrected for this recovery rates.*

3. Environmental tests

In the following section the results of following environmental tests are presented:

- OECD test guideline 303A – Simulation Test - Aerobic Sewage Treatment: Activated Sludge Units
- OECD test guideline 312 – Leaching in Soil Columns
- OECD test guideline 106 – Adsorption - Desorption - Using a Batch Equilibrium Method

The tests were performed according to the OECD Guidelines for testing chemicals. In principle the OECD Guidelines are established and used for testing of chemicals. Based on testing of soluble chemicals, the common metric mentioned in the OECD test guidelines is mass based.

The applicability of the test guidelines for nanomaterials were checked in this study and if necessary modified and adapted. Metrics other than mass, like surface area or number concentration, may be important for the testing of nanomaterials and possibly closer related to their behaviour. But the detection of the other metrics in complex media is nowadays not available for standard tests and shall be target metrics for further tests. Mass will be used until then to get information about the fate and behaviour in complex media as pursued in the present study.

3.1 Laboratory sewage treatment plant – OECD 303A

3.1.1 Methods

Principle of the test

The fate and behaviour of nanoscale TiO_2 in sewage treatment plants (STP) has been assessed in the laboratory sewage treatment plants (LSTP) simulation test according to OECD Guideline 303A (2001). In principle, this method is designed to determine the elimination and primary and/or ultimate biodegradation of water-soluble organic compounds in a continuously operated test system simulating the activated sludge process. But in principle the behaviour of inorganic compounds in STP may also be assessed. In this case the outcome of the study is the distribution of TiO_2 nanomaterial between activated sludge and the effluent rather than biodegradability. An easily biodegradable organic medium (organic synthetic wastewater, OSW) is added as primary source of carbon and energy for the micro-organisms to the LSTP. Each activated sludge plant unit consists of an aeration vessel with a capacity of about 3 L of activated sludge and a separator (secondary clarifier) which holds about 1.5 L. Usually, two continuously operated test units are run in parallel under identical conditions with a mean hydraulic retention time of 6 h and a mean sludge age (sludge retention time) of 6 to 10 days. The (organic) test substance is normally added at a concentration of between 10 mg/L and 20 mg/L dissolved organic carbon (DOC) to one of the units, the second being used as a reference unit 2 (without TiO_2) to determine the biodegradation of the organic medium.

The objective of the study was to determine the distribution of TiO_2 between activated sludge and the effluent and to calculate the overall TiO_2 mass balance of the flow-through activated sludge process.

The testing device of the one LSTP unit and the respective measurement points are shown in Figure 27. The aeration vessels of both units were aerated by sintered cubes and compressed air in order to maintain aerobic conditions and to keep sludge flocs in suspension. Additionally, the sludge was pumped intermittently from the separator to the aeration vessel (16 seconds every 15 minutes, ≈ 400 mL) to recycle ≈ 1.6 L per hour.

Study design

Synthetic drinking water and organic synthetic wastewater

Synthetic drinking water (SDW) according to DIN EN ISO 6341 (Annex III - A3-3 Synthetic drinking water) was used as matrix in order to have defined, comparable and reproducible test suspensions and conditions instead of tap water which is suggested by OECD. A preliminary test resulted in the observation that the buffer capacity of the synthetic drinking water was not sufficient in order to maintain a pH between 7.0 and 8.0. Therefore the 3.5 fold amount of sodium hydrogen carbonate compared to the standard concentration of DIN EN ISO 6341 was added. The SDW was continuously added via a peristaltic tube pump (BVP ISM 444, Ismatec SA Labortechnik-Analytik, Glattburg Switzerland) with neoprene tubing in both units (10.8 L per day respectively 12 L per day in unit 1 and unit 2).

The organic synthetic wastewater (OSW) consisting of peptone, meat extract, and urea was added as a concentrate via a perfusor pump to the activated sludge (Perfusor IV, B. Braun Melsungen AG, Melsungen, Germany). The use of the concentrate was intended to prevent losses through biodegradation before the dosage to the LSTP. As suggested in OECD guideline 303A 2.5 mL per hour was added which corresponds to half of the standard concentration of the OSW for simulating nitrifying conditions (Annex III - A3-4 Organic synthetic wastewater). The final concentration of the OSW corresponded to 50 mg/L DOC. The OSW was renewed every 24 hours

Evaluation and validity criteria

The OECD guideline recommends that preferably at least 15 valid values should be determined in the plateau phase, normally lasting three weeks, for the evaluation of the test result. In the present study a stepwise approach was decided while increasing the dosage of TiO_2 from 1 to 10 mg/L in order to obtain realistic and worst case influent concentrations.

The validity criteria require that the DOC elimination in the reference unit 2 (without TiO_2) is above 80% after two weeks. If the test is performed under nitrifying conditions (as it was in the study) the mean ammonium concentration in the effluents should be < 1 mg/L NH_4-N and the mean nitrite concentration < 2 mg/L NO_2-N.

Preparation of the TiO_2 suspension

The nanoscale TiO_2 (P25) was suspended in synthetic drinking water (SDW) (Figure 26) according to DIN EN ISO 6341 and stabilised with 1 wt% sodium hexametaphosphate (SHP) to reach a standard concentration of 100 mg/L. For this an ultrasonic homogeniser (200 W – Bandelin Sonoplus) was used for 40 min at a volume of 300 mL. The resulting TiO_2 suspension had an average particle size < 250 nm (DLS measurements).

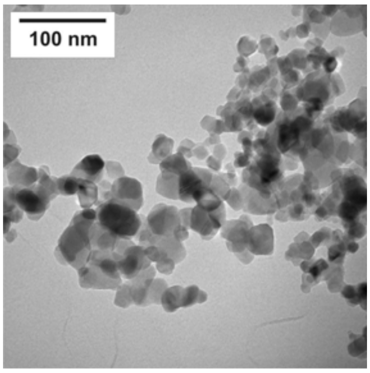


Figure 26: SEM scan of the P25 suspension for the LSTP tests.

Dosage of the TiO_2 suspension

Under continuously stirring (Variomag Mono Direct, H+P Labortechnik, Oberschleissheim, Germany) a tenfold concentrated suspension was added to reach a final concentration of 1 mg/L, 5 mg/L, and 10 mg/L TiO_2 and 1 g/L SHP (final concentration in the aeration vessel) in the aeration vessel by using a peristaltic tube pump (BVP ISM 444, Ismatec SA Labortechnik-Analytik, Glattburg Switzerland) with neoprene tubing.

The SDW contained the 3.5 fold amount of $NaHCO_3$ in order to maintain the pH. The suspension was renewed every 24 h. DLS measurements showed that the TiO_2 agglomerates had an average particle size in suspension < 250 nm diameter also when entering the activated sludge basin after the tube. Dosage into the test unit 1 (with TiO_2) was 1.2 L per day. In summary, a volume of 10.8 L SDW per day and 1.2 L TiO_2 suspensions with SHP per day) (total volume 12 L per day) was loaded through test unit 1 while unit 2 received 12 L SDW per day.

Procedure of the simulation test

The study included two trials with three different TiO_2 concentrations being added to the test unit 1 (with TiO_2) in addition to the OSW. The reference unit 2 (without TiO_2) was only fed with OSW (also without additive) and served as functional control of the test performance.

The study had to be divided into 2 trials due to a leakage in unit 1 (with TiO_2) when changing from 5 mg/L to 10 mg/L TiO_2 concentration. Trial 1 therefore consisted of an adaptation phase of seven days followed by seven days dosage of 1 mg/L TiO_2 and seven day dosage of 5 mg/L TiO_2 (final concentration in the influent). Trial 2 consisted of seven days dosage of 10 mg/L TiO_2. Dosage was carried out under continuous stirring of a ten-fold concentrated TiO_2 suspension (see above). The activated sludge was taken on April 26, 2010 (trial 1) and on May 24, 2010 (trial 2) from the municipal wastewater treatment plant Breisgauer Bucht, which treats predominantly domestic wastewater of 600.000 inhabitant equivalents. In total 2750 mL (trial 1) and 2000 mL (trial 2) activated sludge were added to both LSTPs. Dry solid of the activated sludge was determined by weight measurements after 2.5 h drying at 105 °C (mean of triplicate measurements). According to the OECD guideline the start concentration of the activated sludge concentration is 2.5 g/L and should be maintained in the range of 1 – 3 g/L though the removal of the surplus sludge which also determines the sludge age within a range of six days to ten days.

The hydraulic retention time of 6 h and the OWS were designed to maintain nitrifying conditions. After one week acclimatisation the test was run for one week per concentration. Sampling for TiO_2 measurement was carried out daily in the outflow and in the surplus sludge for enabling a TiO_2 balance.

On day 22 trial 1 was terminated, the total volume of the LSTP units was released and the TiO_2 in homogenised sample was determined. The activated sludge of test unit 1 (with TiO_2) was discarded and the activated sludge of reference unit 2 (without TiO_2) was distributed to both LSTP units and supplemented with fresh activated sludge from the municipal STP. The reason for resetting the study was a leakage in unit 1 (with TiO_2) at day 22. The use of the activated sludge from reference unit 2 (without TiO_2), which was pre-adapted to the OSW, was decided in order to avoid another lag phase at the start of trial 2.

Measurements

DLS measurements of the stock suspension

Before the suspension was added to the system, agglomerate size and zeta potential measurements were conducted with a Malvern Zetasizer ZS (the stability criteria were followed) – results see Figure 5.

ICP/OES analysis of the surplus sludge and the effluent

The sludge and the effluent of the test and reference system was analysed with ICP/OES after HCl, HNO_3, HF digestion (chapter 2.2) for balancing. Explorative SEM / EDX scans were conducted of the outflow and the sludge, after the experiment to get information about the behaviour in the systems (size of the agglomerates). For the SEM / EDX scan (Annex III - A3-6 SEM EDX scan of the sewage sludge of the LSTP) 10 µL of the test sample were transferred with a pipette to a sample holder and dried. The test sample was stirred for one minute prior to sampling to generate a homogeneous suspension.

Parameter describing the functionality of the treatment process

For the determination of the dissolved organic carbon (DOC) samples were filtered by 0.45 µm cellulose acetate filters (Weissrand, Schleicher & Schuell, Dassel, Germany). DOC measurements were performed using total carbon analyser (TOC-5000A, Shimadzu Deutschland, Duisburg). The measurement principle follows catalytically aided combustion oxidation at 900 °C after purging the dissolved inorganic carbon (carbonate carbon) with oxygen.

Ammonium, nitrite and nitrate concentrations in the effluent of the LSTP units have been determined photometrically through cuvette test (LCK 304, LCK 341, and LCK 340, HACH LANGE GmbH, Düsseldorf, Germany) as NH_4-N, NO_2-N, and NO_3-N.

The pH, the oxygen concentration in the activated sludge basin, and the temperature has been determined daily (WTW pH 330i, WTW Oxi 730, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

The filtratable solids in the effluents of the LSTP units are an indicator of the efficiency of the sedimentation of the activated sludge in the settling vessel. The filtratable solids were determined following the description of DWA (2003) by filtering volumes of 2 - 5 L through glass fibre filters (MN 85/70, diameter 110 mm, Machery-Nagel GmbH&Co KG, Düren, Germany) with the aid of a vacuum flask and measuring the increase in weight after drying at 105 °C. According to the filter specifications of the supplier this filter has a pore size of about 0.6 µm.

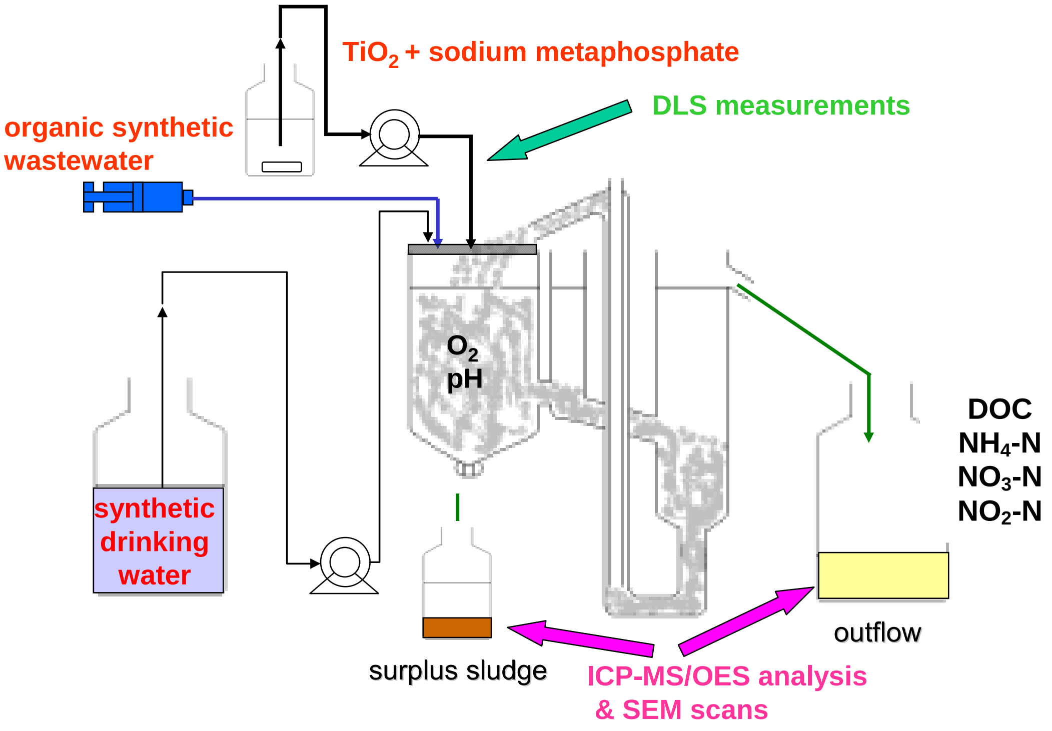


Figure 27: Testing device of the LSTP and measurement points.

3.1.2 Results

Stability of the TiO_2 suspensions

The objective of the study was to simulate natural effects as a realistic worst case. Thus, the TiO_2 dosage in nanoscale form to the activated sludge basin should be guaranteed. Pre-tests pointed out, that stable suspensions of 100 mg/L TiO_2 could only be prepared using 1 wt% of sodium hexametaphosphate and sonication. The resulting stock suspension and the TiO_2 suspension in the outlet of the peristaltic tube pump proved to be stable as measured on-site by DLS measurements (Malvern Zetasizer ZS). The use of a dispersant was somewhat counterproductive to the sedimentation of the activated sludge because the settlement of the activated sludge in the clarifier, which is an integral part of the treatment process, was reduced.

Ecotoxicological assessment of sodium hexametaphosphate in the fish embryo toxicity (FET) assay DIN EN ISO 15088:2009

The results of the LSTP showed an influence of SHP onto the sludge as an increase of suspended solids (activated sludge flocs) was recorded. Hence to evaluate the toxic potential of sodium hexametaphosphate a FET assay according to DIN EN ISO 15088:2009 was performed. SHP concentrations ranging from 0.01 to 1 wt% were investigated.
At the concentration steps of 0.5 and 1 wt% SHP 100% mortality was determined after 96 h post fertilization. At a lower concentration of 0.1 wt% SHP, which was the highest in the LSTP experiment, 30% mortality was detected and 20% of the larvae were underdeveloped or showed deformations. The next lower SHP concentration of 0.085 wt% showed a mortality of 15%. At 0.065 wt% SHP a mortality of 5% and at 0.05 wt% SHP no mortality after 96 h was calculated. The calculated EC_{50} (50% effect concentration) for SHP were after 48 h 0.27 wt% and after 96 h 0.16 wt% SHP (details in Annex III - A3-2 Ecotoxicological assessment of SHP in the fish embryo toxicity assay DIN EN ISO 15088:2009 - method). These results show that the use of an additive has to be carefully evaluated since it may be beneficial for the suspension preparation, however, it may also have toxic effects onto the test organism.

Functionality of the treatment process

The biodegradation and nitrification of the synthetic wastewater in the unit 1 (with TiO_2) was comparable to that of the reference unit 2 (without TiO_2). In the outflow of the test LSTP an increase of suspended solids (activated sludge flocs) was observed which is explained with the use of the dispersant SHP which had to be added for stabilising the TiO_2 suspension (Figure 28). At the beginning of each of the both trials a temporary decline of the DOC elimination was observed which is probably due to the acclimatization of the activated sludge to the dispersant SHP. After two and three days the sludge was adapted to the SHP and the DOC-elimination reached the plateau levels previous observed (above 97%). In the later progress of trial 1 and trial 2 the biodegradation extent of the OSW was above 95% for both LSTP (with and without TiO_2 + SHP).

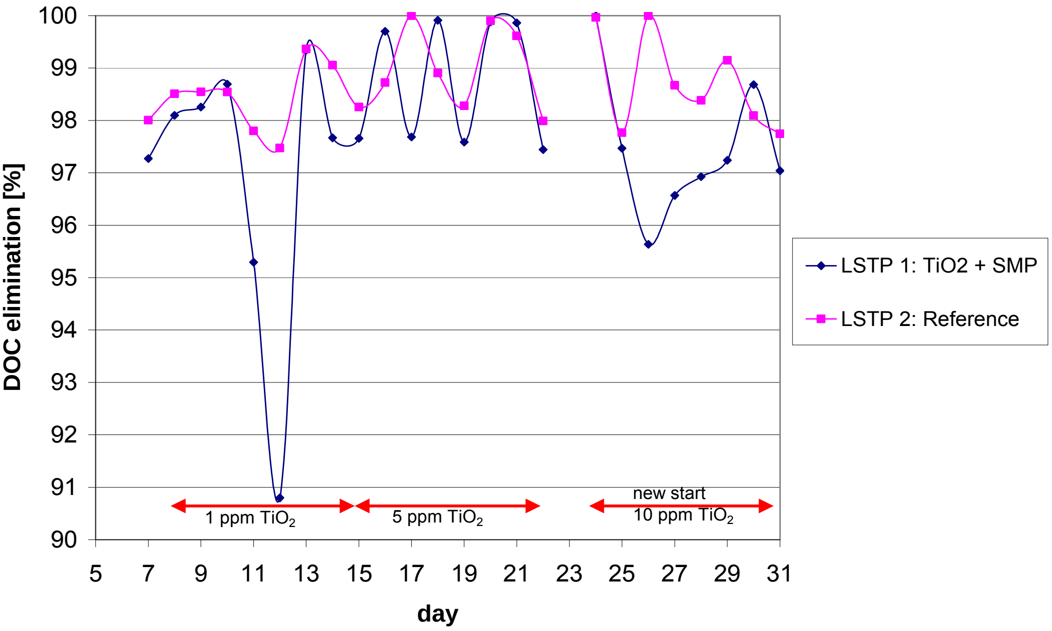


Figure 28: DOC elimination.

Fate of nanoscale TiO_2 in laboratory sewage treatment plants

The TiO_2 measurements in the surplus sludge and in the outflow resulted in technical difficulties of the digestion methods and ICP/MS, respective ICP/OES analysis (chapter 2.2). Finally, the measurements with ICP/OES analysis after HCl, HNO_3, HF digestion resulted in the most reliable results in terms of variability and reliability. Only these measurement results are presented in detail in this report. The balancing was conducted with the measured uncorrected measured data. This means, that the bad recovery of TiO_2 in activated sludge (see Figure 22) was not considered being valid and therefore no correction factor used. The original concentrations as analytically measured were used for the calculation of the mass balance.

A comparison of the TiO_2 concentration in the surplus sludge and in the outflow of the LSTP showed a relatively high correlation ($R^2 = 0.84$) with the TiO_2 concentration in the activated sludge being a factor 23 higher than in the outflow (slope in Figure 29). This is a strong indicator that more than 95% of the nanoscale TiO_2 is adsorbed to the activated sludge.

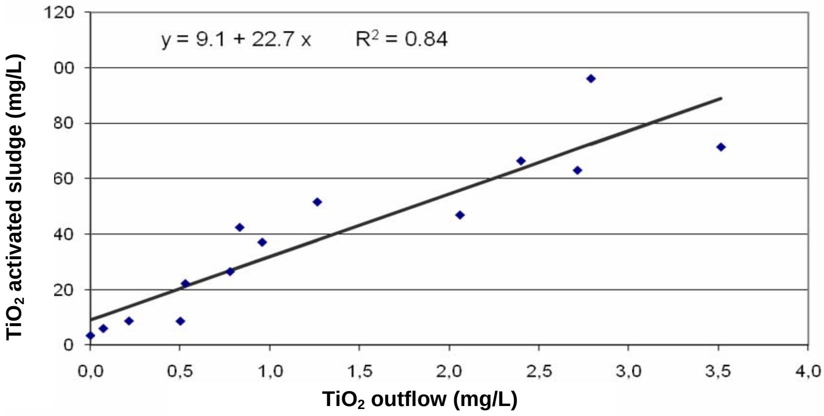


Figure 29: TiO_2 concentration in the activated sludge and in the outflow.

Explorative SEM and EDX scans of the sewage sludge and the effluent showed agglomerates of the P25 in the sludge. Agglomerates in the size range < 200 nm and > 1 µm could be detected relatively frequently in the SEM scans. In the effluent no P25 agglomerates were seen in the SEM scans.

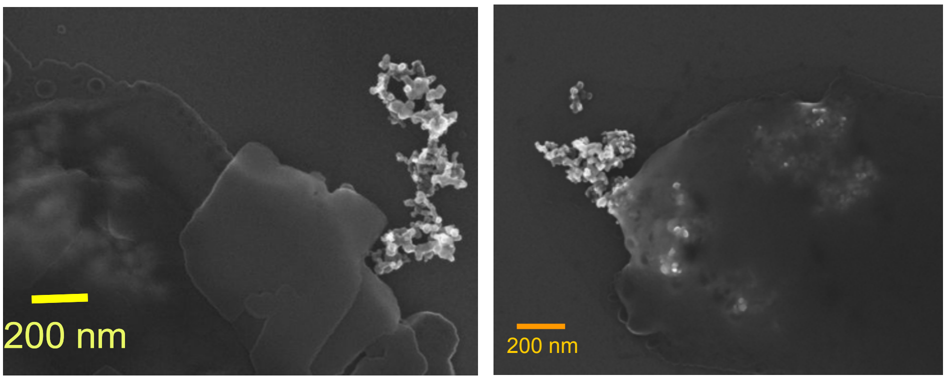


Figure 30: SEM scan of the sewage sludge of the test system of day 31.

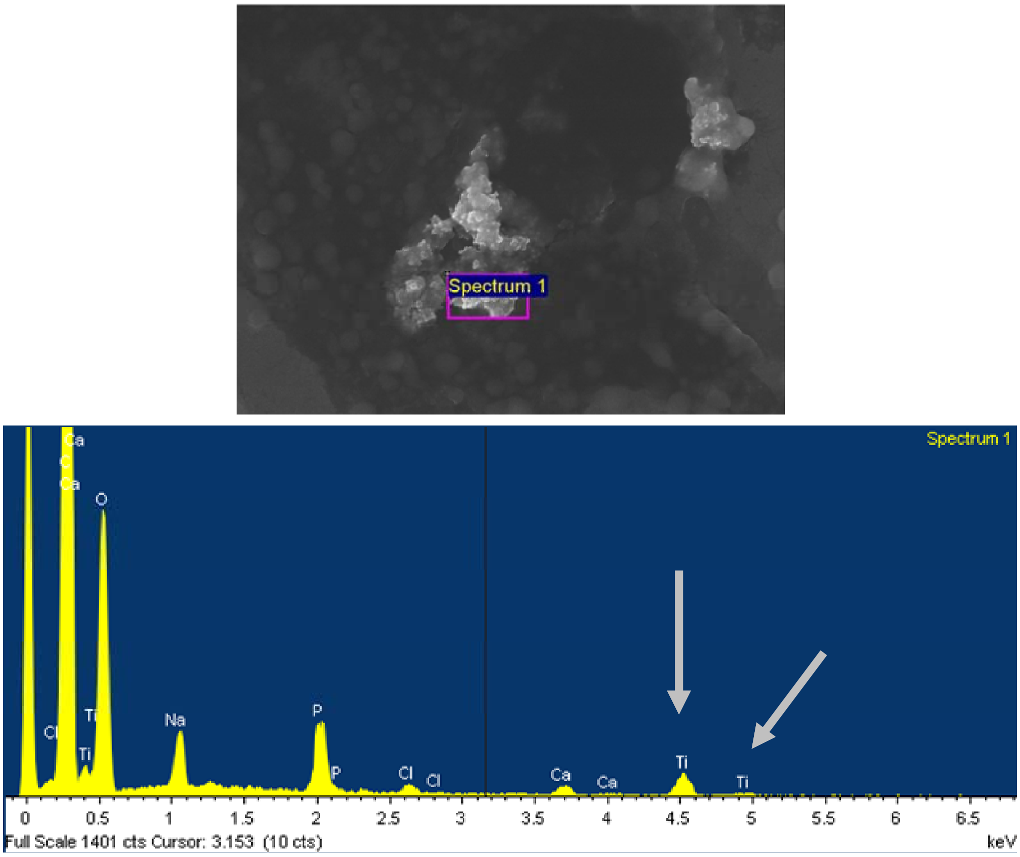


Figure 31: SEM and EDX scan of the sludge of the sewage treatment of day 31.

Balance of nanoscale TiO_2 in laboratory treatment plants

The calculation of the overall TiO_2 balance is a strong instrument for the determination of the reliability of the treatment process as well of the analytics. In principle the TiO_2 introduced into the system should be detected in the activated sludge and in the outflow of the LSTP.

$$\sum TiO_{2\ \mathrm{dosage}}\ [\mathrm{mg}] = \sum TiO_{2\ \mathrm{as}}\ [\mathrm{mg/L}] \times V_{\mathrm{as}}\ [\mathrm{L}] + \sum TiO_{2\ \mathrm{out}}\ [\mathrm{mg/L}] \times V_{\mathrm{out}}\ [\mathrm{L}]$$

where

$\sum TiO_{2\ \mathrm{dosage}}$	is the total amount of TiO_2 added to the activated sludge basin,
$\sum TiO_{2\ \mathrm{as}}, \sum TiO_{2\ \mathrm{out}}$	are the TiO_2 concentrations measured in the activated sludge and in the outflow,
$V_{\mathrm{as}}, V_{\mathrm{out}}$	are the volumes of the activated sludge (surplus sludge and total sludge at the end of the test) and in the outflow.

For the balance the TiO_2 introduced into the system and determined in the activated sludge and in the outflow of the LSTP have been calculated on a daily basis and the absolute values have been cumulated (Figure 32 and Figure 33). For this, a few TiO_2 data have been interpolated from the adjoining TiO_2 concentrations in the outflow and in the activated sludge (see cursive values in Annex III - A3-5 Results ICP OES measurements of the sewage sludge and the effluent). The total amount of TiO_2 added to the LSTP cumulated to 504 mg in trial 1 (dosage 1 and 5 mg/L TiO_2) and 840 mg in trial 2 (dosage 10 mg/L TiO_2) – Annex III.

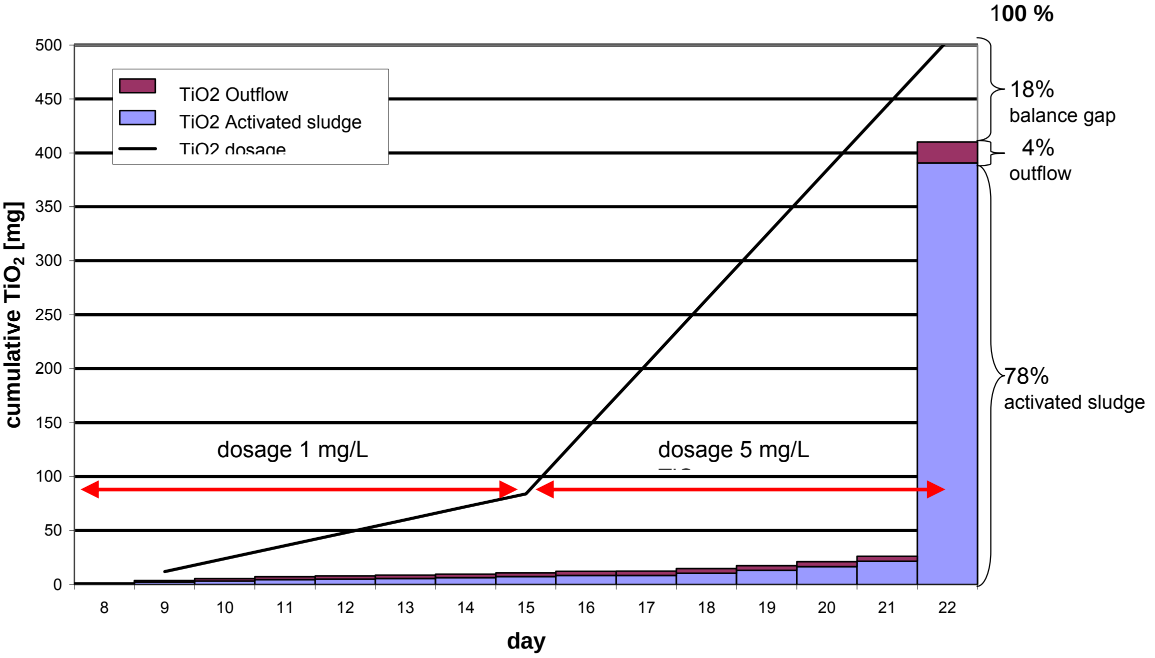


Figure 32: Cumulative release of TiO_2 from laboratory STP (trial 1). Data from day 8 to day 21 represent the TiO_2 removal with the surplus sludge from the LSTP, at day 22 the remaining TiO_2 present in the total activated sludge of the LSTP has been considered.

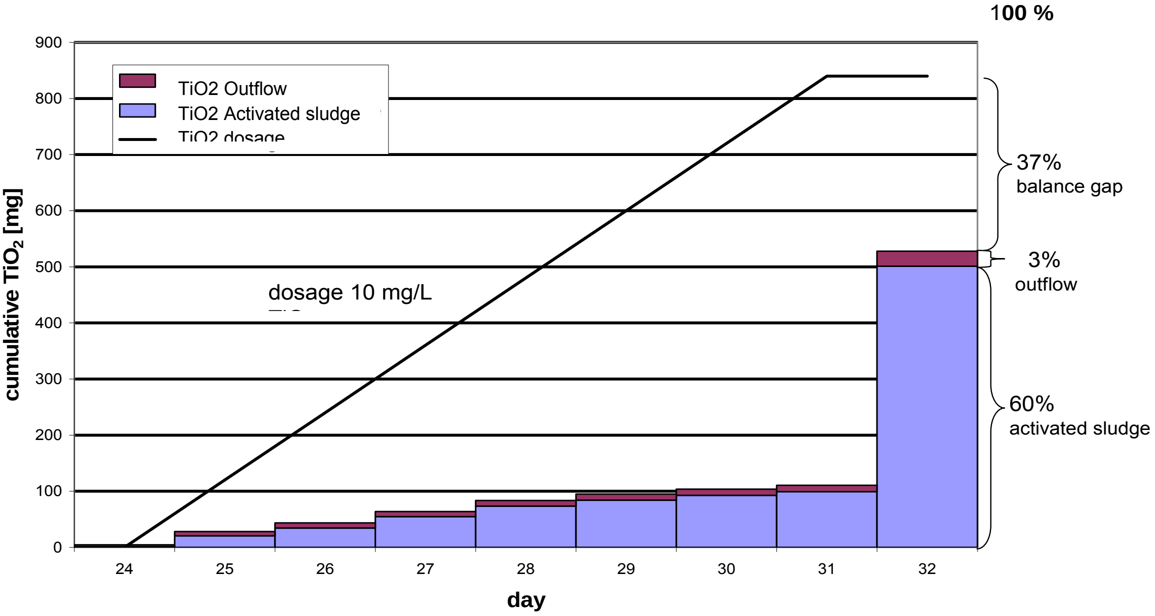


Figure 33: Cumulative release of TiO_2 from laboratory STP (trial 2). Data from day 8 to day 21 represent the TiO_2 removal with the surplus sludge from the LSTP, at day 22 the remaining TiO_2 present in the total activated sludge of the LSTP has been considered.

As expected, most of the nanoscale TiO_2 was adsorbed to the activated sludge. Therefore the increase of the cumulative amount was determined by the test duration and the volume removed as surplus sludge. At the end of the test the remaining total volume of the LSTP unit (5.5 L) multiplied by the TiO_2 concentration of the homogenised mixed sample of both the activated sludge basin and the separator attributed the main part to the overall balance. In the outflow about 4% (trial 1) and 3% (trial 2) of the TiO_2 introduced have been detected which corresponds quite well with the correlation of the TiO_2 concentration in the activated sludge and in the outflow. However, the overall balance showed a considerable gap of 18% (trial 1) and 37% (trial 2). This gap can partially be explained by an average detection efficiency of about 90% and the uncertainty of the measurements.

3.1.3 Discussion

Only few data on the behaviour of nanoscale TiO_2 in STP are available so far (Gottschalk 2010, Kiser et al., 2009). The clearance efficiency of TiO_2 in sewage treatment plants (STP) has been determined to be in the order of 70 - 85% (mainly attributed to particles > 0.7 µm) while in the outflow predominate particles below 0.7 µm were detected (Kiser et al., 2009). Comparable results were found for cerium oxide (Limbach et al., 2008). Sorption to activated sludge is considered as a major removal mechanism for nanoparticles.

According to a probabilistic material flow analysis of nano-TiO_2 the most relevant TiO_2 flow to the environment (30.7 – 33.8 t/a or 95%) is waste water. The main part of this amount is removed and eliminated in waste incineration plants (26.6 – 29.22 t/a) while 4.1 – 4.5 t/a entering surface waters (Gottschalk et al., 2010).

In the presented study the fate and behaviour of nanoscale TiO_2 (P25 with an average particle size in suspension < 250 nm) in the laboratory sewage treatment plants simulation test (LSTP) according to OECD Guideline 303A (2001) has been investigated.

Stability of the TiO_2 suspensions

In synthetic drinking water an additive (1 wt% sodium hexametaphosphate, SHP) was necessary to prepare a stable P25 TiO_2 suspension for 24 h. The result is a highly artificial medium which considerably differs from natural water and which might also have impacts to the overall efficiency of the treatment process. The influence of SHP could have been compensated by dosing the same SHP concentration into the reference unit 2.

Functionality of the treatment process

The dosage of TiO_2 with 1 wt% SHP did not cause an impact on the overall efficiency of the treatment process. The DOC-elimination and nitrification was not affected by TiO_2 and the dispersant. The values obtained were far better than those described in the validity criteria for OECD 303A (Annex III - A3-1 Standard operation procedure – Nanoscale TiO_2 in laboratory sewage treatment plant simulation test according to OECD 303A (2001)). However, the filterable solids were considerably higher in unit 1 (with TiO_2) which is explained by the use of a SHP as dispersant. Anyhow, compared to observations in technical STP the concentrations of filterable solids were still within an acceptable range. In technical STP filterable solids above 30 mg/L are considered as a malfunction (DWA 2003).

Fate of nanoscaled TiO_2 in laboratory sewage treatment plants

The results show that the TiO_2 in the outflow is mainly adsorbed to activated sludge. The TiO_2 concentration in the outflow only explained 3 - 4% of the total nanoscale TiO_2 introduced. This raises the question in which form the TiO_2 is present in the outflow. The particle sizes of the TiO_2 in the outflow could not be determined by DLS measurements due to other particles present in the samples resulting from the OSW and activated sludge. There was the presumption that the TiO_2 content in the outflow mainly depends on the sludge overflow. The calculated TiO_2 (mg/L) in the outflow is derived from the TiO_2 of the activated sludge (mg/L) multiplied by the filterable solids (mg/L) and divided by the dry solids of the activated sludge in the aeration vessel (mg/L) (Figure 34).

At the start of the test small amounts of TiO_2 are introduced together with the activated sludge into both LSTP. In the reference LSTP, reference unit 2 (without TiO_2) the TiO_2 in the effluents were below the detection limit of 5 µg/L. In the activated sludge the TiO_2 concentration slowly dropped down due to the removal of the surplus sludge. The overall TiO_2 balance of the reference unit 2 (without TiO_2) resulted, that an amount of 19.5 mg TiO_2 (trial 1) and 27.2 mg TiO_2 (trial 2) was present in the activated sludge of the reference unit 2 (without TiO_2) (absolute values). This background concentration corresponded to 5% of the total TiO_2 amount found in the test unit 1 (with TiO_2) (391 mg in trial 1 and 501 mg in trial 2, see Annex III - A3-5 Results ICP OES measurements of the sewage sludge and the effluent). The background concentration of TiO_2 introduced with the activated sludge inoculum was therefore not considered in the evaluation.

Table 8: Overall results and comparative data.

	Results	**Validity criteria OECD**	**Reference data**
DOC elimination	96% - 100% in both units		
Nitrification	NH_4-N < 0.2 mg/L NO_2-N < 0.2 mg/L NO_3-N = 17 - 18 mg/L	Under nitrifying conditions: NH_4-N < 1 mg/L NO_2-N < 2 mg/L	Inter-laboratory test: NH_4-N < 0.37 mg/L NO_2-N < 0.27 mg/L NO_3-N = 21 mg/L
Filtrateable solids	With TiO_2 4 - 17 mg/L Without TiO_2 1 - 2 mg/L		Typical values in the outflow of technical STP ~ 10 mg/L Malfunction (sludge overflow) from 30 - 50 mg/L

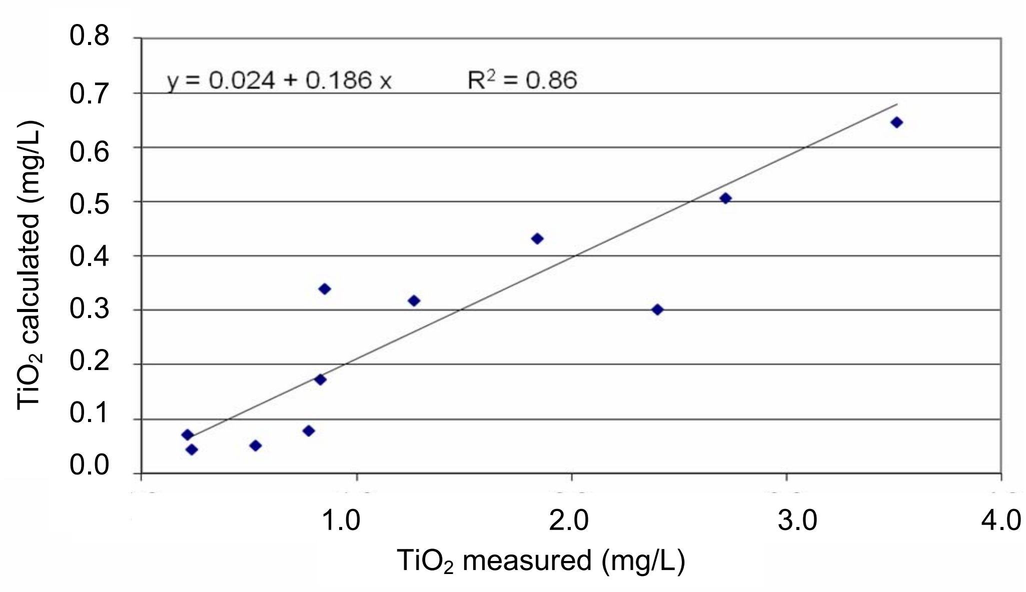


Figure 34: Measured and calculated TiO_2 concentration in the unfiltered outflow.

The correlation of the measured TiO_2 in the outflow of unit 1 and the TiO_2 calculated from the filterable solids in the effluent and the TiO_2 concentration in the activated sludge was quite good ($R^2 = 0.86$) but the measured TiO_2 in the outflow was about four times higher than the calculated TiO_2 resulting from the filterable solids. This is a strong indication that the TiO_2 concentration in the outflow has to attribute to smaller particles which are not removed by the glass fibre filter (pore size 0.6 µm). Thus, the TiO_2 concentration in the outflow is mainly determined by the presence of smaller particles below 0.6 µm and not by the sludge overflow as measured by the filterable solids (> 0.6 µm). Similar results were described by Kiser et al., (2009) and Limbach et al., (2008) for other nanoscale material (cerium oxide).

The overall TiO_2 balance showed a gap of 18% (trial 1) and 37% (trial 2). In biodegradability testing a mass balance is performed when ^{14}C-labelled test substances are used. The OECD Guideline 314 “Simulation tests to assess the biodegradability of chemicals discharged in wastewater” recommends target recoveries of radioactivity from the test matrix of 85 – 110% (average). For individual samples a range from 75 to 115% should be reached. However, these ranges should not be used as criteria for acceptance of the tests for nanomaterials due to higher analytical uncertainties. While the results of trial 1 almost reach these requirements the balance in trial 2 does not. There are a number of possible reasons for the balance gap:

- The recovery experiments for P25 in activated sludge showed a sufficient recovery rate of 92% at lower concentrations (10 mg/L TiO_2). At higher concentrations (above 40 mg/L TiO_2) the recovery rate was below 50% which may be explained with matrix effects (see 2.2.1 Recovery in different matrices). Although these results are considered as outliers, the observation may explain the higher gap in the balance in trial 2 of the experiment compared to that of trial 1. The highest TiO_2 concentration in activated sludge measured at the end of trial 2 was 96 mg/L and thus in the same range as the concentrations tested in the recovery experiment.
- While the OECD 303 A test is usually performed at one definite concentration the dosage of the nanoscale TiO_2 was split into three concentrations of 1 mg/L, 5 mg/L and 10 mg/L TiO_2 in order to obtain as much information as possible. It is not sure whether the dosage at one standard concentration might be more convenient for the evaluation.
- The overall balance is mainly determined by one single value, the TiO_2 concentration in the sludge at the end of the test. Any variations in that concentration have a decisive influence on the overall results.
- The processing of the LSTP has uncertainties in terms of the representativeness of the sampling, especially of the surplus sludge, which tends to settle or to accumulate on glass surfaces. Note that in the standard procedure of the OECD 303A test exclusively the concentrations in the effluent are considered in the evaluation. The digestion of TiO_2 for subsequent ICP/OES analytics required very hard conditions (use of hydrofluoric acid). Although a good recovery rate of TiO_2 suspensions was obtained the influence of the matrix activated sludge is not fully understood. There might be an underestimation of TiO_2 in the activated sludge samples but also in the outflow where the TiO_2 might also be adsorbed to suspended solids.

In the sludge of the LSTP with explorative SEM and EDX scans the P25 was detected as agglomerates (from < 200 nm to > 1 µm in size). In the effluent of the LSTP no P25 could be identified.

From this we conclude that the mass balance result obtained is in the range of uncertainty for such an experiment. Overall we find for the uncorrected values that 3 - 4% of the mass of nanoscaled TiO_2 particles is not retained in a normally running sewage plant.

3.2 Leaching experiments in soil columns – OECD 312

3.2.1 Background

Man-made substances can reach soil ecosystems directly via application (e.g. agrochemicals) or indirect via waste water, sewage sludge, or air wet / dry deposition. After they reach the soil ecosystem, information on their mobility and movement (leaching) into deeper soil layers or the groundwater and on their potential for transformation, are important for a risk assessment. The OECD test guideline 312 is one of the test methods to be applied but was not tested for use for nanomaterials in detail yet. Besides testing the method also the results obtained are of importance. They deliver information on the behaviour of nanomaterials in soils including possible influences of surface coatings.

3.2.2 Methods and materials

Glass columns filled with three different air-dried, sterilised and sieved natural soils were used. The test run in duplicate with one reference system.

Glass columns

For the experiments glass columns with an inner diameter of 4 cm and a height of 35 cm, were used. The columns were filled to a height of 30 cm, which equates to an amount of 560 g - 660 g soil matter.

Nanomaterials

In Table 1 information about the three tested TiO_2 materials, PC105, P25 and UV Titan M262, is given. The materials differ mainly in their crystalline form, particle size in suspension, and surface coating.

Soils

Three different natural reference soils with varying pH, texture and cationic exchange capacity (CEC), were used for the study:

- A01 - Dystric Cambisol (loamy sand, medium acid, very light humic)
- A06 - Cambic Rendzina (silty clay loam, very sub-acid, medium humic)
- A04 - Gleyic Podsol (loamy sand, medium acid, medium humic)

The reference soils were provided by the Fraunhofer Institute in Schmallenberg, Germany (www.refesol.de). Before use, all soils were air dried for 48 h at 21 °C and sieved through a 2 mm mesh.

Analysis data of the used natural soils are given below (Figure 35). For more detailed information (e.g. Organic Carbon content, breakthrough curves) of the used soils see Annex III - A3-10 Detailed analysis data of the employed soils.

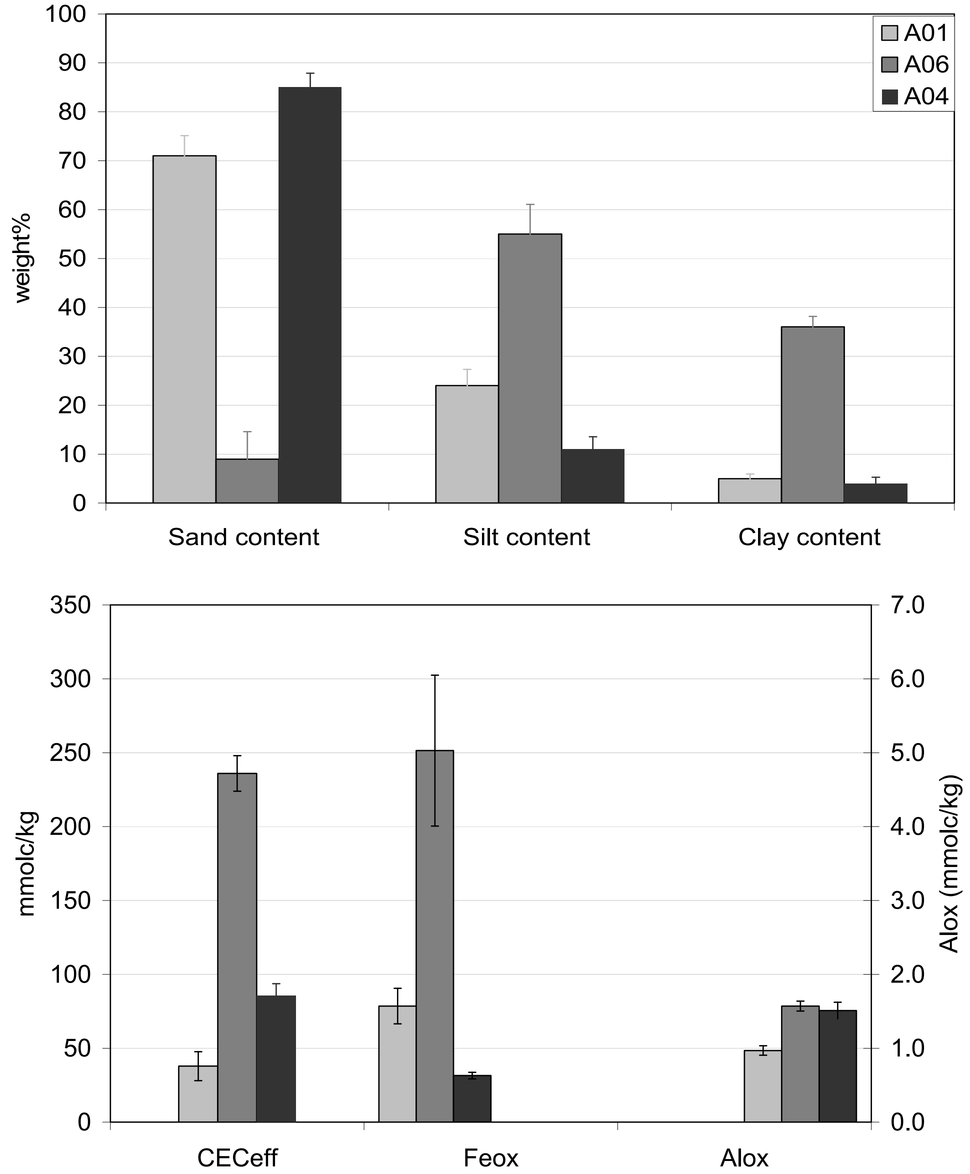
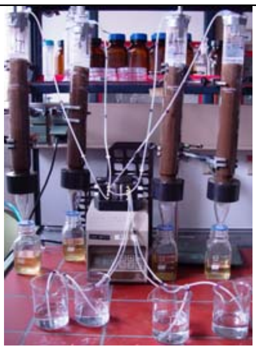
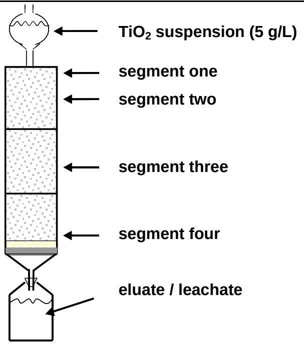


Figure 35: Sand, silt and clay content of the soils – A01 Dystric Cambisol, A06 Cambic Rendzina and A04 Gleyic Podsol (upper figure); cation exchange capacity (CEC), Ironoxalat (Feox), Aluminumoxalat (Alox) of the used soils (lower figure) - www.refesol.de.

Study design

The main steps of the soil transport tests are summarized in Table 9. Detailed information about the study design (SOP) can be found in Annex III - A3-8 Standard operation procedure – Nanoscale TiO_2 in soil columns according to OECD 312 (2004).

Table 9: Overview of the study and experiment design.

1.	2.	3.	4.
Columns were filled with the reference soils. Afterwards the soils were pre-wetted with a 0.01 M $CaCl_2$ solution from the bottom to the top, to displace most of the air in the soil pores.	The suspension with the nano-material (5 g/L) was applied on the top of the column → total of 500 mg TiO_2	0.01 M $CaCl_2$ solution (200 mm / 48 h) was continuously applied to the column, simulating a heavy rainfall event. The eluate was sampled over 48 h.	After leaching the columns were sectioned in four segments: - 1: top layer 0 -1 cm, - 2: 3 - 4 cm, - 3: 16 - 17 cm, - 4: bottom layer 29 - 30 cm Each segment had a height of 1 cm. The segments were air dried, homogenised by grinding and chemically analysed. Representative samples were analysed with SEM and EDX.
			 

Initial study

A first test with the nanomaterial added as solid material to the soil was performed before the main experiments were conducted, to derive information about the transport behaviour of the dry material. The application of the solid material, even though described in the OECD guideline was later not pursued to ensure reproducible particle size distributions being introduced with particle sizes below 250 nm diameter.

The test run was conducted with 60 mg P25 mixed in 60 g A01 soil, before the mixture was added to the soil column. Afterwards artificial rain was applied for 48 h as for the main study. The eluate and the segments were analysed with TXRF (Total X-Ray Fluorescence Spectroscopy). No mobility was detected (results Annex III - A3-12 TXRF results of the first leaching experiment).

For the main experiment the nanomaterials were suspended in water to simulate the most likely exposure path (via the sewage treatment plant or surface water), so a different, maybe higher mobility could be expected. Using this procedure also allows a better control and comparability of the particle size distributions applied in the tests.

3.2.3 Results of the soil leaching tests

Every test was performed in duplicate (column one and column two). All presented values are corrected for the natural Titanium background concentration of the soils. The calculation of the amount of the TiO_2 in the corresponding segments of the soil columns can be found in the SOP for this test (Annex III - A3-8 Standard operation procedure – Nanoscale TiO_2 in soil columns according to OECD 312 (2004)). The mobility is analysed in consideration of material, soil type and taking into account that only two columns were tested per set-up.

Representative SEM and EDX analysis of segment one were conducted for all materials. Additionally representative SEM / EDX scans were conducted of other eleven segments. For the SEM / EDX scan, a minimum of 26 soil grains were analysed. Identification of transported TiO_2 via SEM / EDX included always both, morphological and chemical analysis, to differentiate background and nanomaterial. If Ti was detected, an area close to the detected Ti was scanned to be sure that additional Ti was detected and not the background Ti of the soil itself. This is exemplarily shown for soil A01 and PC105 (Figure 39).

Eluate

In the collected eluate of all columns no values above the limit of detection were determined. An average amount of 250 ml was transported through the soil column and was collected. If this and the LOD (< 5 µg/L) is considered, a maximum of 0.04% of the added material could have leached through the columns. Due to these findings only a low, inter-segment mobility of the nanomaterials is expected.

Dystric Cambisol – A01

The soil columns treated with PC105 showed only in the first segment a significant amount of PC105. All further segments contained no Titanium above the background concentration of that soil (Table 10 and Figure 36). With SEM / EDX no additional TiO_2 in segment three was detected.

The soil columns treated with UV Titan M262 showed a similar behaviour like PC105, but for column one a possible transport from the first to the second segment is indicated (Figure 37). Still no detectable transport beyond the upper few centimetres was seen (Table 10), but with SEM / EDX isolated TiO_2 agglomerates were detected in segment four (Figure 40). No transport is indicated by the second column treated with UV Titan M262.

The soil columns with P25 showed high Titanium in the top segment of column two. In segment one and segment two of column one only 5% and 2% of the added TiO_2 was detected, respectively (Figure 38 & Table 10). The low concentration in the upper segments of column one is assumed to be an outlier, because a) the soil was white with clearly high TiO_2 content and b) no TiO_2 was found in the eluate. From the results of column two and the obvious outlier for segment one in column one we conclude that no significant transport of P25 was detected. Except segment one no additional TiO_2 could be detected with SEM / EDX (25 analyses) of segment three of soil A01 treated with P25.

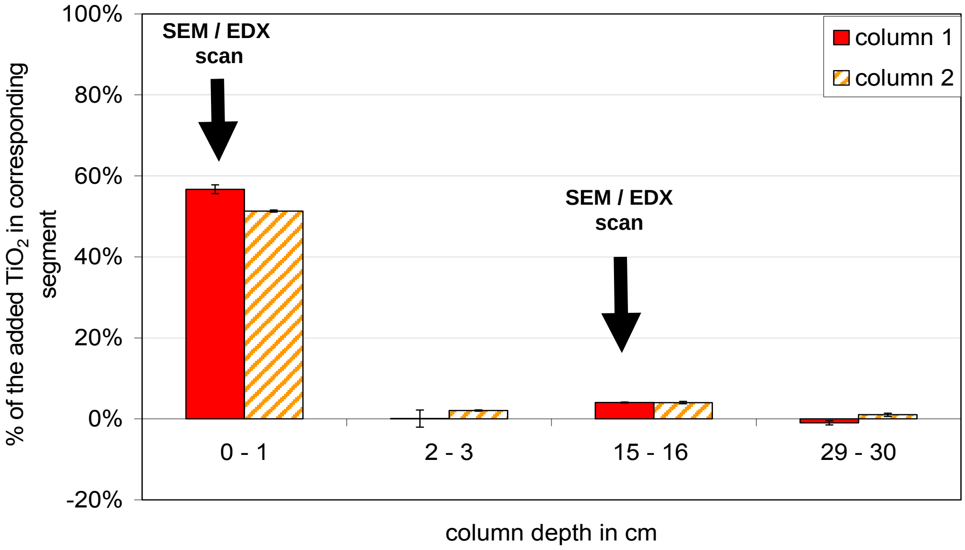


Figure 36: Soil column run with PC105 and soil A01 Dystric Cambisol. Natural Ti in soil A01 0.19% (1.9 g/kg). Error bars = max and min; n = 2.

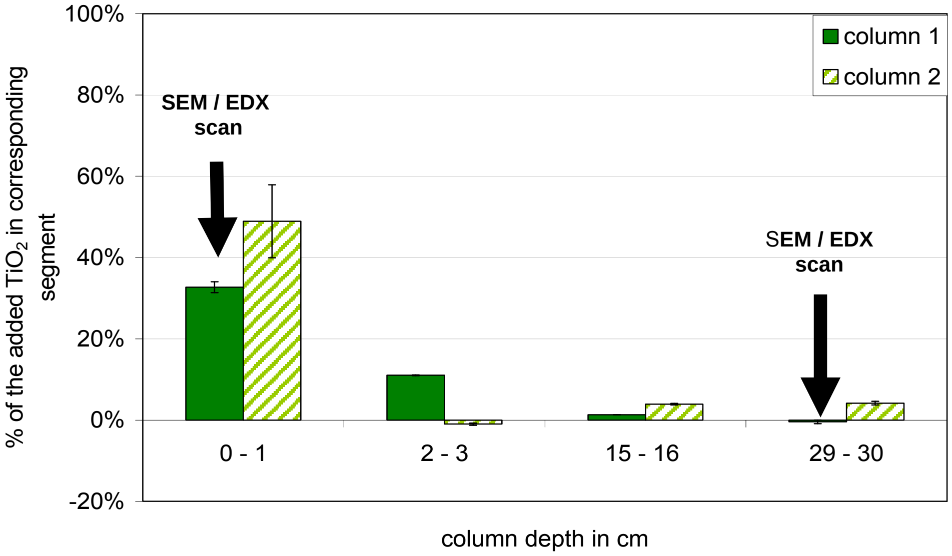


Figure 37: Soil column run with UV Titan M262 and soil A01 Dystric Cambisol. Natural Ti in soil A01 0.19% (1.9 g/kg). Error bars = max and min; n = 2.

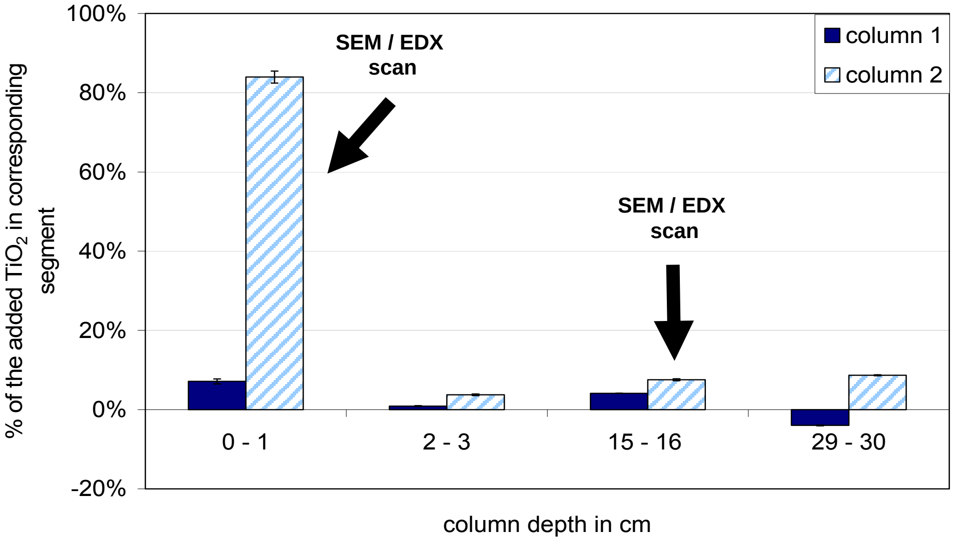


Figure 38: Soil column run with P25 and soil A01 Dystric Cambisol. Natural Ti in soil A01 0.19% (1.9 g/kg). Error bars = max and min; n = 2.

In Table 10 the concentrations of the three tested materials are presented as percent in the segment of the initial concentration. The data are background corrected. The highest recovery was found in the first segment of the soil column independent of the added TiO_2 material. Only the coated material UV Titan M262 indicated a transport down to segment two (13% recovery of the added mass).

Table 10: PC105, UV Titan M262 and P25 in the four analysed soil column segments of soil A01 in % of the added mass of 500 mg (TiO_2). All values are corrected for the Titanium concentration in the natural soil. The double analysis of each segment showed reproducibility better than 0.8 mass-%.

	PC105 recovery (% of added mass)		**UV Titan M262 recovery (% of added mass)**		**P25 recovery (% of added mass)**	
	column one	column two	column one	column two	column one	column two
1	57	54	36	51*	5	83
2	-2	3	13	-1	2	1
3	1	2	-2	1	-1	2
4	0	1	0	5	0	1

* The dublicate analysis showed a difference of 5 mass-%

The total recovery in the four segments should always be significantly below 100 % in sum since only the four segments were analysed and not the whole column, more specifically four times 1 cm of the 30 cm column length. The same occurs for the other tested soils.

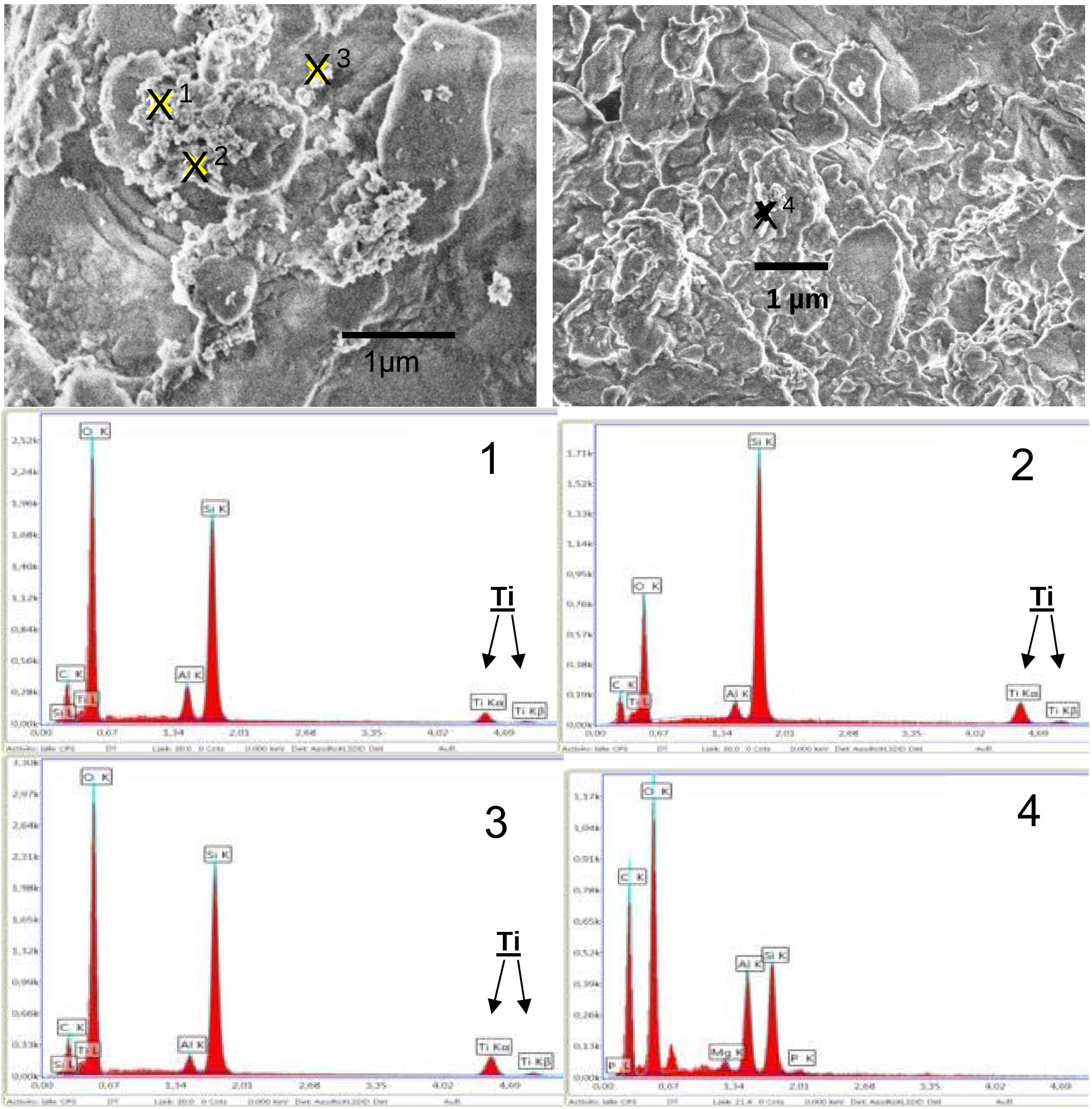


Figure 39: SEM / EDX scans of segment one of soil A01 treated with UV Titan M262. The lower right scan shows the negative control with no Ti detected (scan 4).

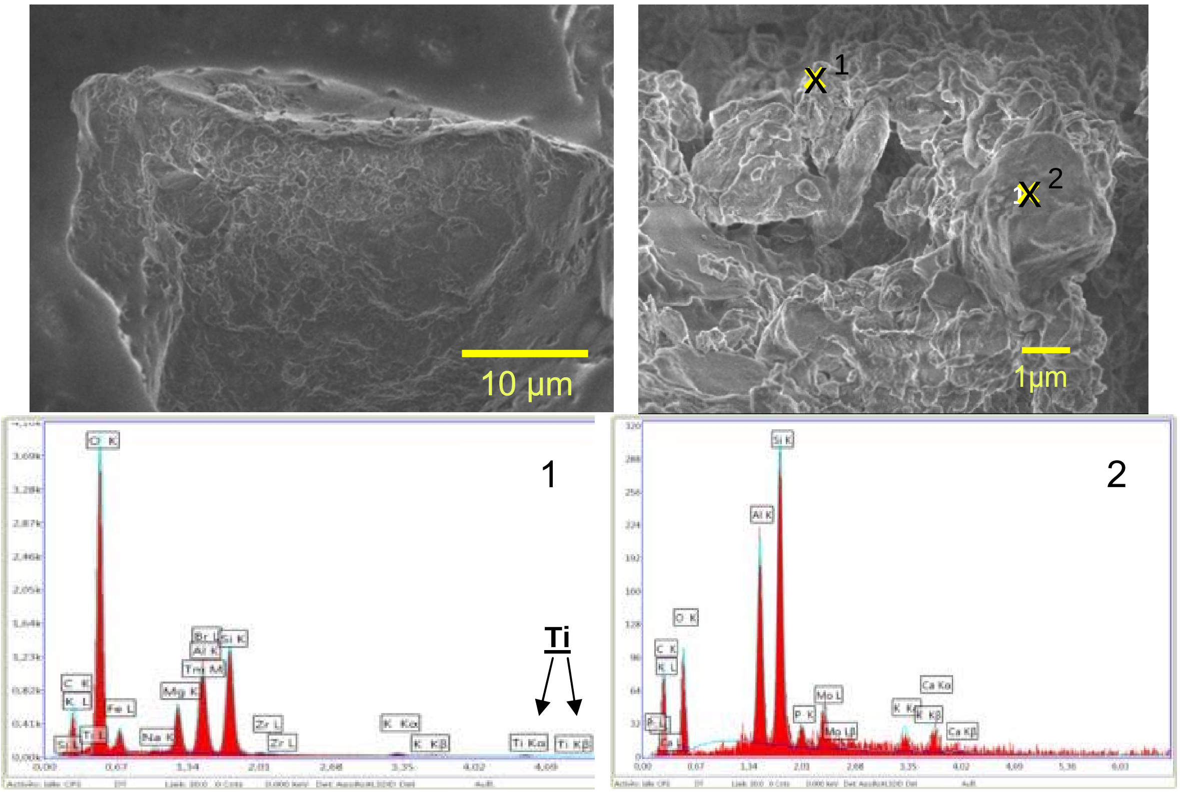


Figure 40: SEM / EDX scans of segment four of soil A01 treated with UV Titan M262. The right scan shows the negative control with no Ti detected (scan 2).

➔ *All three different TiO_2 nanomaterials showed similar behaviour with transport limited to the upper few centimetres for this soil type. No additional Titanium was chemically detected in the lower soil segments as well as in the eluate. Only for UV Titan M262 a transport was indicated for the first two segments. With SEM / EDX scans in segment four isolated TiO_2 (UV Titan M262) agglomerates were detected. It is conceivable that only single agglomerates were transported, and due to this were below the detection limit of the chemical analysis*

Cambic Rendzina - A06

PC105 showed always highest recovery in the first segment. Mobility is indicated in column one, showing a value above background in the second segment (Figure 41), but in the range of the uncertainty of the chemical analysis (recovery of PC105 was 133% ± 1%). But this observation is confirmed by the SEM / EDX scans (Figure 42). A transport of isolated TiO_2 agglomerates down to the segment four was observed in column one (Figure 43). No transport was chemically detected in column two (Figure 41).

In contrast, the soil columns treated with UV Titan M262 indicated a transport down to the fourth segment, for both samples (Figure 44) even though not unambiguously. The Ti concentrations of segments three for both columns was partially in the range of the background Titanium concentration. With SEM / EDX (26 analyses) except segment one (Figure 46) no additional TiO_2 could be detected in segment four in column two.

The soil column test with P25 resulted in highest Titanium concentrations in segment one with 80% and 76% of the added nanomaterial staying in the upper first centimetre, in column one and two (Figure 45). Hence no transport – based on the chemical analysis – was determined. However, with SEM / EDX the detection of isolated TiO_2 agglomerates was possible in segment four of column one (Figure 47), which indicates an isolated TiO_2 agglomerate transport in this soil column.

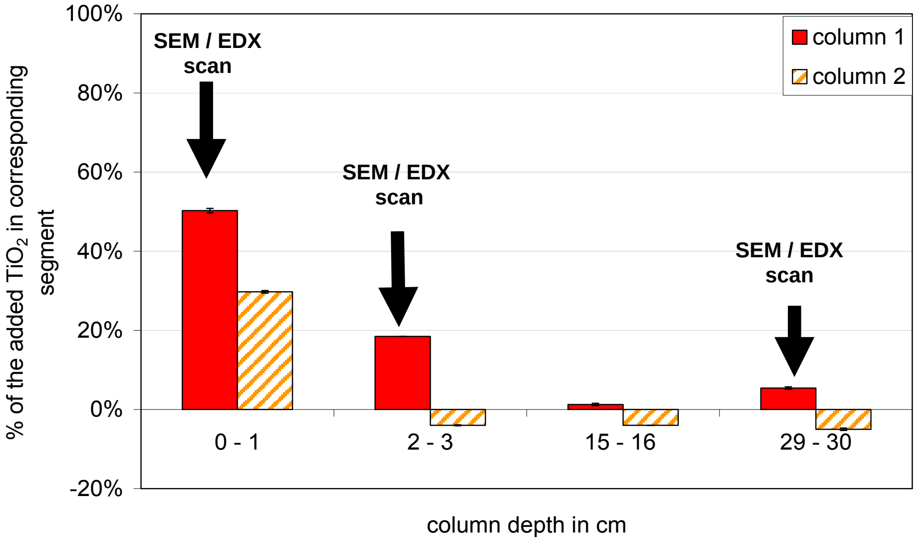


Figure 41: Soil column run with PC105 and soil A06 Cambic Rendzina. Natural Ti concentration in soil A06 0.42% (4.2 g/kg). Error bars = max and min; n = 2.

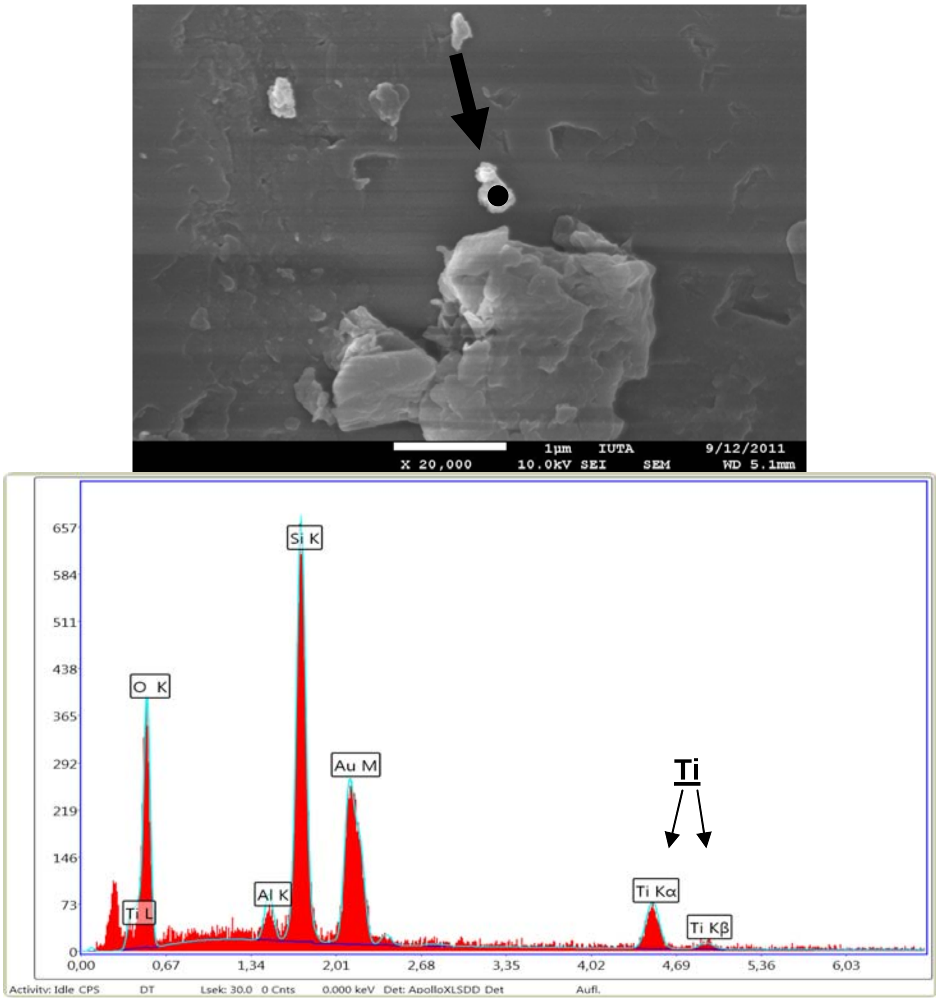


Figure 42: SEM / EDX scans of segment two of soil A06 treated with PC105.

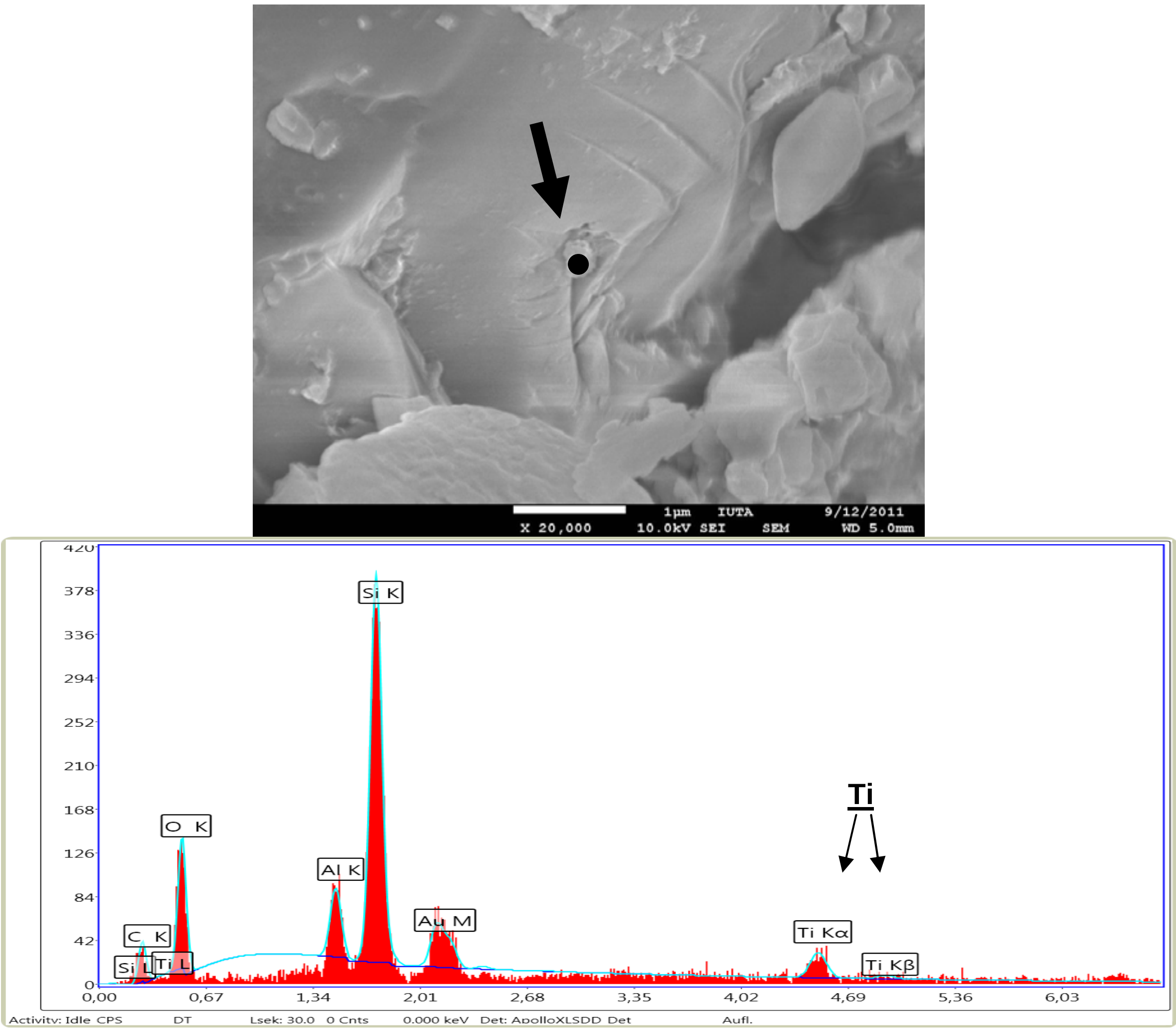


Figure 43: SEM / EDX scans of segment four of soil A06 treated with PC105.

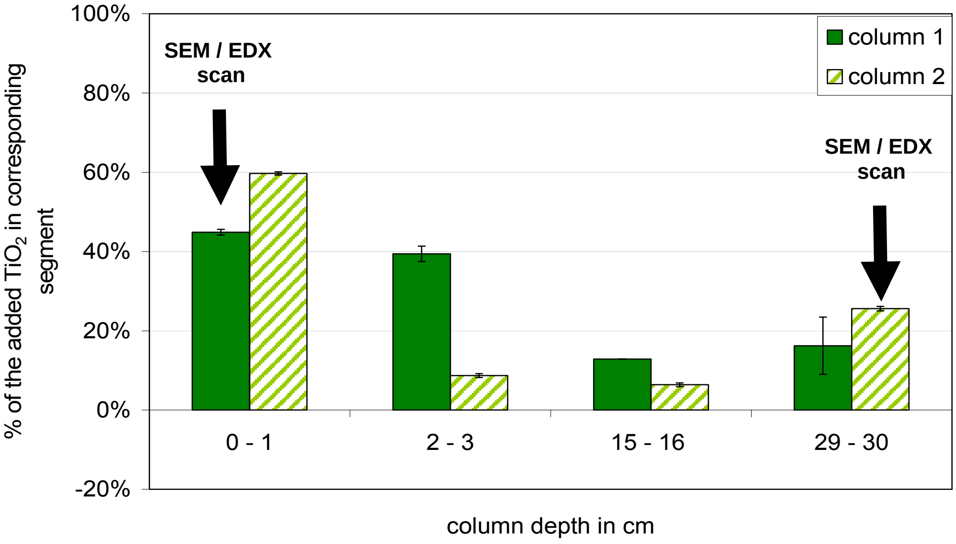


Figure 44: Soil column run with UV Titan M262 and soil A06 Cambic Rendzina. Ti background concentration of soil A06 was 0.42% (4.2 g/kg). Error bars = max and min; n = 2.

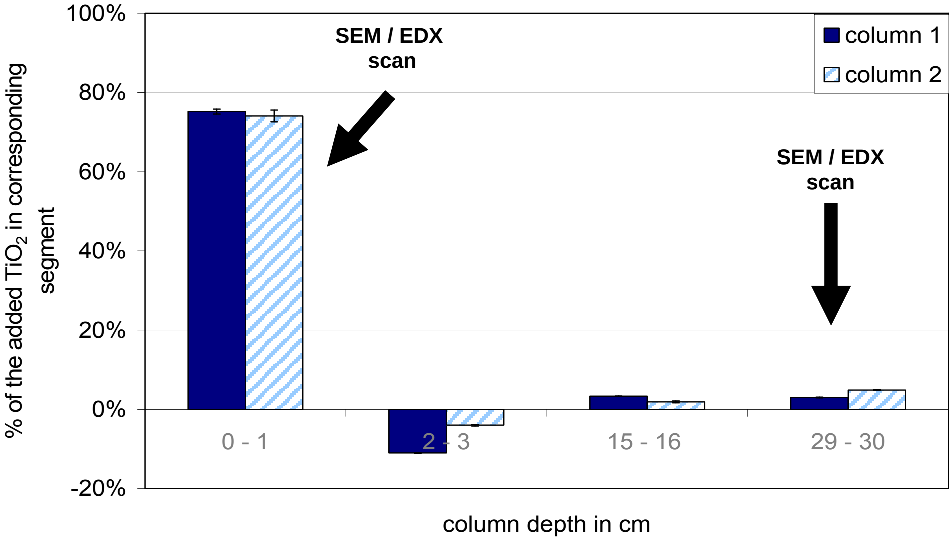


Figure 45: Soil column run with P25 and soil A06 Cambic Rendzina. Natural Ti concentration in soil A06 0.42% (4.2 g/kg). Error bars = max and min; n = 2.

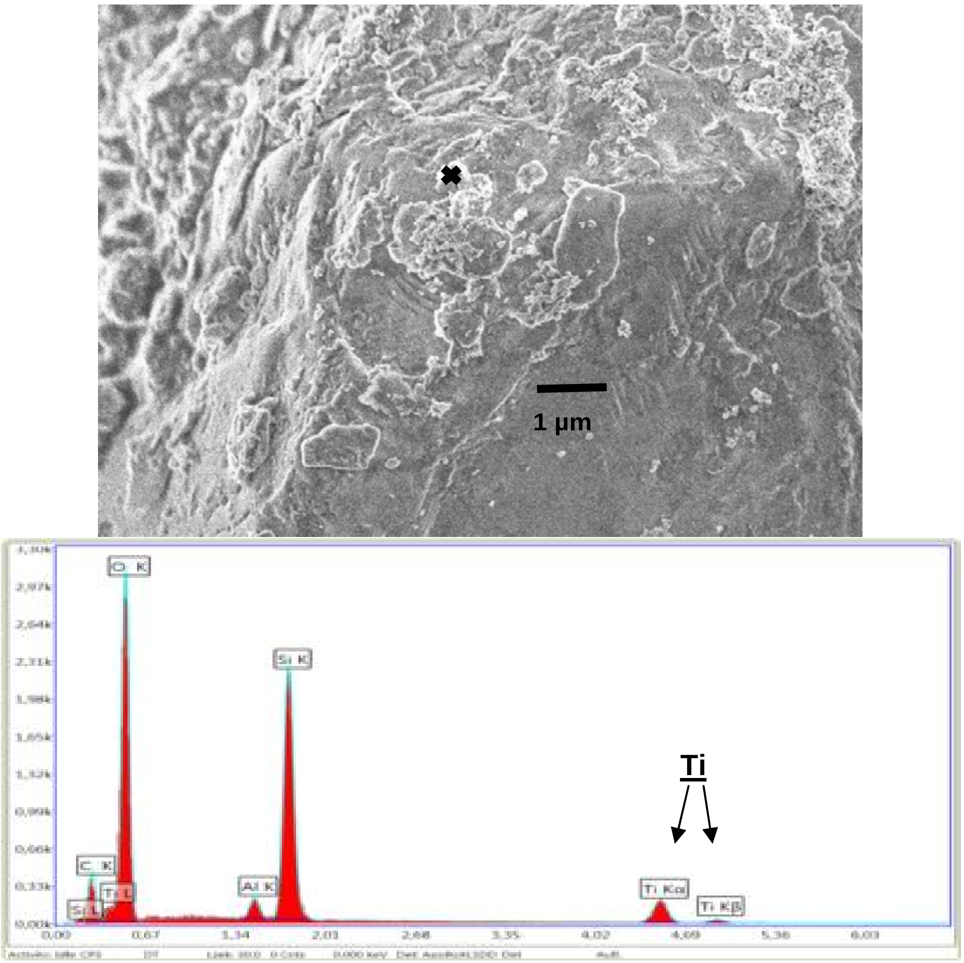


Figure 46: SEM / EDX scans of segment one of soil A06 treated with UV Titan M262.

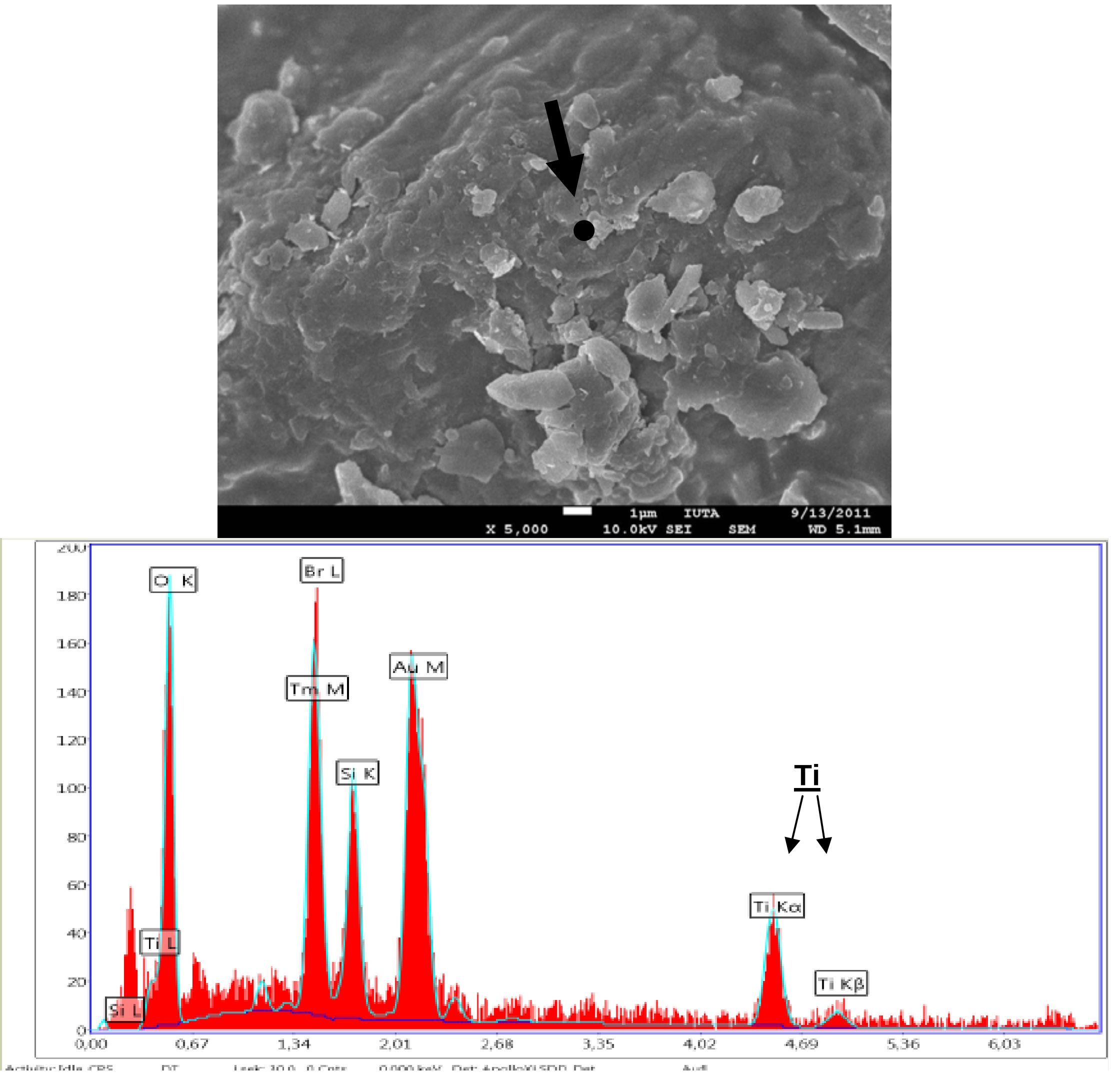


Figure 47: SEM / EDX scans of segment four of soil A06 treated with P25.

In Table 11 the concentrations of the three tested materials in the segments are given in percent of the initial concentration. The data are soil Ti background corrected. The highest fractions of the added Titanium nanomaterial were always found in the first segment of the soil column independent of the added TiO_2 material. Only the coated material UV Titan M262 indicated a transport down to segment two in column one (14% of added material) and segment four in column two (19% of added material).

Table 11: PC105, UV Titan M262 and P25 in the four analysed soil column segments of soil A06 in % of the added mass of 500 mg (TiO_2). All values are corrected for the Titanium concentration in the natural soil. The dublicate analysis of each segment showed reproducibility better than 0.8 mass-%.

	PC105 recovery (% of added mass)		**UV Titan M262 recovery (% of added mass)**		**P25 recovery (% of added mass)**	
	column one	column two	column one	column two	column one	column two
1	45	36	39	57	80	76
2	3	-11	14	1	0	0
3	0	-11	7	1	-2	-1
4	0	-12	7	19	-1	0

As it was mentioned for soil A01 only four segments of 1 cm depth each were analysed per column. This means that the remaining TiO_2 is very likely in the soil between the segments, most likely between segment one and two. Also some of the TiO_2 may adhere to the column wall and was lost for soil transport and analysis and due to this the total recovery was below 100%.

- → *All three different TiO_2 nanomaterials showed the highest amount of the applied nanomaterial in the first segment of the soil columns, likewise to soil type A01.*
- → *With chemical analysis no transport of P25 and PC105 beyond the first few centimetres was detected.*
- → *However with SEM / EDX isolated TiO_2 agglomerate transport, partly down to segment four were shown in some columns.*
- → *A transport for UV Titan M262 was indicated down to segment four for both columns, but no additional Titanium was detected in the eluate.*

Gleyic Podsol - A04

The high water retention potency of this soil hindered the run off during the experiment. Consequently the drainage had to be triggered by application of a short suction power. The pathway of the suspension which could be detected afterwards was mostly along the glass column wall (Annex III - A3-13 Gleyic Podsol – A04: results of the soil leaching test) independent on the type of the nanomaterial. The detected TiO_2 concentration in the soil column could be influenced by the observed transport at the glass column wall. Therefore results obtained for this soil type have a very high uncertainty and no valid conclusions can be drawn. Hence the results were not used for further interpretation. Indicative results of this test are presented and summarised in (Annex III - A3-13 Gleyic Podsol – A04: results of the soil leaching test).

3.2.3 Discussion and summary

All soil types showed for all tested materials the highest TiO_2 concentration in the first segment of the soil columns. No significant transport of PC105 and P25 nanomaterial in any of the tested types was detected with bulk chemical analysis. This was slightly different for UV Titan M262. Some mobility beyond the upper few centimetres could be seen, highest likeliness of transport for soil type A06 (Table 12).

The detected results for the Gleyic Podsol have a very high uncertainty. Adhesion and transport along the glass wall of the materials was visually detected and an effect on the analysis cannot be excluded and were not used for the further interpretation.

With SEM / EDX the detection of isolated TiO_2 agglomerates in nearly all segments was frequently possible. It is conceivable that a) the bulk chemical analysis was not sensitive enough for the detection of this transport taking the natural background into account and b) that single agglomerates are transported within the soil column.

Table 12: Summary of the detected transport (based on results of the chemical analysis) for every tested material and soil A01 and A06.

Soil	PC105		UV Titan M262		P25	
	column one	column two	column one	column two	column one	column two
A01	-	-	+	-	-	-
Conclusion	no significant transport detected		transport indicated, between the first and second segment		no significant transport detected	
A06	(+)	-	+	(+)	-	(+)
Conclusion	chemically no significant transport detected. SEM / EDX indicate a transport of single agglomerates		transport indicated, down to segment four		chemically no transport detected. SEM / EDX indicate a transport of single agglomerates	

+ = transport
(+) = transport indicated
- = no transport within the uncertainty limits

In consideration of these results, it is concluded that the size of the particles did not affect the mobility, because P25 and UV Titian M262 showed a comparable agglomerate size in the suspension, with no transport detected for P25 but some mobility for UV Titan M262. Optically a transport of PC105 (with the highest agglomeration size (DLS measurements: 500 nm, SD = 4.62) was detected down to segment four of soil type A06. Furthermore the suspensions showed a comparable zeta potential of +26 mV (SD = 1.06) for UV Titan M262, +23 mV (SD = 0.4) for P25 and +29 mV for PC105 at pH 5. No size and zeta potential measurements of the suspension could be performed in the systems, so that no statements about agglomerate sizes in the soil matrix can be made.
It is conceivable that the coating of the UV Titan M262 (aluminiumoxide and dimethicone) could have affected the transport behaviour in the tested soil systems.

Two scenarios for UV Titan M262 are hypothesized:

1. The top layer of the coating (dimethicone) is assumed to be washed off during suspension preparation, as shown by Auffan et al., (2010). Due to this Al_2O_3 remains as actual surface material (UV Titan M262 showed the same IEP as Al_2O_3 (pH 9)). It is conceivable that the cation exchange with alumina was hampered compared with single charged molecules like sodium, mainly for soils with high clay content – A06 and with this an increased mobility could be possible.
2. The washed off dimethicone in the suspension could affect the transport (hydrophobic behaviour), which could lead to a higher mobility compared to PC105 and P25.

The hindered mobility of non-coated, positively charged nanomaterials can be expected for soils with mostly negatively charged minerals (cation exchange) in the tested pH ranges.

In contrast to these results of low to no mobility in the presented tests, Fang et al., (2009) and Duester et al., (2011) detected a transport of the applied Ti nanomaterials in different natural soils.
Fang et al., (2009) detected basically a clay and pH dependent transport of the tested non-coated TiO_2 nanomaterial. High transport behaviour in soils was related to a large soil particle texture and a low ionic strength. A reduced transport was related to increased clay content and salinity. Duester et al., (2011) also detected a transport of negatively charged TiO_2 nanomaterials (size < 100 nm) in a floodplain soil with a pH ~ 8.

The main and assumed to be crucial contrasts of both studies compared to ours leading to a possible mobility of nanomaterials is the use of negatively charged and smaller nanomaterials at higher pH values of the soils. Additionally they used a smaller amount of soil and shorter columns for their tests and also suspensions with lower concentrations. This, as well the smaller particle size could have affected the mobility of the nanomaterials by reduced clogging and filtering effects. The negative charge of the particles may lead to a stronger electrostatic repulsion (negatively charged soil matrix), resulting in a (higher) mobility. Finally the higher pH values (6.15 – 8.58) and due to this a higher CEC compared with our soils, except soil A06, may have been another reason for the detected mobility in the studies by Fang et al., (2009) and Duester et al., (2011).

The used high concentration of 5 g/L in our study could have induced coagulation and clogging effects as soon as the suspension was applied to the surface of the soil columns, which could reduce a possible transport of the materials (visually a white layer of the applied materials was identified at the top of every column). However with the used experimental setup and the high background concentration of Titanium in natural soils, no use of a lower concentration was seen to be suitable (detection of added Ti in the last segment of the soil must be possible).

3.3 Adsorption experiments – OECD 106

3.3.1 Background and principal approach

Understanding adsorption and desorption processes of the soil matrix and material is of interest to understand possible environmental mobility. In detail, information on these processes may allow first mobility estimates, to derive information on possible changes in surface functionalities, and to estimate availability in the liquid phase. Only very little of these information are currently available for nanomaterials.

Therefore the adsorption of P25 to five different soil types was tested based on the OECD guideline 106 (adsorption / desorption using a batch equilibrium method; OECD, 2000). In addition the adsorption of UV Titan M262 was determined taking the results from the leaching experiment in soil columns into account which showed possible mobility of this material in soils.

Principal approach

A defined soil / suspension mixture is shaken for a defined time. Afterwards the mixture is centrifuged to differentiate between adsorbed and non adsorbed material. Therefore the supernatant is analysed for its nanomaterial content. Based on the assumption that the nanomaterial not detected in the supernatant was adsorbed by the soil, the adsorbed amount is calculated (Figure 48).

In a first study (tier 1) the soil / suspension ratio and shaking time was tested for tier 2. The adsorption by five different soils was analysed (tier 2). Adsorption isotherms should be calculated based on the results of tier 2 in tier 3.

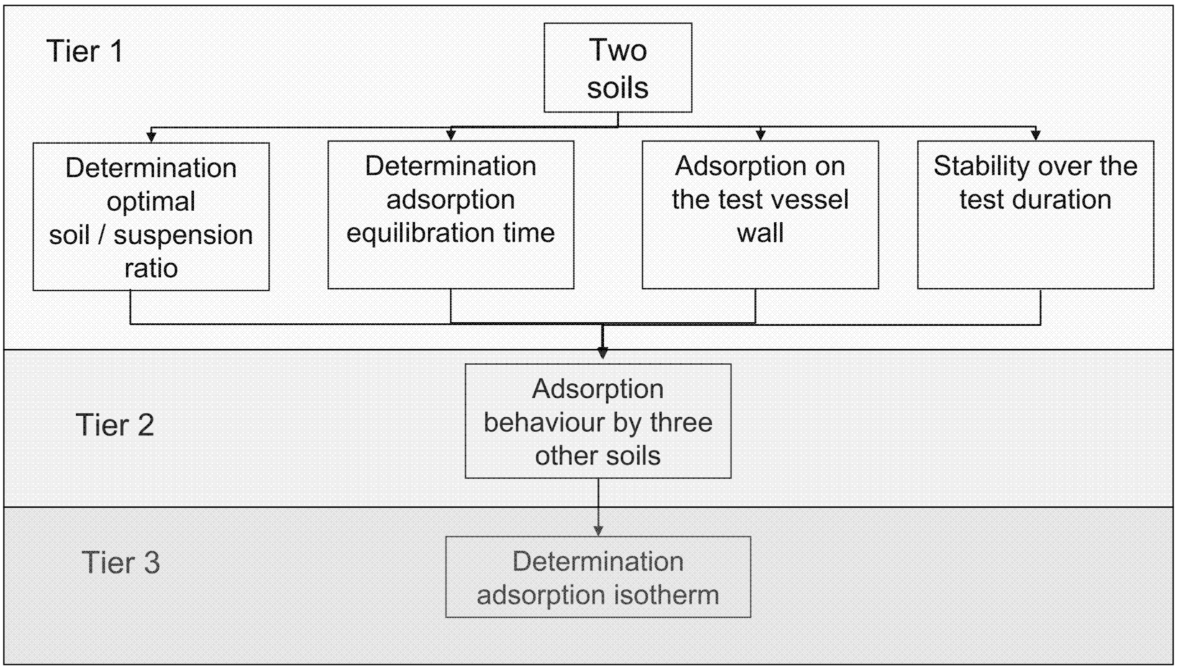


Figure 48: Scheme of the principal approach of OECD Test 106.

3.3.2 Methods

Soil / suspension ratios were tested with a concentration of 1 g/L for P25 and UV Titan M262. The suspensions were prepared using the developed SOP (Annex III - A3-9 Standard operation procedure – Nanoscale TiO_2 adsorption behaviour according to OECD 106 (2000)). Before the suspension was added to the soil, the air dried soil was equilibrated for 12 h with a 0.01 M $CaCl_2$ solution. After the equilibration the suspension was added to the soil / 0.01 M $CaCl_2$ solution mixture. The soil / suspension mixture was shaken before fractionated by centrifugation (10 min at 2700 g). Subsequent 15 mL of the supernatant were transferred using a pipette and analysed with ICP/OES after HCl, HNO_3, HF digestion (s. chapter 2.2). The residual liquid was decanted to determine the volume of the liquid fraction for the calculation of the TiO_2 concentration in the supernatant after the experiment.

The adsorbed amount of the test substance at the soil was calculated (detailed information can be found in the SOP for the test - Annex III) as the difference between the initial amount of the TiO_2 (TiO_2 initial) of the suspension and the amount in the supernatant at the end of the experiment (TiO_2 end). This equation assumes that all liquid stays liquid and is not adsorbed by the soil.

$$adsorption\ [\%] = \frac{TiO_2\ initial - TiO_2\ end}{TiO_2\ initial} \times 100$$

TiO_2 initial = the initial amount in µg
TiO_2 end = measured Titania mass in the supernatant after centrifugation in µg

All experiments were performed in duplicate. The pH of the soil and the soil / suspension mixture was detected (see **Table 16Fehler! Verweisquelle konnte nicht gefunden werden.** to check possible pH influence to the cation exchange capacity of a soil and along with this the adsorption described by Scheffer and Schachtschabel (2002)).

To determine the adsorption of the tested nanomaterials, the optimal soil / suspension ratio and equilibration time was identified in a preliminary study. Therefore three different mixing ratios (1/1, 1/5, 1/25) were tested and the mixture was shaken for 4 h, 8 h, 24 h and 48 h to identify the needed equilibration time and adequate mixing ratio (Table 13). Two soils (A01 & A06) with varying clay content and pH were used for this first test.

Table 13: Tested mixing ratios and shaking times.

mixing ratio	soil	material	shaking time in hours
1/1 – 50 g soil / 50 mL suspension	A01 & A06	5 mg P25	4, 8, 24 and 48
1/5 – 10 g soil / 50 mL suspension			
1/25 – 2 g soil / 50 mL suspension			

3.3.3 Result

First test to determine suitable test concentrations

Three different tests had to be conducted to identify and define the best test conditions:

- Desorption / leaching of the soil using the SOP (Annex III - nanoscale TiO_2 adsorption behaviour according to OECD 106 (2000)) of the test procedure to derive the background contribution from the corresponding soils, due to the known high background concentration of Titanium in natural soils and a possible influence of a leachable fraction.
- Testing the adsorption / desorption behaviour for a given soil type with varying time of shaking and mixing ratio TiO_2 stock suspension / soil mass.
- Identification of the pH conditions and how the test suspension influenced this value.

Testing the adsorption / desorption behaviour for a given soil type with varying time of shaking and mixing ratio TiO_2 stock suspension / soil mass

The soils A01 and A06 were tested with three different soil / suspension ratios (1/1, 1/5 and 1/25) and analysed after different equilibration time (Figure 49 and Figure 50) to determine the most suitable concentration for the actual adsorption test. For this first test only P25 was used as nanomaterial. Table 14 and Table 15 are presenting the results of the desorption / leaching tests with DI water to determine the background of leachable Ti in the tested soil.

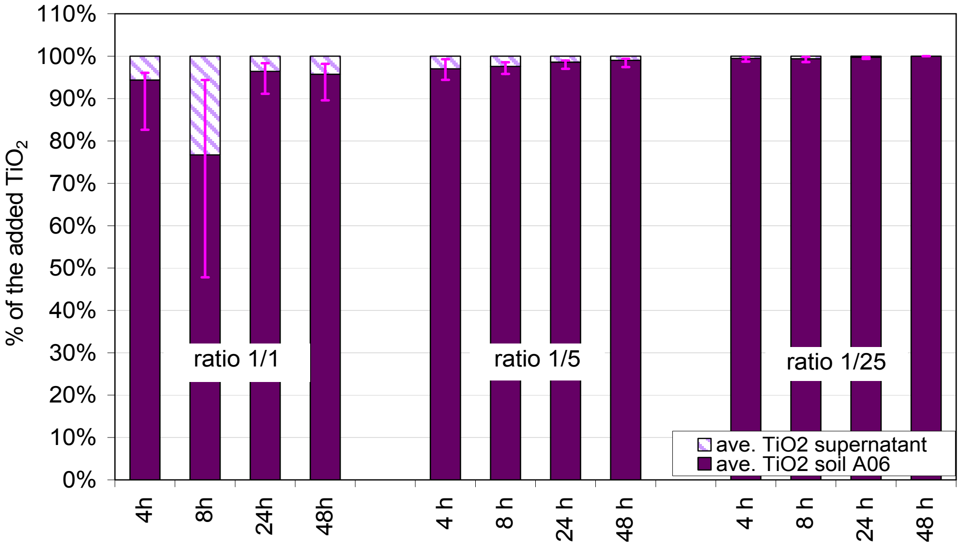


Figure 49: Average determined partitioning of TiO_2 between supernatant and soil A06 for three ratios and four different equilibration times. Error bars = max and min; n = 2.

Table 14: Average of the leachable Titanium content of the natural soil A06 after 48 h shaking; n = 2.

	Ti content in µg/L ratio 1/1	Ti content in µg/L ratio 1/5	Ti content in µg/L ratio 1/25
Blank value	271 ± 37%	153 ± 45%	114 ± 35%

Figure 49 and Table 14 show the results for soil A06. It is evident that already after 4 h the main part of the added P25 was adsorbed to the soil matrix, for all tested ratios (except ration 1/1 after 8 h, which we assumed as an outlier, against the background of the other results). The detected concentration of the P25 was in the range of the background values. The ratio 1/1 showed the highest TiO_2 concentration in the supernatant and the mixture 1/25 the lowest.

The adsorption behaviour of soil A01 (Figure 50 and Table 15) shows a somewhat comparable behaviour to A06, because soil A01 also shows that the TiO_2 concentration in the supernatant can be viewed as equilibrated already after 4 h taking uncertainties into account. Still a higher variance in the results can be identified along with an indication of lower adsorption capacity for this soil type compare to A06. The ratio 1/1 shows the highest TiO_2 concentration in the supernatant and ratio 1/25 the lowest as was the case for soil A06.

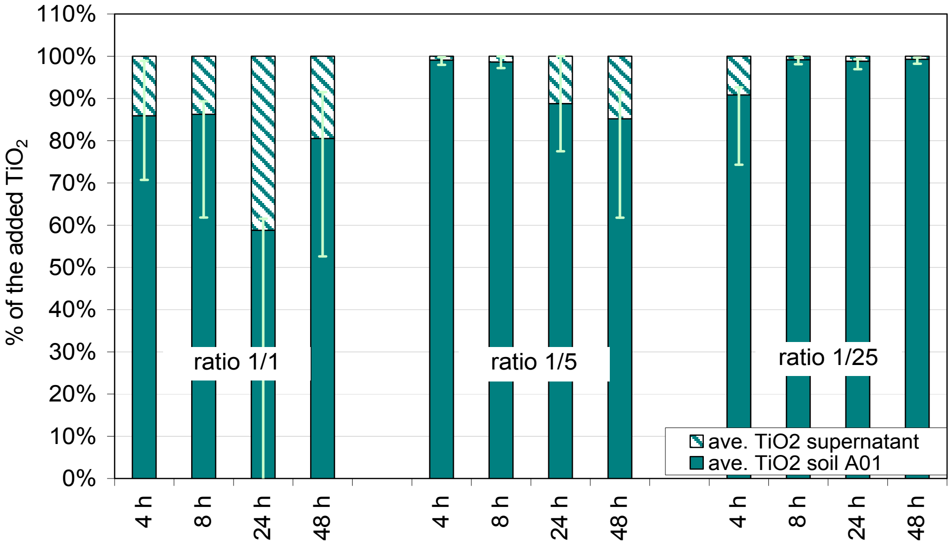


Figure 50: Average determined partitioning of TiO_2 between supernatant and soil A01 for three ratios and four different equilibration times. Error bars = max and min; n = 2.

Table 15: Leachable Titanium content of the natural soil A01 after 48 h shaking.

	Ti content in µg/L ratio 1/1	Ti content in µg/L ratio 1/5	Ti content in µg/L ratio 1/25
Blank value	739 ± 27%	83 ± 45%	61 ± 12%

The trend in Figure 49 & Figure 50 indicate higher absorption of the nanomaterial with decreasing soil content which is contrary to what is expected. No clear explanation can be given in the moment. Still, both the analytical uncertainty and the experimental variability, as can be seen in the figures, show that the trend is within the overall experimental uncertainty.

Identification and influence of the pH conditions of / on the test suspension

The pH can influence the adsorption capacity of a soil; due to this we measured the pH of the soil / suspension mixture directly before and after shaking (**Table 16Fehler! Verweisquelle konnte nicht gefunden werden.**). The pHs of the columns were measured in the supernatant after the centrifugation step.

Table 16: pHs of the supernatant of the samples (mixture soil and nanomaterial P25) after different equilibration times and centrifugation for soil A06 and soil A01. BV (blank value) = same mixture but without TiO_2.

	4 h	**8 h**	**24 h**	**48 h**	**BV 48 h**
soil A06					
50 g	6.3 ± 0	6.2 ± 0.1	6.2 ± 0	6.3 ± 0.1	6.3 ± 0.1
10 g	6.1 ± 0.1	6.3 ± 0.2	6.5 ± 0.1	6.5 ± 0	6.2 ± 0.1
2 g	6.5 ± 0.1	6.4 ± 0	6.4 ± 0	6.6 ± 0.2	6.4 ± 0.1
soil A01					
50 g	5.4 ± 0.1	5.4 ± 0	5.3 ± 0	5.2 ± 0.1	5.3 ± 0.1
10 g	5.3 ± 0.1	5.3 ± 0.2	5.1 ± 0	5.4 ± 0.1	5.4 ± 0.1
2 g	5.4 ± 0.1	5.2 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.1

The average of the pH for soil A06 was pH 6.3 (CV 3%) and for soil A01 5.3 (CV 2%). The amount of soil (2 g or 50 g) had no significant influence on the pH of the supernatant within the range of uncertainty. The shaking time showed no significant effect on the pH for soil type A01 or A06. The blank values (supernatant of the soil / 0.01 M $CaCl_2$ solution mixture) also showed no soil amount dependency, the pH for soil A06 was around 6.3 (CV 2%) and for soil A01 5.4 (CV 2%).

The pH of the $CaCl_2$ solution was around 5.2 (CV 3%) and of the P25 suspension 5.0 (CV 1%). The mixture of $CaCl_2$ and P25 showed a significant increase of the pH by pH 1.2 -1.4 to 6.4 (CV 1%).

- ➔ *The highest adsorption was found for the soil/suspension ratio with the lowest soil amount for both tested soil types. Still, adsorption was in nearly all cases > 95 % and normally > 97%.*
- ➔ *No significant differences in time and mixing ratio were identified in the pre-tests. Therefore it was decided to use a soil / suspension ratio of 1/5 (as the middle ratio tested) and a shaking time of 4 h (minimum time needed for equilibration) for the following tests.*
- ➔ *A leachable Ti fraction is present in natural soils being dependent on the soil types and amount of soil added to the suspension. This soluble fraction may explain the observed tendency of higher adsorption efficiencies for lower soil concentrations.*
- ➔ *With increasing soil mass added into a fixed volume of liquid the fraction of leachable Titanium decreased indicating a saturation of the supernatant.*
- ➔ *To make allowance of the uncertainty of the results the supernatant was analysed for Titanium additionally after 1 h, 2 h and 8 h in Stage 2 of the test.*
- ➔ *The leachable soil Titanium of the tested soils A01 and A06 leading to a background concentration in the supernatant were analysed in more detailed in the following study, by testing further soil / water mixing ratios.*

A leachable Ti fraction was presented in the tested mixing ratios. To get more information about the leachable fraction of the soil types A01 and A06, additional analysis with different soil / water mixing ratios (**Table 17**) were conducted.

Table 17: Tested mixing ratios after an equilibration time of 24 h.

mixing ratio	Soil
75 g soil / 50 mL 0.01 M $CaCl_2$ solution	A01 and A06
50 g soil / 50 mL 0.01 M $CaCl_2$ solution	
10 g soil / 50 mL 0.01 M $CaCl_2$ solution	
2 g soil / 50 mL 0.01 M $CaCl_2$ solution	

In **Figure 51** the amount of the leachable TiO_2 content of soil A06 and A01 which was found in the supernatant after the experiment is shown.

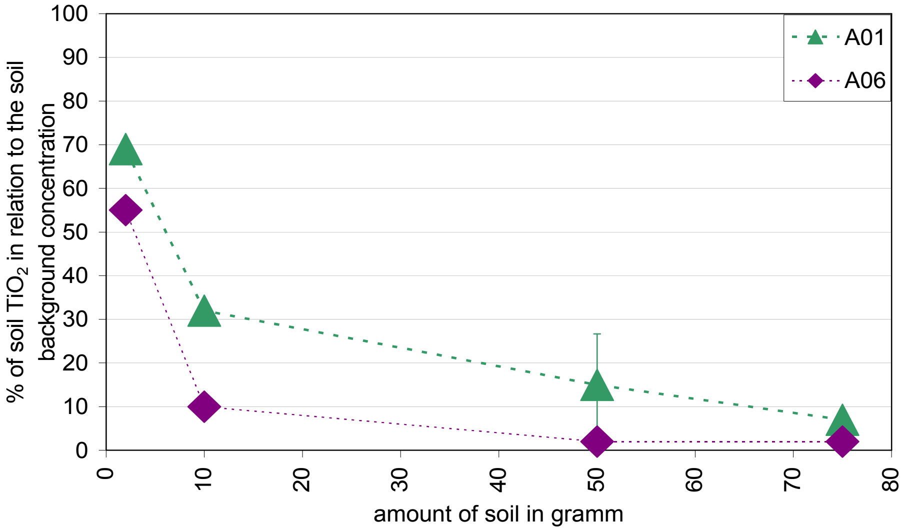


Figure 51: Percent of the leached TiO2 of the tested soil types A06 (diamonds) and A01 (triangles) as function of the soil added. Error bars = max and min; n = 2 for 75 g; n = 4 for 50 g, 10 g and 2 g.

Both soils A06 and soil A01 showed the same behaviour, with increasing soil amount the leachable TiO_2 from the soil decreased. Soil type A06 always shows a lower leachable fraction compared to soil type A01 for all tested values.

Tier 2 - Adsorption kinetics at one concentration of the test substance

Based on the results of the first tests with soil A01 and A06 a soil / suspension ratio of 1/5 and a contact time of 4 h was chosen for the following tests. Beside soil A01 and A06 which were used for the preliminary test, three further natural reference soils with varying pH, texture and cationic exchange capacity (CEC) were used for the study:

- A02 - Stagnic Luvisol (silt loam, sub-acid, light humic)
- G03 - Eutric Cambisol (silt loam, medium acid, medium humic)
- G05 - Gleyic Fluvisol (silt loam, strongly acid, strongly humic)

These reference soils were also provided by the Fraunhofer Institute in Schmallenberg, Germany (www.refesol.de). Before use, all soils were air dried for 48 h at 21 °C and sieved by a 2 mm mesh.

Analysis data of the used natural soils are given below (Figure 52). For more detailed information (e.g. Organic Carbon) of the used soils see Annex III - A3-10 Detailed analysis data of the employed soils. The pH of soil A02 was 6.63 (CV 2.4%), for soil G03 5.64 (CV 1.2%) and 4.78 (CV 1.2%) for soil G05.

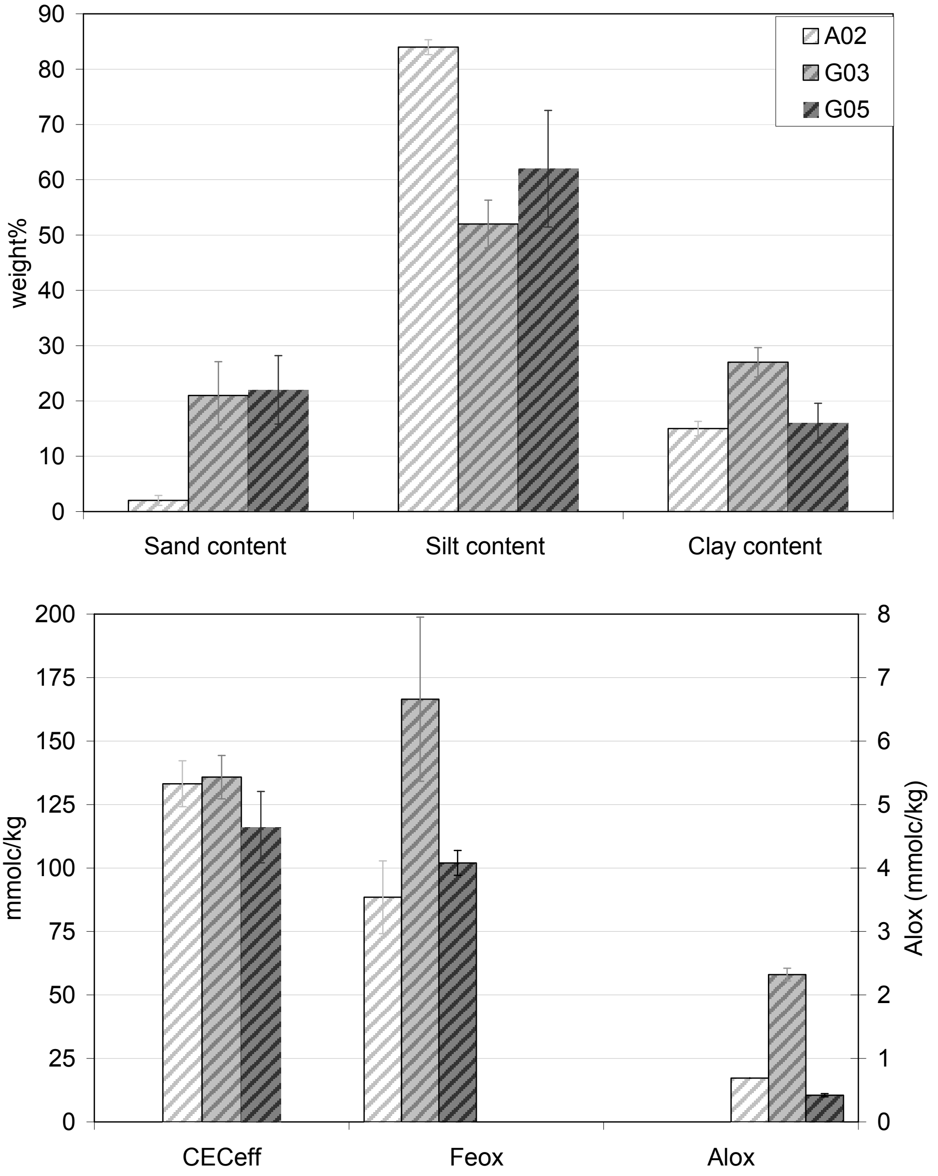


Figure 52: Sand, silt and clay content of the soils – A02 = Stagnic Luvisol, G03 = Eutric Cambisol and G05 = Gleyic Fluvisol (upper figure); cation exchange capacity (CEC), Ironoxalat (Feox), Aluminumoxalat (Alox) of the used soils (lower figure); (www.refesol.de).

For the determination of the adsorption kinetics the above described soil types were tested with P25 as well as UV Titan M262. To obtain more information about the adsorption behaviour testing was extended to the soil types A01 and A06.

Analogue to the first test the soils were equilibrated with 0.01 M $CaCl_2$ solution for 12 h. After that the P25 and UV Titan M262 suspension was added (2.5 mg/L in the system). The mixture was subsequently shaken for 1 h, 2 h, 4 h and for three new tested soil types A02, G03 and G05 also for 8 h for P25 and for 4 h for UV Titan M262 (Table 18). Afterwards the mixture was centrifuged and the supernatant analysed of its Titanium content, using ICP/OES after HCl, HNO_3, HF digestion (see chapter 2.2 Chemical analysis) .

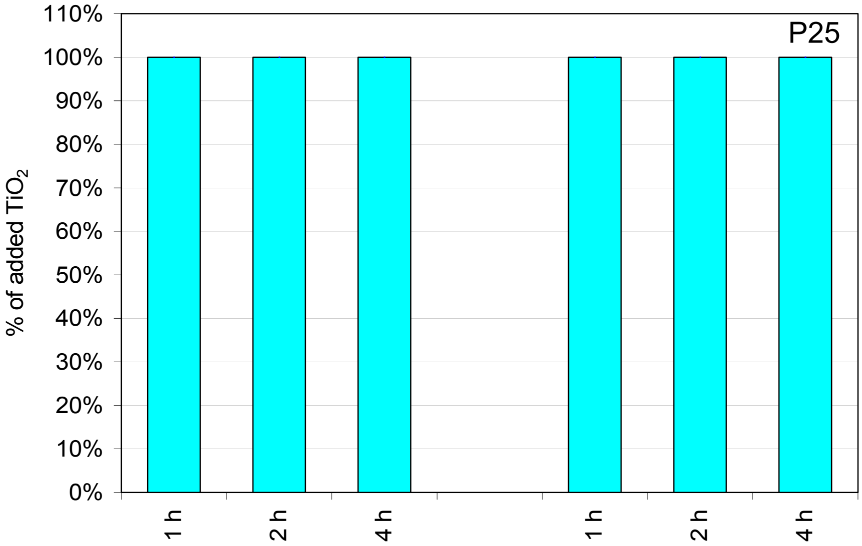
The adsorbed amount of the particles was calculated and analysed using equation described in section 3.3.2 Methods and in detailed described in Annex III - A3-9 Standard operation procedure – Nanoscale TiO_2 adsorption behaviour according to OECD 106 (2000).

Table 18: Tested materials and soils for the adsorption kinetics.

soil	mixing ratio	material	equilibration time in hours
A06	1/5 – 5 g soil / 25 mL suspension	2.5 mg P25 and UV Titan M262	1, 2 and 4
A01			
A02			1, 2, 4 and 8
G03			
G05			

The soil types mixed with UV Titan M262 showed for all samples no Titanium concentration > 1% of the added material in the supernatant after centrifugation (Figure 53 - Figure 57). For P25 for soil type A06, A01 and G05 (Figure 53, Figure 54 and Figure 57) the same behaviour as for UV Titan M262 was detected. No Titanium concentration > 1% of the added material was detected in the supernatant after centrifugation. For soil type A02 and G03 no Titanium concentration > 5% - namely 3 - 4 % and 4 - 5% - of the added material was detected in the supernatant after centrifugation (Figure 55 and Figure 56). Still, the amount is very small and no further information can be derived from these measurements.

It was also shown, that, dependent on the soil type, a leachable Ti fraction exist. Soil type G03 and G05 showed no leachable Ti concentration > 5 µg/L (LOD) in the supernatant. The highest leachable fraction was detected for soil type A02 with 375 µg/L, than soil type A06 with 40 µg/L followed by soil type A01 with 11 µg/L all after 4 h equilibration time.



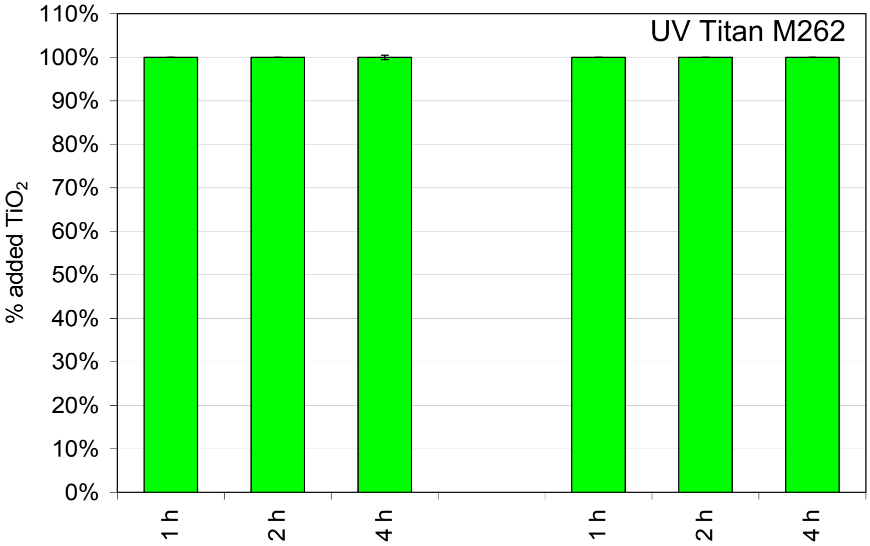
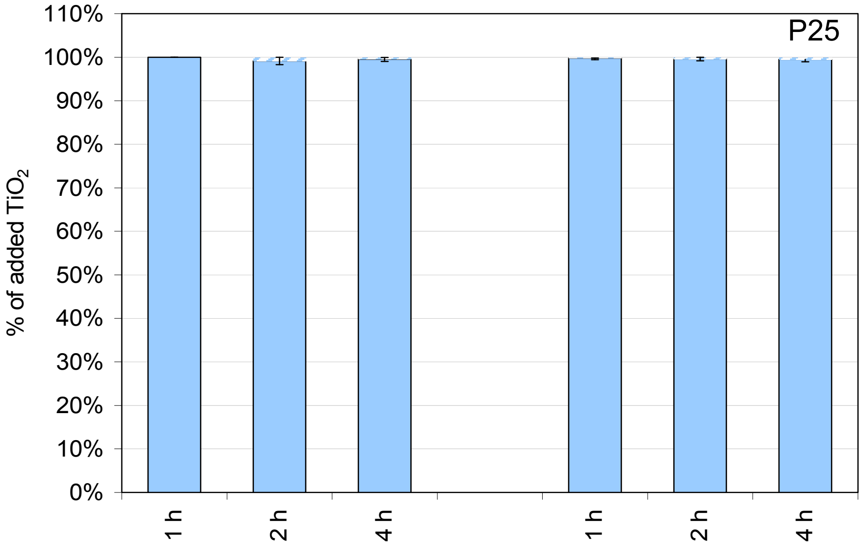


Figure 53: P25 (upper figure) and UV Titan M262 (lower figure) fractionation between soil A06 and supernatant for the mixing ratio 1/5 and equilibration times. Shaded part measured TiO_2 in the supernatant (here < 1%), full coloured part calculated TiO_2 fraction in the soil. Error bars = max and min; n = 2.



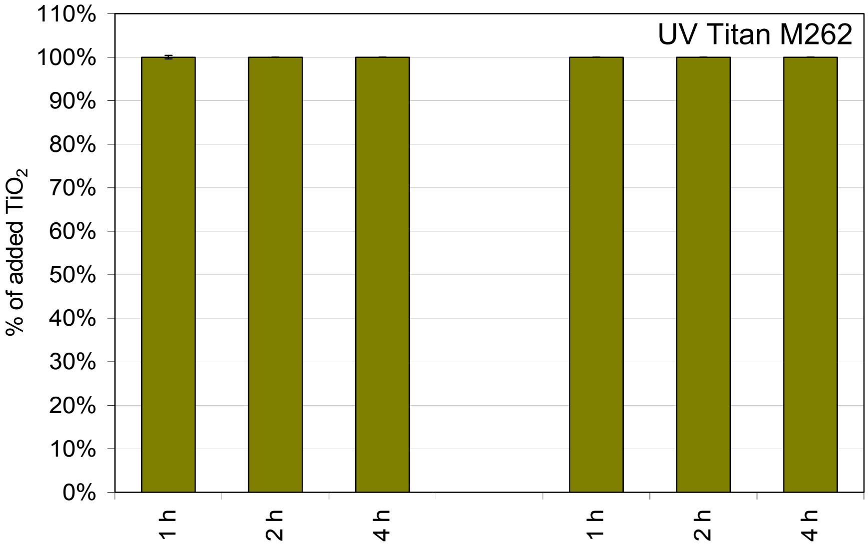
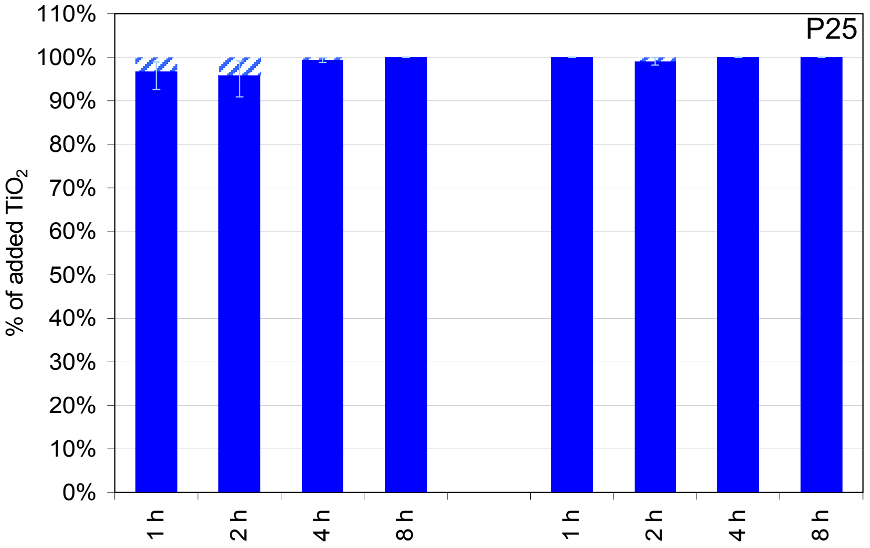


Figure 54: P25 (upper figure) and UV Titan M262 (lower figure) fractionation between soil A01 and supernatant for the mixing ratio 1/5 and equilibration times. Shaded part measured TiO_2 in the supernatant, full coloured part calculated TiO_2 fraction in the soil. Error bars = max and min; n = 2.

The results for soil A06 and soil A01 are in agreement with the results of the preliminary study. For the mixing ratio 1/5 and equilibration time of 4 h for both soils no increased TiO_2 concentration was detected in the supernatant.



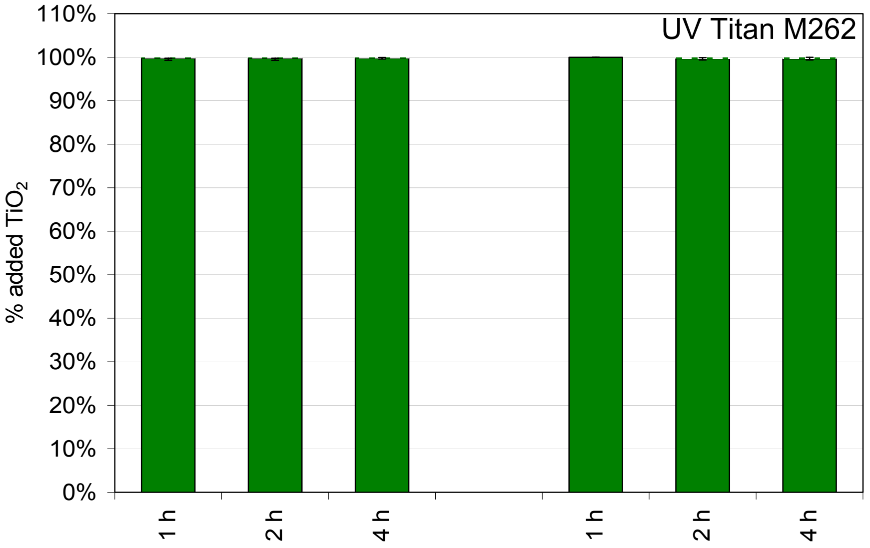
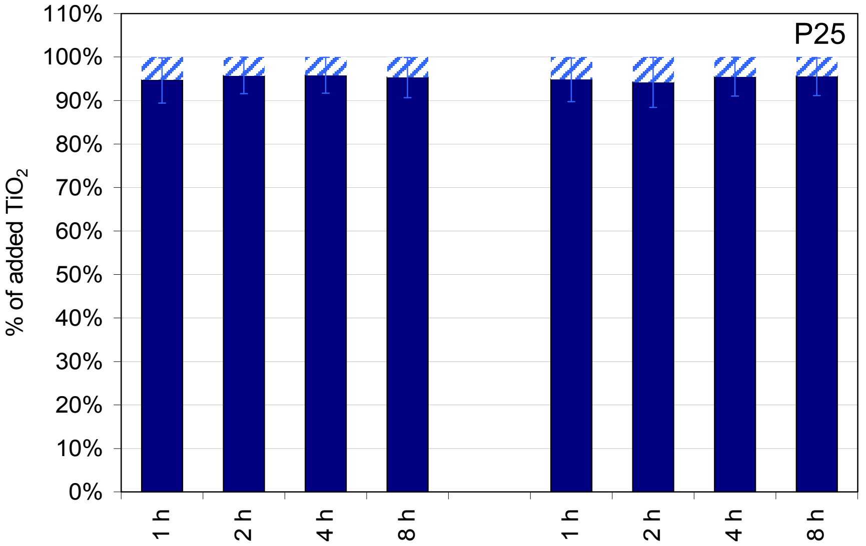


Figure 55: P25 (upper figure) and UV Titan M262 (lower figure) fractionation between soil A02 and supernatant for the mixing ratio 1/5 and equilibration times. Shaded part measured TiO_2 in the supernatant, full coloured part calculated TiO_2 fraction in the soil. Error bars = max and min; n = 2.



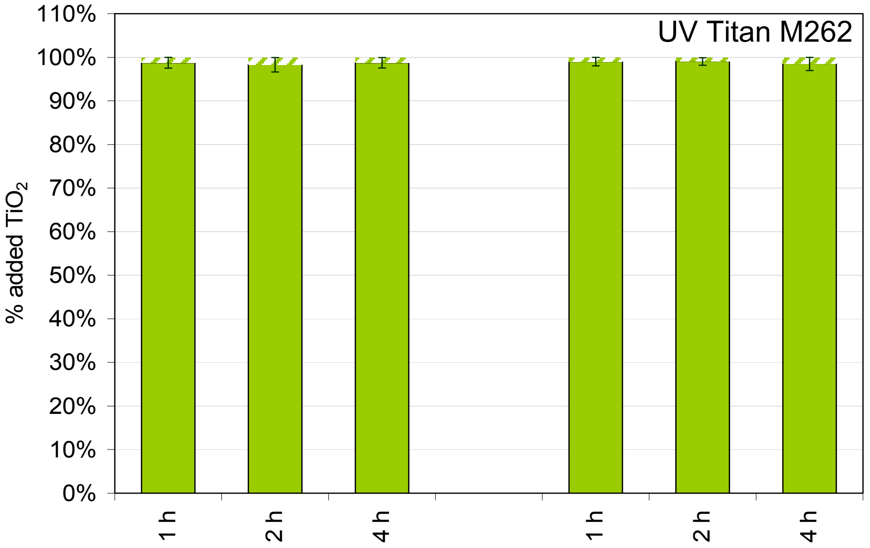
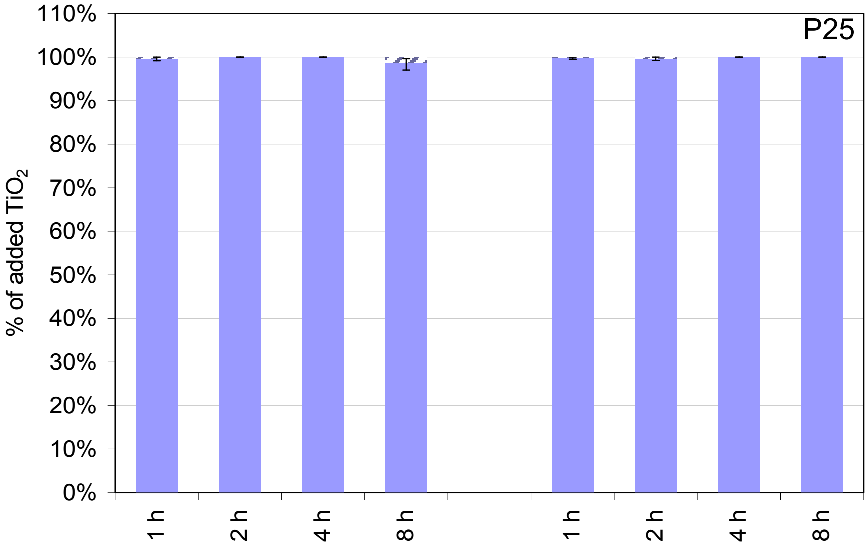


Figure 56: P25 (upper figure) and UV Titan M262 (lower figure) fractionation between soil G03 and supernatant for the mixing ratio 1/5 and equilibration times. Shaded part measured TiO_2 in the supernatant, full coloured part calculated TiO_2 fraction in the soil. Error bars = max and min; n = 2.



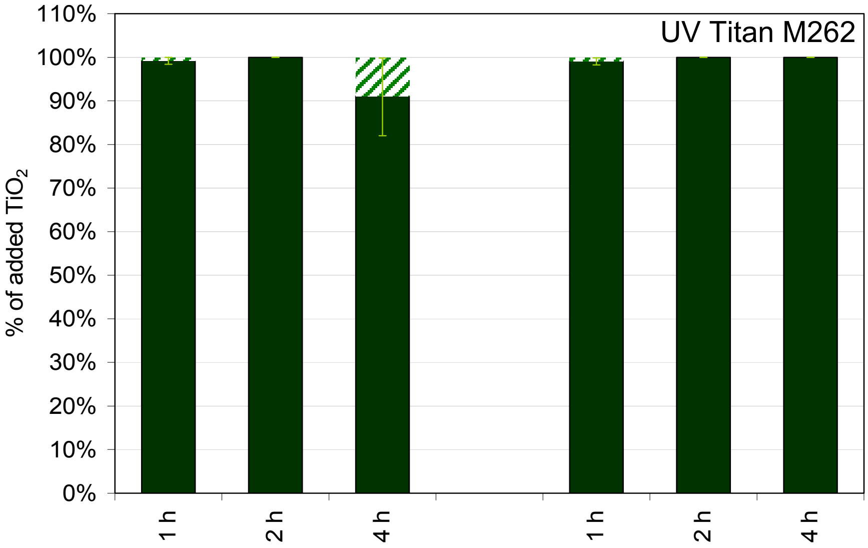


Figure 57: P25 (upper figure) and UV Titan M262 (lower figure) fractionation between soil G05 and supernatant for the mixing ratio 1/5 and equilibration times. Shaded part measured TiO_2 in the supernatant, full coloured part calculated TiO_2 fraction in the soil. Error bars = max and min; n = 2.

3.3.4 Discussion and summary

For all tested soils with both tested nanomaterials no significant concentrations of the nanomaterial was detected in the supernatant and no adsorption isotherms could be calculated.

For the differentiation between adsorbed and non adsorbed particles by the soil matrix, the soil / suspension mixture was centrifuged to separate the solid from the aqueous phase. In contrast to soluble chemicals a loss of particles by centrifugation without adsorption on the soil matrix occur, for any particle size but mainly for larger particles. This can be important if agglomeration of the particles occurs, during the test.

To derive first estimates for the settling behaviour of particles Stokes' law can be used to calculate the velocity of small particles in a centrifugal field.

$$v_S = \frac{(\rho_s - \rho_f) \cdot z \cdot g \cdot d^2}{18 \cdot \eta_f} \qquad Re < 0.5$$

v_S	settling velocity
ρ_s	density of solid
ρ_f	density of fluid
z	relative centrifugal force
g	gravitational acceleration
d	particle diameter
η_f	dynamic viscosity of fluid
Re	Reynolds number of particle

For the above mentioned centrifuge with 2700 g all TiO_2 particles larger than 177 nm settled and therefore are removed from the supernatant.

It is conceivable that by contact of the TiO_2 suspension with the soil / water mixture agglomeration of the particles occurred and due to this it is conceivable that during the centrifugation step the TiO_2 agglomerates were separated from the liquid fraction without adsorption processes. The assumption that the amount of the materials which was not detected in the supernatant has to be absorbed by the soil is not true for nanomaterials.

With the tested TiO_2 materials no differentiation in the solid phase between settled and adsorbed particles is possible, which can lead to uncertain results.

→ *We conclude that the OECD test guideline 106 cannot be employed to derive information on adsorption – desorption isotherms at least for the nanomaterials tested here.*

→ *Furthermore the test procedure itself is ambiguous in view of how any test results may be interpreted especially in view of separation of suspended nanomaterials along with soil particles by using a centrifuge or filter. Hence we recommend this test method not to be used for testing of nanomaterials.*

4. Relevance of results for nanosized TiO_2, input scenarios and environmental mobility

Several pathways of TiO_2 nanomaterial into the environment exist and recent studies have shown the release of nanoscale particles from products during their life cycle in the environment (Kaegi et al., 2008, 2010, Hsu and Chein 2007). The importance of the pathway via water treatment plants and sewage sludge into surface waters and soils, as investigated with the OECD test guidelines in this study, is currently discussed, due to the product applications. Consequently the U.S. EPA, 2010 already hypothesized if TiO_2 nanomaterial enters the municipal sewage systems it might be present in both waste streams (solid and liquid). Hence the TiO_2 nanomaterial may be transported to the soil via sludge disposal on agricultural or public areas. The fate of the TiO_2 nanomaterial from here is even less known and several scenarios exists. Certainly TiO_2 can be viewed to be persistent in the environment. One scenario currently investigated is the transport of TiO_2 nanomaterial through the soil column to the ground water and subsequently entering the food chain via e.g. drinking water. Therefore TiO_2 nanomaterial will enter the environment and ecosystems. Whether the TiO_2 nanomaterial might differ in its behaviour (reactivity, mobility etc.) from conventional product or natural TiO_2 is largely unknown yet and has to be investigated (U.S. EPA, 2010).

Suspension preparation

The nanomaterials were suspended in different liquid media for the environmental tests to simulate the most likely path of entry in the environment. The first TiO_2 suspension preparation experiments (stirring, ultrasonic bath) based on the suspension procedure mentioned in Hyung et al., (2007) and Hund-Rinke et al., (2010). With procedures given in those papers (stirring or ultrasonic bath) no stable suspension could be prepared. Therefore the use of an ultrasonic homogeniser was tested and found to be necessary (chapter 2.1). With the use of an ultrasonic homogeniser a stable suspension could be prepared in DI water. In a liquid media with a higher ionic strength (SDW for the LSTP experiments), no stable suspension could be prepared unless an additive was employed (chapter 2.1). The destabilisation (agglomeration) effect of increased ionic strength is in good agreement with results from other studies (von der Kammer et al., 2010; Kottelat et al., 2009; French et al., 2009; Domingos et al., 2009, Zhang et al. 2009).

Laboratory sewage treatment plant

In this study a very good clearance efficiency of the laboratory sewage treatment plant was determined. The major part of the added TiO_2 nanomaterial was adsorbed to the sewage sludge, and only 3 – 5 % of the added nanomaterial was found in the effluent. These results are in agreement with other recent studies. Kiser et al., (2009) detected 70 - 85% (mainly attributed to particles > 0.7 µm) adsorbed to the sewage sludge, while in the outflow predominantly particles below 0.7 µm were found. With 2 - 5% of the added cerium oxide in the outflow comparable results to the study presented here (3 - 4%) were found for cerium oxide in a laboratory sewage treatment plant (Limbach et al., 2008). Sorption to activated sludge is therefore to be considered as a major removal mechanism for nanomaterials in sewage treatment plants.

The study from Jarvie et al., (2009) demonstrated the different behaviour of bare and functionalised SiO_2 nanomaterials (with Tween 20 - non-ionic tenside) in wastewater. They detected main differences in the behaviour of different bare and functionalised materials. The functionalisation of a material is important for its following fate in the environment. In our study P25, a non functionalised anatase / rutil TiO_2 nanomaterial was tested. If different functionalisations ore other crystalline structures effect the behaviour was not tested and could not be estimated. This has to be tested in further studies.

Leaching in soil columns

Only little Information is available on the fate and behaviour of nanomaterials in soil ecosystems. So far six studies investigating transport behaviour of TiO_2 nanomaterials in natural or artificial soil systems have been published to the knowledge of the authors. One further study was found studying the behaviour of aluminiumoxide nanomaterials. For a summary of their findings see Table 19.

The studies by Leocanet et al., (2004) and Guzman et al., (2006) generated basic information about the transport behaviour in artificial porous system. The results of these studies are difficult to extrapolate to natural systems.

The studies by Solovitch et al., (2010), Fang et al., (2009, 2011) and Duester et al., (2011) indicate a transport of the added TiO_2 material. As it was mentioned before the main difference of these studies compared to ours leading to a possible mobility of nanomaterials is the use of negatively charged and smaller Nanomaterials. Solovitch et al., (2010) and Darlington et al., (2009) showed that a contrary zetapotential (Nanomaterial positive and matrix negative) reduced or hindered the transport.

Additionally they used a smaller amount of soil and shorter columns for their tests and also a minor concentrated suspension (reduced agglomeration probability). This, as well the smaller particle size could have affected the mobility of the nanomaterials by reduced clogging and filtering effects.

Adsorption behaviour

Kottelat et al., (2010) tested the adsorption of cerium oxide nanomaterials to soils, based on the OECD test guideline 106, as contribution of Switzerland to the OECD programme for “Safety Testing of a Representative Set of Manufactured Nanomaterials”. They come to the same conclusion as we did, that is, the OECD Guideline 106 is not applicable for the testing of Nanomaterials, due to the problem that no differentiation between adsorbed and settled material is possible.

Fang et al., (2009) analysed the stability of TiO_2 nanomaterial suspensions in soil water. They detected that dependent on the soil type, the suspensions was stable over ten days. Comparable results were found by Domingos et al., (2009) and Yang et al., (2009). They had shown, that some humic acids could stabilise suspension. We conclude that the type of the soil could effect the stability of TiO_2 nanomaterial suspension and therefore their behaviour.

Table 19: Improved overview on peer reviewed studies on the mobility of metal oxide primarily TiO_2 nanomaterials in porous media.

Study	**Lecoanet et al., 2004**	**Guzmann et al., 2006**	**Solovitch et al., 2010**	**Darlington et al., 2009**	**Fang et al., 2009**	**Fang et al., 2011**	**Duester et al., 2011**
Tested Nanomaterial	TiO_2 (Anatase)	TiO_2	TiO_2	Al_2O_3	TiO_2	TiO_2	TiO_2 & Sb_2O_3
Application form	polydisperse suspension ~ (agglomerates: 198 nm)	polydisperse suspension – (agglomerates < 150 nm – at pH 5 settled agglomerates suspended with pH 7 water)	suspension – (agglomerates: ~ 150 nm)	powder and suspension (agglomerates = 204 nm - 1.5 µm)	suspension (agglomerates: ~ 100 nm)	suspension (agglomerates: ~ 100 nm)	suspension (agglomerates 99 nm)
Testmatrix / -system	acrylic column; spherical silicate beads 300 – 425 µm	two dimensional glass porous system ; 700 µm	plexiglass column; natural sand	glass columns; natural soil and sand	glass columns; 12 different soils	glass column; 4 different soils	cylinder; natural floodplain (natural porous system)
Setup conditions	pH 7; negative zeta potential	zeta potential pH dependent; IEP pH 5.5; varying pH values of the suspension pH 1, 3 , 7 (pH 5), 10, 12	positive & negative zeta potential (dependent pH)	positive & negative zeta potential, pH 7	varying pH > 6 & negative zeta potential	varying pH > 6 & negative zeta potential	pH > 7 & negative zeta potential
Method	controlled flow from top to the bottom → detection in the effluent	controlled flow from top to the bottom → detection in the effluent	controlled flow from top to the bottom → detection in the effluent	suspension pumped from bottom to the top → detection in effluent	saturate system pumped from bottom to the top → detection in effluent	saturate system pumped from bottom to the top → detection in effluent	application on the top → detection in pore water

Results	a. transport detected → in dependency of Darcy velocity	a. transport detected (except pH 7 (pH 5) – settled agglomerates – zero point of charge)	a. transport detected → if the material and the sand matrix showed the same zeta potential (negative)	a. transport detected → inversely size related	a. detected transport → correlated with large soil particles and low ionic strength	a. transport detected → Carrier effect by Titania particles leads to a higher mobility of copper	a. transport for Sb_2O_3 transport indicated for TiO_2
		b. transport effected by pH and therefore by size and zeta potential	b. retention (up to 95%) → if the material showed a positive and the matrix negative zeta potential	b. clogging effect by powder application; increasing agglomeration with increasing ionic strength → decreased transport; Dominant factor for transport = zeta potential – similar zeta potential of soil and material → transport	b. transport is negative correlated with clay content, dissolved organic carbon, and salinity		

5. Recommendation on the OECD test guidelines

The fate and behaviour of nanoscale particles in STPs is of decisive importance for their environmental risk assessment. Therefore three OECD test guidelines were tested for their applicability for nanomaterials. The following three sections summarise our recommendations with regard to these guidelines.
Overarching all test guidelines is the issue on the way of applying the nanomaterials in these guidelines. Extensive tests and developments have been done towards standard dispersion protocols when applying suspensions. Following recommendations resulted:

Little changes in pH, ion concentrations etc. may have significant effects on the stability of a suspension. Therefore we recommend that:

- clean solvents, including harmonised distilled water, should be used,
- all deviations should be recorded,
- an appropriate dispersion action (shaking, stirring, sonication) shall be chosen,
- suspension stability evaluation shall at minimum be based on observation of sedimentation and size distribution measurements,
- size distribution in the suspension should be reported,
- and those should be related to the application in the test guideline.

A round robin test based on the above criteria showed good inter-laboratory comparability if the same techniques and suspension media were applied. No specific recommendation can be given for use of nanomaterial powders in the tests.

5.1 Applicability of the OECD test guideline 303A for testing nanoscale particles

The use of laboratory sewage treatment plants (LSTP) is one option for assessing the fate and behaviour of nanoparticles in STPs, but few data about its applicability to nanomaterials is available so far. Therefore, the OECD simulation test according to OECD Guideline 303A (2001) was evaluated using nanoscale TiO_2. The results indicate that the OECD guidance on testing the efficiency of sewage treatment plants is in principle applicable to assess the behaviour of nanoscale particles. The test guideline allows enough modifications to adapt the design to the question. Still certain criteria specific for nanomaterials have to be set to allow for comparability and interpretability of the results obtained.

- The test should be run under nitrifying conditions as in most technical STP, in order to assess the impact of nanomaterials to activated sludge. Here, the nitrifying bacteria are among the most sensitive of activated sludge. Hence the recoding of corresponding data should be recommended.
- A clear statement should be included that other stages of the biological wastewater treatment, such as primary sedimentation, denitrification, and/or the filtration of the effluent from the clarifier is not simulated with this test system and might be influenced by the nanomaterial.
- The use of Synthetic Drinking Water (SDW) instead of tab water is recommended in order to have reproducible test conditions and to achieve better comparability between laboratories.
- The dosage of nanoscale particles should be made separately from that of the organic synthetic wastewater in order to avoid any agglomeration of the particles. The use of a suitable dispersant may be considered, but its potential impact on the treatment process has to be assessed.
- When the impact of the dispersant has been determined in a pre-test to be acceptable, the reference LSTP unit may also be fed with the same concentration of the dispersant as the test LSTP unit. However, a negative impact of the dispersant on the clearing efficiency might counteract the overall validity of the test.
- The determination of the filterable solids in the effluents of the LSTP is recommended offering an additional tool for describing the influence of the dispersant. Also at least indicative analyses on the nature and partitioning of the nanoscale particles in the effluent, whether adsorbed to filterable solids or not, should be conducted.
- The main sampling points for subsequent chemical analysis include the activated sludge next to the effluents of the LSTP. The calculation of an overall balance of the nanomaterial in question is recommended as a quality control. The OECD 303 A should include a paragraph describing the principle of calculating such a balance and achievable recovery rates. So far the possibility to establish a mass balance is only referred to in Annex III of OECD “guideline 303 A” for poorly water soluble or volatile test substances. OECD guideline 314 “Simulation tests to assess the biodegradability of chemicals discharged in wastewater” might serve as an example for the description of a mass balance.

→ *We recommend that the above mentioned points be added to the OECD test guideline 303 A for use with nanomaterials. Provided this addition we find the test guideline to be applicable for nanomaterials, here specifically Titanium Dioxide.*

5.2 Applicability of the OECD test guidelines 312 for testing nanoscale particles

The OECD test guideline 312 tests the mobility of chemicals, here specifically nanomaterials in soil columns to derive information on possible environmental transport. We found the OECD test guideline 312 to be principally also applicable to assess the behaviour of nanomaterials. The test guideline allows a series of proposed modifications making it appropriate. Certainly at some stages of application difficulties occur, and adaptations should be considered for following points:

- Soil selection
- Concentration and detection
- Application of the nanomaterial

Soil selection

In the test guideline several criteria for soil selection are provided with regard to pH values, texture and organic content. But no limitations concerning their water permeability are made.
In this study a backwater soil (Gleyic Podsol A04) was used whereupon difficulties occurred due to the lack of breakthrough by gravity for water. A once applied suction power overcame this problem and triggered a run off of the material. Unluckily the transport was mainly along the glass column wall, as could be seen by the deposition of TiO_2 along the column, which in turn made a statement about the mobility of a material in soils difficult.

→ *We recommend limiting the use to soil types with normal to low retention potential in soil column tests which allow water transport by gravity. If other soil types are employed, needing the aid of suction power, all data should be carefully evaluated for transport mainly along the soil column walls.*

Concentration and detection

The amount of the substance to be applied in the test is defined in the guideline to be high enough to enable detection of at least 0.5% of the applied dose in any single segment. Alternatively, the dose may correspond to the maximum recommended use rate – real exposure concentration (single application).
The latter recommendation could not be followed due to missing information on additional, anthropogenic TiO_2 nanomaterial concentrations in the environment. Only modelled concentration (Gottschalk et al., 2010) are available, which predict an increase of anthropogenic Ti in soils of around 1.3 µg/kg*a. For soils treated with sewage treatment plant sludge an increase of around 89 µg/kg*a is predicted. The corresponding soil concentrations cannot be used for the soil column experiments considering the high Titanium background and the needed detection limit.
To ensure that the added Ti could be detected in any segment of the soil column an amount of 500 mg TiO_2 was used based on the assumption of equal distribution. This corresponds to a concentration of 5 g/L of the stock suspension, whereof 100 mL were applied to the soil column. It is conceivable that the used concentration of the material may affect the mobility (increased agglomeration, increased filtration and pore clogging).

→ *We recommend the test scenario to be clearly defined for better comparison between different nanomaterials. In the here chosen case of TiO_2 relatively high, worst case scenario concentrations had to be employed possibly leading to high agglomeration and pore clogging. If another nanomaterial with significantly lower concentration is used possibly a higher transport rate could be determined. Hence information on concentration dependent effects in soil column test for nanomaterials is needed.*

→ *We recommend that for the simulation of a more realistic scenario the test design should be adapted for the application of lower concentrated suspensions over a longer time period.*

- → *We recommendet that the sampling of different allliquots of the eluate can be necessary, to achieve a higher concentration in the sample.*
- → *We recommend some detailed tests using also other detection methods in the eluate, like Field Flow Fractionation coupled with a mass spectrometer (FFF-MS) or Surface-Enhanced Raman Spectroscopy (SERS) which are able to detect nanomaterials in matrices with a high background of other materials. However these detection methods are expensive and the detailed studies should be conducted mainly for method evaluation purposes. More information about detection methods can be found in Tiede et al., 2008.*
- → *SEM / EDX analysis is a useful tool to detect the transport of isolated TiO_2 agglomerates and tiny amounts of Ti and their shape.*

Application of the nanomaterial

No specific recommendation can be given with regard to the way the nanomaterial should be applied to the soil column.

In this study the material was applied to the soil columns in form of a suspension, a likely path of entry into the environment. The use of a suspension allows reproducible conditions of the applied particle size distribution. With a suspension a homogeneous application could be warranted, whereas reproducible homogeneous spiking with a dry powder may be challenging. Still, premixing of the dry powder with a soil section may also be used.

5.3 Applicability of the OECD test guidelines 106 for testing nanoscale particles

The OECD test guideline 106 was designed for the testing of the adsorption behaviour of soluble chemicals to a soil matrix. By testing nanomaterials following difficulties occurred during the test:

- Agglomeration of the nanomaterial during the test
- No separation between adsorbed and non adsorbed materials

No adsorption isotherms could be determined for nanomaterials with this method.

Influence of agglomeration on the results

Agglomeration of the test material may occur when mixing the suspension with the soils due to changes in the ionic strength, pH and destabilising compounds (chapter 2.1). This may also be the case in natural conditions. Increases in particle size may result in the separation of the suspended particulate nanomaterial along with the soil fraction using filtration or centrifugation. Both centrifugation and filtration remove nanomaterial agglomerates from the supernatant. Hence no differentiation of adsorbed or agglomerated nanomaterial can be made using the OECD test guideline 106 and no information can be correctly obtained for adsorption coefficients and isotherms.

→ *We conclude that the OECD test guideline 106 is principally not applicable for nanomaterial testing.*

6. Summary

The focus of this report is the determination, fate and behaviour of nanoscaled Titanium dioxide (TiO_2) in the environment. Nanoscaled TiO_2 is an industrial product being used in various applications such as sunscreens, coatings and paints which make a release into the environment likely. To be able to assess possible impacts of nanomaterials like TiO_2 corresponding test procedures, here specifically OECD test guidelines were tested for the applicability and the results obtained interpreted. More specifically the OECD test guidelines OECD 303A – Laboratory Sewage Treatment Plants, OECD 312 – Leaching in Soil Columns and OECD 106 – Adsorption / Desorption in Soils were tested. Three different types of Titanium dioxide materials uncoated (P25 and PC105) and hydrophic coated (UV Titan 262) were investigated to also derive information on possible influences of coating on the environmental behaviour.

Prerequisites for conducting the tests were evaluation of the analytical methods to be employed, in this case specifically the chemical analysis for Titanium and the preparation of stable suspensions for OECD 303A, 312 and 106.

Analytical methods employed in this study were mainly particle size and zeta potential measurements in liquids, electron microscopy coupled with elemental analysis based on X-ray and a wet chemical quantitative analysis of Titanium. Especially the latter was difficult to establish due to the low detection limit and relatively high reproducibility needed for the various matrices used in the tests and the high background concentration in the tested environmental matrices. Finally an Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP/OES) was employed for Titanium detection after digestion. Also various digestion methods had to be tested. In the end one digestive method using HCl, HNO_3 and HF were employed for suspensions and the sewage sludge, while a sulphuric acid treatment in the microwave was used for the soil matrix. The recovery determined for the sewage sludge and suspensions was determined to be on average 90%, whereas that for the soil matrix 100%. The latter showed a much higher uncertainty of about 50% than that for sewage sludge and liquid samples (±20%). In all matrices a trend of increasing recovery from PC105, P25 to UV Titan M262 was seen. This trend was not taken into account due to the high uncertainties of the analysis results.

The second prerequisite, stable suspensions for the environmental tests according to their needs, was pursuit by testing different mixing types from stirring to ultrasonic fingers, mixing times and pHs. Basically, no single suspension preparation method can be recommended due to different needs in the environmental tests, e.g. the possibility to use dispersion agents or not. Hence separate protocols were developed. In brief: P25 was dispersed in synthetic drinking water using 1 wt% sodium hexametaphosphate with an ultrasonic homogeniser to derive a stable suspension over 24 hours. It is important to note that drinking water could not be used since the stability of the P25 suspension showed high variance with different types of tab water. Hence the recommendations of OECD 303A using drinking water could not be employed.

In the different tested environmental compartment (soil pH 5 – 7, sewage treatment plant pH 7) varying pH values occur, due to this the stability of the suspensions was tested at different pH values. No dispersion agent was used in the soil column tests (OECD 312) or the adsorption / desorption soil tests (OECD 106). Still stable suspensions were achieved in deionised (DI) water with a pH of 5, which was employed in the soil tests OECD 312 and 106. In DI water for P25 no pH effect on the stability of the suspension could be detected at the tested values (pH 5, 7, 9 and 10). For PC105 with increasing pH an agglomeration of the material was observed. For UV Titan M262 a comparable behaviour to PC105 was seen with the highest instability of the suspension at pH 9 (isoelectric point).

The first environmental test was conducted in the laboratory sewage treatment plant with P25 and increasing input concentrations of the nanomaterial over 30 days. Balancing the inflow and outflow of Titanium combined with the sewage sludge analyses a high retention in the sludge was determined. Only a small amount of the Titanium dioxide added to the system (3 - 5 mass-%) was found in the effluent of the system. It is concluded that overall the OECD test guideline 303 A can be employed if specific points are taken into account.

The second environmental test, the leaching experiments in the soil columns was conducted with all three Titanium dioxide materials and three different soil types with varying composition. For P25 and PC105 no significant mobility deeper than the upper few centimetres was determined. Only for UV Titan M262 a transport was indicated down to the fourth segment of the soil columns. Still, no Titanium above the detection limit was measured in the leachate of the columns. With SEM / EDX a transport of isolated TiO_2 agglomerates of all materials was detected in nearly all segments. It is conceivable that the bulk chemical analysis was not sensitive enough for the detection of this transport taking the natural background into account. All together it seems that soils do adsorb the main part of nanomaterials quite effectively or the high concentration employed lead to larger, more immobile agglomerates. Also a kind of clogging of the pores of the soil could have affected the transport. The latter may be of less importance since the water flow through the column was not significantly affected.

No specific recommendation can be given with regard to the way the nanomaterial should be brought onto the soil column. We decided to follow a likely path of environmental entry by using a suspension, which also allows for reproducible conditions in view of particle sizes. Still a premixing of the nanomaterial with a soil section may also be used.

Overall the OECD Method 312 can be used for the testing of nanomaterials. Still, a clear definition of exposure scenarios should be given to mimic more realistic concentrations and avoid different findings due to different concentrations employed. Therefore, specific analytical tools may have to be developed to allow simulations at lower concentrations. At least for TiO_2 the analysis is a challenge in view of the soil background concentration.

The adsorption experiments of P25 and UV Titan M262 showed that no added material could be detected in the supernatant, independent on the type of the material. The assumption that the amount of the materials which was not detected in the supernatant after the test has to be adsorbed by the soil is not true for nanomaterials, because the centrifugation step, to differentiate between the adsorbed and the suspended fraction, lead also to enhanced sedimentation of the agglomerated materials. No differentiation between adsorbed, settled or non adsorbed nanomaterial is possible. The aim of OECD 106 is to obtain a sorption value which can be used to predict partitioning under a variety of environmental conditions. This guideline is not applicable for the testing of nanomaterials, because no differentiation between adsorbed, settled and non adsorbed materials is possible.

Both, OECD 303A and 312 showed high retention of the TiO_2 nanomaterials tested. It seems that the material was immobilised significantly by the sewage sludge and the soil matrix. Nevertheless, for both matrices a small fraction was seen to be mobile. About 3-5% of the added TiO_2 to the laboratory sewage treatment plant was leaving this cleaning step. The subsequent treatment steps will possibly reduce this amount before it is released into the environment. In the soil column experiments for one material, UV Titan M262 mobility was indicated by bulk chemical analysis. SEM / EDX analysis on the other hand frequently identified single TiO_2 agglomerates in the soil matrix. Investigations on which fraction is possibly mobile should be pursued.

Detailed further recommendations on the use of the test guidelines and on the results of the measurements are given in the corresponding sections of the report.

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Annex I

A1-1 Standard operation procedure - Dynamic Light Scattering (DLS) measurements of particle size and zeta potential of Titanium dioxide (TiO_2) – nanomaterial suspensions in aqueous media

1. **Aim of the SOP**
2. **Background**
3. **Preliminary results**
4. **Measuring procedure**

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is intended to provide information for the size and zeta potential measurement of Titanium dioxide suspension for environmental testing within the Project 3709 65 417 and to enable reproducible results in different laboratories
The SOP describes the procedures which are suitable for dynamic light scattering size and zeta potential measurement.

2. Background

Size measurement by dynamic light scattering

Dynamic light scattering is a technique to determine the size distribution profile of small particles in suspension. Performing a dynamic light scattering in aqueous media requires the knowledge of the refractive index of the particle and the particle’s absorption. Furthermore the knowledge about the characteristics of the medium is necessary.

Necessary Instruments

- Dynamic light scattering instrument
- Liquid handling apparatus

Used instruments

In this project dynamic light scattering instruments (Zetasizer ZS DTS 3600 and HPPS, Malvern, England, DelsaNano C - Beckman Coulter, Krefeld, Germany) were used to measure TiO_2 P25, PC105 and UV Titan M262 nanoparticles size in an aqueous suspension.

Zeta potential measurement by laser Doppler Electrophoresis (LDE)

The zeta potential can be used to assess the charge stability of a disperse system. The zeta potential is the force that controls the charge interactions in a suspension. It can be measured by applying an electric field across the dispersion. The zeta potential correlates with the magnitude of particle’s migration velocity towards the electrode of the opposite charge. This can be measured with laser Doppler anemometry. With the knowledge of the dispersant viscosity and the use of Smoluchowski or Huckel theories the zeta potential can be calculated.

3. Preliminary results

Preliminary results show that larger particles in the suspension have a higher impact on the size measurement than smaller particles. Hence large particles are over represented in the size measurement. The obtained results can be presented as intensities, number and volume concentrations of the particles. However the intensity data is the basic information obtained from the measurements, is least influence by assumptions and hence should be used. For measurement result comparisons Z.average value of the diameter should preferably be used. The cumulate analysis gives two values, the mean size of the particles in suspension and a polydispersity index. This result is comparable with other methods of analysis for spherical, monomodal samples with a polydispersitiy index value of 0.1. For samples with an increased polydispersity index the Z.average size can be used for comparative purposes. For samples with a polydispersity index value over 0.5 a Z.average comparison is not recommended. A distribution analysis should be applied to determine the peak positions. To compare the results the Z.average value for the diameter and the polydispersity index (PDI) should be reported.

4. Measuring procedure

To gain reproducible measurements the dynamic light scattering measurements were standardised in this project. All chemical properties were shared between the project partners. For Malvern products a software internal SOP was generated for the measurement. The suspensions were sampled according to the recommendations of the DLS instruments manufacturers. Suspensions were sampled from the upper water column with a pipette without homogenisation.

Malvern Instruments: Size measurement

Create a new SOP for measuring a TiO_2 suspension

Sample:
Material:
Name: TiO_2 P25
RI: 2.7
Absorption: 0.01
Dispersant
Dispersant: Water
Temperature: 25 °C
Viscosity: 0.8872
RI: 1.33
General Options
Sample viscosity options: use dispersant viscosity as sample
Temperature
Temperature: 25 °C
Equilibration time: 15 min
Cell
Cell type: Disposable sizing cuvette
Measurement
Measurement angle: 173 Backscatter
Measurement duration: 1*60 sec
  Measurement:
    Number of Measurements: min 3 (recommended 10)
    Delay between measurements: 2 sec
Advanced
Measurement duration
  Extend duration for large particles: No
Measurement setting:
  Positioning method: seek for optimal position
  Automatic attenuation selection: Yes
Data processing
Analysis model: General purpose

DELSA Nano ZS - Beckmann Coulter

Measurement Parameter	
General Conditions	
File Save:	Auto
Repetition:	3 (recommended 10)
Manuel Temperature Setting:	No
Auto Print:	Manuel
Equilibrating:	Yes
Statistical Summary	Yes
Equilibration:	15 min
Waiting time:	2 sec
Size Measurements	
Dust Limit	5
Upper Dust Limit	10
Lower dust Limit	100
Minimum Intensity	3000
Pinhole (µm)	50
Analysis Parameter	
General	
Analysis method:	CONTIN
Side cut left	0
Side cut right	0
Display	
Graph x Axis:	Manual 4000 nm
Graph Y Axis	AUTO
Others	
Fitting range	G2(τ)
G2(τ)max	2
G2(τ)min	1.003
Noise cut level (%)	0.3
Molecular wight Analisys Const	
Molecular weight	NO
Cell Parameter	
General	
Measurement item	Size
Measurement type	Type 2
Cell name	Disposable Cuvette
Details	
Correlation type	Log
Size Measurements	
Accumulation Time	70
Dilutent properties	
General	
Dilutent name	Water
- Properties	
RI	1.33
Viscosity	0.89
Dielectrical constant	78.3

Test report according to ISO 22412:2008

The test report shall contain at least the following information:

a) Average particle size, d_{DLS}, - here Z.average - being the mean and standard deviation of at least three repeated measurements*;
b) The PI, being the mean and standard deviation of at least three repeat measurments
c) If the ,mean values of d DLS and PI are concentration dependent, their values extrapolated to infinite dilution or the value obtained at the lowest concentration;
d) All the information required for the complete identification of the sample, including details of particle shape and homogeneity;
e) The sampling method used, if known;
f) The test method used, together with reference to this International Standard;
g) The instrument type and number;
h) The dispersion conditions:
1) Dispersing liquid and its cleaning procedure,
2) Concentration of particulate material,
3) Dispersing agents and their concentration,
4) Dispersing procedure,
5) Sonication conditions: frequency and applied power (if necessary);
i) The measurement conditions:
1) actual concentration investigated,
2) viscosity and refractive index of the dispersion liquid,
3) temperature of the sample,
j) analyst identification:
1) name and place of laboratory,
2) operator´s name and initials,
3) date;
k) All operation details not specified in this International Standard, or regarded as optional, together with details of any incident that may have influenced the result(s).

* ISO 13321:1996 specifies six repeat measurements. Experience with the method specified in this International Standard indicates that three measurements are sufficient.

A1-2 Comparison of the different DLS - instruments

Actually for size distribution measurements using DLS no standard / reference materials are required due to a quality assurance. Nevertheless the reproducibility and correctness is commonly checked by an internal standard of PSL particles. Beside this internal standard, revealing for all in this project applied instruments a good reproducibility and correctness, an inter-instrument comparison measurement using Titan suspensions were performed. After suspension preparation the size distribution of a 100 mg/L Titan suspension was measured (Figure 58).

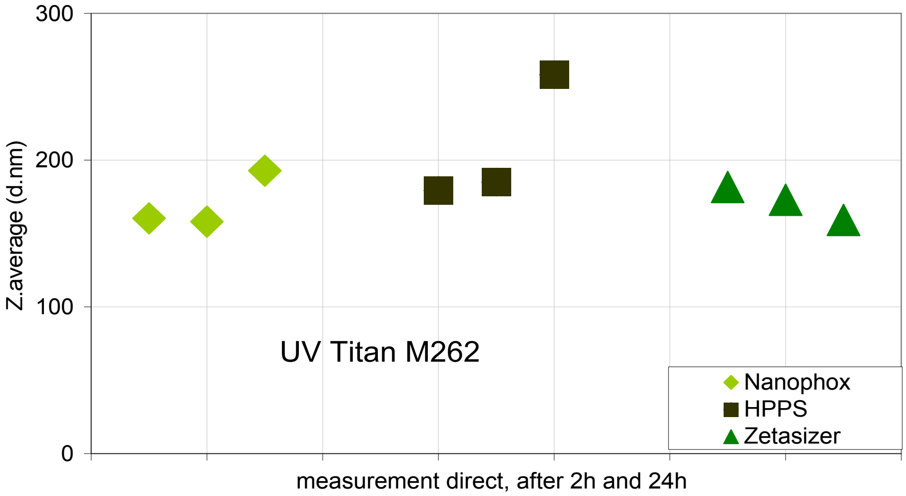
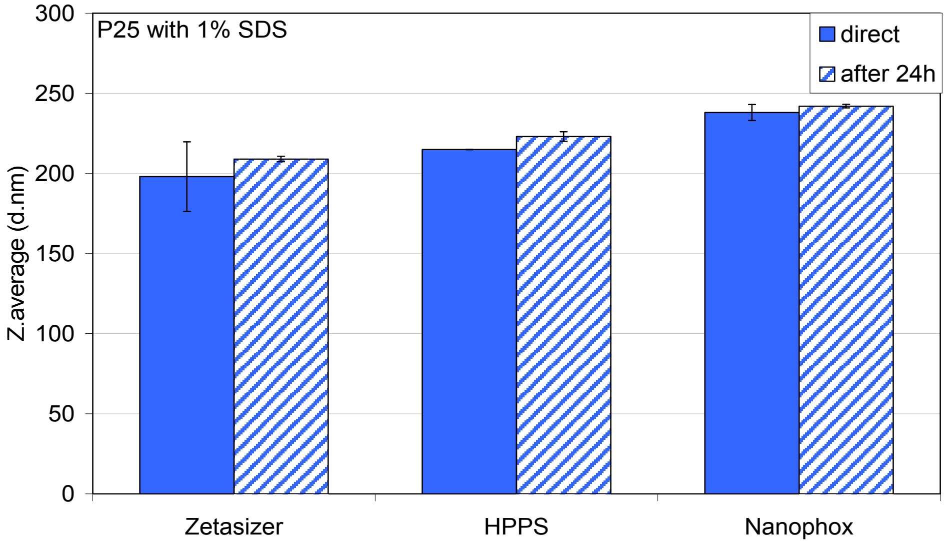


Figure 58: Exemplarily results of different DLS measurements of 100 mg/L UV Titan M262 after 10 min sonication. Measurement of three different instruments, direct, after 2 h and 24 h.

The measurements confirmed the before by internal standard checked good reproducibility. All three instruments revealed no significant size distribution differences of the tested suspension. After 2 h slight differences which are probably caused by settlement and agglomeration effects were detectable.

A1-3 Comparison of sodiumdodecyl sulfate (SDS) and sodium hexametaphosphate (SHP) DLS measurements of P25 suspensions measured with different DLS – instruments



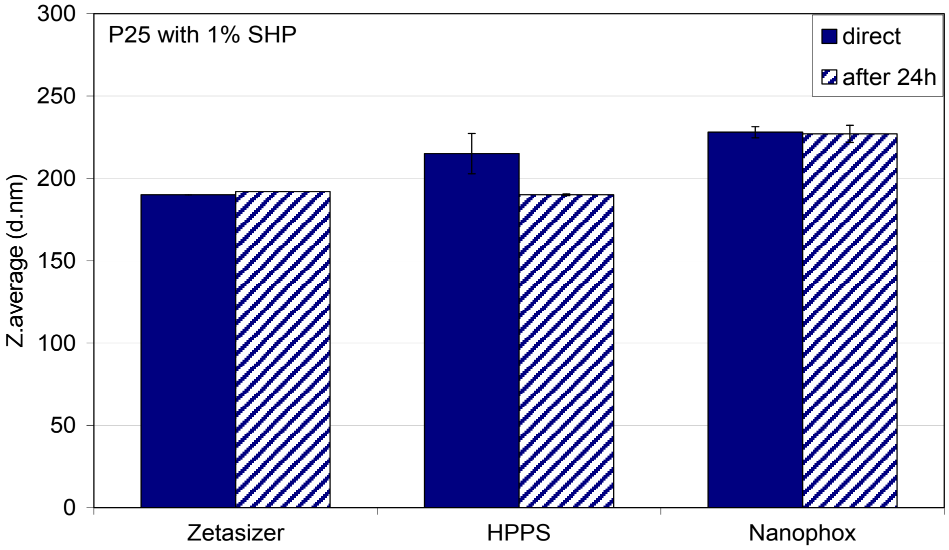


Figure 59: DLS measurements of 50 mg/L P25 in synthetic drinking water using 1 wt% sodiumdodecy sulfate (upper figure) and sodium hexametaphophate (lower figure) after sonication (ultrasonic homogenizer 200 Watt, 10 min, pulse 0.2 / 0.8), direct and after 24 hours. Error bars = SD; n = 3.

The measurements confirmed that SDS and SHP showed a stabilization effect on the P25 suspension in synthetic drinking water. It is also shown that all three instruments revealed a good comparability of the tested suspension.

A1-4 Standard operating procedure – Preparing P25 suspensions in synthetic drinking water

1. **Aim of the SOP**
2. **Background**
3. **Preliminary results**
4. **Preparing suspension**

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is intended to provide information for the preparation of a stable nanoscale Titanium dioxide suspension for environmental testing within the Project 3709 65 417 and afford reproducible results in different laboratories (comprehensible proceedings).

The SOP describes the proceedings which are suitable for preparing a stable nanoparticle suspension in this project.

2. Background

Suspension Requirements

The suspension must be stable at least for 24 h (a variance of 10% is accepted). An appropriate stability of a suspension is declared as a constant particle size distribution, concentration and zeta potential.

Stability criteria
- Optical observation (no visible sedimentation of the particles)
- Size of the particles in the suspension
- Zeta potential
- Particle concentration
- pH value of the suspension
- Conductance of the suspension

Necessary Instruments
- A sensitive analytical balance.
- Sonication equipment with sufficient rated power.
- Sensitive instrument detecting the particle size distribution and the zeta potential in aqueous media.

Used instruments

In this Project sonication equipment (Bandelin Sonoplus HD2200 ultrasonic homogeniser 200 W, Sonotrode VS70T) was used to disperse TiO_2 P25 nanoparticle in an aqueous suspension.

The size of the particles in suspension and the zeta potential of the suspension were measured using DLS instruments (Zeta Sizer - Malvern instruments; Nanophox – Particle Metrix).

3. Preliminary results

Preliminary results show that a sufficient stability of the suspension can not be warranted if only sonication for dispersion were used. The use of an additive was necessary to get a stable suspension for a minimum of 24 h.

In this project sodium hexamethaphosphate was found as an appropriate additive leading to a stable suspension.

4. Preparing suspension

- For preparing suspension a synthetic tap water was used (DIN EN ISO 6341).

- 1 Vol.-% of sodium hexamethaphosphate (additive) was weighted in a 400 mL beaker glass and filled up with 300 mL synthetic tap water.
- Dissolution of the additive by sonication in a water bath (until it dissolved).
- A defined amount of the nanoparticles were weighted in a second 400 mL beaker glass (a variance of 1 % is accepted).
- For this study 30 mg to generate a stock dispersion with a concentration of 100 mg/L was used.
- The 300 mL solution with the additive was filled carefully in the second beaker glass with the nanoparticles.
- The beaker glass with the nanoparticle suspension was sonicated with a Bandelin Sonoplus HD 2200 ultrasonic homogeniser for 30 minutes* with a pulse of 0.2 / 0.8.
- For sonication the beaker glass with the suspension was put in a bigger beaker glass with cold/ice water to minimize the heating of the suspension during the sonication.
- The horn of the ultrasonic homogeniser was dipped into the suspension and placed in the middle of the beaker glass with a distance between horn and bottom of the beaker glass of 1 cm.
- The horn of the ultrasonic homogeniser was dipped into the suspension – after use the horn was cleaned with ethanol and afterwards with deionised water.
- After sonication the suspension was characterised to their particle size distribution – using a DLS instrument.
- The stock dispersion was diluted to the target concentration.
- After dilution the suspension was stirred and characterised again to their number size distribution - using a DLS instrument.

* the sonication time must be adapted to the volume of the prepared suspension, diameter of the beaker glass, the concentration of the nanoparticles and the rated power of the ultrasonic instrument.

A1-5 Standard operating procedure – Preparing Titanium dioxide suspensions in deionised water

1. Aim of the SOP
2. Background
3. Preliminary results
4. Preparing suspension

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is the preparation of a stable nanoscale Titanium dioxide suspension for environmental testing within the Project 3709 65 417 and afford reproducible results in different laboratories (comprehensible proceedings).

The SOP describes the proceedings which are suitable for preparing a stable TiO_2 nanoparticle suspension in this project for P25, PC105 and UV Titan M262.

2. Background

Suspension Requirements

- The suspension must be stable at least for 24 h (a variance of 10% is accepted).
- An appropriate stability of a suspension is declared as a constant particle size distribution, concentration and zeta potential.

Stability criteria

- Optical observation (no visible sedimentation of the particles)
- Size of the particles in the suspension
- Zeta potential
- Particle concentration
- pH value of the suspension
- Conductance of the suspension

Necessary Instruments

- A sensitive analytical balance.
- Sonication equipment with sufficient rated power.
- Sensitive instrument detecting the particle size distribution and the zeta potential in aqueous media.

Used instruments

In this Project sonication equipment (Bandelin Sonoplus HD2200 ultrasonic homogeniser 200 W, Sonotrode VS70T) was used to disperse TiO_2 nanoparticle in an aqueous suspension.

The particle size and the zeta potential of the suspension were measured using DLS instruments (Delsa-Nano CS – Beckman Coulter / Zeta Sizer ZS - Malvern Instruments; Nanophox – Sympatec, size only).

3. Preliminary results

Preliminary results show that a sufficient stability is warranted if the suspension (100 mg TiO_2 material / 100 mL deionised water in a 250 mL beaker glass) was sonicated for 10 minutes.

4. Preparing suspension

- For preparing suspension deionised water was used (pH 5.0 - variance of 10%).
- A defined amount of the nanomaterial - here 100 mg of the solid material was weighted in a 250 mL beaker glass (a variance of 1% is accepted).
- After this 100 mL of deionised water was carefully added to the material.
- The beaker glass with the nanoparticle suspension was sonicated with a Bandelin Sonoplus HD 2200 ultrasonic homogeniser for 10 minutes* with a pulse of 0.2 / 0.8.

- The horn of the ultrasonic homogeniser was dipped into the suspension and placed in the middle of the beaker glass with a distance between horn and bottom of the beaker glass of approximately 1 cm.
- For sonication the beaker glass with the suspension was put in a bigger beaker glass with cold/ice water to minimize the heating of the suspension during the sonication.
- After use the horn was cleaned with ethanol and afterwards with deionised water.
- After sonication the suspension was characterised to its size distribution – using a DLS instrument.

* the sonication time must be adapted to the volume of the prepared suspension, diameter of the beaker glass, the concentration of the nanoparticles and the rated power of the ultrasonic instrument.

A1-6 SEM scans of the TiO_2 suspension

In the following section SEM scan of the suspensions which were used for the leaching experiment are presented. For all Titania materials an agglomeration can be seen.

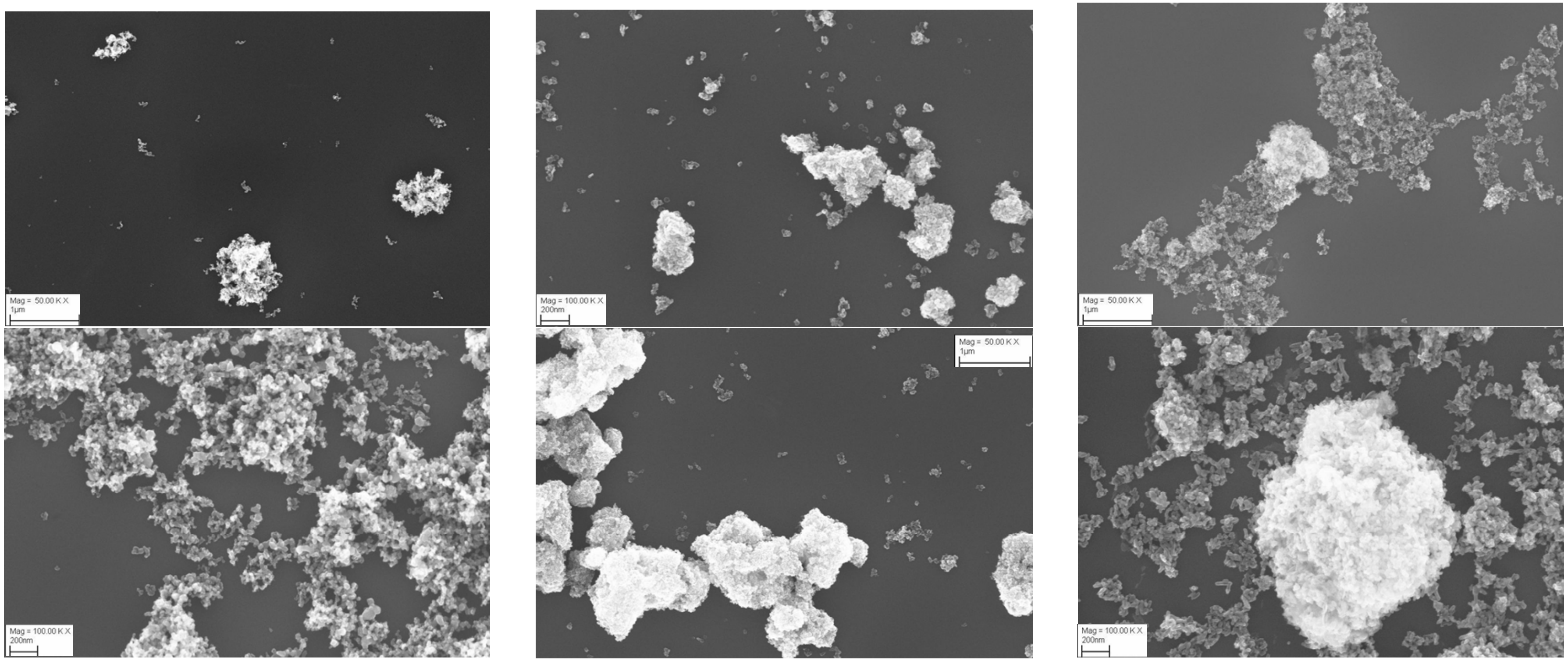


Figure 60: SEM scans of the P25 (left) PC105 (middle), UV Titan M262 (right) suspension (5 g/L) with different magnifications 50,000 and 100,000. Dispersion based on the SOP.

A1-7 pH dependent measurements of PC105 and UV Titan with and without sodium hexametaphosphate

PC105

The PC105 samples with 100 mg/L showed a pH value (6 - 9) dependency of the particle size. With increasing pH a decrease in the particles size distribution and a higher stability was detected. Samples at pH 6 showed very large particle sizes directly after preparation of the suspension; those large particles tended to settled immediately. Furthermore all samples showed sedimentation after 24 h, resulting in a reduced Z.average. In DI water the pH value was not stable within the time frame (Figure 61).

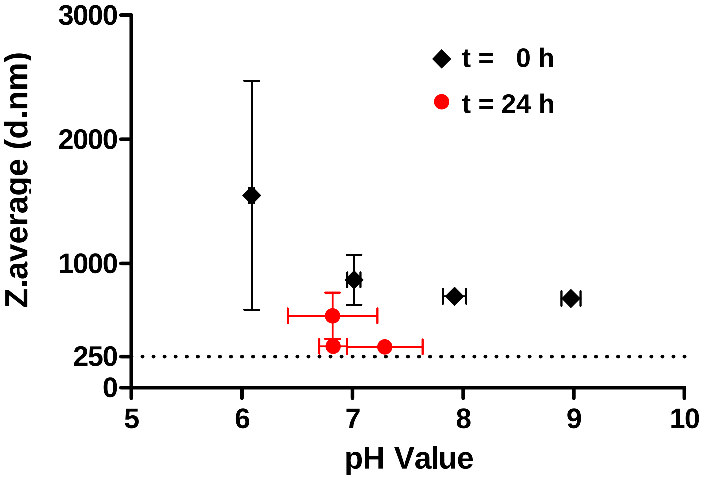


Figure 61: pH value dependent particle sizes of 100 mg/L PC105 in 100 mL DI after sonication for for 5 min (200 W). Error bars are SD. Measured 0 h and 24 h after sonication; n = 3.

The stabilisation effect of sodium hexametaphosphate in SDW was shown for P25. The stabilisation effect was additionally tested for PC105 at different pH values.
The PC105 samples with a concentration of 100 mg/L using 1 wt% SHP showed no pH dependency of the particle size in suspension. Still, the mean particle diameter as expressed by the Z. average is decreasing significantly over the first 24 h. This may be explained by sedimentation of larger agglomerates (Figure 62). The pH value was stable within the time frame of 24 h.

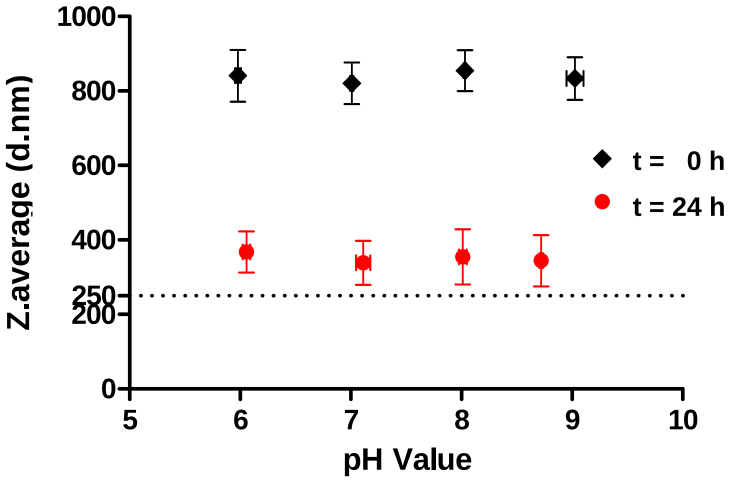


Figure 62: pH value dependent particle sizes of PC105 (100 mg/L) in 100 mL synthetic drinking water and 1 wt% SHP after sonication for 5 min. Error bars are SD. Measured at 0 h and 24 h; n = 3.

UV Titan M262

The suspension of UV Titan M262 showed in the first few seconds a hydrophobic behaviour of the material, but during sonication the hydrophobic behaviour changed and the material could be suspended in water. This is an indication that the hydrophobic hydrosilicon coating of the material was, at least partially, removed during suspension preparation.

The particle size of UV Titan M262 was influenced by the pH value of the suspension. Suspensions with a pH of 8 and pH 9 showed lager particle sizes (> 500 nm) directly after dispersion, with the highest degree of agglomeration at pH 9 (IEP).

After 24 h the agglomerated material was settled and only smaller particles remained in suspension. As a consequence the Z.average value was reduced at 24 h. Another observation is that in DI water (pH 7) the pH value is not stable within the time frame of 24 h (Figure 63). This corresponds with the results of the same media for PC105 (Figure 64). A reason for this observation could be the solution of CO_2 of the ambient air (open system) in the media which result in a pH value of 7. The pH remains at this level as an equilibrium state.

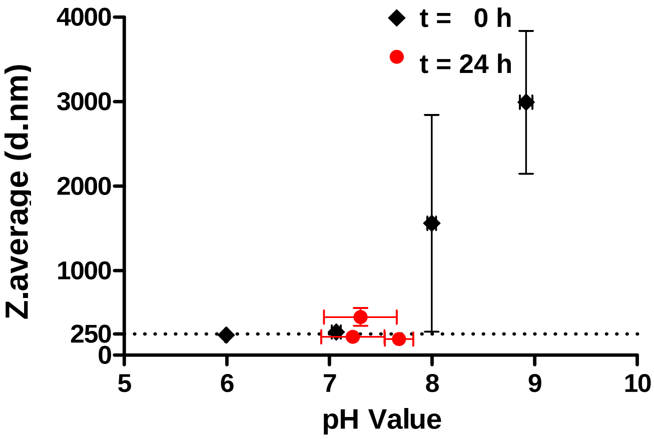


Figure 63: pH value dependent particle size of 100 mg/L UV Titan M262 in 100 mL DI water after sonication for 5 min. Error bars are SD. Measured at 0 h and 24 h; n = 3.

The stabilisation effect of sodium hexametaphosphate in SDW was shown for P25 and PC105 and was also tested by different pH for UV Titan M262.
The UV Titan M262 samples with a concentration of 100 mg/L using 1 wt% SHP showed no pH dependency of the particle size in suspension, but sedimentation during the 24 h. Due to this at t = 24 h smaller particles were measured, hence reducing the Z.average (Figure 64). The pH value was stable within the time frame of 24 h.

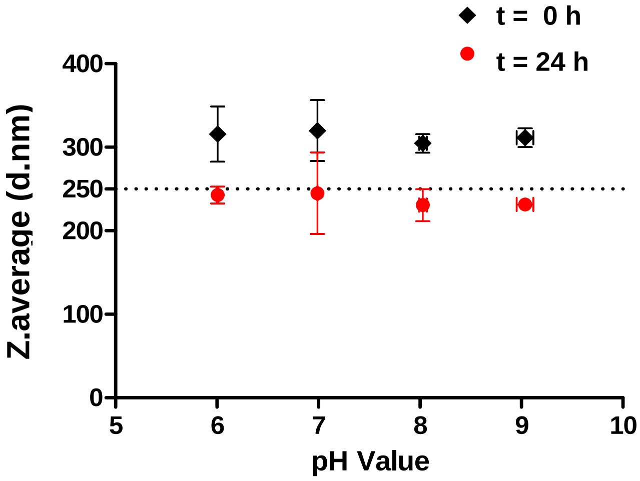


Figure 64: pH value dependent particle size of 100 mg/L UV Titan M262 in 100 mL synthetic drinking water with 1 wt% SHP after sonication for 5 min. Error bars are SD. Measured at 0 h and 24 h; n = 3.

→ *With the addition of 1 wt% SHP no pH dependent instability occurred for PC105 and UV Titan M262.*

Annex II

A2.1 Standard operating procedure - Analytic procedure

1. **Aim of the SOP**
2. **Background**
3. **Analytical procedure - Digestion**
4. **Analytical methods – Detection of Titanium**

1. Aim of the SOP

The aim of this Standard Operating Procedure is to describe the Titanium analysis of samples spiked with TiO_2 suspensions. The methods described here include analysis of soil samples of different composition, activated sludge samples, and liquid samples as e.g. outflow samples from a laboratory scale waste water treatment facility. Preparation of the suspensions is described in SOP - "Preparing Titanium dioxide (TiO_2) - P25 nanoparticle suspension in synthetic drinking water" and "Preparing Titanium dioxide (TiO_2) - nanoparticle suspension in deionised water.

All analysis were performed for within the project 3709 65 417. Sampling strategies and sampling protocols are not a part of this SOP.

2. Background

Preparation

Titanium is a ubiquitous metal which may be present in several chemical forms at least in trace concentrations. Therefore, all glassware was machine cleaned and rinsed with doubly quartz distilled water. Cleanliness was monitored by analysis of blank.

Calibration

Calibration was done using commercial standard solutions. Doubly quartz distilled water was used to prepare calibration solutions from traceable titanium standards (CertiPUR 1.70243.0100) from Merck, Darmstadt, Germany. The standard solution is traceable to standard reference material (SRM) of the National Institute of Standard and Technology (NIST, USA). The standard solution contains 1004 ± 5 mg/L Ti as $(NH_4)_2TiF_6$ in H_2O.

All samples were digested and analysed at least in duplicate. Negative and reference material controls, as far as available, were analysed together with project samples. Limit of detection (LOD) was estimated by analysis of calibration standards of known concentration. At least five calibration points were measured. All measurements were done using external calibration. Calibration curves were completely linear with $R^2 > 0.95$. For measurements performed by ICP/MS the LOD was < 1 µg/L and for ICP/OES the LOD was < 5 µg/L.

Reference materials

For the purposes of this project, SRM 2709a "San Joaquin Soil" was purchased. This reference material was selected because it contains a certified value for Titanium (0.342 ± 0.024%). However, certification of the titanium concentration was done using neutron activation analysis and x-ray fluorescence**.**

3. Analytical procedure - Digestion

Aqueous samples: Digestion using aqua regia/HF

Titantium dioxide in aqueous matrices is digested using a mixture of aqua regia and HF. Using this method, the organic matrix is probably destroyed by aqua regia and titanium is transformed into a stable fluoride salt.

Up to 10 mL of sample (depending on the type of sample) were pipetted into a PET test tube for centrifugation. Samples were concentrated to about 100 µL using a commercial concentration apparatus (Turbova, Zymark, Germany) at 70°C under a light nitrogen stream. After addition of aqua regia 2.4 mL HCl (36 - 38%, Ti < 0.2 ppb, J.T. Baker) and 0.8 mL

HNO_3 (69%, Ti < 0.5 ppb) 0.8 mL of HF (48%, Roth supra, Ti < 1 ppb) was added and the mixture was vortexed for 1 min. The reaction vessels were placed in an ultrasonic bath (Sonorex RK 510S) and digestion was finished within 30 min. Caps of the reaction vessels must not be closed tightly because reaction gases may be formed.
For the destruction of residual HF, 1 mL of boric acid solution (4%, Merck, Germany), was added. Finally, the digestion solution still containing solids was centrifuged at 4000 rpm for 20 min.

Soil, sediment and sludge samples: Microwave / H_2SO_4 digestion
An exact amount of sediment (about 200 mg) is placed in a Teflon reaction vessel. 5 mL H_2SO_4 (95%, Roth supra, Ti < 1 ppb) is added. The reaction vessel is placed in an automated microwave digestion apparatus (MLS Ethos plus) and digested by heating up to 218°C within 1 h and holding the temperature for 30 min. Samples are allowed to cool to room temperature and are filled up to a defined volume. Samples are centrifuged at 4000 rpm for 20 min, and supernatant is passed through a 0.45 µm membrane filter.

4. Analytical methods - Detection of Titanium

Analysis by inductively coupled plasma optical emission spectrometry (ICP/OES)
Titanium was detected at several wavelengths including 337,280 nm and 336,122 nm.
All analysis was done using an ICP/OES system “Vista MPX” of Varian (now Agilent; USA) equipped with a conical glass vaporizer and a cyclone vaporizer. Measurement of samples digested by aqua regia was done using an inert V-slit vaporizer and an inert Sturman-Masters cyclone vaporizer.

A2.2 $KHSO_4$ digestion method

The “classical method” for the digestion of TiO_2 minerals is digestion in molten $KHSO_4$. This method is described in textbooks whilst there is no standardised method available. Heating of $KHSO_4$ to 250 °C releases water leaving potassium bisulphate or pyrosulphate behind. The latter serves as oxide ion acceptor and therefore serves as the final digestion agent. Higher temperatures will lead to the formation of SO_3 and decomposition of the digesting agent. During the first part of the project soil samples were analysed using digestion in molten $KHSO_4$. To this end, 50 mg of soil or sediment were placed in a platinum digestion vessel. Three grams of $KHSO_4$ were added and mixed with the sample. This mixture was heated up three times until white fumes are formed. The resulting cake is dissolved under heating in 4 mL concentrated sulphuric acid (24%). The white coloured solution is transferred to a volumetric flask and filled up to 1 L. Usually, samples were diluted 1:10 for the analysis.
By using this method the salt concentration in media increased and due to this the H_2SO_4 and HCl, HNO_3, HF digestions were sued for the analysis of the environmental samples.

A2.3 Comparison measurements (IME and HMM) of different soils

In the knowledge of the authors certified reference materials / soils for Ti using a H_2SO_4 digestion and ICP/OES detection method don't exist. Consequently a direct comparison for the detected and referred Ti values is not appropriate. However for quality assurance of the analytical soil data inter laboratory comparison measurements of four different soils using ICP/OES after H_2SO_4 microwave digestion were performed. The laboratory of the IME (Fraunhofer Institute for Molecular Biology and Applied Ecology) and the HMM (Heidelberg University Hospital, Department of Infectiology - Hygiene and Medical Microbiology) digested and analysed two soil types A01 and G03, as well two reference soil materials SRM 2709a (San Joaquin Soil, NIST) and the BCR 142 (light sandy soil, JRC) (Figure 60).

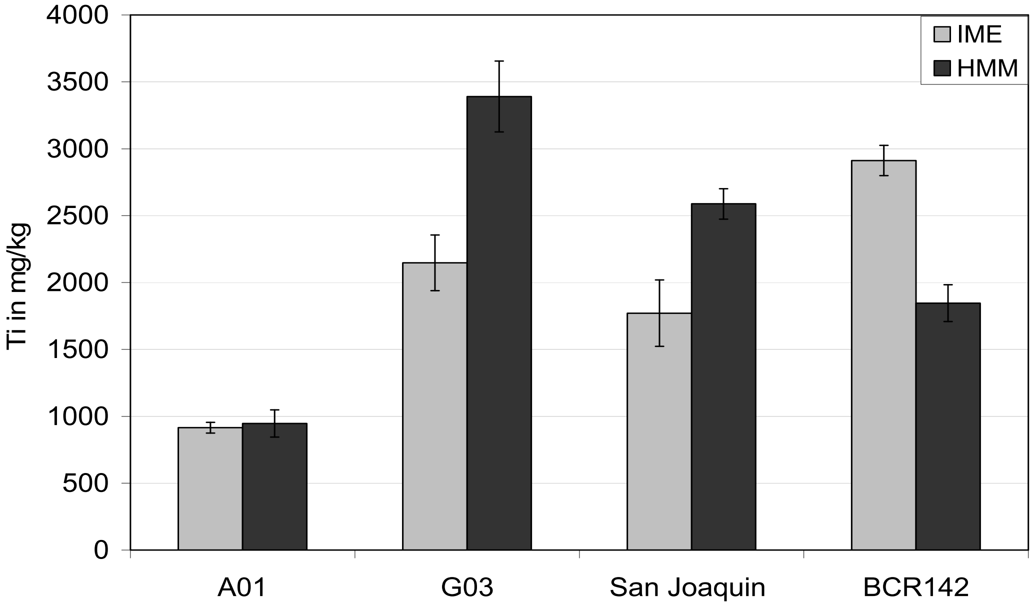


Figure 65: Comparison measurement of four different soils to their Ti content using ICP/OES; IME n = 3, HMM n = 6.

The measurements confirmed the analytical difficulties for Ti revealing just for A01 a good reproducibility. For G03 and the two reference materials inter laboratory concentration differences were found up to 45%. Beside the detection method and possible interferences / matrix effects also the digestion method might be a reason for this unsatisfying reproducibility.

Annex III

A3-1 Standard operation procedure – Nanoscale TiO_2 in laboratory sewage treatment plant simulation test according to OECD 303A (2001)

1. Aim of the standard operating procedure (SOP)
2. Principle of the test
3. Validity of the rest
4. Description of the test method
5. Procedure
5. Evaluation
7. References
8. Annex

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is intended to provide information for the performance of the laboratory sewage treatment plants (LSTP) simulation test within the Project 3709 65 471. The SOP describes the proceedings which are suitable for assessing the fate and behaviour of nanoparticles in sewage treatment plants.

2. Principle of the test

The OECD 303A has been designed to determine the elimination and the primary and/or ultimate biodegradation of water soluble organic compounds in a continuously operated test system simulating the activated sludge process. In principle, the behaviour of inorganic compounds in STP such as nanoparticles may also be assessed with this test system. In this case the outcome of the study is the distribution of nanoparticles between activated sludge and the effluent rather than biodegradability.

An easily biodegradable organic medium (organic synthetic wastewater, OSW) is added as primary source of carbon and energy for the micro-organisms to the LSTP. Each activated sludge plant unit consists of an aeration vessel with a capacity of about 3 L of activated sludge and a separator (secondary clarifier) which holds about 1.5 L. Usually, two continuously operated test units are run in parallel under identical conditions with a mean hydraulic retention time of 6 h and a mean sludge age (sludge retention time) of 6 to 10 days. The test unit 1 receives the test item while the reference unit 2 serves as functional control to determine the biodegradation of the organic medium. Normally the (organic) test substance is added at a concentration of between 10 mg/L and 20 mg/L dissolved organic carbon (DOC) to the test unit. The test concentration for inorganic materials depends on the detection limit for the material, the background concentration, and the expected concentration in the environment.

3. Validity of the test

The test is valid if the degree of DOC or COD elimination in the control unit is > 80% after two weeks and no unusual observations have been made.

If the test is performed under nitrifying conditions (as it is suggested in order to simulate standard treatment conditions), the mean concentration in the effluents should be < 1 mg/L ammonia-N and < 2 mg/L nitrite-N.

4. Description of the test method

Apparatus

The test system consists of a test unit and a reference unit of a laboratory activated sludge plant (Husmann unit, Figure 66). Storage vessels of sufficient size for the influents and effluents are needed, as well as pumps to dose the influent.

Each activated sludge plant unit consists of an aeration vessel with a known capacity of about 3 L of activated sludge and a separator (secondary clarifier) which holds about 1.5 L; an airlift pump is used to recycle the activated sludge from the separator to the aeration vessel intermittently at regular intervals.

For aeration of the sludge in the aeration vessels of both systems, suitable techniques are required, for example sintered cubes (diffuser stones) and compressed air. The air is cleaned by passing through a washing flask filled with deionised water. Sufficient air must pass through the system to maintain aerobic conditions and to keep sludge flocks in suspension at all times during the test. For dosing the synthetic drinking water (SDW) and the nanoparticle susupension peristaltic tube pumps and for the concentrated OSW perfusor pumps are used.

Filtration apparatus

Membrane filters of suitable porosity (nominal aperture diameter 0.45 µm) for the determination of the dissolved organic carbon (DOC). The filters are washed with approx. 60 mL deionised water to remove leachable organic carbon.

Glass fibre filters with a diameter of about 110 mm and a pore size of about 0.6 µm for the determination of the filtratable solids with the aid of a vacuum flask and a vacuum pump.

Analytical equipment

- Total carbon analyser for measuring DOC (Dissolved Organic Carbon) and TOC (Total Organic Carbon)
- pH electrode
- Oxygen electrode
- Thermometer with min / max display
- Cuvette tests for photometrical determination of ammonium, nitrite and nitrate or other detection devices

Water

According to OECD 303 A the organic medium should be dissolved in tap water. The use of synthetic drinking water (SDW) instead of tap water is recommended in order to have reproducible test conditions which allow better comparability within laboratories. Therefore SDW according to DIN EN ISO 6341 is used as matrix. In order to increase the buffer capacity to a sufficient level the 3.5 fold amount of sodium hydrogen carbonate compared to that concentration referred to in the DIN EN ISO 6341 is added.

The SDW is prepared as following:

	Stock solution		Added in 1 L	Added in 12 L	Final concentration
D1	$CaCl_2 \times 2\ H_2O$	294 g/L	1 mL	12 mL	2.0 mmol/L
D2	$MgSO_4 \times 7\ H_2O$	123.3 g/L	1 mL	12 mL	0.5 mmol/L
D3	KCl	2.3 g/L	2.5 mL	30 mL	0.075 mmol/L
D4	$NaHCO_3$	25.9 g/L	8.5 mL	102 mL	2.62 mmol/L
B	K_2HPO_4 *)	33.5 g/L	0.417 mL	5 mL	80.14 µmol/L

*) Solution B corresponds to the inorganic part of the organic synthetic wastewater

Organic medium

The OECD 303 A describes the organic synthetic wastewater (OSW) which consists of bacto-pepton, meat extract and urea. Because the OSW should be fed separately from the SDW, in order to minimize its biodegradation before entering the LSTPs, a concentrated OSW is made up according to DIN 38412-26 (1994) and continuously dosed via a perfusor pump.

The OSW is prepared as following:

SolutionA	Stock solution (g/L)	Dosage 2.5 mL per hour Final concentration (mg/L)
Bacto –pepton	32	80
Meat extract	22	55
Urea	6	15
NaCl	1.4	3.5
$CaCl_2 * 2 H_2O$	0.8	2
$MgSO_4 * 7 H_2O$	0.4	1
K_2HPO_4	To be added with the SDW	
Resulting DOC		≈ 50

Dosage of nanomaterial suspension

The dosage of the nanoparticles is considered the critical part of the experiment. The suspension should be separately added to the aeration vessel. The stability of the nanoparticle suspension may be reached through a suitable dispersant and energy input through ultrasonic homogeniser and continuously stirring. For example a stock solution of 100 mg/L TiO_2 in SDW with 1% sodium hexametaphosphate (SHP) is prepared and 1,200 mL of the suspension is added continuously to the aeration vessel with a peristaltic pump. The preparation of the TiO_2 stock solution is described in the SOP "Preparing Titanium Dioxide (TiO_2) – UV Titan M262 nanoparticle suspension". The overall procedure should be thoroughly described and the particle distribution of the suspension should be determined immediately before entering the aeration vessel.

In project 3709 65 471 three different TiO_2 concentrations were added by preparing the nanoparticle stock solution with 100 mg/L TiO_2 and diluting it with suitable amounts of SDW with 1% SHP to achieve ten-fold concentrated feeding suspension which continuously added to the aeration vessel as follows:

Nanoparticle stock solution with 100 mg/L TiO_2 (mL)	Dilution with SDW with 1% SHP (mL)	Concentration of feeding suspension (mg/L TiO_2)	Final concentration in the test (mg/L TiO_2)
120	1080	10	1
600	600	50	5
1200	0	100	10

The final concentration of the dispersant SHP in the test was 1 g/L.

5. Procedure

Preparation of the inoculum

As inoculum activated sludge of a municipal biological waste water treatment plant, which receives predominantly domestic sewage, is used. The inoculum is kept aerated at room temperature and used within 24 h. The concentration of suspended solids is determined by filtering a definite amount of the activated sludge through a paper filter and measuring the increase in weight after drying at 105 °C. The starting concentration of dry matter is about 2.5 g/L and should be maintained in the range of 1 - 3 g/L by the removal of the surplus sludge which also determines the sludge age within a range of 6 days to 10 days.

Dosage of organic medium

The test system is run in a room with a controlled temperature of 20 - 25 °C. The synthetic drinking water (SDW) and the organic synthetic wastewater (OSW) are renewed daily and dosed separately.

A sufficient volume of SDW is prepared. Initially the aeration vessel and the separator are filled with the SDW and the inoculum is added. The aeration is started such that the sludge is kept in suspension and in an aerobic state and the dosing of the influent and the recycling of the settled sludge is started. The SDW is pumped out of thoroughly cleaned storage vessels into the aeration vessels of the test and control unit with a rate of 0.5 L/h. Thus, the hydraulic retention time is 6 hours.

The concentrated organic synthetic wastewater (OSW) is dosed into the aeration vessels of the test and control unit with a rate of 2.5 mL/h.

To confirm the dosage rates, the daily amount SDW and OSW dosed is measured by noting the reduction in volume of the solutions in the storage vessels.

Dosage of nanoparticle suspension

The dosage of the TiO_2 nanoparticle suspension is carried out under continuous stirring. The suspension is pumped into the aeration vessels of the test unit with a rate of 1.2 L/day (50 mL/h).

The addition of the test substance is started after an adaptation phase (in this test 7 days), in which the system has stabilised and is removing the DOC of the organic medium efficiently (about 80%).

Handling of activated sludge

The concentration of activated sludge solids is kept in the range of 1 to 3 g/L (dry weight) and the mean sludge age in the range of 6 to 10 days. Therefore, about 100 - 300 mL surplus sludge is removed daily, and the suspended solids in the aeration vessels are determined at least three times a week by weight measurements after 2.5 h drying at 105 °C.

Throughout the test, any sludge adhering to the walls of the aeration vessel and the separator is removed daily, so that it is resuspended.

The settled sludge from the separator is recycled to the aeration vessel by intermittent air lift pumping (16 seconds every 15 minutes, ≈ 400 mL, ≈ 1.6 L/h).

Sampling and analysis

(See Figure 67 in the 8. **Annex** of this SOP)

The pH, the oxygen concentration in the activated sludge basin, and the temperature is determined daily. It is ensured that oxygen is always available (> 2 mg/L) and that the temperature is kept in the required range (20 °C to 25 °C). The pH is kept at 7.5 ± 0.5.

The DOC in the influents to the control and test vessels and in the collected effluents is measured daily. For this he samples are filtered through cellulose acetate filters of pore size 0.45 µm and analysed in a total carbon analyser.

Three times a week the ammonium, nitrite and nitrate concentrations in the effluent of the LSTP units are determined photometrically through cuvette test as NH_4-N, NO_2-N, and NO_3-N .

The filtratable solids in the effluents of the LSTP units are an indicator of the efficiency of the sedimentation of the activated sludge in the settling vessel. The filtratable solids are determined alt least three times a week, by filtering volumes of 2 - 5 L through glass fibre filters (pore size about 0.6 µm) with the aid of a vacuum flask and measuring the increase in weight after drying at 105 °C.

Analysis of TiO_2

DLS measurements of the stock suspension

Before the suspension is added to the aeration vessel, the size distribution is determined through measurements of the zeta potential measurements using a Malvern Zetasizer ZS.

ICP/OES analysis of the surplus sludge and the effluent

For balancing, the sludge and the effluent of the test and reference system are analysed with ICP/OES after digestion with HCl, HNO_3 and HF. Explorative SEM / EDX scans are conducted of the outflow and the sludge after the experiment to get information about the behaviour in the systems (size of the agglomerates).

6. Evaluation

The percentage of DOC elimination of the organic medium is calculated using the equation:

$$D_t = \frac{C_m - E_o}{C_m} \times 100$$

where D_t = % elimination of DOC of the organic medium at time t
C_m = DOC of the organic medium in the influent (mg/L)
E_o = measured DOC value in the effluent at time t (mg/L)

The calculation of the overall TiO_2 balance is a strong instrument for the determination of the reliability of both the procedure of the treatment process and of the analytics. In principle the TiO_2 introduced into the system should be detected in the activated sludge and in the outflow of the LSTP.

$$\Sigma\, TiO_{2\ dosage}\ [mg] = \Sigma\, TiO_{2\ as}\ [mg/L] \times V_{as}\ [L] + \Sigma\, TiO_{2\ out}\ [mg/L] \times V_{out}\ [L]$$

where $\Sigma\, TiO_{2\ dosage}$ is the total amount of TiO_2 added to the activated sludge basin,

$\Sigma\, TiO_{2\ as}$, $\Sigma\, TiO_{2\ out}$ are the TiO_2 concentrations measured in the activated sludge and in the outflow,

V_{as}, V_{out} are the volumes of the activated sludge (surplus sludge and total sludge at the end of the test) and in the outflow.

7. References

DIN 38412-26 (1994) Abbau- und Eliminations-Test für Tenside zur Simulation kommunaler Kläranlagen (L 26).

OECD 303 A (adopted 22.1.01) Simulation Test - Aerobic Sewage Treatment: 303 A: Activated Sludge Units. OECD Guidelines for the testing of chemicals.

8. Annex

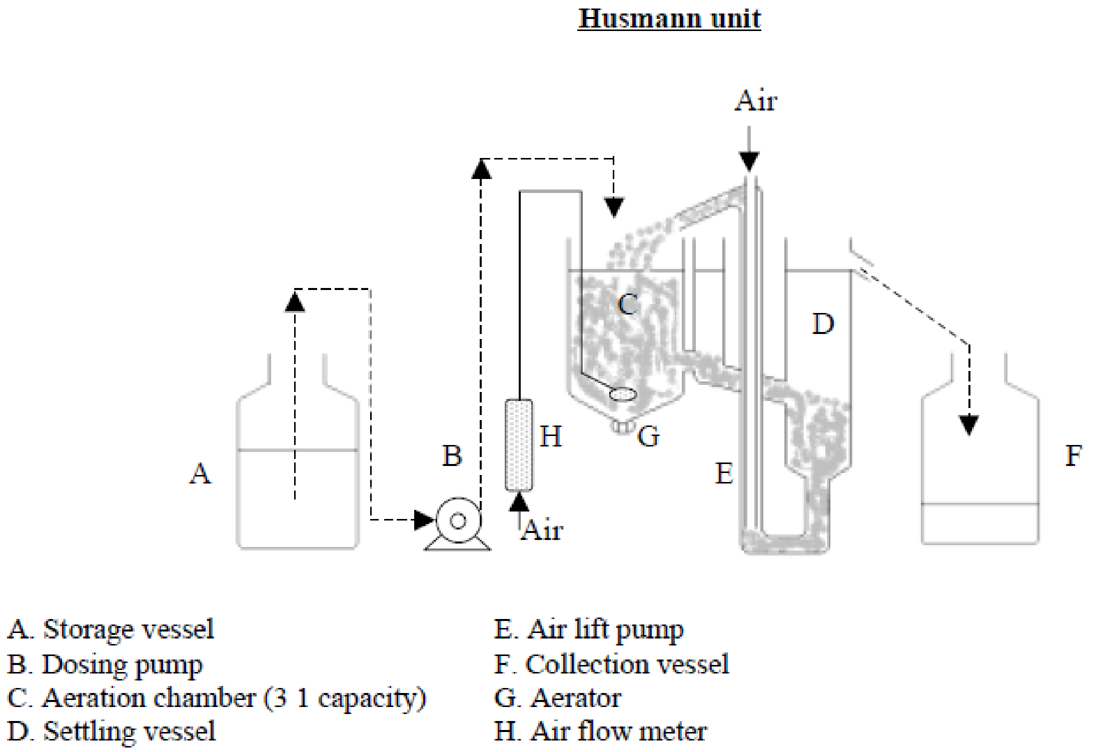


Figure 66: Equipment used for the test.

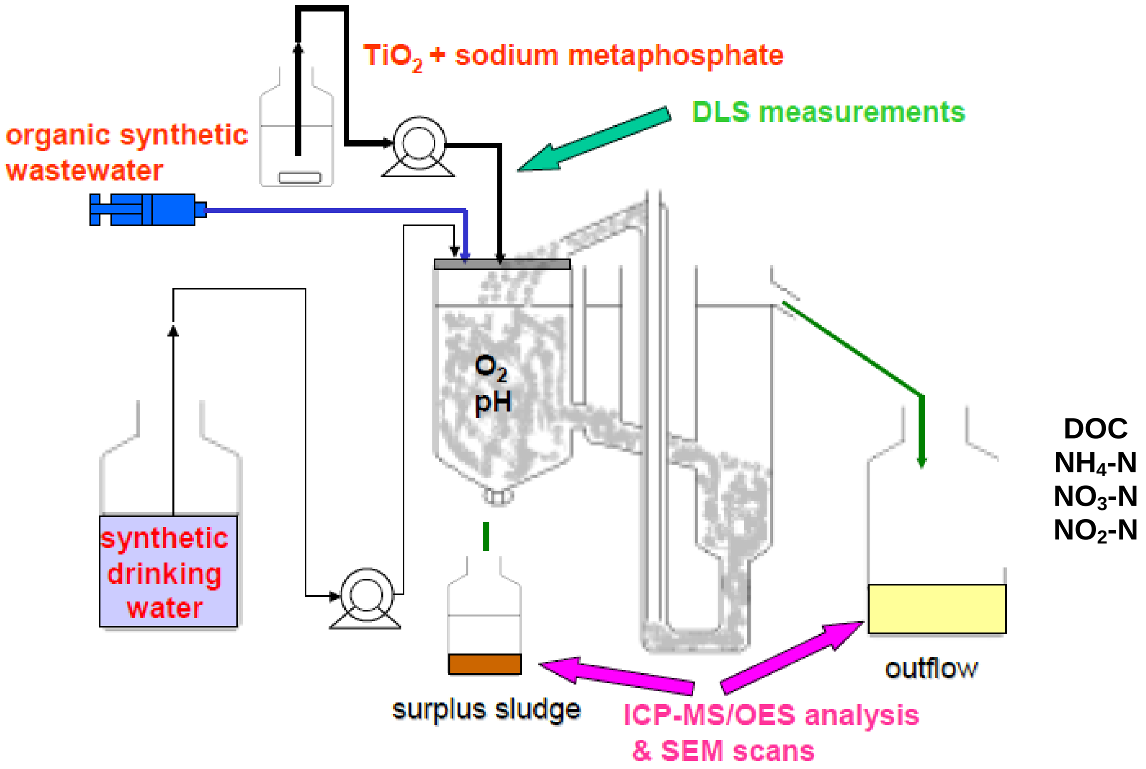


Figure 67: Testing device of the LSTP and measurement points.

A3-2 Ecotoxicological assessment of SHP in the fish embryo toxicity assay DIN EN ISO 15088:2009 - method

For ecotoxicological assessment of the nanoparticles in SDW an evaluation of SHP was necessary. As Titanium dioxide particles agglomerate in SDW due to the high ion concentration as well as the influence to bivalent cations the addition of SHP is required to achieve stable suspensions. However this substance has to be ecotoxicologically evaluated. First hints for an adverse effect were observed in the OECD Guideline test 303A. The Fish Embryo Toxicity assay was conducted to determine the toxicity.

Fish Embryo Toxicity Test (FET) - Zebrafish broodstock and egg production

Groups of 20 three-month old zebrafish (*Danio rerio*) with a ratio of 3:2 (males to females) were used for egg production. Spawning trays consisting of a flat basin and a metal net covering to which artificial plants are attached are placed into the aquaria. The artificial plants serve as a breeding stimulant and substrate while the metal net covering prevents the fish from feeding on their own spawn. Mating, spawning and fertilisation take place within 30 min after the onset of light in the morning.
Animals are fed with commercially available artificial diets (e.g., TetraMin™ flakes; Tetra, Melle, Germany) and live *nauplius larvae* of *Artemia sp*. once daily *ad libitum*.
The assay is carried out following the German Standard DIN EN ISO 15088:2009 for the FET with zebrafish.

The FET was initiated as soon as possible after fertilization of the eggs and not later than 3 h post fertilisation (128 cell stage). To identify fertilized eggs, a binocular with a minimum magnification of 25 was used. Freshly spawned eggs exhibit the following structures: The chorion surrounds the perivitelline space, which contains the yolk. The blastodisc is located at the animal pole of the yolk.

SHP solutions were tested in seven different concentrations prepared with synthetic tap water (ISO 6341-1996, 1996). Synthetic tap water in the absence of the chemical was used as a negative control. As a standard positive control, 3.7 mg/L 3.4-dichloroaniline (Sigma-Aldrich GmbH, Steinheim) (mortality > 10%) was used.
Evaluation of the test was carried out with an inverted microscope at magnifications of 40 and 100. After an exposure time of 48 h and 96 h, lethal and sublethal endpoints were recorded. As a modification to the DIN 20 fish eggs at each concentration were selected to evaluate the toxicity of SHP.

Table 20: Lethal and sublethal effects in the fish embryo toxicity assay.

Lethal endpoints	Sublethal endpoints
Coagulation Not detached tail No somites No heartbeat	No spontaneous movement Epiboly Reduced heartbeat rate No blood circulation Reduced blood circulation Oedema No eye anlage Underdeveloped Deformation Less / no pigmentation No eye pigmentation No / malformed fins Deformation of the spine Curved spine

Results

Sodium hexametaphosphate (SHP) showed toxic effects in the FET assay in concentrations ranging from 0.085 to 1 wt%. In the two independent replicates a 50 % effect concentration of 0.27 wt% were calculated in the timeframe of 48 h and 0.16 wt% for 96 h.

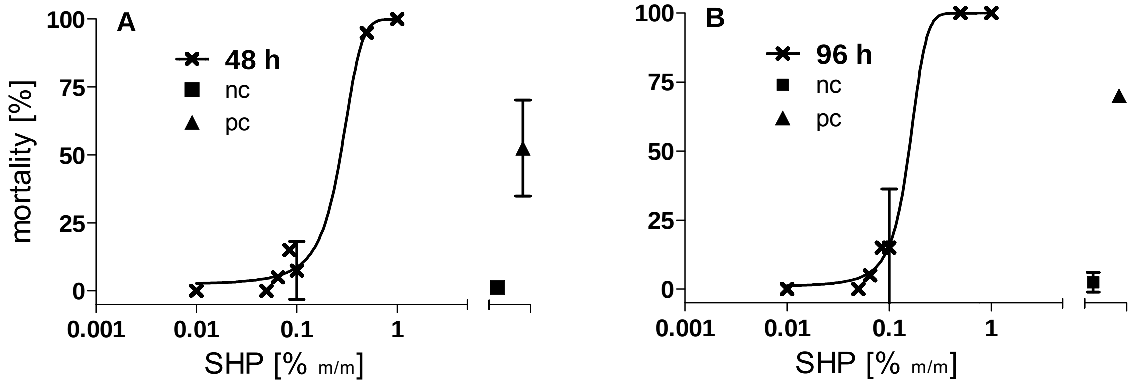


Figure 68: The fish embryo toxicity assay results for SHP. Seven SHP concentrations 0.01, 0.05, 0.065, 0.085, 0.1, 0.5 and 1.0 wt% of SHP in SDW are presented as percentage mortality after **A** 48 h and **B** 96 h after fertilization; n = 1. The concentration of 0.1 wt% SHP was performed twice and is indicated with its SD. On the right are the negative control (nc) and positive control (pc) displayed; n = 2.

The toxicity of SHP in the fish embryo toxicity assay was evaluated in two steps. At the first step a screening by the concentrations of 0.01, 0.1, 0.5 and 1% were evaluated. At the second step intermediate concentrations of 0.05, 0.065, 0.085 and 0.1 wt% SHP were evaluated. In each concentration 20 eggs were exposed.

At the concentration steps of 0.5 and 1 wt% SHP 100% mortality was determined after 96 h post fertilization. At a lower concentration of 0.1 wt% SHP in the first test no mortality was detected, whereas in the second test 30% mortality was detected. In addition in the second test 20% of the larvae were underdeveloped or showed deformations. They had a weak hearth beat and weak circulation.

These results are within the variance of the FET assay. Furthermore the concentration of 0.1 wt% is in a concentration range where the toxicity manifests. Hence a tighter concentration range should be evaluated to describe the steep increase in the dose response curve. A literature research showed that SHP has a toxic effect on bacteria and chicken embryos. Furthermore an overview of the safety assessment of sodium hexametaphosphate was presented by Andersen, (2001), Post et al., (1963), Verrett et al., (1980). In this report the toxicity varies in different studies with a recommendation to use < 0.2 wt% SHP.

The next lower SHP concentration of 0.085 wt% showed a mortality of 15%. At 0.065% a mortality of 5% and at 0.05% no mortality after 96 h was calculated. The calculated EC_{50} (50% effect concentration) for SHP were after 48 h 0.27% and after 96 h 0.16 wt% SHP (Figure 68).

Influence of the sodium hexametaphosphate concentration onto the stability of P25 suspensions in SDW

SHP showed a stabilizing effect on the nanomaterial suspension. Based on these results the concentration of SHP was determined which was sufficient to achieve a stable P25 suspension for at least 24 h.

The tested concentrations were 0.01, 0.1, 0.5 and 1.0 wt% SHP in synthetic drinking water (Figure 69). The lowest concentration of 0.01% was not sufficient to produce a stable suspension over 24 h. The higher concentrated suspensions of 0.1, 0.5 and 1.0 wt% SHP were stable at least for 72 h.

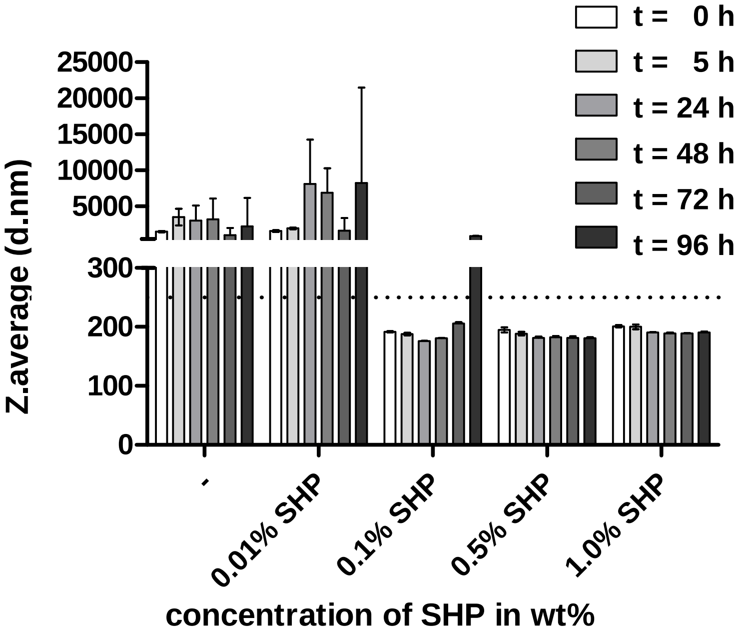


Figure 69: Size analysis of 100 mL synthetic drinking water with 50 mg/L P25 and 0, 0.01, 0.1, 0.5 and 1 wt% SHP sonicated with 200 W for 10 min. Results are presented as Z.average of five measurements of the column (nm) as bars; n = 5. Measured time points are 0 h, 5 h, 24 h, 48 h, 72 h and 96 h. Target Z.average of 250 nm is indicated with the dashed line.

Based on this results different P25 concentrations were tested with 0.1 wt% SHP. The low concentrations of 0.5, 1.0 and 5.0 mg/L of P25 resulted in not reproducible measurement with high standard deviations as well as high particle size values, which could be affected by the detection limit of the dynamic light scattering instrument. These results show that a DLS measurement of very low titanium oxide concentrations is problematic. A concentration range from 5 to 100 mg/l was determined in repeated measurements to be reproducible in this study. A stable particle suspension can be produced in the concentration range of 0.1 to 1 wt% SHP. An explanation for this effect can be the softening of the water by sequestering magnesium and calcium. The concentration of 1 wt% SHP for the preparation of the stock suspension was chosen as a 10 fold surplus to maintain sufficient SHP to achieve a softening effect in the dilutions steps for OECD 303A test.

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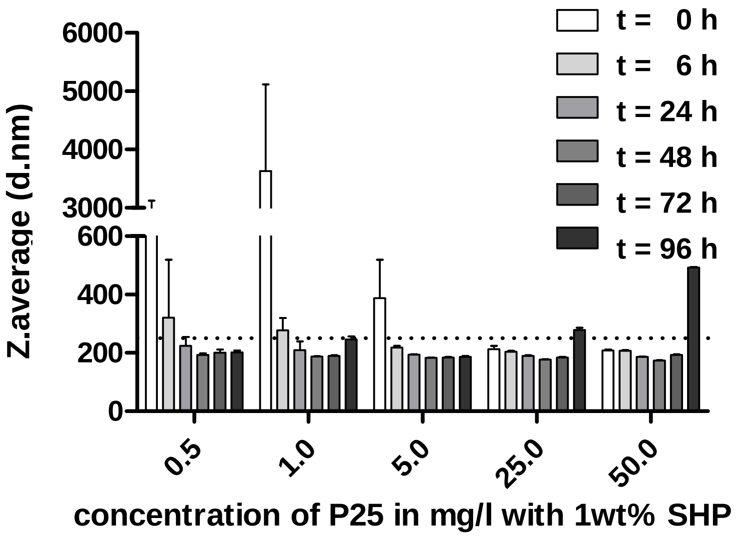


Figure 70: Size analysis of 100 mL synthetic drinking water with 0.5, 1, 5, 25 and 50 mg/L P25 and 1 wt% SHP sonicated with 200 W for 15 min. Results are presented as Z.average of five measurements in nm; n = 5. Measured time points are 0 h, 6 h, 24 h, 48 h, 72 h and 96 h. Z.average is presented as bars with SD. Target Z.average of 250 nm is indicated with the dashed line.

A3-3 Synthetic drinking water

Additionally, crucial information of the chemical composition of the synthetic drinking water which was also used in the ecotoxicity tests (e.g. OECD 202, OECD 203) is given in the following Table 21.

Table 21: Synthetic drinking water.

	Stock solution	Mineral medium
DF1	$CaCl_2 \times 2\ H_2O$ 294 g/L	1 mL into 1000 mL ≈ 2.0 mmol/L
DF2	$MgSO_4 \times 7\ H_2O$ 123.3 g/L	1 mL into 1000 mL (equates 0.5 mmol/l)
DF3	KCl 2.3 g/L	2.5 mL into 1000 mL (equates 0.075 mmol/l)
DF4 *)	$NaHCO_3$ 25.9 g/L	2.5 mL into 1000 mL ≈ 0.75 mmol/L In the study a 3.5 fold amount (8.5 mL into 1000 mL) has been added in order to stabilize the pH.
Solution B **)	K_2HPO_4 33.5 g/L	5 mL into 12 L

*) The higher concentration of DF4 is justified because OECD 303 A also allows the addition of $NaHCO_3$.

**) Solution B corresponds to the inorganic part of the organic synthetic wastewater

A3-4 Organic synthetic wastewater

In Table 22 the chemical composition of the organic synthetic waste water are presented, which were used for the LSTP experiments.

Table 22: Organic synthetic wastewater.

Solution A	Stock solution* (g/L)	Dosage 2.5 mL per hour Final concentration (mg/L)
Bacto –pepton	32	80
Meat extract	22	55
Urea	6	15
NaCl	1.4	3.5
$CaCl_2$ * 2 H_2O	0.8	2
$MgSO_4$ * 7 H_2O	0.4	1
K_2HPO_4	*Is not included because it has been added with the synthetic drinking water (see table A1)*	
Resulting DOC		*≈ 50*

* The stock solutions are described in DIN 38412-26 (1994) Abbau- und Eliminations-Test für Tenside zur Simulation kommunaler Kläranlagen (L 26).

A3-5 Results ICP OES measurements of the sewage sludge and the effluent

In the following Tables (Table 23 - Table 25) the results of the ICP/OES analysis, which were used for the balancing are presented in detail. At the end of each trial the total volume of the LSTP was released and the activated sludge mixed with the supernatant for the clarifier basin. The TiO_2 of the homogenised sample is therefore somewhat lower than the last TiO_2 measurement of the surplus sludge taken from the activated sludge basis.

Table 23: TiO_2 concentration in activated sludge and in the outflow after digestion with HCl + HNO_3 + HF and ICP/OES analytic [unit 1 (with TiO_2), reference unit 2 (without TiO_2)]; MV of n = 2.

day	**Dosage TiO_2 mg/L**	**Activated sludge**				**Outflow LSTP**		
		unit 1 mg/L	**SD**	**unit 2 mg/L**	**SD**	**unit 1 mg/L**	**SD**	**unit 2 mg/L**
Trial 1								
5		4.245	0.361					
6		4.485	0.290					
7		3.700	0.453					
8	1	3.445	0.290	3.620	0.566	< 0,005		< 0.005
9	1	5.995	0.064			0.072	0.034	< 0.005
10	1	8.015	0.757	3.625	0.007			< 0.005
11	1	8.705	0.488	3.895	0.587	0.215	0.230	< 0.005
12	1	8.635	0.700			0.501	0.169	< 0.005
13	1			3.575	0.035	0.322	0.028	< 0.005
14	1					0.276	0.012	< 0.005
15	5					0.232	0.016	< 0.005
16	5	22.199	0.424			0.529	0.001	< 0.005
17	5					0.727	0.136	
18	5	42.448	3.040			0.832	0.030	< 0.005
19	5	51.598	0.707	2.945		1.265	0.007	< 0.005
20	5					0.849	0.004	< 0.005
21	5				0.148	1.839	1.260	< 0.005
End		**67.097**	**6.081**	**2.790**	**0.007**	**0.673**		**< 0.005**
Trial 2								
24	10	7.060	0.085	7.550	0.467	0.957	0.001	< 0.005
25	10	26.499	0.173	7.235	0.120	0.778	0.107	< 0.005
26	10	46.898	1.273			2.060	0.028	< 0.005
27	10	66.397	0.424	4.235	0.332	2.400	0.014	< 0.005
28	10	62.997	0.848	3.235	0.474	2.715	0.049	< 0.005
29	10	71.397	9.051	3.440	0.071	3.515	0.035	< 0.005
30	10					3.035	0.148	< 0.005
31		96.046	18.313			2.790	0.071	< 0.005
End		**72.997**	**2.8**	**2.890**	**0.4**			

Table 24: Process parameter and measurements Trial 1 of the LSTP unit 1 and unit 2.

Trial 1																										
day		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**End**		
LSTP unit 1 (with TiO2)																										
Dosage TiO_2	(mg/L)	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	5	5	5	5	5	5	5			
pH		7.2	7.3	7.3	7.3	7.4	7.3	7.2	7.6	7.2	7	7.5	7.3	7.3	7.3	7.2	7.2	7.4	7.2	7.3	7.4	7.3	7.3			
Oxygen	(mg/L)	2	3.3	3.2	5.5	6.3	4.3	4.3	4.4	5	5	4.5	5	5.1	5.4	4.8	4.2	4.5	3.1	6.7	6.4	6.5	6.4			
Temperature	(°C)	23.8	24.1	23.5	23.6	24.1	23.2	23.2	23.2	22.8	22.7	22.7	22.5	22.8	23.3	23.5	23.4	23.7	23.9	23.4	23.2	22.5	23.2			
TiO_2 in activated sludge	(mg/L)						4.2	4.5	3.7	3.4	6	8	8.7	8.6	*12.03*	*15.42*	*18.81*	22.2	*32.3*	42.4	51.6	*70.9*	*99.3*	**67.1**		
Volumen surplus sludge	(mL)		30	130	250	200	300	300	270	200	230	150	150	50	50	50	50	50		50	50	50	50	5500	**sum**	**% of dosage**
TiO_2 amount absolute	(mg)									0.7	1.4	1.2	1.3	0.4	0.6	0.8	0.9	1.1	0	2.1	2.6	3.5	5	369	390.7	78%
Dry solid activated sludge	(g/l)		8.7	2.8		4.1			2		2		1.7			1.9		1.3		1.3			1.4			
Measurements in the outflow																										
DOC	(mg/L)					1.6			1.4	1	0.9	0.7	2.4		0.3	1.2	1.2	0.2		0		0.1	0.1			
NH_4-N	(mg/L)					0.097			0.052		0.078		0.192			0.103		0.081		0.225			0.051			
NO_2-N	(mg/L)					0.152			0.083		0.106		0.12			0.036		0.031		0.094			0.016			
NO_3-N	(mg/L)					17.9			18.8		15.7		17			14.9		15.5		16.4			17.1			
Filtratable solids	(mg/L)												13.4				3.5	3		5.3	8		6.1			
TiO_2 in the outflow	(mg/L)									0	0.07	*0.14*	0.21	0.5	0.32	0.28	0.23	0.53	0.73	0.83	1.26	0.85	1.84			
TiO_2 amount in the outflow	(mg)									0	0.9	1.7	2.6	6	3.9	3.3	2.8	6.3	8.7	10	15.2	10.2	22.1			
LSTP reference unit 2 (without TiO_2)																										
pH		7.3	7.2	7.2	7.3	7.4	7.3	7.3	7.6	7.5	7.3	7.7	6.9	7.7	7.6	7.6	7.1	7.6	6.1	7.3	7.5	7.7	7.7			
Oxygen	(mg/L)	3.3	3.7	4.2	2.8	5.8	5.1	5	6.3	5.3	5.1	5.2	6.2	5.6	5.7	5.2	4.6	5.7	5.2	5.1	2.6	5.8	6.3			
Temperature	(°C)	23.4	24.8	25.3	25.4	24.8	24.1	24	23.7	23.4	23.7	23.3	22.9	22.4	23.9	24.1	24.2	24.3	24.6	23.9	23.9	23	23.6			
TiO_2 in activated sludge	(mg/L)									3.6	*3.6*	3.6	3.9	*3.7*	3.6	*3.5*	*3.4*	*3.3*	*3.2*	*3.1*	2.9	2.8	*2.7*	*2.7*		
Volumen surplus sludge	(mL)		30	130	250	200	300	300	270	200	230	150	150	50	50	50	100	100	50	50	50	50	50	5500	**sum**	**% of unit 1**
TiO_2 amount absolute	(mg)									0.7	0.8	0.5	0.6	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.1	0.1	0.1	14.9	19.5	5%
Dry solid activated sludge	(g/l)		4.2	2.9		4			1.7		1.9		1.8			2.5		1.9		1.7			2.2			
Measurements in the outflow																										
DOC	(mg/L)					2.2			1	0.7	0.7	0.7	1.1		0.3	0.5	0.9	0.6		0.5		0.1	0.2			
NH_4-N	(mg/L)					0.069			0.036		0.056		0.092			0.066		0.074		0.08			0.269			
NO_2-N	(mg/L)					0.135			0.019		0.101		0.105			0.095		0.1		0.148			0.26			
NO_3-N	(mg/L)					20.2			11.2		16.5		18.1			16.8		23.7		29.1			15.1			
Filtratable solids	(mg/L)												1.4				0.6	0.7		1.8	0.5		2.3			
TiO_2 in the outflow	(mg/L)									<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005		<0.005	<0.005	<0.005	<0.005			
Data in cursive have been extrapolated																										

Table 25: Process parameter and measurements Trial 2 of the LSTP unit 1 and unit 2.

Trial 2										
day		24	25	26	27	28	29	30	31	End		
LSTP unit 1 (with TiO2) - restart												
Dosage TiO_2	(mg/L)	10	10	10	10	10	10	10				
pH		7.4	7.2	7.2	7.3	7.3	7.3	7.3				
Oxygen	(mg/L)	6.5	6.3	6.5	6.6	6.1	6.2	6.4				
Temperature	(°C)	23.1	23.3	22.8	23.2	23.6	24.0	23.9				
TiO_2 in activated sludge	(mg/L)	7.1	26.5	46.9	66.4	63.0	71.4	*83.7*	96.1	73		
Volumen surplus sludge	(mL)	170	730	300	300	300	150	100	70	5500	**sum**	% of dosage
TiO_2 amount absolute	(mg)	1.2	19.3	14.1	19.9	18.9	10.7	8.4	6.7	401.5	500.7	60%
Dry solid activated sludge	(g/l)		3.7		3.5	1.9		1.9				
Measurements in the outflow												
DOC	(mg/L)	0.0	1.3		1.7	1.5	1.4	0.7	1.5			
NH_4-N	(mg/L)		0.046			0.049						
NO_2-N	(mg/L)		0.035			0.030						
NO_3-N	(mg/L)		13.8			18.8						
Filtratable solids	(mg/L)		10.7		15.7	15.6	17.2	14.5	12.4			
TiO_2 in the outflow	(mg/L)	1.0	0.8	2.1	2.4	2.7	3.5	3.0	2.8			
TiO_2 amount in the outflow	(mg)	11.5	9.3	24.7	28.8	32.6	42.2	36.4	33.5			
LSTP reference unit 2 (without TiO_2)												
pH		7.7	7.7	7.7	7.6	7.6	7.6	7.6				
Oxygen	(mg/L)	6.9	6.4	7.0	5.6	4.9	4.6	5.5				
Temperature	(°C)	23.5	22.9	22.3	24.0	24.4	24.8	24.8				
TiO_2 in activated sludge	(mg/L)	7.2	*5.9*	*4.6*	4.2	3.2	3.4	*3.3*	*3.2*	2.89		
Volumen surplus sludge	(mL)	470	730	300	50	300	150	100	70	5500	**sum**	% of unit 1
TiO_2 amount absolute	(mg)	3.4	4.3	1.4	0.2	1.0	0.5	0.3	0.2	15.9	**27.2**	5%
Dry solid activated sludge	(g/l)		3.5		2.8	1.8		1.6				
Measurements in the outflow												
DOC	(mg/L)	0.0	1.1		0.7	0.8	0.4	1.0	1.1			
NH_4-N	(mg/L)		0.036			0.053						
NO_2-N	(mg/L)		0.040			0.098						
NO_3-N	(mg/L)		13.3			17.6						
Filtratable solids	(mg/L)		2.4		0.8	1.8	2.0	2.3	1.1			
TiO_2 in the outflow	(mg/L)	<0.005		<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005		
Data in cursive have been extrapolated												

A3-6 SEM EDX scan of the sewage sludge of the LSTP

In the following Figures (Figure 68-69) representative SEM and EDX scans of the sewage sludge are presented, exemplarily for day 28 and 31 the last day of the experiment.

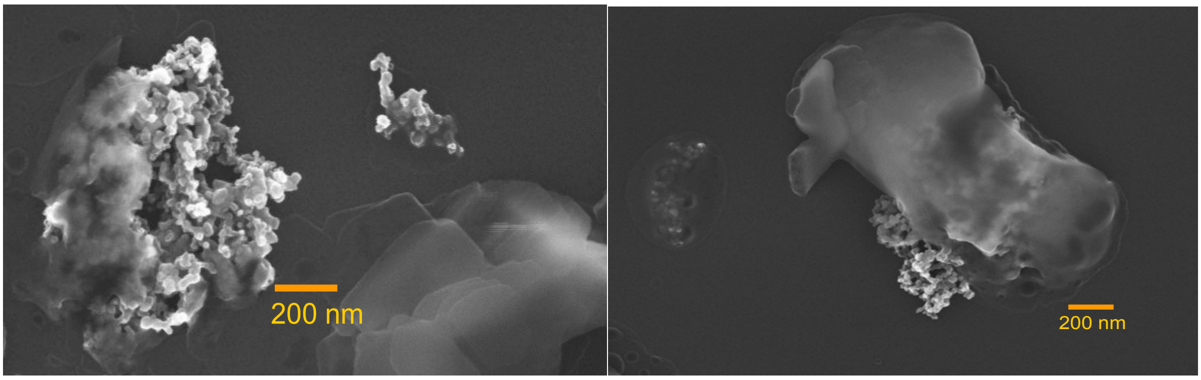
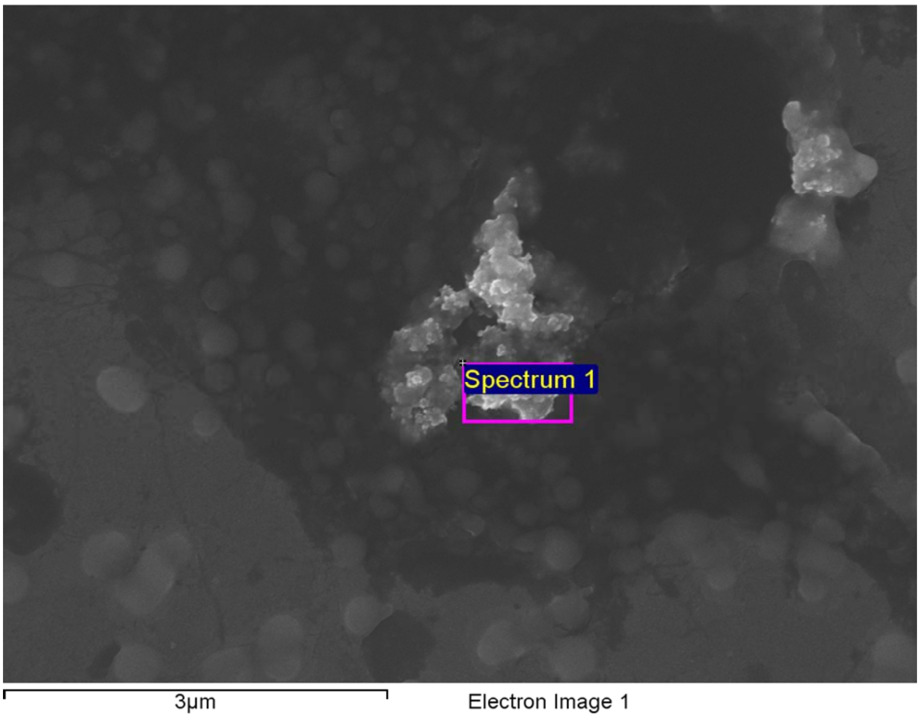
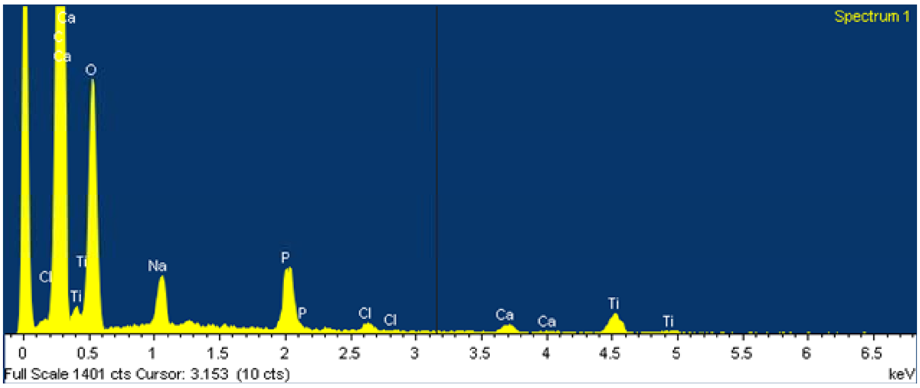


Figure 71: SEM scans of the sewage sludge of the test system at day 28.





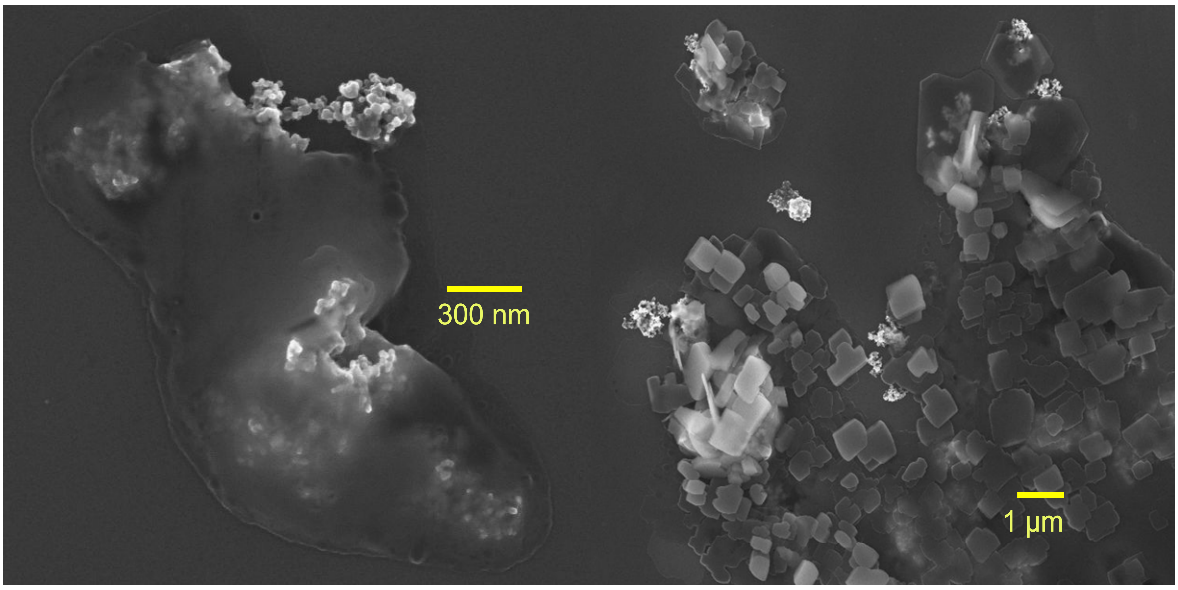


Figure 72: SEM scans of the sewage sludge of the test system at day 31.

A3-7 Photodocumentation - laboratory sewage treatment plant

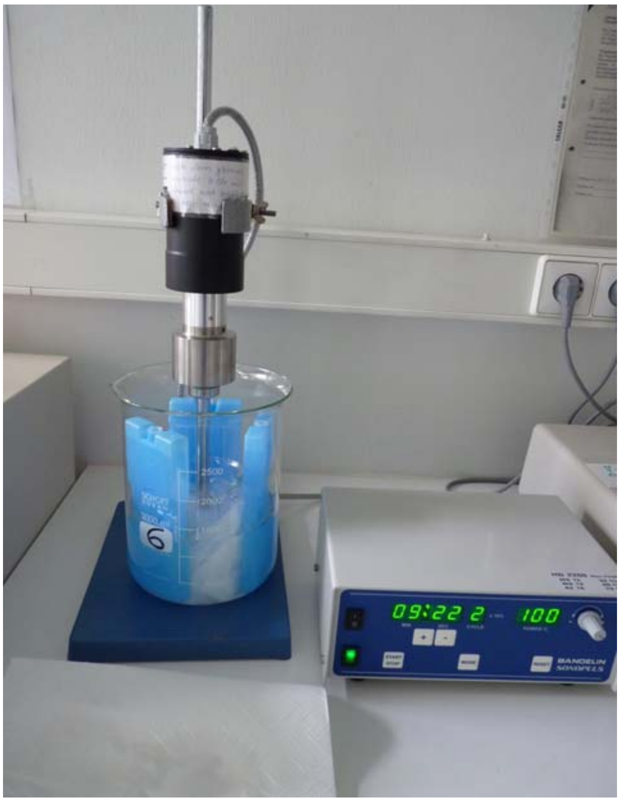


Figure 73: Preparation of the TiO_2 suspension.

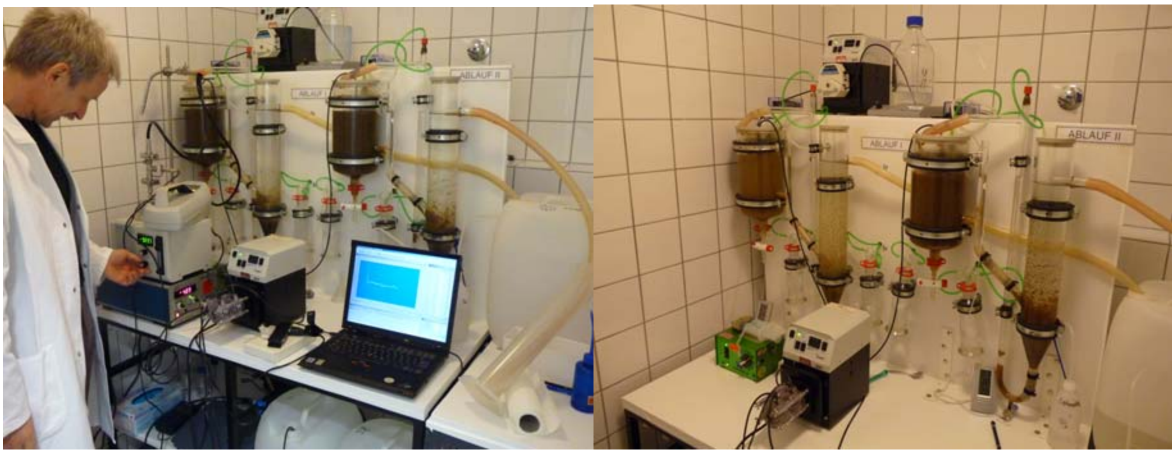


Figure 74: Laboratory sewage treatment plant day 8 start with 1 mg/L TiO_2 (left), day 15 start with 5 mg/L TiO_2 (right).

Figure 75: Laboratory sewage treatment plant day 21 end with 5 mg/L TiO_2 (left) and dosage of the TiO_2 suspension (detail) (right).

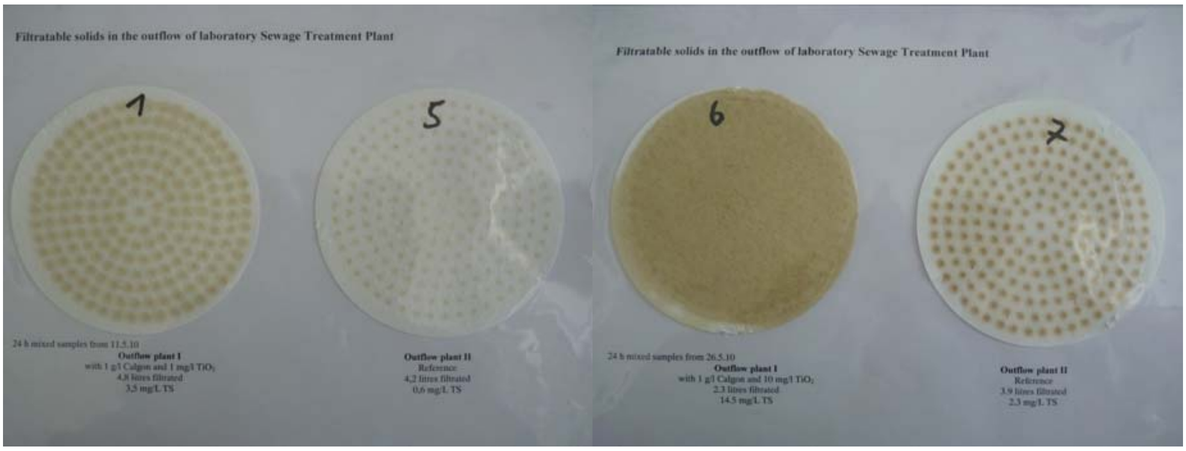


Figure 76: Filtrateable solids in the effluents day 15 (start with 5 mg/L) and day 30 (10 mg/L).

Figure 77: Microscopic analysis of the sludge (left) test system, reference system (right).

A3-8 Standard operation procedure – Nanoscale TiO_2 in soil columns according to OECD 312 (2004)

1. **Aim of the standard operating procedure (SOP)**
2. **Principle of the test**
3. **Description of the test method**
4. **Calculation of the recovery of TiO_2 in the different segments of the column**

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is intended to provide information for the performance of the leaching experiments of TiO_2 nanomaterials in soils within the Project 3709 65 471. The SOP describes the proceedings which are suitable for assessing the fate and behaviour of nanomaterials in a soil column.

2. Principle of the test

Columns of inert material (here glass) were packed with soil. The soil was saturated and equilibrated with a 0.01 M $CaCl_2$ solution (artificial rain) and allowed to drain. Afterwards the suspension was applied to the column. The mobility of the nanomaterial was detected by analysis of the eluate and four segments of the soil.

3. Description of the test method

The glass column were filled with air dried, sieved soil (< 2 mm) to a height of 30 cm. The soil was added to the column in small portions with a spoon.

The soil weight of the duplicate test columns should be similar (< 10% variance). Afterwards the soil was pre-wetted with a 0.01 M $CaCl_2$ solution from the bottom to the top. Until the 0.01 M $CaCl_2$ solution reached a height of 34 cm the 0.01 M $CaCl_2$ solution was allowed to drain by gravity.

Thereafter 100 mL of the TiO_2 suspensions (prepared after the SOP for TiO_2 suspension preparation in DI water), in a concentration of 5 g/L were applied to the soil column surface using a peristaltic pump. The rate of the peristaltic pump was calculated to warrant the application of 251 mL liquid (nanomaterial suspension + artificial rain).

The eluate was collected over the whole period. The eluate was homogenised for 5 min using an ultrasonic bath (200 W) and 2 * 25 mL were extracted (pipette) for chemical analysis.

After the columns were allowed to drain, the soil columns were sectioned into four segments. From each of these four segments a sample was extracted using a spoon.

from the top of the column	
segment one	0 – 1 cm
segment two	2 – 3 cm
segment three	15 – 16 cm
segment four	29 – 30 cm

The test was performed in duplicate with one blank.

4. Calculation of the recovery of TiO_2 in the different segments of the column

The recovery of the added TiO_2 was calculated as the detected concentration in relation to the added concentration.

First the average of the measured concentration (ave.measured Ti in mg/kg) was corrected for the average background concentration (ave,background Ti in mg/kg).

$$TiO_{2cor}(mg/kg). = ave.measuredTi - ave.backgroundTi$$

$TiO_{2cor.}$ (mg/kg) = background corrected TiO_2 concentration in mg/kg

With this value - $TiO_{2\ cor,}$ - the concentration in the extracted segment was calculated ($TiO_{2segment}$) based on the amount of the extracted segment in milligram *(amount of segment)*.

$$TiO_{2segment}(mg/kg) = TiO_{2cor}.*\ amount\ of\ segment$$

With this value ($TiO_{2segment}$ in g/kg) the recovery (TiO_{2reco}) in relation to the initial concentration was calculated as recovery in percent.

$$TiO_{_2reco}.(\%) = \left(TiO_{2segment} *initial\ concentartionTiO_2\right)*100$$

A3-9 Standard operation procedure – Nanoscale TiO_2 adsorption behaviour according to OECD 106 (2000)

1. Aim of the standard operating procedure (SOP)
2. Principle of the test
3. Description of the test method
4. Calculation of the adsorbed amount

1. Aim of the Standard Operating Procedure (SOP)

The aim of this Standard Operating Procedure is intended to provide information for the performance of the adsorption / desorption of TiO_2 nanomaterials in a batch equilibration test within the Project 3709 65 471.

2. Principle of the test

The test is designed to evaluate the adsorption of a chemical on different soil types with a varying texture, organic content and pH. The test comprises three tiers.

Tier 1: Preliminary study in order to determine:

- soil / solution ratio;
- equilibration time for adsorption and the amount adsorbed substance at equilibrium
- adsorption of the test substance on the surfaces of the test vessels and the stability of the test substance during the test period.

Tier 2: Screening test: based on the results of tier 1 the adsorption is studied in five different soil types.

Tier 3: Determination of Freundlich adsorption isotherms to determine the influence of concentration on the extent of adsorption on soils.

3. Description of the test method

Tier 1 - Preliminary study

Two soils with different texture, organic content and pH were used.

Three different mixing ratios

- 50 g soil and 50 cm^3 suspension (ratio 1/1);
- 10 g soil and 50 cm^3 suspension (ratio 1/5);
- 2 g soil and 50 cm^3 suspension (ratio 1/25),

were tested after four points in time:

4 h, 8 h, 4 h and 48 h.

Every experiment was tested in duplicate with one blank, where only 0.01 M $CaCl_2$ solution was tested with the defined amount if soil.

Before the suspension was added to the soil / suspension mixture, the soils were equilibrated for 12 h with 45 mL of 0.01 M $CaCl_2$ solution.

The suspension of the TiO_2 nanomaterial (1 g/L) was prepared (prepared after the SOP for TiO_2 suspension preparation in DI water).

After 12 h 10% of the amount of the solution (here 5 mL) were added to the soil / solution mixture.

The soil/suspension mixture was then shaken for 4 h, 8 h, 24 h and 48 h.

After the defined time the mixture was centrifuged (2700 g) to separate the adsorbed from the non-adsorbed fraction.

After centrifugation 15 mL of the supernatant were extracted (pipette) for chemical analysis and the remaining amount of the supernatant was decanted to determine the volume of the liquid fraction for the calculation of the adsorbed amount.
Adsorption on the surface of the test vessel and stability of the test substance
The lost of the material to the test vessel was tested, by measuring the concentration of the material in suspension without soil after a shaking time of 48 h.

Tier 2 - Adsorption kinetics at one concentration of the test substance
Based on the results of tier 1 three additional soils were tested.
The defined soil / suspension ratio and equilibration time (here 1/5 and 4 h) was tested.
Because no significant amount of the added TiO_2 was found in the supernatant, two additional time points were studied, 1 h and 2 h.
The sample preparation followed the preparation mentioned in tier 1.

4. Calculation of the adsorbed amount

Since the supernatant is analysed for the Titanium content first the absolute amount of Titanium in this is calculated:

TiO_2 in supernatant (µg) = Ti (µg/L in supernatant) * supernatant (L) * 79.87/47.87

With the known amount of TiO_2 added to the suspension calculated according to following equation:

TiO_2 added (µg) = TiO_2 (µg/L in stock suspension) * stock suspension (L)

With these to values the percent of TiO_2 remaining in the suspension is calculated as follows:

TiO_2 susenpded (%) = TiO_2 in supernatant (µg) / TiO_2 added (µg)

The remaining TiO_2 is assumed to be in the soil.

TiO_2 soil (%) = TiO_2 added (%) – TiO_2 in supernatant (%)

Results

→ No differentiation between adsorbed, settled and non adsorbed material was possible,due to this the calculation of adsorption isotherms could not be conducted.

A3-10 Detailed analysis data of the employed soils

In the following Tables (Table 26 - 31) detailed analysis of the soils which were used for the experiments are presented.

Table 26: Detailed analysis data of the used soils – Soil A01 Dystric Cambisol – AV = Average, COV = coefficient of variation. A = field.

Dystric Cambisol – A01			
	AV	**COV**	**Unit**
Sand	71	5.8	(weight%)
Silt	24	13.8	(weight%)
Clay	5	19	(weight%)
pH	5.67	4	
OC	0.93	24.7	(%)
N	882	11.5	(mg/kg)
CEC_{eff}	37.9	25.8	(mmolc/kg)
Fe_{ox}	1.57	15.3	(mmolc/kg)
Al_{ox}	0.95	6.5	(mmolc/kg)
Watercapacity*	264		(mL/kg)

eff = effective, ox = oxalate, * only IME laboratory

Table 27: Detailed analysis data of the used soils – Soil A06 Cambic Rendzina – AV = Average, COV = coefficient of variation. A = field

Cambic Rendzina – A06			
	AV	**COV**	**Unit**
Sand	9	62.3	(weight%)
Silt	55	11	(weight%)
Clay	36	6	(weight%)
pH	6.78	0.8	
OC	2.46	11.2	(%)
N	2814	4.9	(mg/kg)
CEC_{eff}	236.0	5.1	(mmolc/kg)
Fe_{ox}	5.03	20.3	(mmolc/kg)
Al_{ox}	1.57	4.3	(mmolc/kg)
Watercapacity*	591		(mL/kg)

eff = effective, ox = oxalate, * only IME laboratory

Table 28: Detailed analysis data of the used soils – Soil A04 Gleyic Podsol – AV = Average, COV = coefficient of variation. A = field.

Gleyic Podsol – A04			
	AV	**COV**	**Unit**
Sand	85	3.4	(weight%)
Silt	11	23.3	(weight%)
Clay	4	32.6	(weight%)
pH	5.14	5.7	
OC	2.91	3.8	(%)
N	1616	1.0	(mg/kg)
CEC_{eff}	85.7	9.4	(mmolc/kg)
Fe_{ox}	0.63	7.0	(mmolc/kg)
Al_{ox}	1.51	7.5	(mmolc/kg)
Watercapacity*	346		(mL/kg)

eff= effective, ox=oxalate, * only IME laboratory

Table 29: Detailed analysis data of the used soils – Soil A02 Stagnic Luvisol – AV = Average, COV = coefficient of variation. A = field.

Stagnic Luvisol – A02			
	AV	**COV**	**Unit**
Sand	2.00	45.00	(weight%)
Silt	84.00	1.60	(weight%)
Clay	15.00	8.70	(weight%)
pH	6.63	2.40	
OC	1.30	4.90	(%)
N	1500.00	2.00	(mg/kg)
CEC_{eff}	133.20	6.80	(mmolc/kg)
Fe_{ox}	3.54	16.20	(mmolc/kg)
Al_{ox}	0.69	0.80	(mmolc/kg)
Watercapacity*	419.00		(mL/kg)

eff = effective, ox = oxalate, * only IME laboratory

Table 30: Detailed analysis data of the used soils – Soil G03 Eutric Cambisol – AV = Average, COV = coefficient of variation. G = grassland

Eutric Cambisol – G03			
	AV	**COV**	**Unit**
Sand	21	29	(weight%)
Silt	52	8.3	(weight%)
Clay	27	9.8	(weight%)
pH	5.64	1.2	
OC	3.85	4.5	(%)
N	4179	9.1	(mg/kg)
CEC_{eff}	135.8	6.3	(mmolc/kg)
Fe_{ox}	6.66	19.4	(mmolc/kg)
Al_{ox}	2.32	4.4	(mmolc/kg)
Watercapacity*	768		(mL/kg)

eff = effective, ox = oxalate, * only IME laboratory

Table 31: Detailed analysis data of the used soils – Soil G05 Gleyic Fluvisol – AV = Average, COV = coefficient of variation. G = grassland.

Gleyic Fluvisol– G05			
	AV	**COV**	**Unit**
Sand	22	28.1	(weight %)
Silt	62	17	(weight %)
Clay	16	22.4	(weight %)
pH	4.78	1.2	
OC	3.08	7.5	(%)
N	3446	3.2	(mg/kg)
CEC_{eff}	116.1	12.1	(mmolc/kg)
Fe_{ox}	4.08	4.8	(mmolc/kg)
Al_{ox}	0.42	6	(mmolc/kg)
Watercapacity*	584		(mL/kg)

eff = effective, ox = oxalate, * only IME laboratory

A3-11 Breakthrough curve of the used soils for the leaching experiment

Breakthrough experiments are commonly performed a priori to define or test possible transport processes in general. Consequently before the leaching experiments were conducted with the nanomaterials, breakthrough curves of the soils A01, A06 and A04 with a 0.1 M NaCl solution were performed.

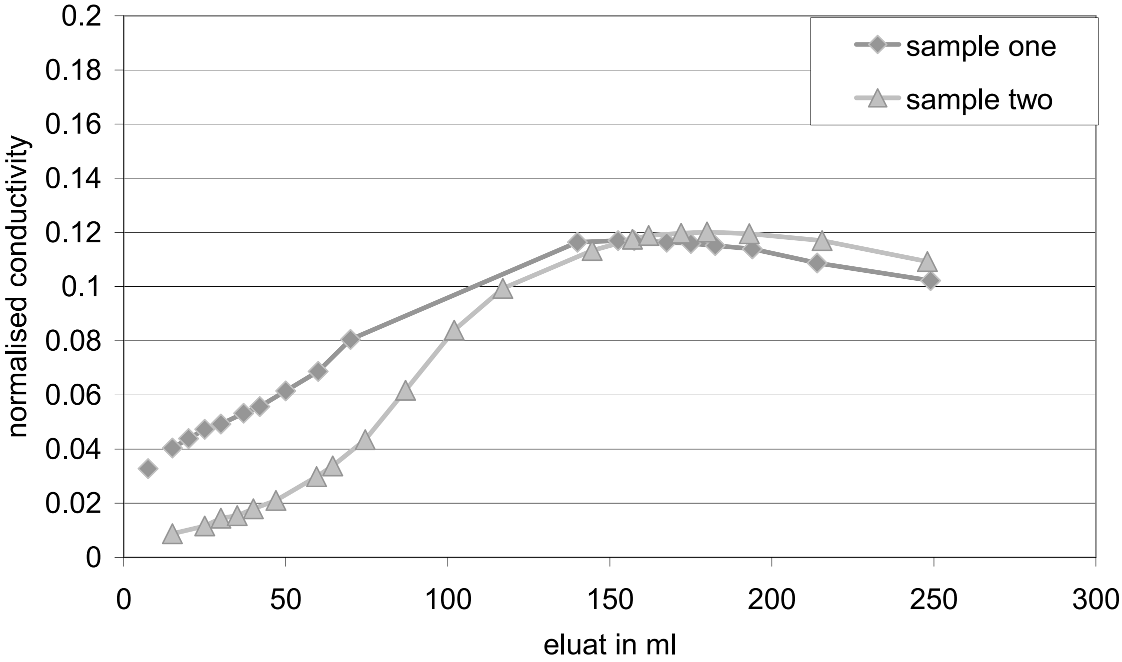


Figure 78: Breakthrough curve of soil A01 normalised of the initial conductivity.

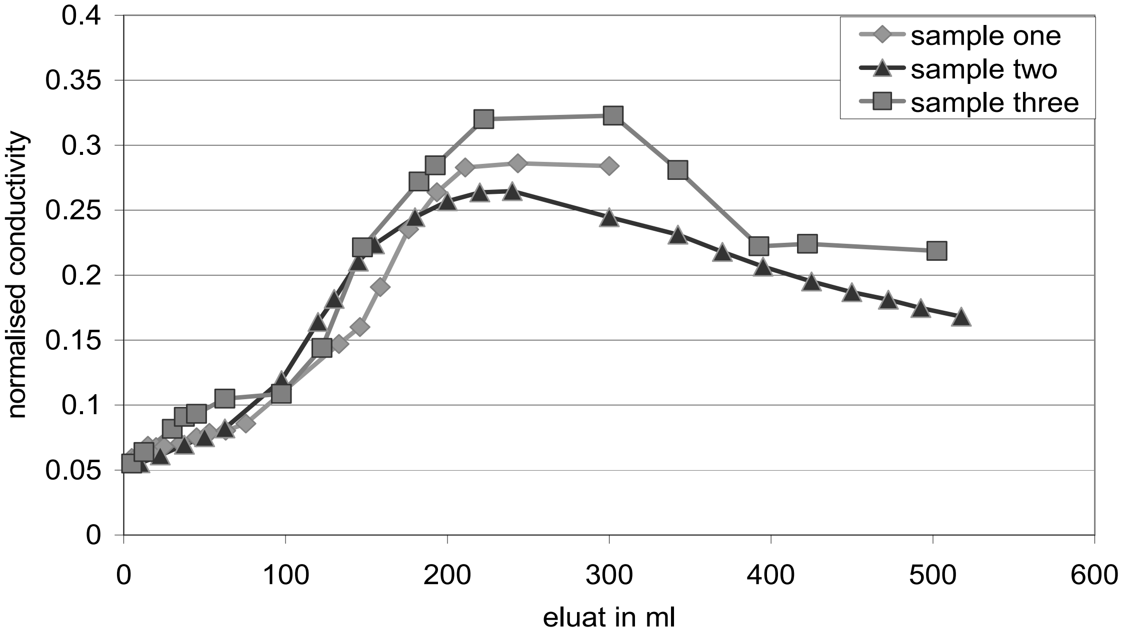


Figure 79: Breakthrough curve of soil A06 normalised of the initial conductivity.

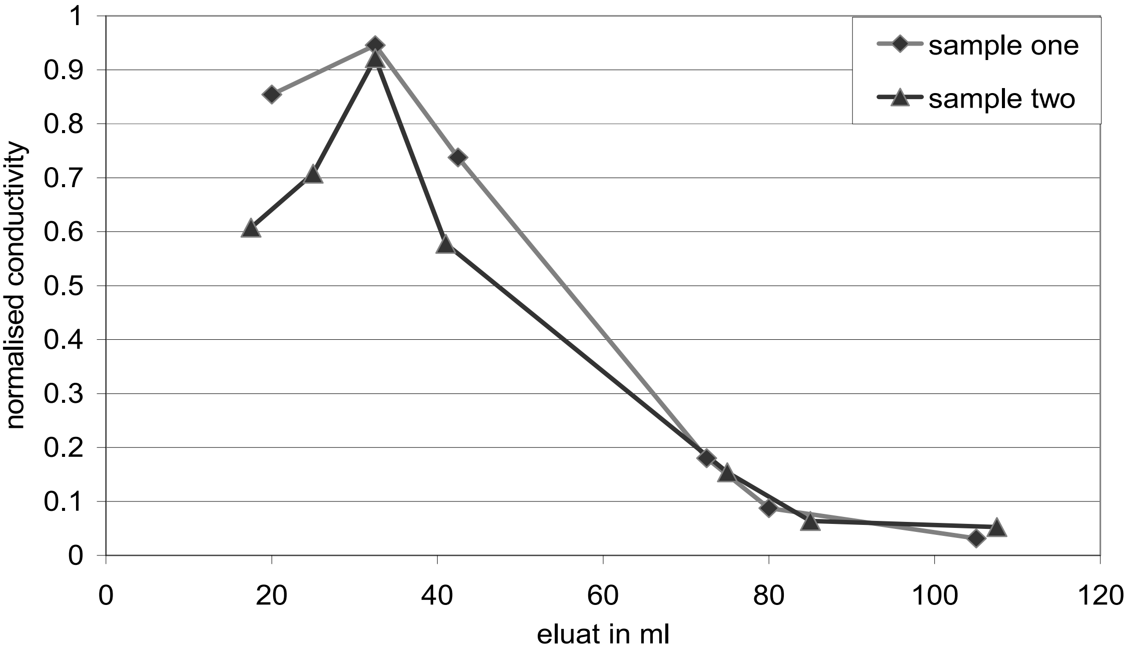


Figure 80: Breakthrough curve of soil A04 normalised of the initial conductivity. The drainage was triggered by application of a short suction power.

A3-12 TXRF results of the first leaching experiment

As a first indication of possible TiO_2 transport processes in soils TXRF (Total X-Ray Fluorescence) analysis of the four segments (as described previous) was performed for the first leaching experiment. The TXRF analysis results revealed no transport (Figure 78).

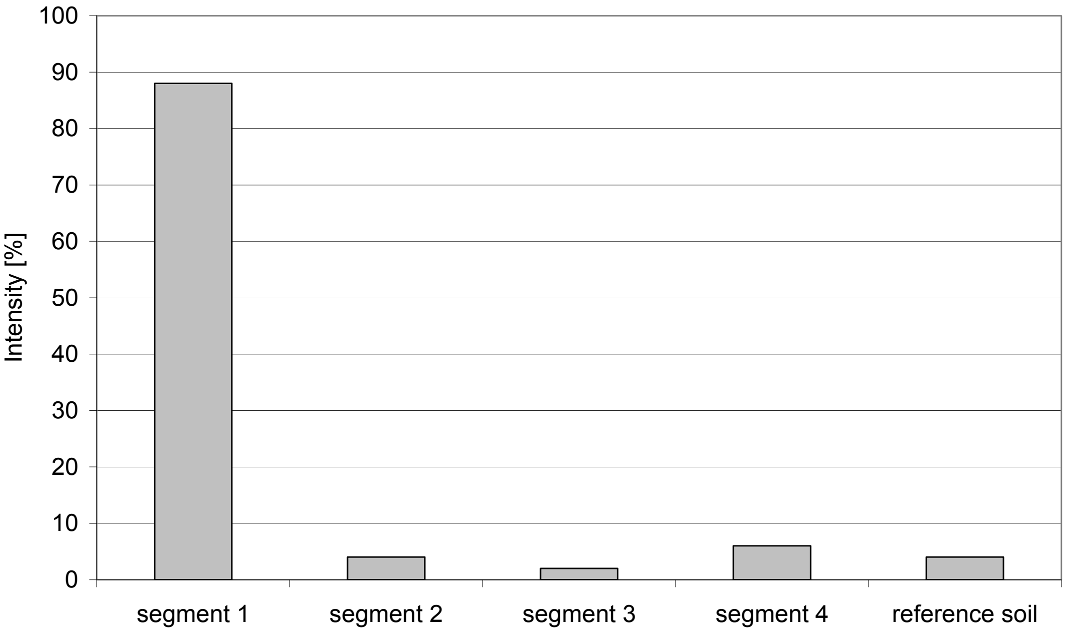


Figure 81: TXRF Titanium signal intensity of the segments for soil A01. No recovery in the eluate was detected. For the sample preparation 10 mg soil were mixed with 500 µL DI water and an aliquot of 10 µL was analysed.

A3-13 Gleyic Podsol – A04: results of the soil leaching test

To avoid adulterated results columns were taken from the centre of the segment, but an influence of this to the following results could not be excluded. Hence all data have to be interpreted with care and the overall behaviour may not be viewed as naturally occurring.
Despite the drainage along the wall no additional Ti was found in the eluate indicating significant detainment of the nanomaterial in the small soil segments that the suspension had to pass.
The A04 soil columns treated with PC105 showed no transport based on bulk chemical analysis but with SEM / EDX the detection of additional TiO_2 was possible in segment two of column two (Figure 83).

Both A04 soil columns treated with UV Titan M262 indicated a transport. For column one a transport to segment four was indicated, but the detected values are within the range of uncertainty (recovery of UV Titan M262 97% ± 12%) (s. chapter 2.1). It is conceivable that a part of the material is mobile and gets to segment four, but the largest part is immobile and stays in segment one. For column two a transport of the nanomaterial is indicated with a decreasing recoveries from top to bottom of the column. With SEM / EDX the detection of isolated TiO_2 agglomerates was possible in segment two of column two (Figure 84 & Figure **85**).

The bulk chemical analysis of the soil columns treated with P25 showed no significant transport for column one. Also for column two no mobility beyond the upper five centimetres could be detected. The low concentration determined in segment one of this column was probably not correct, since an accumulation of the added TiO_2 in the first segment was clearly visible (white layer on the top of the first segment). With SEM / EDX the detection of isolated TiO_2 agglomerates was possible in segment two of column two (Figure 86).

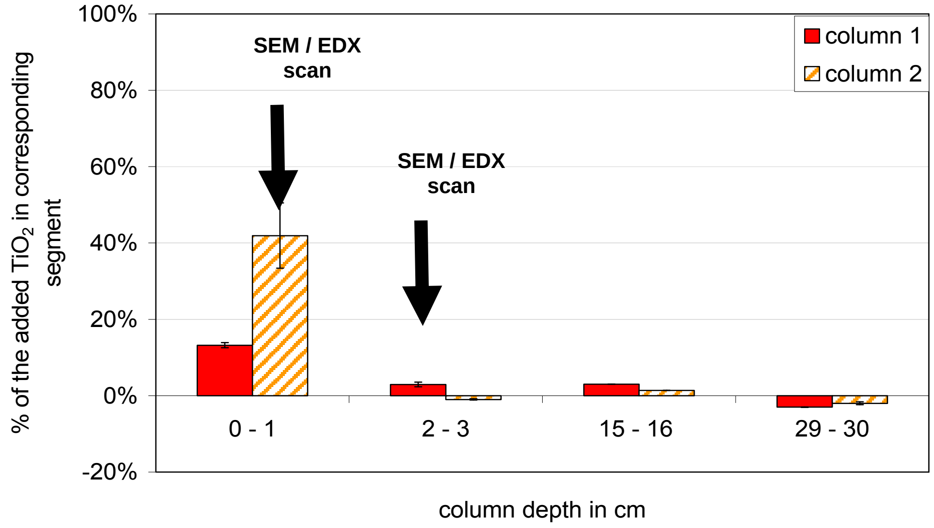


Figure 82: Soil column run with PC105 and soil A04 Gleyic Podsol. Ti background concentration of soil A04 was 0.11% (1.1 g/kg). Error bars = max and min; n = 2.

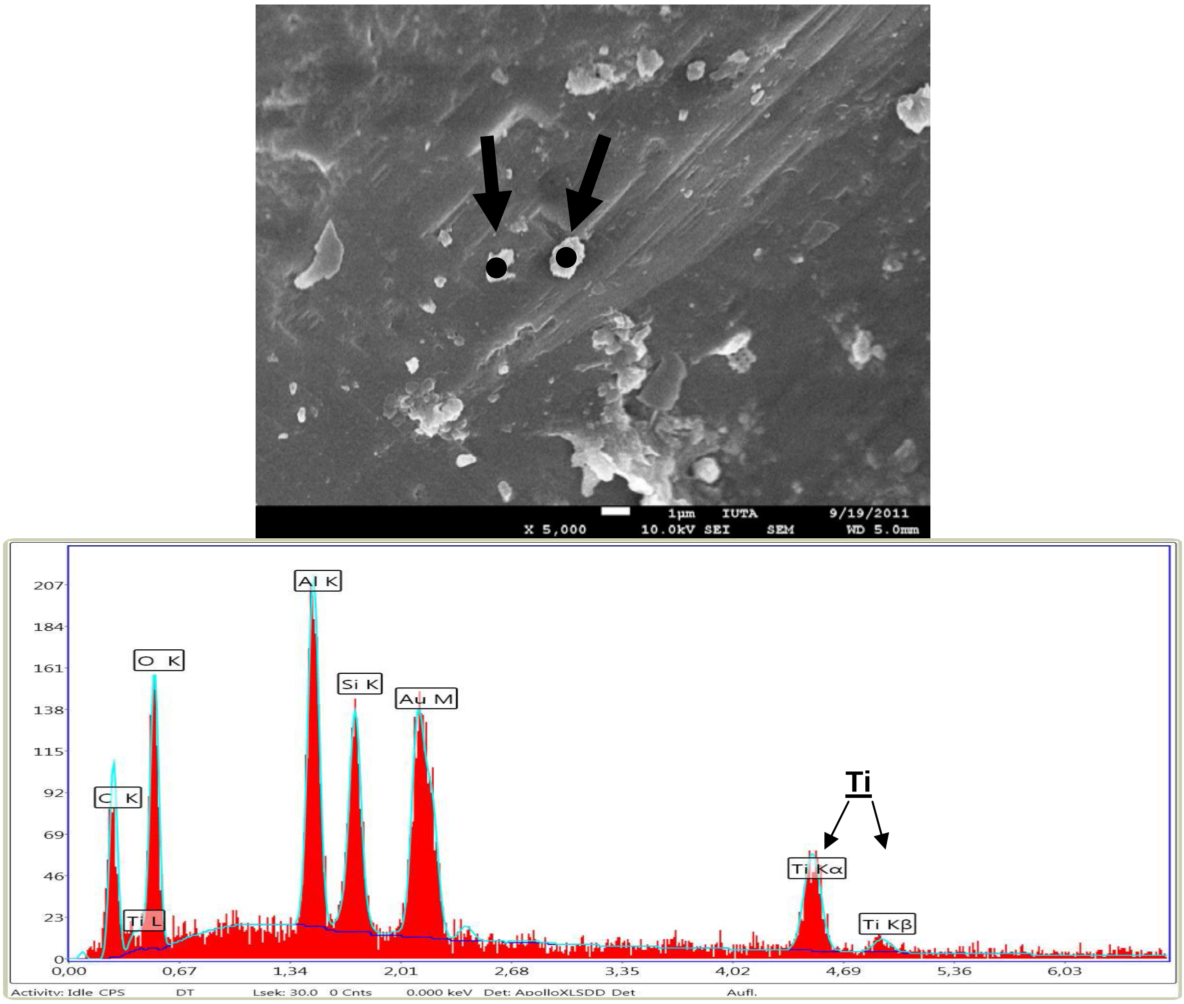


Figure 83: SEM / EDX scans of segment two of soil A04 treated with PC105.

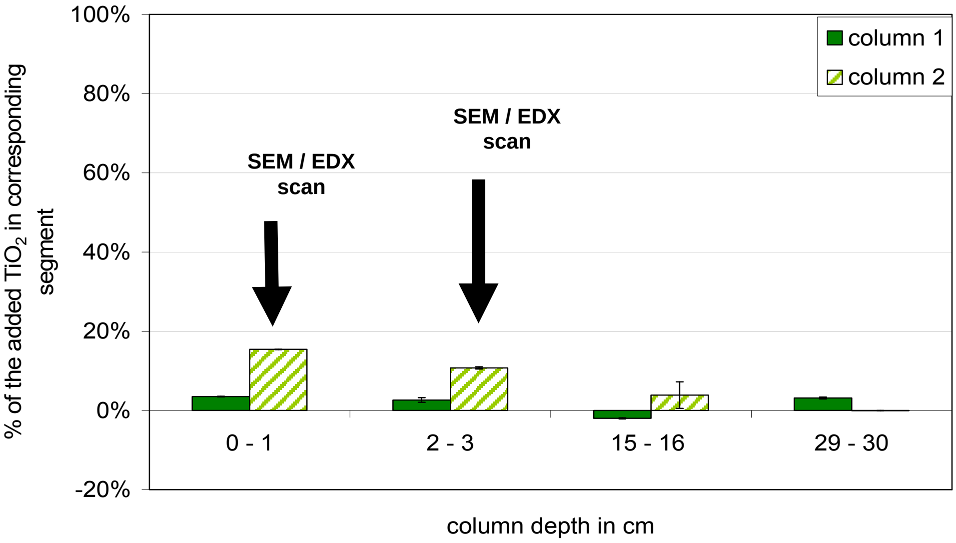


Figure 84: Soil column run with UV Titan M262 and soil A04 Gleyic Podsol. Ti background concentration of soil A04 was 0.11% (1.1 g/kg). Error bars = max and min; n = 2.

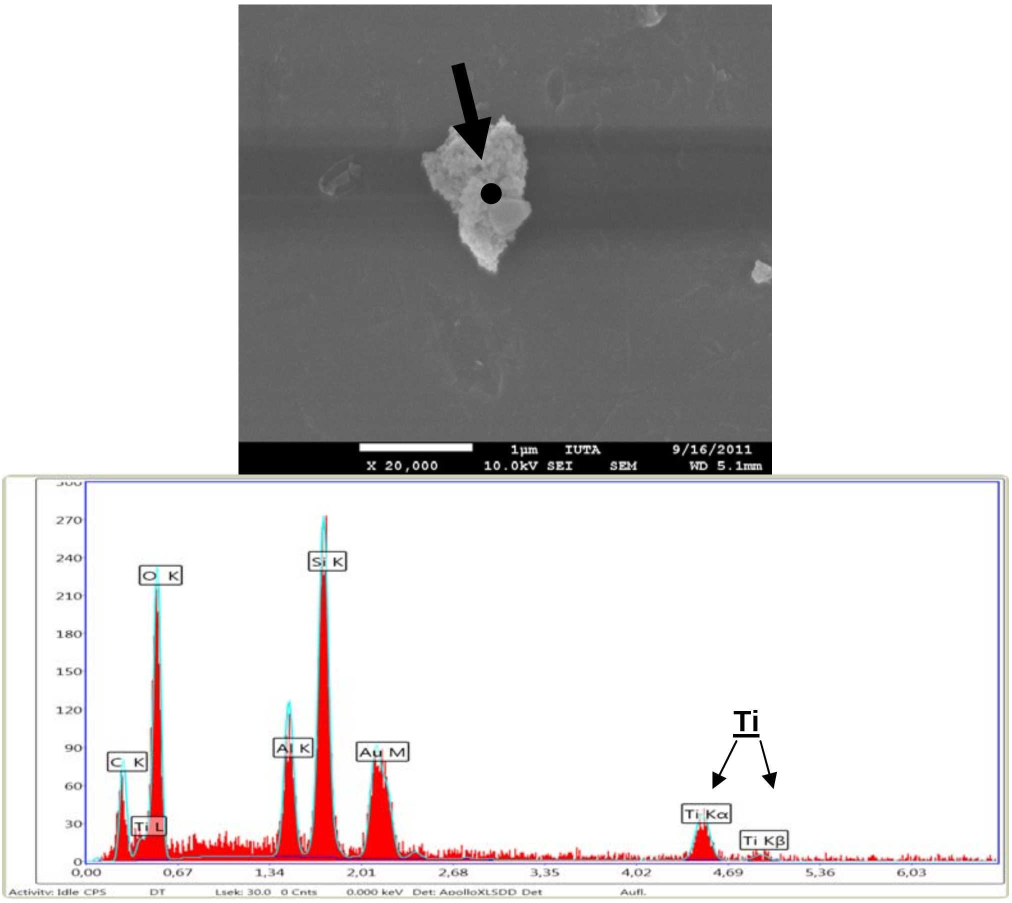


Figure 85: SEM / EDX scans of segment two of soil A04 treated with UV Titan M262.

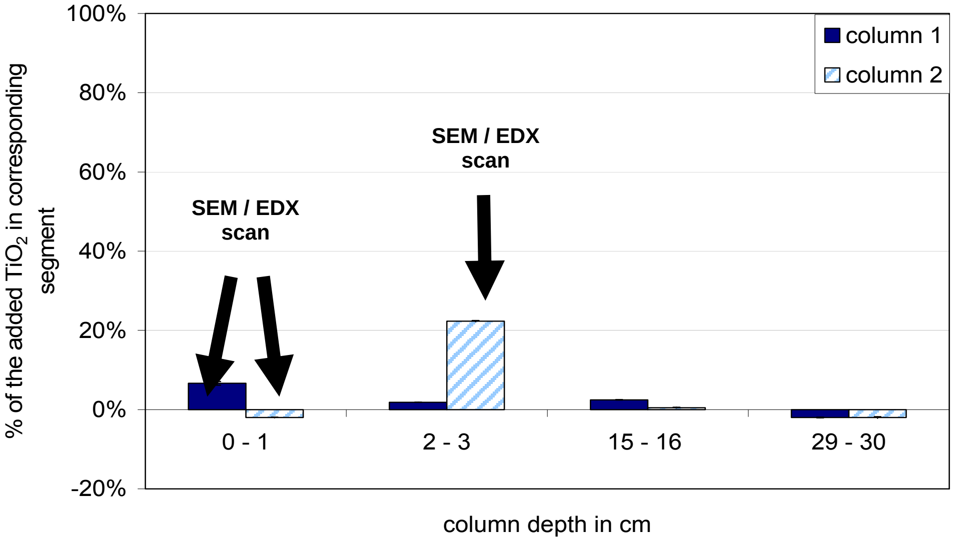


Figure 86: Soil column run with P25 and soil A04 Gleyic Podsol. Ti background concentration of soil A04 was 0.11% (1.1 g/kg). Error bars = max and min; n = 2.

In Figure 86 the mass fractions of the three tested nanomaterials in soil A04 is presented as percent of the added TiO_2 mass. The data are Ti background corrected. Please note that the results related to soil A04 have to be handled with great care since most of the transport took place along the walls of the glass columns. Neglecting this and taken only the inner part of the soil column for analysis highest mass fractions were found in the first or second segment of the soil column independent of the added TiO_2 material. Only the coated material UV Titan M262 indicated a transport down to segment two (10% recovery, column one) and segment four (6%, column two). This was conceived with SEM / EDX scans. For PC105 with SEM / EDX an isolated transport of TiO_2 agglomerates down to segment two was shown.

Representative SEM and EDX of segment one for all materials were conducted.

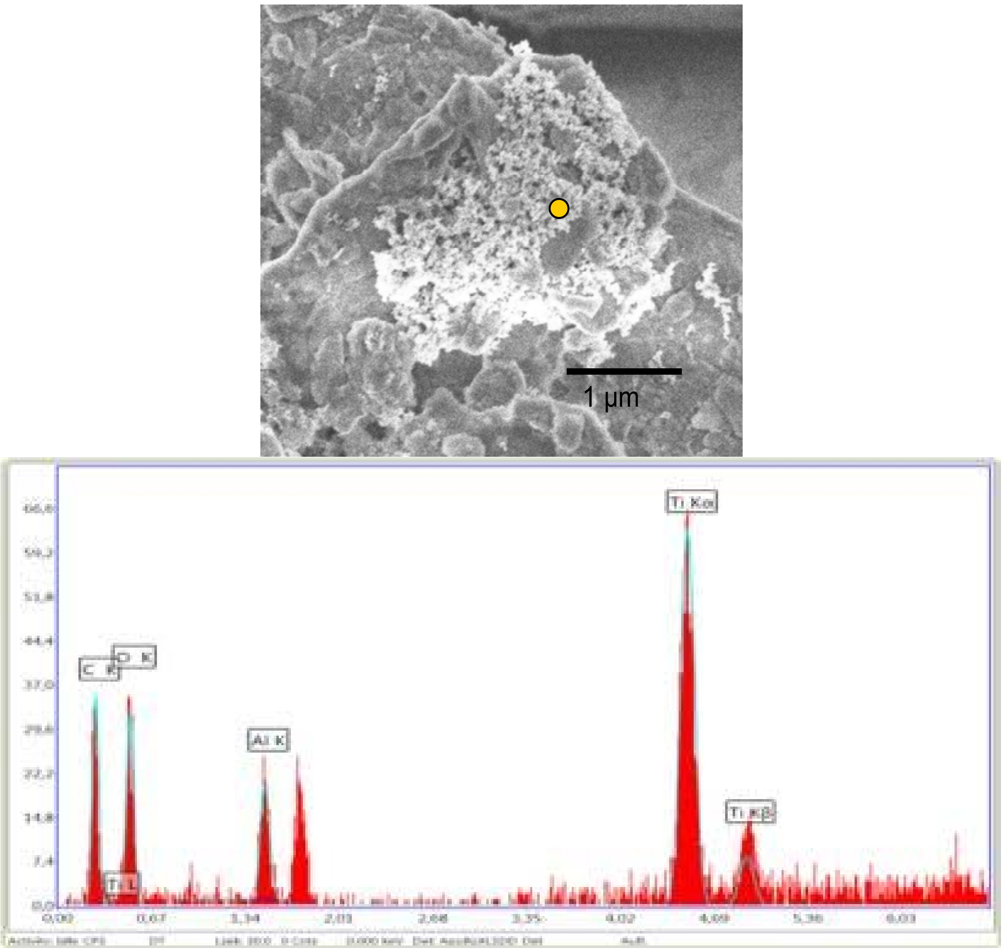


Figure 87: SEM / EDX scans of segment one of soil A04 treated with UV Titan M262.

Table 32: PC105, UV Titan M262 and P25 in the four analysed soil column segments of soil A04 in % of the added mass of 500 mg (TiO2). All values are corrected for the Titanium concentration in the natural soil. The double analysis of each segment showed a reproducibility better than 0.8 mass-%.

	PC105 recovery (% of added mass)		**UV Titan M262 recovery (% of added mass)**		**P25 recovery (% of added mass)**	
	column one	column two	column one	column two	column one	column two
1	11	50	4	17	6	1
2	1	0	0	10	1	20
3	1	0	0	5	3	1
4	0	0	6	1	0	0

- ➔ *With chemicals analysis no transport of P25 and PC105 was shown for this soil type, but with SEM / EDX a transport of PC105 could be identified.*
- ➔ *For UV Titan M262 a transport was indicated, but not as clear as for soil A06. However with SEM / EDX a transport of P25 down to segment two was identified.*

A3-14 SEM EDX Scans of the segments of different treated soil columns

In the following figures (Figure 88 - Figure 90) SEM and EDX scans of the first segment of the soil columns with soiltype A01 are presented.

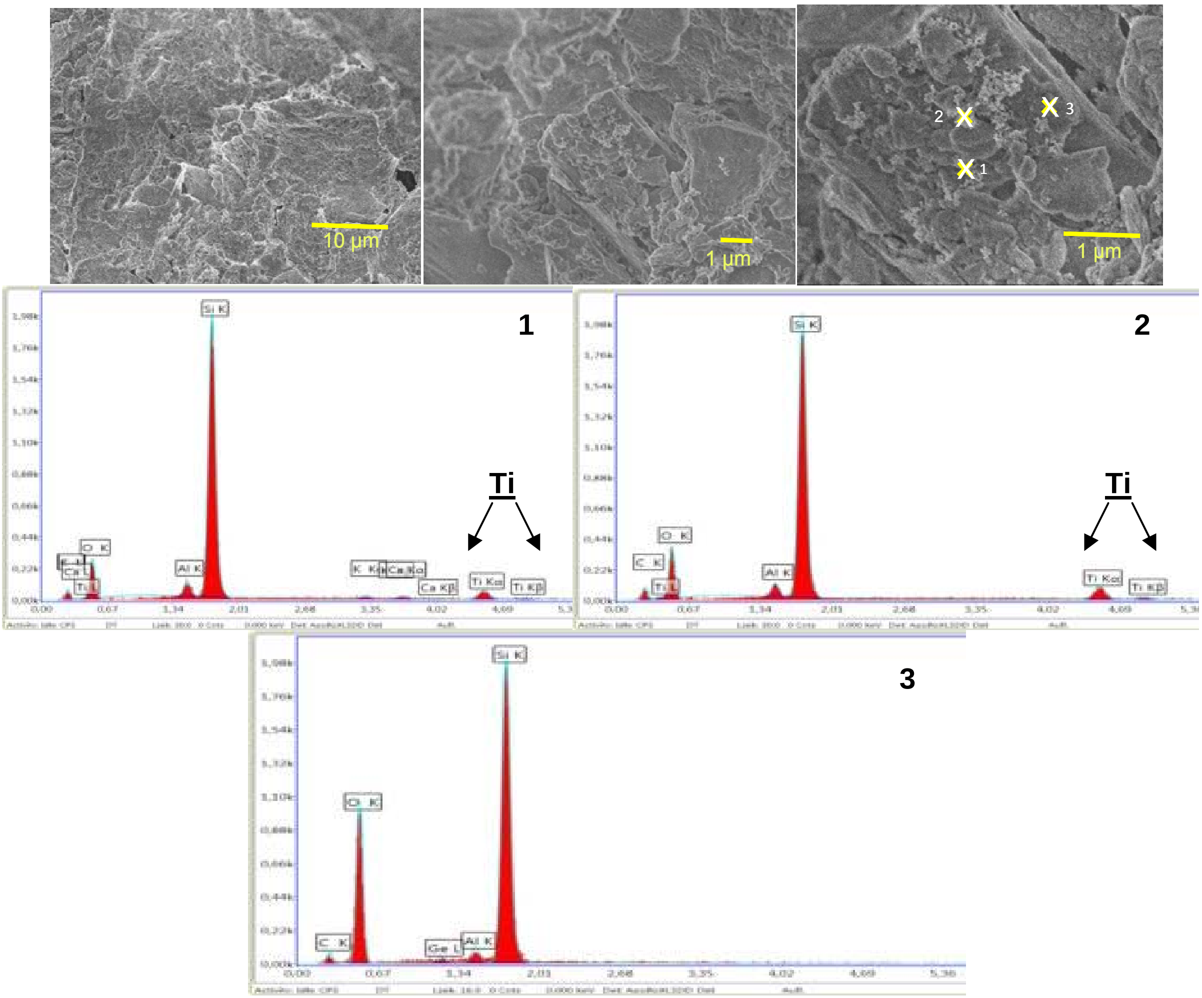


Figure 88: SEM / EDX scans of segment one of soil A01 treated with P25.

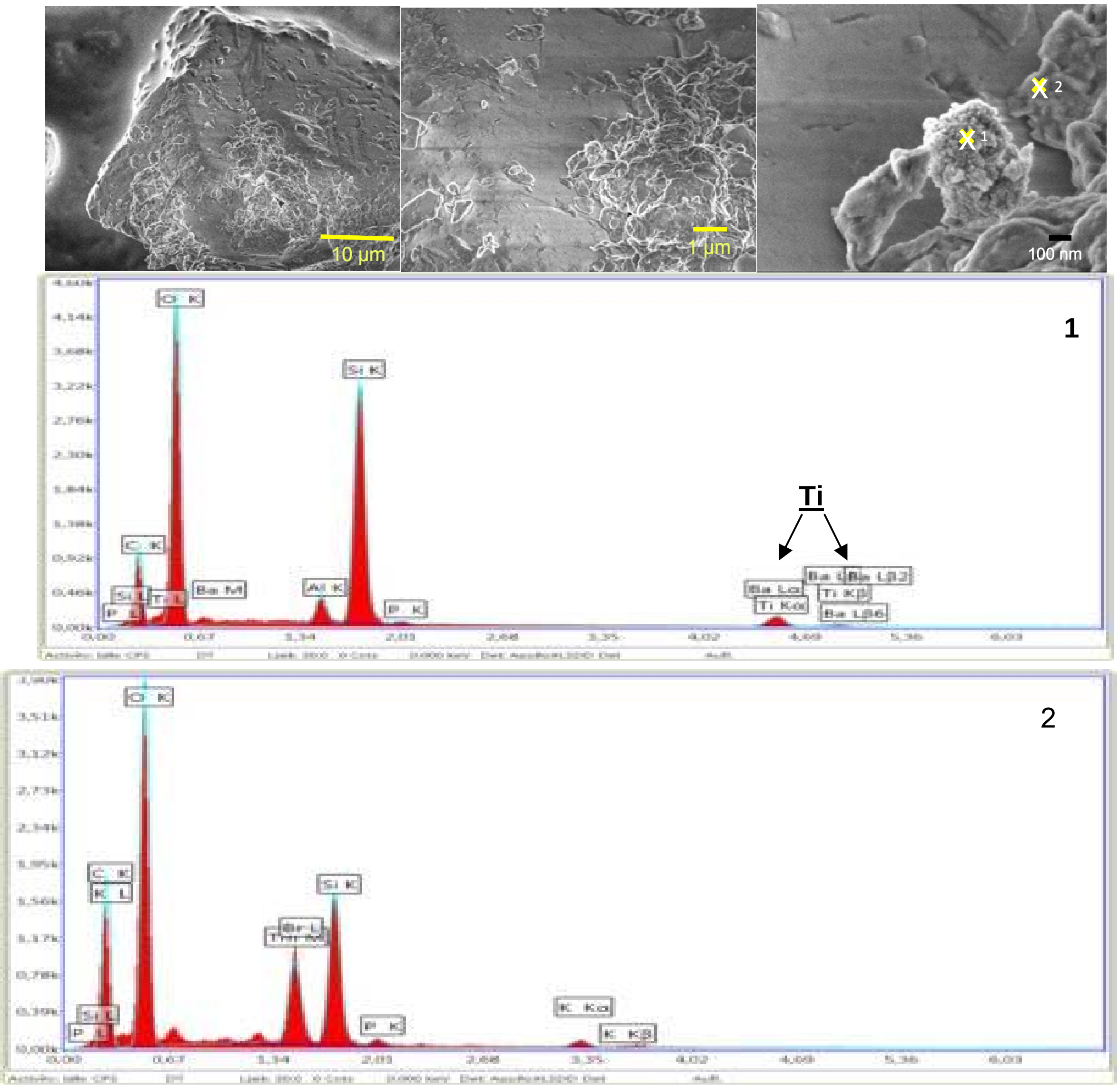


Figure 89: SEM / EDX scans of segment one of soil A01 treated with PC105.

SEM / EDX scans of segment four of soil A01 treated with UV Titan M262 overall 26 particles were analysed. Visually TiO_2 particles could be detected and for two particles EDX detected also Ti.

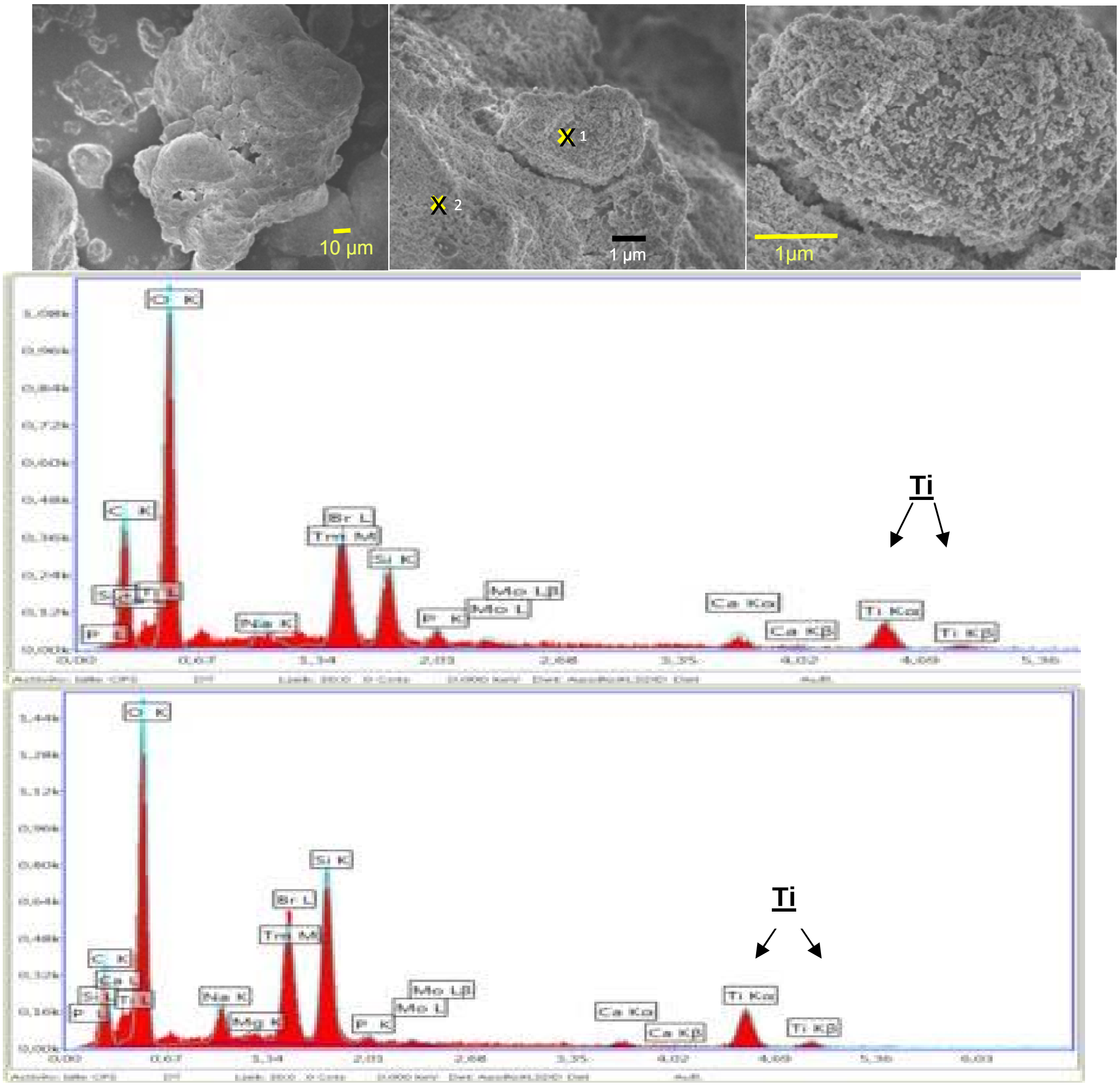


Figure 90: SEM / EDX scans of segment one of soil A01 treated with UV Titan M262.

In the following figures (Figure 91 - Figure 93) SEM and EDX scans of the first segment of the soil columns with soiltype A06 are presented.

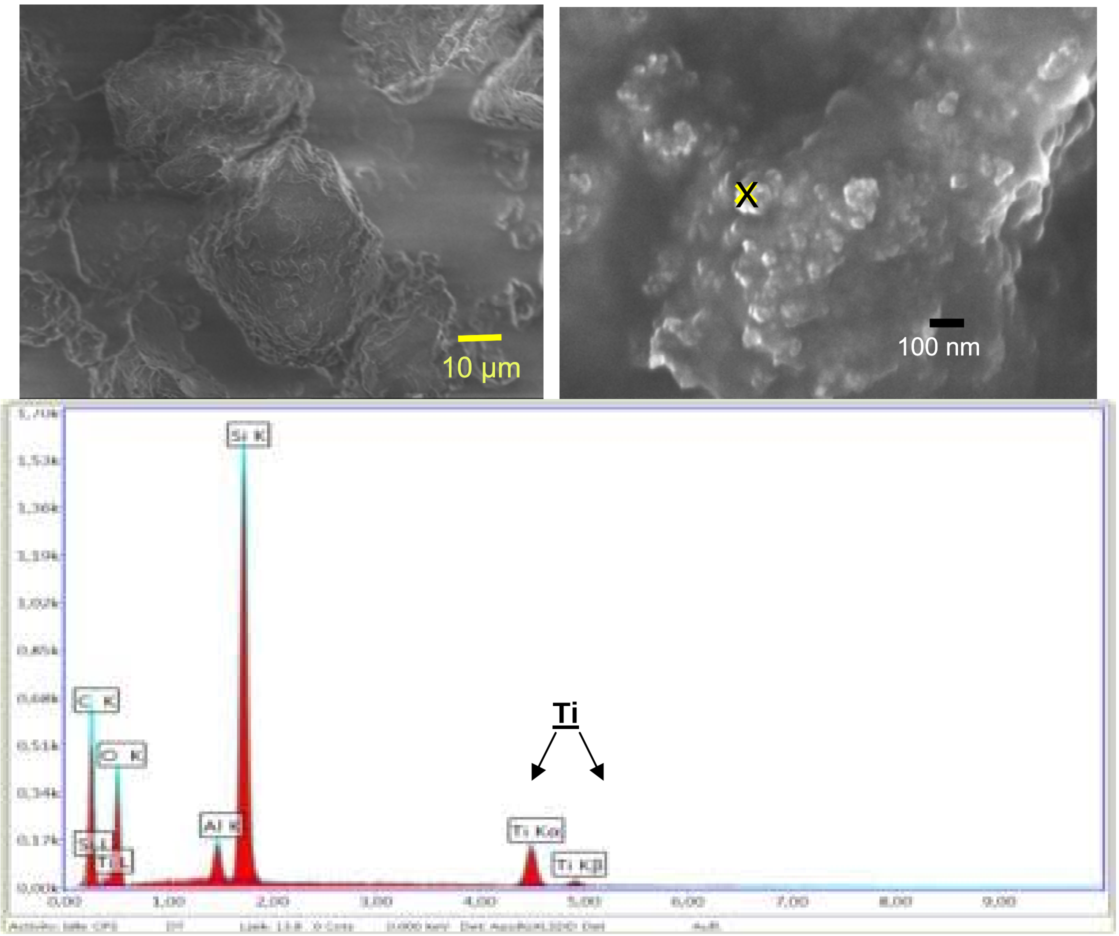


Figure 91: SEM / EDX scans of segment one of soil A06 treated with UV Titan M262.

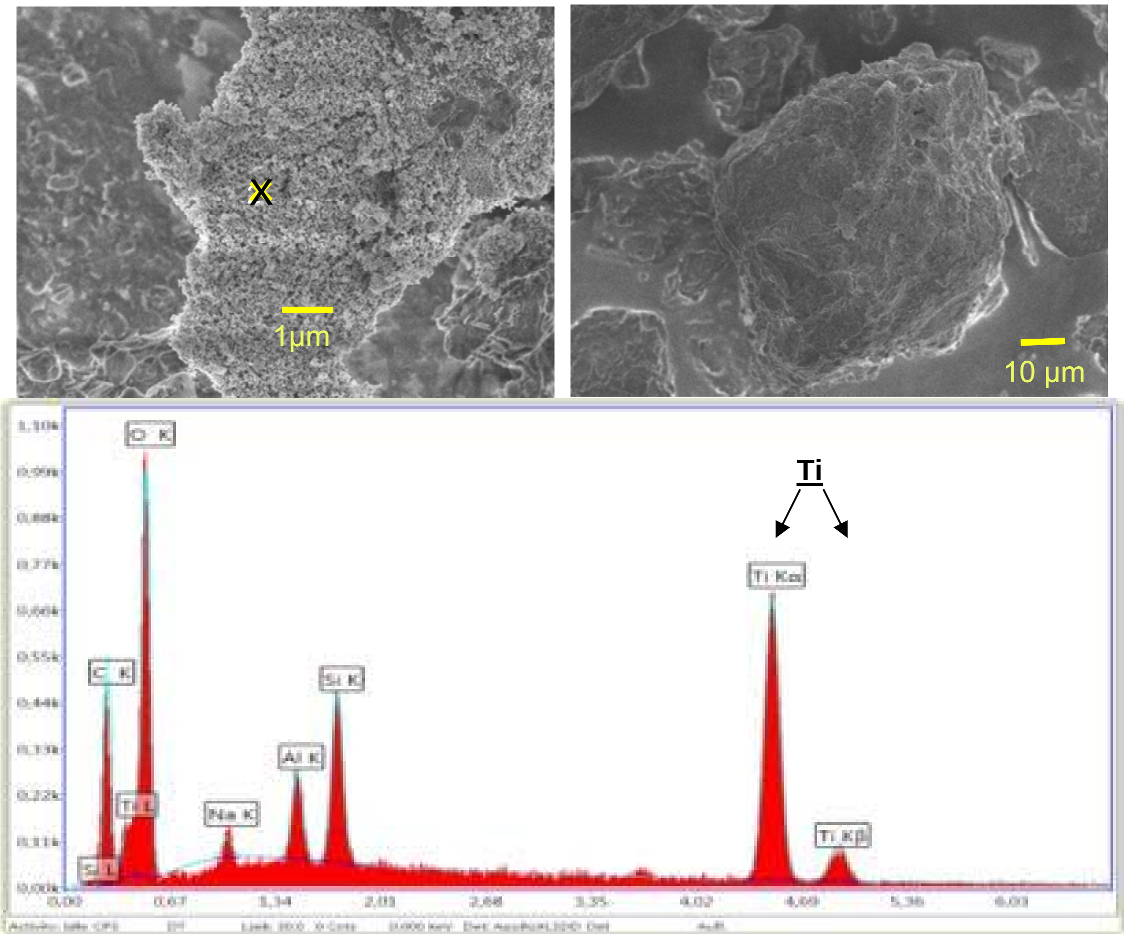


Figure 92: SEM / EDX scans of segment one of soil A06 treated with P25.

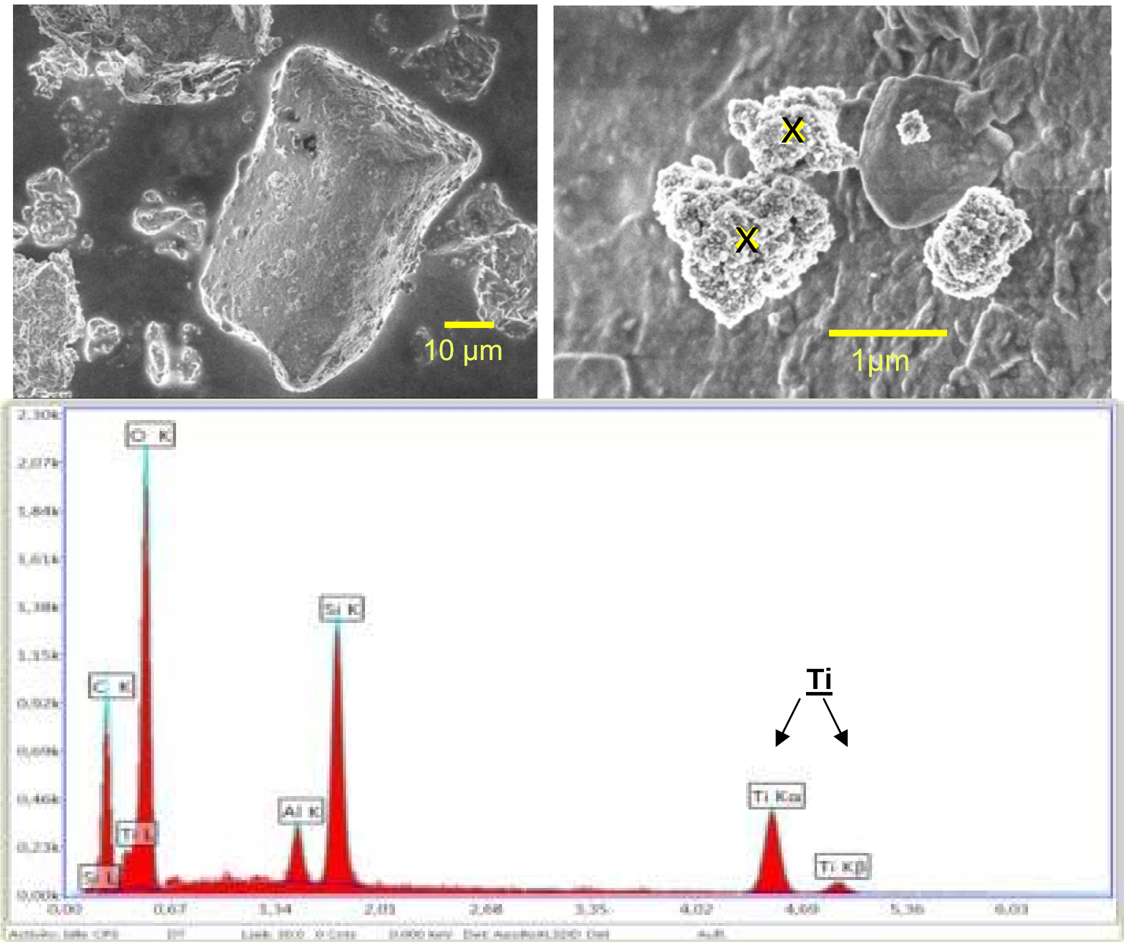


Figure 93: SEM / EDX scans of segment one of soil A06 treated with PC105.

SEM / EDX scans of segment four of soil A06 treated with UV Titan M262. Overall 25 particles were analysed, TiO_2 couldn’t be identified neither visually nor with EDX.

A3-15 Photodocumentation – leaching experiments in soil columns

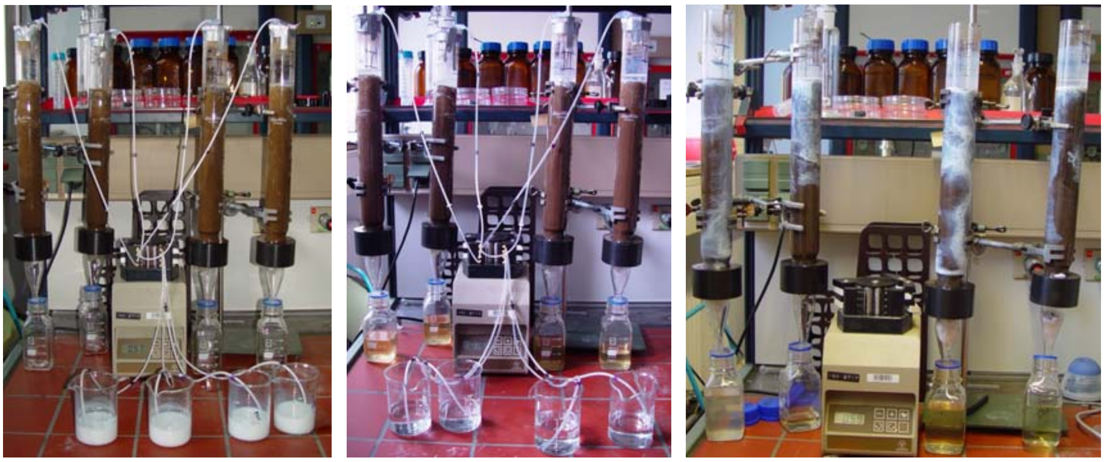


Figure 94: Application of the TiO_2 suspension to the soil columns (left), afterwards the artificial rain was applied (middle), the eluate was collected after 48 h (right) – soil A04 treated with UV Titan M262.

Figure 95: Soil columns after the test, before they were differentiated in different segments. Treated with UV Titan M262 – soil column filed with soil A01 (top), A06 (middle) and A04 (bottom).

Annex IV

A4-1 Occupational measurements

Institut für Energie- und Umwelttechnik e. V. (IUTA)
Bereich:
Luftreinhaltung &
Nachhaltige Nanotechnologie
Bliersheimer Straße 60
47229 Duisburg

Umweltrisiken von Nanomaterialien: Untersuchung des Verhaltens ausgewählter Nanomaterialien unter Umweltbedingungen in Abhängigkeit von Form, Größe und Oberflächengestaltung

UBA-Projekt FKZ 3709 65 417

Laufzeit: 1. Oktober 2009 bis 01. November 2011

Bericht Nr. LP 58/2010

Expositionsbezogene Partikelmessungen am Arbeitsplatz beim Zubereiten und Zudosieren einer TiO_2-Suspension zur Laborkläranlage

Bearbeiter:
Heinz Kaminski
Carmen Nickel
Thomas Kuhlbusch

Duisburg, 10. Mai 2010

Hintergrund
Im ersten Teil des Forschungsvorhabens wurden nanoskalige Materialien in Suspensionen gebracht und charakterisiert. Basierend auf den Charakterisierungen der Testmedien wird im weiteren Verlauf des Vorhabens das Verhalten der eingesetzten Nanomaterialien in der Umwelt mit den Schwerpunkten Wasser und Boden entsprechend der OECD-Richtlinien untersucht. Dazu wird u. a. nanoskaliges Titandioxid in Suspension gebracht und einer Laborkläranlage zugesetzt, um die biologische Abbaubarkeit von TiO_2 in einer Kläranlagensimulation nach OECD 303A zu untersuchen.
Zur Klärung der Fragestellung, ob eine Exposition bei der Herstellung dieser TiO_2-Suspensionen und beim Zudosieren zur Kläranlage durch eventuell freiwerdendes TiO_2 besteht, wurden Messungen zur Partikelfreisetzung am Arbeitsplatz beim Umgang mit TiO_2 durchgeführt. Untersucht wurden dabei im Einzelnen die Zubereitung der Suspension (Einwiegen und Dispergieren des TiO_2) und das Zudosieren der Suspension zur Kläranlage. Für diese Untersuchungen wurden ein Partikelzähler, der Partikelanzahlkonzentrationsänderungen erfassen kann, und ein Partikelsammelgerät, welches Partikel auf Substraten für weitere Untersuchungen abscheidet, eingesetzt.

Messgeräte und Qualitätssicherung

Partikelzähler

Die Partikelmessungen wurden mit dem tragbaren Partikelzähler CPC (Condensation Particle Counter) Modell 3007 (TSI Inc.) durchgeführt. Bei den Partikelmessungen werden die eingesaugten Partikel im CPC durch eine mit Isopropanol gesättigte Umgebung geleitet. In der daran anschließenden Kühlstrecke dienen die Partikel als Kondensationskerne und das kondensierende Isopropanol lässt die Partikel anwachsen. Die somit angewachsenen Partikel können in der anschließenden Optikeinheit erfasst und gezählt werden. Der CPC bestimmt die Gesamtpartikelanzahl im Größenbereich von 10 nm bis 1.000 nm in einem Konzentrationsbereich von bis zu 10^5 Partikeln/cm^3 mit einer zeitlichen Auflösung von einer Sekunde.

Partikelsammler

Als Partikelsammler wurde der NAS (Nanometer Aerosol Sampler) Model 3089 (TSI Inc.) verwendet. Dieser elektrostatische Präzipitator wurde speziell zur Abscheidung von Partikeln im Nanometerbereich entwickelt. Das Verfahren beruht auf der unipolaren Aufladung der Partikel mit nachfolgender Abscheidung auf einem Substrat oder Probenträger mithilfe eines elektrischen Feldes. Dieser Präzipitator sammelt die Probe auf speziellen Glaskohlenstoffträgern, die dann in Bezug auf Morphologie und Zusammensetzung mittels rasterelektronischer Mikroskopie (REM) und energiedissipativen Röntgenanalyse (EDX) untersucht wurden.

Qualitätssicherung

Zur Sicherstellung, dass der Partikelzähler nanoskaliges TiO_2 sowohl aus einer Staubung als auch aus durch Freisetzung aus einer wässrigen Suspension detektieren kann, wurden im Labor zwei Tests durchgeführt.
Zuerst wurde TiO_2 im Labor in den luftgetragenen Zustand versetzt und dem Partikelzähler kurzzeitig angeboten. Dazu wurde der Behälter (Aeroxide TiO_2 P25, Chargennr. 4168112198) undefiniert geschüttelt und kurzzeitig geöffnet. Der zeitliche Partikelkonzentrationsverlauf für diesen Test ist in Abbildung 1 dargestellt. Deutlich erkennbar sind die Konzentrationsanstiege nach dem kurzen Öffnen des Behälters (Kennzeichnung Start Staubung) und ebenso die Konzentrationsrückgänge durch Verdünnungseffekte verursacht durch die Laborabluftanlage.

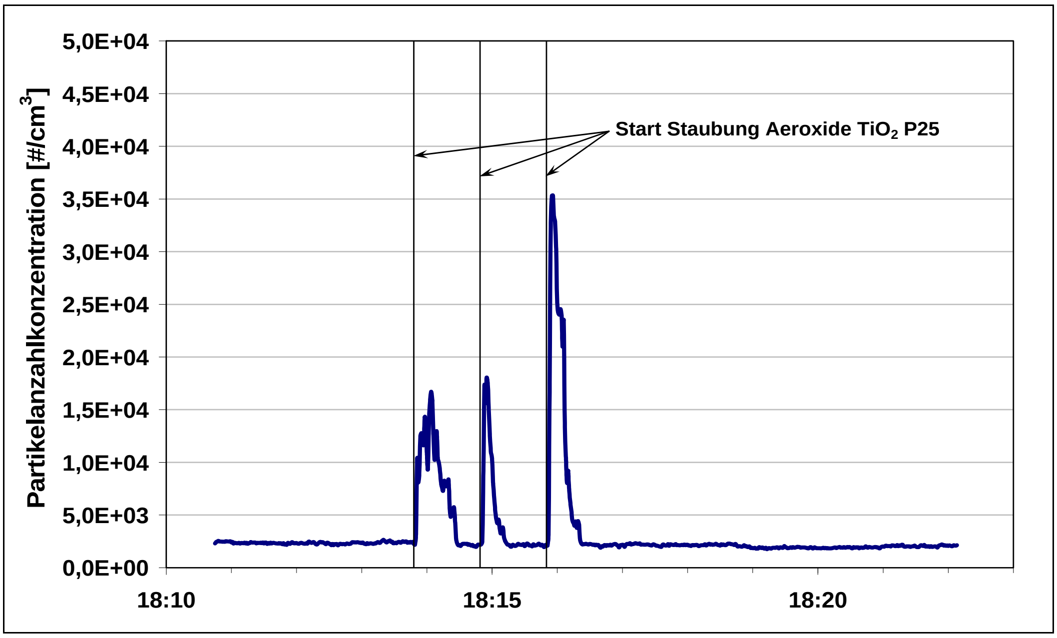


Abbildung 1: Reaktion des CPC auf Konzentrationsänderungen während der Staubung

Als weiterer Test wurde Aeroxide TiO_2 P25 aus einer wässrigen Suspension mittels Partikelgenerator in ein Ausgleichsgefäß verdüst und vermessen. Der Partikelkonzentrationsverlauf ist in Abbildung 2 dargestellt. Auch bei diesem Test reagierte der CPC sofort auf Partikelanzahlkonzentrationsänderungen, sowohl beim Start als auch beim Stopp der Partikelgenerierung. Die mithilfe eines SMPS (Scanning Mobility Particle Sizer) ermittelte Partikelgrößenverteilung ist ebenfalls in Abbildung 2 als eingebettetes Diagramm aufgetragen. Danach lag der Modalwert der Suspension bei 200 nm.

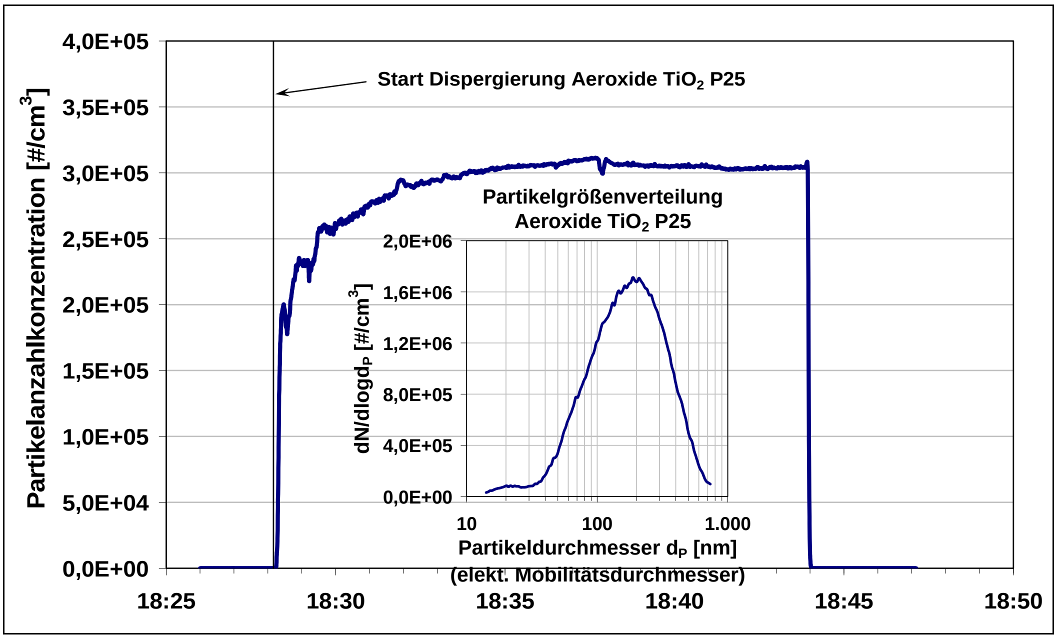


Abbildung 2: Reaktion des CPC auf Konzentrationsänderungen während der Partikelgenerierung mittels Partikelgenerator

Damit war die Eignung des CPC gegeben, freiwerdende Partikel bei der Zubereitung der TiO_2-Suspension und der Zudosierung der Suspension in die Kläranlage zu detektieren.

Zusätzlich sollten während der Zubereitung und Zudosierung Partikel auf Probenträger gesammelt und mittels Elektronenmikroskopie untersucht werden. Um die Tauglichkeit der eingesetzten Methode für den Nachweis von TiO_2 zu zeigen, wurden Titandioxidpartikel (Aeroxide TiO_2 P25) auf einem Glaskohlenstoffträger aufgebracht und mittels REM/EDX untersucht. Bei diesem Test konnten Titandioxidpartikel bis zu einer unteren Größe von etwa 30 nm eindeutig identifiziert werden, siehe nachfolgende Abbildung 3.

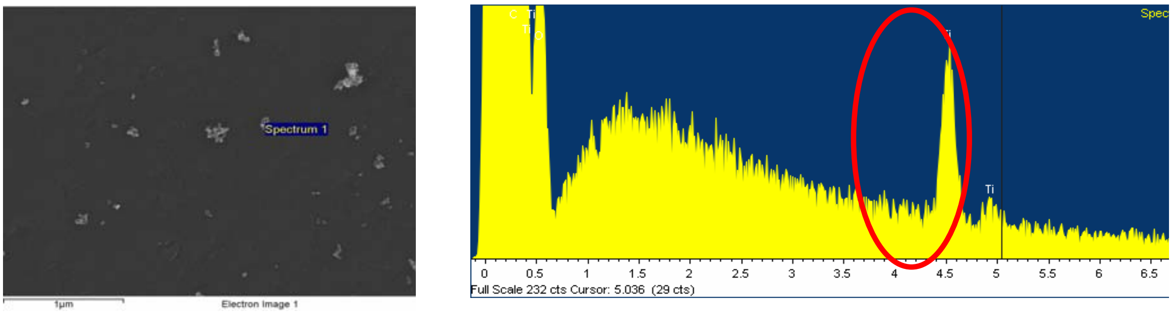


Abbildung 3: Ausschnitt der REM-Aufnahme des Glaskohlenstoffträgers mit erkennbaren Partikeln (links) und Ergebnis der EDX-Analyse mit deutlicher Titan-Identifizierung

Messungen und Ergebnisse

Partikelanzahlkonzentration

Die eigentlichen Partikelmessungen zur Überprüfung, ob eine Partikelfreisetzung während der Zubereitung oder Zudosierung der TiO_2 Suspension stattfindet, wurden in den Laboren der Hydrotox GmbH durchgeführt. Zur Herstellung der Suspension wurden dazu zunächst 3 x 30 mg Aeroxide TiO_2 P25 eingewogen. Während des Einwiegens wurde die persönliche Laborschutzausrüstung verwendet (Laborkittel, Einweghandschuhe, Filtermaske mit Filterklasse FFP3). Den Aufbau der Messgeräte während des Einwiegens ist in Abbildung 4, den dazugehörigen Partikelkonzentrationsverlauf in Sekundenwerten ist in Abbildung 5 zu sehen. Danach ergibt sich während des Einwiegens kein signifikant erkennbarer Konzentrationsanstieg, der auf eine deutliche Freisetzung von Partikeln hinweisen würde. Tendenziell konnte eine abnehmende Partikelkonzentration beobachtet werden. Vor dem Einwiegen lag die mittlere Partikelkonzentration bei 8.520 $\#/cm^3$ ± 440 $\#/cm^3$ (Zeitintervall von 8 min), während des Einwiegens bei 7.130 $\#/cm^3$ ± 520 $\#/cm^3$ (Zeitintervall von 13 min). Die leicht abnehmende Konzentration während des Einwiegens könnte dadurch begründet sein, dass die Labortür erst kurz vor Wiegestart geschlossen wurde und somit eine Beruhigung der Laborluft mit verminderter Luftverwirbelung stattfand.

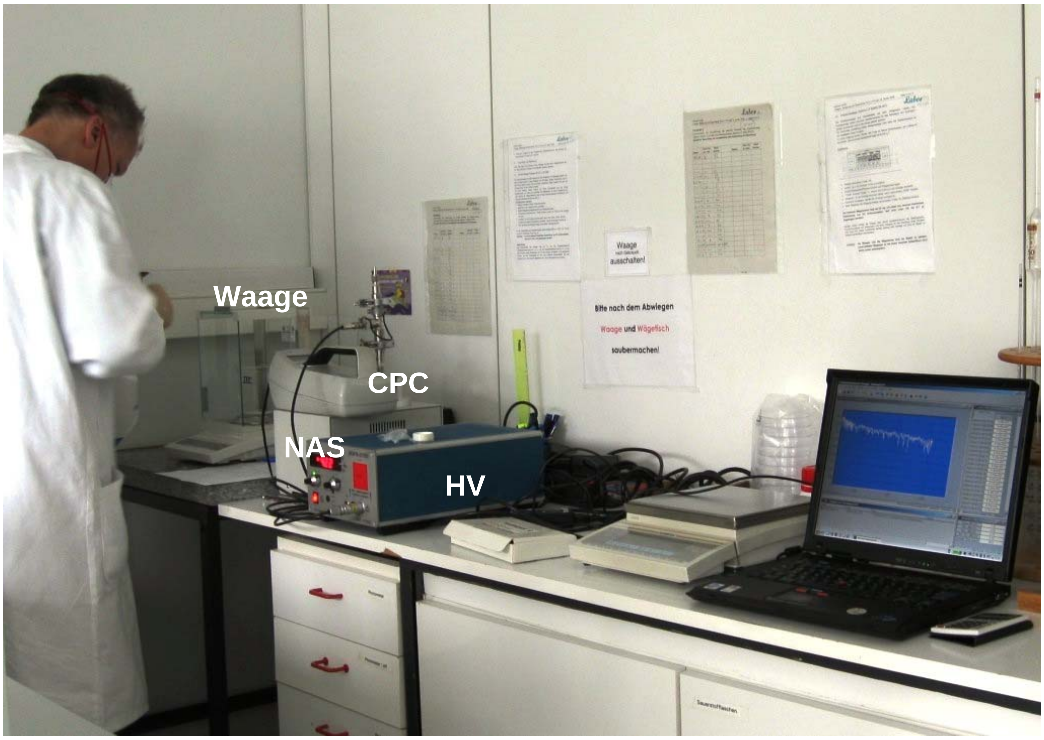


Abbildung 4: Einwiegen des TiO_2 für die Suspension

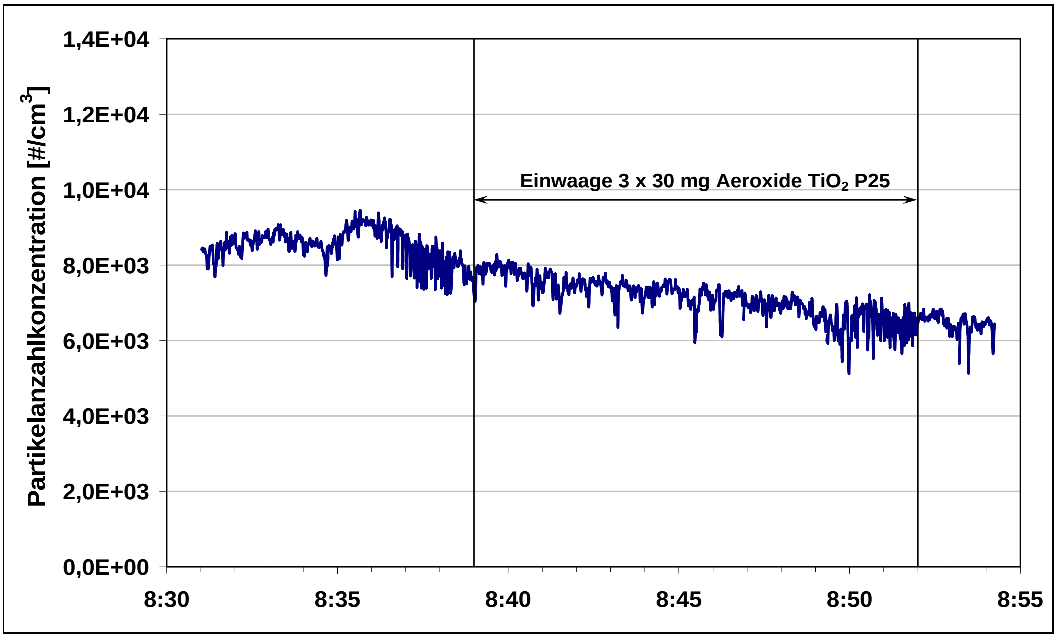


Abbildung 5: Partikelanzahlkonzentrationsverlauf während des Einwiegens von TiO_2

Nach dem Einwiegen wurden 30 mg Titandioxid mit 300 mL synthetischem Leitungswasser gemischt und mittels Ultraschall (Sonopuls HD 2070, Bandelin electronics GmbH) in einem Laborabzug homogenisiert.

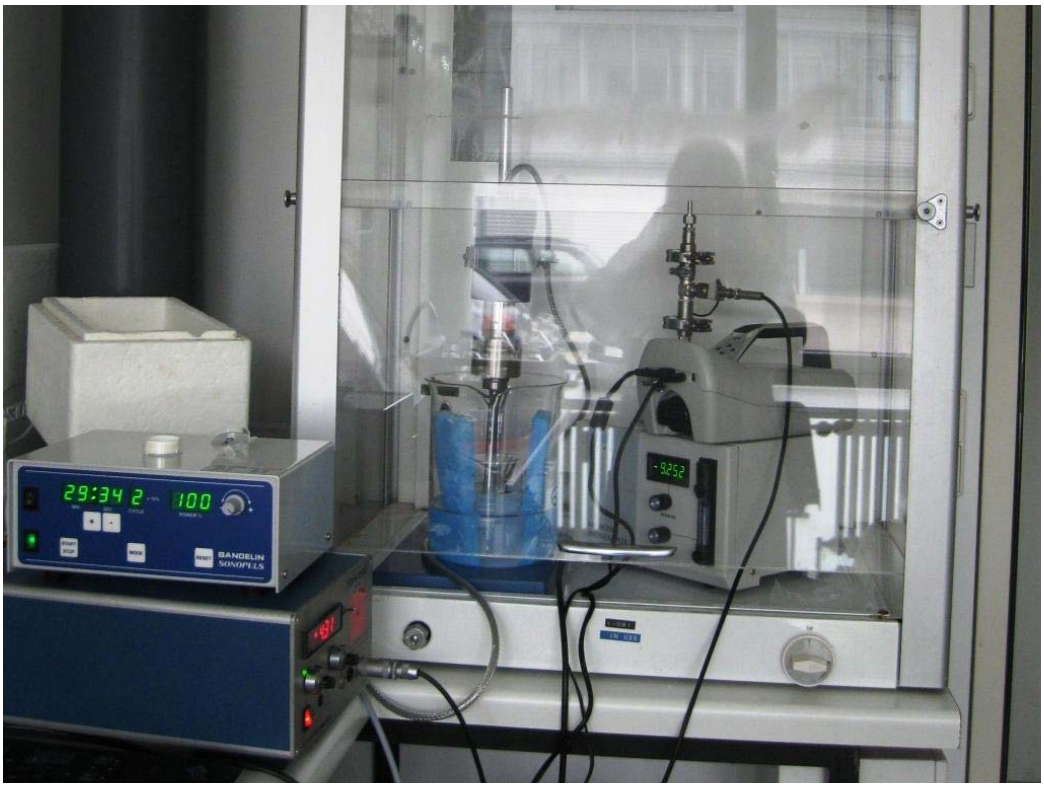


Abbildung 6: Aufbau der Messgeräte beim Homogenisieren mittels Ultraschall im Laborabzug

Abbildung 6 zeigt den Aufbau der Messgeräte im Laborabzug. Während des Homogenisierens wurde die Lüftung im Laborabzug abgeschaltet, damit eventuell freiwerdende Partikel auch von den Messgeräten erfasst werden können. Die Homogenisierungsdauer betrug 30 min bei 200 W Leistung. Der zeitliche Verlauf der Partikelkonzentration (Sekundenwerte) während des Homogenisierens ist in Abbildung 7 aufgezeichnet. Ein signifikanter Anstieg der Partikelanzahlkonzentration durch Freisetzung von Partikeln ist nicht erkennbar. Zu erkennen ist vielmehr ein gleichmäßiger, leicht abfallender Konzentrationsverlauf während der Ultraschallbehandlung. Die mittlere Partikelanzahlkonzentration lag bei 6.440 #/cm^3 ± 320 #/cm^3. Deutlich zu sehen ist auch ein Anstieg der Konzentration nach dem Ende der Ultraschallbehandlung durch Öffnen des Laborabzugs und Entnahme der Suspension bzw. durch Hantieren an den Messgeräten.

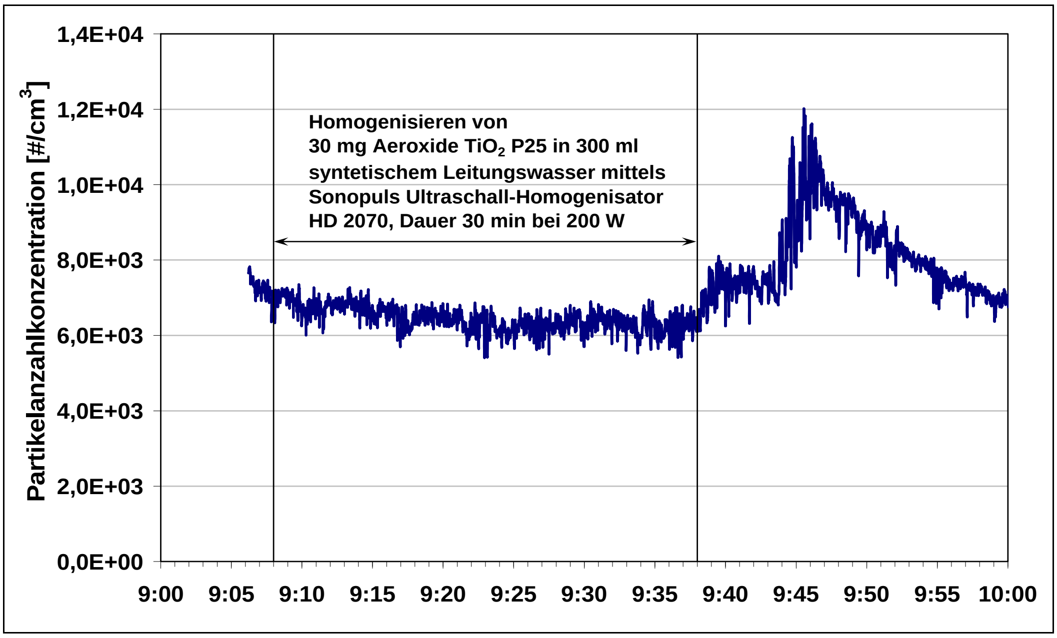


Abbildung 7: Partikelanzahlkonzentrationsverlauf während der Ultraschallbehandlung der TiO_2 Suspension

Nach der Zubereitung der TiO_2 Suspension wurden die Messgeräte an der Laborkläranlage aufgebaut und eine Peristaltikpumpe zur Förderung der TiO_2 Suspension installiert. Die Laborkläranlage ist in Abbildung 8, der zeitliche Partikelanzahlkonzentrationsverlauf während der Messung ist in Abbildung 9 gezeigt. Nach dem Start der Suspensionszudosierung wurde für weitere 4¾ Stunden die Partikelkonzentration aufgezeichnet. Während dieser Zeit lag die mittlere Partikelkonzentration bei 3.930 #/cm^3 ± 824 #/cm^3. Wiederum konnten keine signifikanten Partikelkonzentrationserhöhungen detektiert werden, die einer Freisetzung aus der Laborkläranlage zuzuordnen wären. Die aufgezeichneten Konzentrationserhöhungen können dem Öffnen der Labortür und somit dem Partikeleintrag von außen während der Partikelaufzeichnung zuordnet werden.

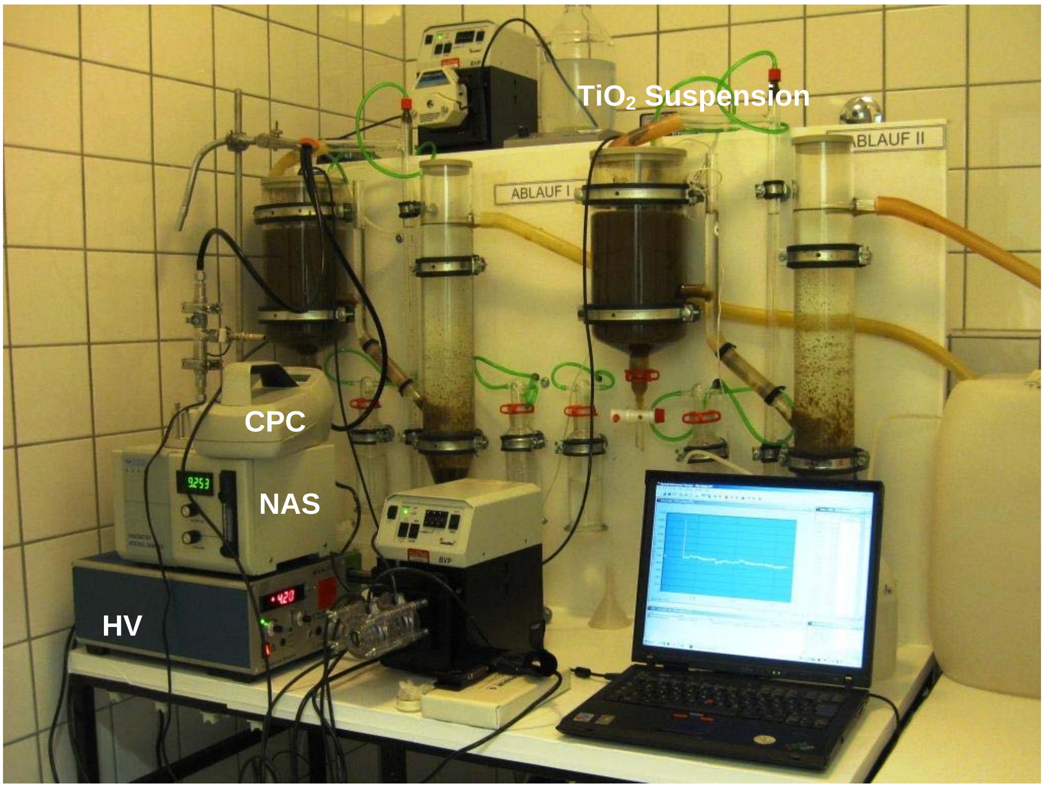


Abbildung 8: Laborkläranlage

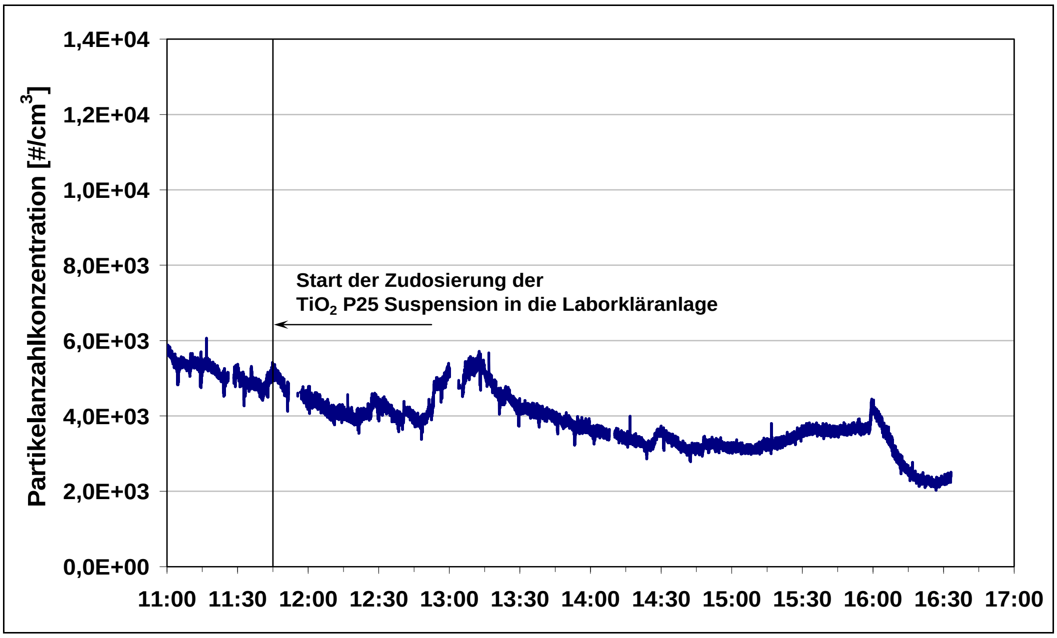


Abbildung 9: Partikelanzahlkonzentrationsverlauf während der Zudosierung der TiO_2 Suspension in die Kläranlage

REM / EDX - Untersuchungen

Während der Partikelmessungen wurden gleichzeitig Partikel auf Sammelsubstraten (Glaskohlenstoffträger) mithilfe des NAS abgeschieden. Dabei wurde jeweils der Probenträger mit einem Volumenstrom von 2,5 l/min beprobt. Zu Steigerung der Sammeleffizienz wurden die Partikel vor der Abscheidung unipolar (positiv 4,2 kV) geladen, um anschließend im elektrischen Feld (negativ 9,2 kV) zum Probenträgerzentrum gelenkt zu werden. Die Sammelzeiten betrugen beim Einwiegen 14 min, beim Homogenisieren 30 min und beim Zudosieren zur Kläranlage 285 min.

Die REM-Untersuchungen der drei Glaskohlenstoffträger mittels EDX-Analyse ergaben keinen positiven Befund an Titanpartikeln. Die Untersuchungen wurden dabei im zentralen Belegungsbereich der Träger durchgeführt, wo die Mehrzahl der Partikel abgeschieden wurde. Neben einigen Salzpartikeln wurden hauptsächlich Agglomerate gefunden, die mit hoher Wahrscheinlichkeit im Wesentlichen aus Kohlenstoff bestehen (beispielsweise Ruß). Weiterhin wurden vereinzelt silizium- und kalziumhaltige Partikel gefunden.

Fazit

Zur Untersuchung der Fragestellung, ob eine Exposition bei der Herstellung einer TiO_2-Suspension und beim Zudosieren zur Kläranlage durch freiwerdendes TiO_2 besteht, wurden Messungen zur Partikelfreisetzung am Arbeitsplatz beim Umgang mit TiO_2 durchgeführt. Untersucht wurden dabei im Einzelnen die Zubereitung (Einwiegen und Dispergieren des TiO_2) und das Zudosieren der Suspension zur Kläranlage. Zum Einsatz kamen dabei ein Partikelzähler und ein Partikelsammelgerät.

Als Ergebnis dieser Untersuchung kann zusammengefasst werden, dass sowohl bei der Zubereitung als auch bei der Zudosierung der TiO_2-Suspension zur Kläranlage keine Partikelkonzentrationserhöhungen detektiert werden konnten, die im signifikanten Bereich lagen. Weiterhin ergaben auch die REM / EDX Untersuchungen keinen Hinweis auf das Vorhandensein von Titanpartikeln auf den Probenträgern.