The Influence of Sewage Sludge Treatment on the Fate of Nonylphenol in Sludge-amended Soils

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der Rheinisch-Westfälischen Technischen Hochschule Aachen zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

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Publications

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Contents

Abbreviations

BMJ	Bundesministerium der Justiz
BOD	Biochemical Oxygen Demand
BPA	Bisphenol A
CE	Centrifuged (non-conditioned) sludge
CEC	Council of the European Communities
COD	Chemical Oxygen Demand
CRF	Constitutional Rights Foundation
DGGE	Denaturing Gradient Gel Electrophoresis
DM	Dry Matter
DNA	Deoxyribonucleic acid
DOM	Dissolved Organic Matter
dw	dry weight
EC	European Commission
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EI	Electron Ionization
EPA	Environmental Protection Agency
EPS	Extracellular Polymeric Substances
EU	European Union
FA	Fulvic Acids
FE	Ferric chloride – conditioned and centrifuged sludge
FT	Freeze-thawed and centrifuged sludge
GC-MS	Gas Chromatograph(y) – Mass Spectrometer(try)
HA	Humic Acids
IR	Infrared (spectroscopy)
ISA	Institut für Siedlungswasserwirtshaft
K_a	Acidity constant
k_d	Partition coefficient
K_{oc}	Organic carbon partition coefficient
K_{ow}	Octanol-Water partition coefficient
LANUV	Landesamt für Natur, Umwelt und Verbraucherschutz
LQ	Liquid (non-dewatered) sludge
LRRM	Land Resource Recycling Management Inc.
LM	Lime – conditioned and centrifuged sludge
LSC	Liquid Scintillation Counter(ting)

N	Non-sterilized (soil or sludge)
ND	Not Determined
NP	Nonylphenol (in part I), p353-Nonylphenol (in parts II, III, IV)
*NP	[ring-U-14C]-p353-Nonylphenol
NPEOs	Nonylphenol Ethoxylates
NRW	Nordrhein Westfalen
OECD	Organisation for Economic Cooperation and Development
OM	Organic Matter
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-p-dioxins
PCDF	Polychlorinated Dibenzofurans
PCR	Polymerase Chain Reaction
PEC	Predicted Environmental Concentration
PFRP	Processes to Further Reduce Pathogens
PNEC	Predicted No Efect Concentration
PO	Polymer – conditioned and centrifuged sludge
PSRP	Processes to Significantly Reduce Pathogens
PTFE	Polytetrafluorethylen
RA	Radioactivity
S	Sterilized (soil or sludge)
SIC	Selected Ion Current
SO	Soil (non-incubated)
TAE	Tris-Acetate-EDTA
TCLP	Toxicity Characteristic Leaching Procedure
TOC	Total Organic Carbon
TVS	Total Volatile Solids
v	volume
VDH	Virginia Department of Health
VS	Volatile Solids
w	weight
WHC	Water Holding Capacity
WM	Wet Mass
ww	wet weight
WWTPs	Wastewater Treatment Plants

Preface

There are two ways to introduce a research work. The first is to refer to the broader scientific topic to which it belongs, expose its specific targets and its potential applications. The second is to describe how the idea about the specific concept came up. This preface implements both, after a short general introduction.

Conventional wastewater treatment in its most popular form, that of activated sludge, involves microorganisms, which feed on and transform organic compounds present in the influent. A significant part of the incoming organic load is transformed to or retained by the produced biomass. The biomass removed as excess sludge during this process is considered as “waste”. This “waste”, rich in nutrients and organic material, is produced in extreme amounts, and thus meaningful to recycle.

At the same time, agriculture is a practice demanding vast amounts of resources, and even characterized – in view of the increasing human population and the changes in the dietary habits – by escalating needs. The use of sewage sludge as a fertilizer and soil conditioner is thus a very reasonable practice; nevertheless, a practice with considerable risks. Spreading, along nutrients and organic matter, concentrated heavy metals, organic pollutants, radionuclides and pathogenic organisms to agricultural land may start a chain of phenomena leading to adverse effects in human health and the environment.

Eliminating risk factors from sewage sludge would definitely be of great interest. Focusing on organic pollutants, a recent study showed that by liming sludge before dewatering – a common conditioning method – significant portions of organic pollutants that are weak acids (e.g. the endocrine disruptor Bisphenol A) may partition to the water phase, which can then be adequately treated. Two questions were derived after this study: a) do sludge conditioning treatments have any further indirect impacts on the risk associated with organic pollutants and b) can treatments like liming be considered advantageous, or are there drawbacks, as it concerns the fate of remaining organic pollutants of sludge in soil? That’s how more or less the idea of studying the impact of sludge treatment on the fate of pollutants in amended soils came up.

To put the study in the right frame, one should return back to the problematic of the organic pollutants in sewage sludge and deal with the related research needs: “How risky is the disposal of sludge to cropland?” “How can the associated risks be reduced?”

Estimating the risks of sludge recycling on agricultural soil is necessary for deciding about the best sludge management per case (other options, like incineration, are available). For the amendment of sludge on a specified soil system, the risk from each organic pollutant will always depend on its concentration level and its bioavailability to living organisms present in the system. Practically, it has been proposed that for each major pollutant the most sensitive exposure pathway to organisms should be determined, and used to define the maximum acceptable concentration in soil. For a safe recycling, the levels in sludge should be low enough, to keep the final concentration

in the amended soil below these threshold limits. Bioavailability is on the other hand an important parameter, to evade an overestimation of the amount of "active" compound. Pollutants levels in sludge and soil, and – probably in the future – their bioavailability are parameters which can be determined before recycling sludge.

The risk will however depend also on the fate of each pollutant on the soil environment. The fate will affect total and bioavailable levels in the period following soil fertilizing, as well as exposure pathways. Moreover, it will determine the concentration of pollutants in soil by the time of next sludge application. The fate of organics in soil is though difficult to predict.

The development of mitigation technologies to a high extent resembles that of risk assessment. In the case of the specific problem, reducing the risks may be achieved either by reducing pollutants levels in sludge, by controlling their availability, or by controlling their further fate in soil, which – ideally – should be predictable under different conditions. The final outcome of research in these fields should theoretically be a model that takes into account all correlations between fate and involved environmental factors and that predicts the behavior of organic compounds under different scenarios. In this frame, uncovering crucial physicochemical and/or biological factors which are determinant to fate processes would actually provide a very useful tool.

Since the sludge matrix comprises the direct environment of sludge pollutants in soil, sludge is expected to have a considerable influence on the mobility and the biological degradation of organic pollutants. Sludge composition may be highly variable, according to its origin and type (geographical and seasonal, municipal or industrial, etc.) and the treatment it has been posed to. From these subfactors, sludge treatment is clearly the one that can be more easily assessed and controlled. Conditioning and dewatering of sludge, which are in focus of the current work, comprise typically the last steps of sludge treatment. Although they may have a critical impact in natural processes taking place in sludge-amended soils, their role, as concerns the thereby degradation, binding and leaching of organic chemicals, has been, till now, not studied.

The actual target of this work was to specify the correlation between on one side the application of conditioning and dewatering treatments on sewage sludge and on the other side the persistence and mobility of sludge-born organic chemicals in sludge-amended soils. Nonylphenol was selected as the test-substrate. It can be regarded that it served as a model organic pollutant, but also as a major toxicant of excess sludge (although the last years its concentration in sludges has been found to be decreasing, as a result of measures applied by the corresponding industry sectors).

Furthermore, to make it possible to extrapolate the findings to other pollutants or to different conditions than those of the performed experiments, an investigation on the mechanism behind the observed effects was carried out.

Summarizing, the specific topic of the current work in the useful form of a question would be expressed as: "In which way, how much, and why does the application of various conditioning and dewatering techniques on sewage sludge influence the fate of Nonylphenol in sludge-amended soils?"

Note about the Theoretical Part: The ability to *imagine* a picture of the composition and processes comprising the complex system studied, as well as possible real scenarios, would be very helpful in discussing any experimental data or when deriving general conclusions. Collecting data towards this direction (and thus some times relatively detailed) was the purpose of the theoretical part of this work: What exactly is or can be "treated sludge"? And thus what is raw sludge or wastewater? Which processes are involved in the fate of an organic chemical in soil? And, furthermore, how is sludge amendment actually practiced and regulated? Finally, what is in particular known about Nonylphenol, in regard of its fate in the sludge-soil system?

I. INTRODUCTION

1. Sewage sludge

1.1 Origin and composition

1.1.1 Wastewater

Water is an invaluable resource for human life. After its use, either biological or connected to the human activities, it is recuperated in the form of wastewater. Before it becomes again available as a high quality resource, wastewater must be purified, hence closing the water cycle.

Wastewater can be divided into four main types: a) *black water*, containing urine, faeces and potentially toilet flash water, b) *grey water*, generated by household activities, like kitchens, laundries, showers etc., c) *storm water*, the run-off from urban and agricultural lands and d) *industrial wastewater*. In practice wastewater is handled as mixture of different types. *Domestic wastewater* refers to combined grey and black water, *urban/municipal wastewater* contains domestic wastewater plus storm water and often industrial wastewater, while *hospital wastewater* is also considered as a separate, special wastewater case. Terms referring to the collecting system are also used: S*eptage* refers to wastewater ending up in a septic tank and accordingly *sewage* is each wastewater driven to a sewerage collecting system.

Municipal wastewater composition apparently varies significantly, depending on its exact sources. With a water content over 99.9%, total impurities (typically 700 mg/L) can be divided into different categories, as shown in Fig. 1. According to this typical distribution, organic and inorganic matter consist each approximately 50% of total solids (suspended plus filterable).

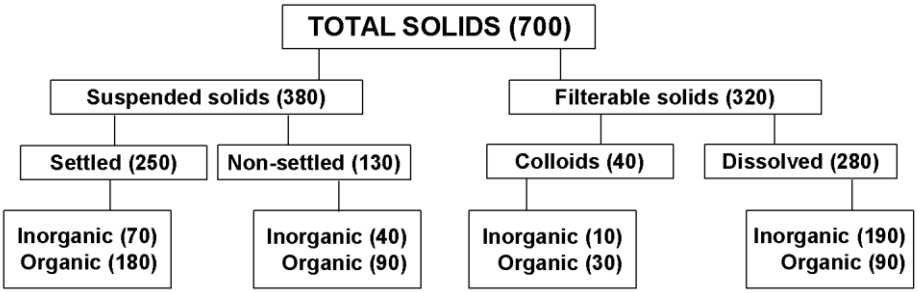


Figure 1. Typical distribution of urban wastewater solids (mg/L) (Maystre and Petiipeierre, 1976).

Sand, silt, and clay are the basic components of the suspended inorganic matter. Dissolved inorganic matter at typical concentrations in waste water principally consists of salts (predominantly calcium, magnesium, sodium, potassium, iron, and manganese salts of carbonate, bicarbonate, chloride, sulfate, nitrate, and phosphate), as well as simple compounds of N, P and S (e.g. ammonia,

hydrogen sulfide etc.) (Qasim, 1985; Vavizos, 1995). Toxic inorganic chemicals in municipal wastewater include heavy metals, as well as other compounds, like cyanides and asbestos (Qasim, 1985) or nanoparticles (Choi et al., 2008).

Major organic components of municipal wastewater are proteins (40-60%), carbohydrates (25-50%) and fatty compounds (fats, oils, and grease, ca. 10%). Organic degradation products of these compounds are also present, including aminoacids, alcohols, fatty acids, phenols, indole, methane etc. A high variety of synthetic organic compounds comprise minor components of wastewater, though responsible for undesired characteristics such as non-biodegradability, foaming and toxicity (Vavizos, 1995). A report of the European Commission on pollutants on urban wastewater (ICON, 2001) refers to the following classes of organic pollutants: Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), surfactants, Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/PCDF), plasticizers, flame retardants, preservatives, antioxidants, solvents, fragrances, pesticides, herbicides and pharmaceutical products.

A general outline of wastewater composition is usually given by a number of important chemical quality parameters. Typical values and ranges of these parameters, for municipal wastewater, are presented in Table 1. Concentrations of inorganic and organic pollutants lie normally on μg/L ranges, with heavy metals being present in clearly higher amounts than single organic molecules (Paxéus, 1996; Wilderer and Kolb, 1997; ICON, 2001).

Table 1. Typical values of important chemical quality parameters for municipal wastewater (Qasim, 1985).

Chemical quality parameter	Typical value	Range
TOC (Total Organic Carbon, mg/L)	150	80 - 290
BOD_5 (Biochemical Oxygen Demand, mg/L)	210	110 - 400
COD (Chemical Oxygen Demand, mg/L)	400	200 - 780
Total Nitrogen (mg/L)	40	20 - 85
Organic Nitrogen (mg/L)	20	8 – 35
Ammonia (as N, mg/L)	20	12 – 50
Nitrite and nitrate (as N, mg/L)	0	0 – small
Total Phosphorus (mg/L)	8	4 – 15
Organic Phosphorus (as P, mg/L)	3	1 – 5
Inorganic Phosphorus (as P, mg/L)	5	3 – 10
pH	7.0	6.5 – 7.5
Alkalinity (as $CaCO_3$, mg/L)	100	50 - 200

Municipal wastewater contains also microorganisms. The biological origin of part of its elements, the contact with sewage piping environment and the presence of organic substrate are some of the factors contributing to the accumulation and growth of bacteria, fungi, protozoa, algae, as well as viruses and higher organisms. An indicative concentration of microorganisms in municipal sewage is 10^{10} Units/L (Vavizos, 1995). Species considered representative of the general state of sewage (such as *E.coli* – indicator of faecal contamination) are commonly used for the hygienic characterization of wastewater.

1.1.2 Wastewater treatment

The cycle of water is a natural process occurring on earth since billions of years, sustained by a number of physicochemical and biological mechanisms. Old methods of wastewater management were relying on these mechanisms, being either simple disposal on the ground or discharge underground, into septic sinks, or into rivers, lakes and the sea (directly or through sewage systems). Sustaining today's human civilization requires however speeding up the overall process. This necessity derives from the high consumption of and needs for good quality water, the cumulation of water usage and disposal in small areas (e.g. by the industry or in cities), the countless number of dangerous chemicals introduced and the improved hygiene standards - all characteristic of the modern society.

Historically the need for treatment of municipal wastewater was better realized after the evident environmental pollution in rivers crossing rapidly expanding cities, in the Europe of the 19th century, and a number of related epidemics (Wiesmann et al., 2007). In Berlin, Hobrecht proposed and built a wastewater collecting and treatment system, which used the principles of soil filtration (Hobrecht, 1884). The drainage water from the implemented wastewater irrigation fields was being discharged into canals or river. After the discovery that the purifying processes in soil were rather due to biological than chemical processes, efforts were made to develop optimized processes, also in order to handle higher wastewater loads. Scientific and technological progress led first to the realization of trickling filters – plants (e.g. in Lawrence-USA, in 1888, and in Hamburg, in 1897). While in 1920, in Sheffield-UK, the first technical-scale activated sludge plant was constructed (Wiesmann et al., 2007).

Already in the 1920s and 1930s (especially in USA), but mainly the decades following the Second World War, there was a rapid application of activated sludge treatment for municipal wastewater in European and American cities (Cooper, 2001). Today's situation can be described by four cases: a) regions, where construction of biological treatment plants or extension of old facilities, in order to cover elimination of organic carbon, is an actual on going priority (e.g. in some countries of eastern, southern and central Europe, DEWA~Europe and UNEP, 2004; EEA, 2005), b) regions, where advanced processes are gaining attention, like nutrients removal, disinfection and use of membranes (e.g. countries of western Europe), c) poor regions, lacking adequate sanitation infrastructure (many sub-Saharian African countries, Snyman, 2004) and d) regions, where on-site treatment still is an often applied technology and a decentralized wastewater management appears as a future trend (remote communities in USA, Australia, Hoover, 2001; Gray, 2003).

In any case, the activated sludge process and its many variants can be considered as the main method of secondary sewage treatment presently (Cooper, 2001).

1.1.3 Activated sludge treatment

Before secondary treatment is applied, most often primary treatment of wastewater is performed, in order to remove the components that can settle or float. More specifically, after screening has been performed and grit has been removed from wastewater, primary sedimentation takes place, where

oil, grease and other floating materials are skimmed from the surface, and the settled solids ("primary sludge") are collected (chemical pre-precipitation is also occasionally applied). Primary sedimentation may remove 30-40% of the BOD_5 of wastewater.

Primary sludge is a grey, slimy material with a typical dry matter (DM) of 3 – 8%. Its composition depends on the characteristics of the catchment area. It consists to a high portion of organic matter (60-90%, TVS – Total Volatile Solids, a measure of combustible organic matter), including faeces, vegetables, fruits, textiles, paper etc. (Qasim, 1985). Main constituents are proteins (20-30% of dry weight, dw), carbohydrates (cellulose and hemicellulose: 10-19% dw), grease and fats (6-35% dw) and lignin (3-7% dw). Primary sludge is also rich in nutrients, as well heavy metals and organic pollutants. The partition of the organic pollutants to the precipitated sludge depends on their properties (e.g. the octanol-water partition coefficient, K_{ow}) and the dissolved organic carbon of the wastewater (DOC) (Katsiyiannis, 2005).

Main purpose of the secondary treatment is to remove soluble organics that escape primary treatment, while further removal of suspended solids is achieved. Although secondary treatment can be achieved also by physicochemical methods, it is typically performed by means of biological processes. Activated sludge belongs to the suspended growth biological treatments (like aerated lagoons and stabilization ponds), in which microorganisms remain in suspension, in contrast to attached growth biological treatments, where the population of active microorganisms is developed on a solid media (Qasim, 1985).

According to the principal mechanism of biological treatment, active biomass comes in contact with wastewater, so that the contained impurities can be consumed as food. A great variety of microorganisms contributes to this process, including bacteria, protozoa, rotifers, nematodes, fungi, algae and others. In the presence of oxygen and nutrients, and under favorable environmental conditions (temperature, pH, contact time, etc.), biodegradable organics are converted into carbon dioxide, water, biomass and other inert products. The transformation of organic impurities is actually achieved due to enzymatic reactions, catalyzed by intra- and extracellular enzymes.

In the activated sludge process, microorganisms are mixed and oxygen is supplied through mechanical aeration. As the microorganisms grow and are mixed, individual organisms flocculate, forming an active mass of microbial floc, the "activated sludge". The mixture of wastewater and activated sludge flows from the aeration basin to a secondary clarifier, where the biomass is settled. A portion of it is returned to the aeration basin, to maintain the appropriate food-to-organisms ratio and thus provide conditions for a rapid breakdown of the organic matter (Qasim, 1985). The excess sludge produced during this process is removed and usually added to the primary sedimentation sludge. The mixed sewage sludge has to be further treated and disposed. Several modifications of the activated sludge treatment exist, differing in mixing and flow pattern in the aeration basin, as well as in the manner in which the microorganisms are mixed with the incoming wastewater (Qasim, 1985).

In general, secondary treatment may remove more than 85% of the BOD_5 and suspended solids. Significant amounts of nitrogen and phosphorus, heavy metals and non-biodegradable organics, as well as bacteria and viruses are not sufficiently eliminated from wastewater, unless

further, tertiary treatment is provided (Qasim, 1985).

Fresh secondary sludge has a typical brown color, characteristic of Fe(III) complexes, which it contains (its color becomes light or dark gray, if it is posed under anaerobic conditions, due to the reduction of Fe^{3+} to Fe^{2+}). It consists of flocs, composed mainly by inactive (dead) bacteria, with a perimeter containing active microorganisms and often a mineral core (Fig. 2). Characteristic of its structure is also the presence of extracellular polymeric substances (EPS), which are excreted by the bacteria and form a kind of outer shell around them. These polymers (consisting of proteins, humic substances, polysaccharides, lipids, nucleic acids etc., Urbain et al., 1993) provide a "glue" material, through which bacteria cells stick together to form the observed flocs. A smaller portion of the EPS remains always unattached in solution as biocolloids (Novak et al., 2003). Accumulation of nutrients and sorption of several organic compounds from wastewater takes also place on their surface (Koppe and Stozek, 1999).

The dry matter content of fresh secondary sludge is typically 0.2%. About 70% of it has organic and about 30% inorganic composition. Major organic compounds of fresh sludge are proteins, followed by polysaccharides and fats, while degradation products like aminoacids, monosaccharides and fatty acids are also present (Koppe and Stozek, 1999). It has been found that the distribution among the various aminoacids and monosaccharides is quite constant among different sludges, independently of the sludge origin, while this doesn't apply for the fatty compounds (different bacteria species have different fatty acids profiles, Thomanetz, 1982). Additional organics, characteristic of bacteria cells composition, are also found, as vitamins, DNA and RNA. In general the composition of fresh sludge resembles to that of bacteria. A typical elemental composition of the organic matter of sludge is as follows: C (39%), N (7%), H (5%), P (2%), S (1%). Hydrophobic components of the wastewater, including pollutants such as PCBs and organochlorine insecticides, adsorb readily onto suspended particulate matter and tend also to accumulate in sewage sludge (Meakins et al., 1994).

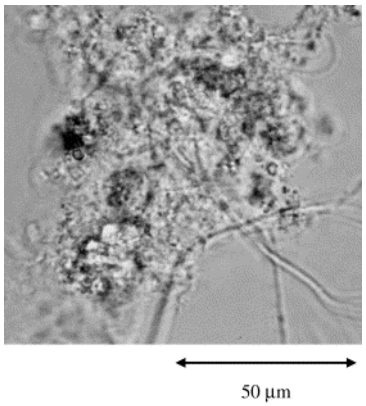


Figure 2. Microscopic photograph of activated sludge (Chu et al., 2001).

In the inorganic fraction of fresh secondary municipal sludge predominate the oxides SiO_2 (30-35%), CaO (20-25%), Al_2O_3 (17-20%), P_2O_5 (12-15%) and Fe_2O_3 (6-7%), which can be either in single or complex mineral forms. Precipitated $Ca_3(PO_4)_2$ is also a common ingredient (Hartmann, 1992). Further cations and heavy metals are found in concentrations of mg/kg to g/kg, depending on the

origin of wastewater and especially the fraction of industrial waste in it.

As mentioned above, secondary sludge is mainly composed from bacteria. Here it must be noted that only species that can stick to flocs can be found in sludge, since single bacteria are removed together with the treated effluent after sludge sedimentation. The exact species present in sludge will depend on wastewater parameters. Nutrients concentration, C:N:P ratio, dissolved oxygen, temperature and presence of toxic compounds (acids, bases, metal ions, phenols, etc.) are factors determining the growth potential of various bacterial species. Except bacteria, organisms often occurring in fresh sludges include protozoa (rhizopodes, ciliates, mastiogophora), rotifers, nematodes and vertebrates (Koppe and Stozek, 1999). Although bacteria are believed to play the most important role in degrading the organic matter during activated sludge treatment, such organisms have indirect roles, and are necessary for the ecological balance of the aerated basins.

Being the actual organic matter degrading component, enzymes in fresh activated sludge are a very important element of its composition. Apart from intracellular enzymes, exoenzymes (extracellular enzymes) play a crucial role in wastewater purification processes. As only small and simple molecules can be transported from the surrounding medium into the bacterial cells, macromolecular organic substrate must first undergo fragmentation (depolymerization and hydrolysis), before being available to bacteria (Chróst, 1991). It has been found that a large proportion of the exoenzymes responsible for this usually rate-limiting step in activated sludge degradation processes, is immobilized in the EPS matrix of activated sludge flocs (Frølund et al., 1995; Goel et al., 1998). The number of enzyme types present in sludge is in general large; it has been estimated that about 5000 enzymes occur in the cell of a typical activated sludge bacteria (Koppe and Stozek, 1999).

1.2 Treatment

1.2.1 Purpose

Sludge treatment is geared to the intended disposal route. Regarding the agricultural use of sludge, treatment targets to:

- reducing its volume, thus decreasing transportation and storage costs
- reducing its fermentability, thereby nuisances, like odour, flies and mosquitoes
- reducing health risks arising from pathogens (ideally also from heavy metals and organic pollutants)

Legislation typically controls the two last aspects. As for the European Union (EU), the Directive 86/278/EEC regulates the reuse practice in agriculture (CEC, 1986). It imposes sludge treatment to reduce its fermentability and pathogen content, while it restricts the heavy metal amount in the sludge, taking into account the content of heavy metals already present in the soil and its pH.

1.2.2 Methods

The EC-Directive in its present form does not specify which treatment of sludge is necessary before agricultural reuse, but rather very generally imposes a "biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and the health hazards resulting from its use". The Directive is currently under revision. In the frame of the latter, in a working document of the European Commission (CEC, 2000) a list of acceptable treatments before agricultural amendment is proposed for the revised legislation. This list (actually non-exhaustive, Schowanek et al., 2004) distinguishes conventional and advanced treatments (Table 2), targeting to specific microbial load standards.

Table 2. Proposed sludge treatment processes [a] (CEC, 2000).

Conventional treatments	Advanced treatments
Thermophilic aerobic digestion (mean retention 20 d.)	Thermal drying
Thermophilic anaerobic digestion (mean retention 20 d.)	Thermophilic aerobic digestion (20 h as batch)
Conditioning with lime (pH≥12 for 24 h)	Thermophilic anaerobic digestion (20 h as batch)
Mesophilic aerobic digestion	Thermal treatment of liquid sludge
Extended aeration	Conditioning with lime (pH≥12 for 3 months or at 55°C for 2 h)
Simultaneous aerobic stabilization	
Storage in liquid form	

[a] For a more specific description including processing parameters, see at the cited reference.

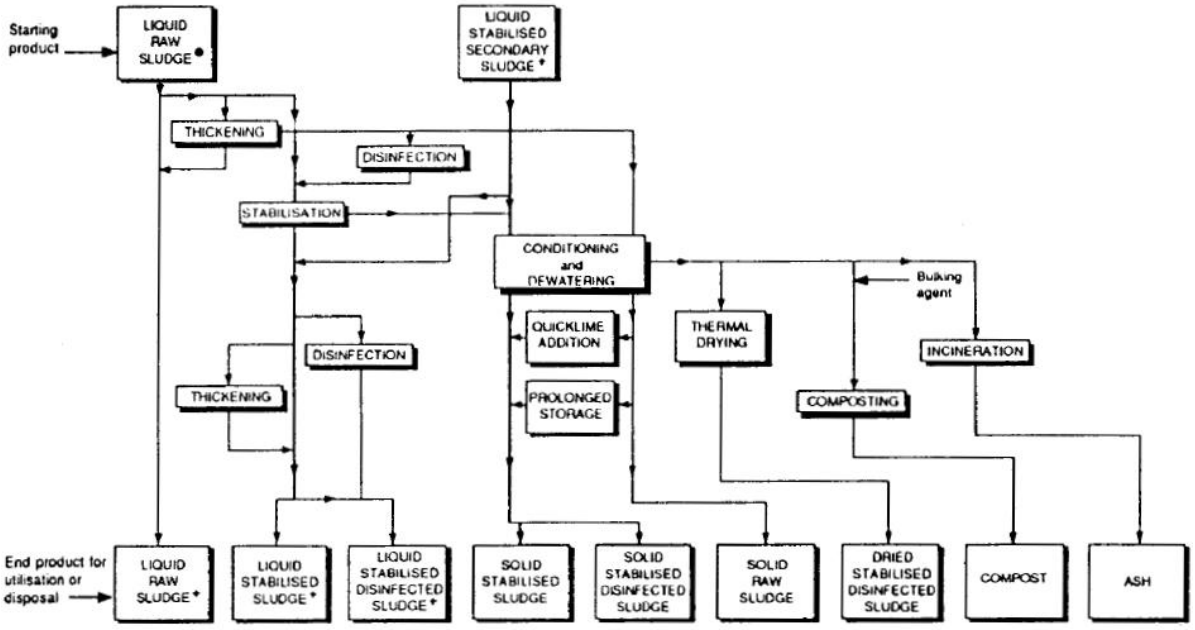


Figure 3. Flow chart of sludge processing options for production of suitable end-products for utilization and disposal (Wild and Jones, 1989).

Typically, a mixture of primary and secondary sewage sludge is treated prior to use in land by a combination of thickening, stabilization, conditioning and dewatering. Additional treatments, for example disinfection or thermal drying, are also applied occasionally. Several methods exist for implementing each of these treatment steps. Actually the general processes involved, the sequence

in which they are applied and the methods that are used vary considerably between Wastewater Treatment Plants (WWTPs), even in the same country. Common general processing options (not necessarily bound to agricultural use) for sludge treatment according to Wild and Jones (1989) are presented in Fig. 3. Further information on the methods used in different countries of the world (mostly European countries and the USA) can be found in Annex I.

Thickening reduces sludge volume and mass by removing excess water. Techniques used are gravity thickening, gravity belt thickening, dissolved air flotation and centrifugation. The moisture quantity removed is much smaller than through dewatering. However, it is often applied prior to conditioning and dewatering, for increasing the performance of the latter. During gravity thickening, a widespread thickening technique, the gravitational forces bring the thickened sludge at the base of the tank, while water is collected at the top.

Stabilisation's aim is basically to reduce the fermentability of the sludge, although the implemented methods contribute also to disinfection. It can be accomplished by biological, chemical or physical means (Qasim, 1985). Some of the available methods are: i) biological: anaerobic digestion (cold, mesophilic, thermophilic), aerobic digestion (cold, mesophilic, thermophilic), dual process (combination of anaerobic and aerobic processes), composting, storage, ii) chemical: lime stabilization, nitrite treatment, iii) physical: heat treatment, pelletizing, wet oxidation, plasma pyrolysis. Anaerobic digestion is the most widely used wastewater sludge stabilization practice, especially in larger treatment works (US-EPA, 1999). It utilizes airtight tanks in which anaerobic microorganisms stabilize the organic matter producing methane and carbon dioxide. Anaerobic digesters are most commonly operated in the mesophilic range (35-40^{o}C) (Qasim, 1985). Digestion is achieved within a period of 20-30 d.

Conditioning of sludge involves chemical and / or physical treatment to enhance water removal during the dewatering step. In fact, some conditioning processes also disinfect sludge and control odours. Chemical conditioning methods are principally associated with mechanical sludge-dewatering systems. Inorganic or organic chemicals can be used (Qasim, 1985).

Among the commonest inorganic chemicals used are ferric chloride and lime (CaO), although ferrous sulphate and aluminium sulphate have also been used. Ferric chloride addition leads to the formation of positively charged iron complexes. These complexes neutralize the negatively charged sludge solids, thus causing them to aggregate. Hydrated lime is usually used in conjunction with ferric iron salts. Lime produces also $CaCO_3$ after reaction with sludge bicarbonates (Qasim, 1985). Currently, ferric chloride and lime are used for specific dewatering methods, i.e. recessed plate filtration and vacuum filter dewatering, where this technology is still employed (Dentel, 2001). Power plant and sludge incinerator ashes (Qasim, 1985), as well as wood chips and sawdust (US-EPA, 1987) have also been used successfully in conditioning of sludge.

Organic polymers (polyelectrolytes) are widely used in sludge conditioning (particularly before centrifugation). They are long-chain, water soluble, specialty chemicals. They differ greatly in chemical composition and functional effectiveness (US-EPA, 1974; US-EPA, 1979, Qasim, 1985). Cationic polymers are the predominant form of organic additives used (anionic and non-ionic additives are also applied). The molecular weight of a conditioning polymer can reach 30 X 10^6 g /

mol, containing over 100,000 monomers. The latter are primarily based on acrylamide, although a variety of chemical modifications are used (Dentel, 2001). The mechanism of action of polymers involves adhesion of sludge particles, thus causing desorption of bound surface water, charge neutralization and agglomeration by bridging between particles (Qasim, 1985).

Chemical flocculants are considered rather expensive, thus alternative physical conditioning methods are of high interest (Dentel, 2001). Methods that have been used include elutriation, thermal conditioning, freeze-thawing, ultrasonic vibration, solvent extraction and irradiation (Qasim, 1985). Freeze-thaw conditioning, at present economically feasible only in cooler climates, where no mechanical refrigeration equipment is needed, has been suggested as the most promising of these techniques (Dentel, 2001). This method relies on the fact that freezing water excludes solid particles ahead of the advancing ice front (Tao et al., 2006). The changes in sludge's flocs are irreversible and after thawing water can be easily removed, already to a high extent by simple gravity drainage (Asthana and Tevari, 1993; Hung et al., 1996; Dentel, 2001).

When land is available, natural dewatering systems can be used, like drying beds, drying lagoons or growing reeds (Qasim, 1985; US-EPA, 1987). These techniques don't require prior conditioning, though they pose odour problems (open exposure). They are normally in use in small treatment facilities, because the capital costs are much lower than for mechanical dewatering processes (Dentel, 2001). Drying period is 10-15 d. for drying beds and 3-6 months for drying lagoons (Qasim, 1985).

Mechanical sludge-dewatering systems include centrifugation, filter press, vacuum filter, horizontal belt filter, electro-osmotic belt filtration, electro-acoustical dewatering, membrane belt filtration etc. (Qasim, 1985; Dentel, 2001). The centrifugal force speeds up the sedimentation rate of sludge solids. Sludge dewatering can be achieved by solid-bowl and basket centrifuges. In a typical solid-bowl unit, the conditioned sludge is pumped into a horizontal or cylindral rotating "bowl". The solids are spun to the outside of the bowl, where they are scraped out by a screw conveyor (Qasim, 1985). There is an outlet for clarified water, while the separated solids on their way to the discharge end undergo further dewatering through special positioning of the screw helix (Degussa) (Fig. 4).

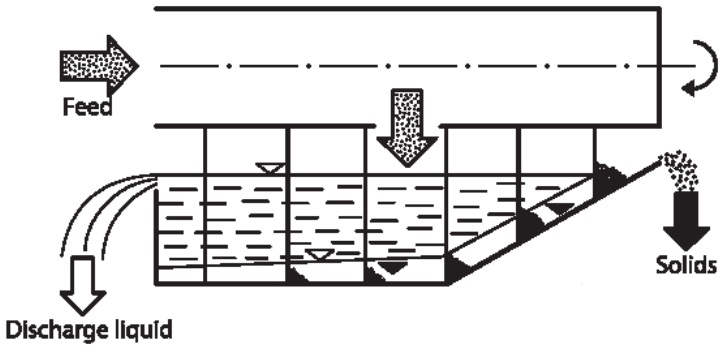


Figure 4. Solid-bowl centrifuge for sludge dewatering (Degussa).

Disinfection of sludge can be achieved by physical methods, like pasteurization, irradiation, thermal pyrolysis, boiling, etc. or addition of chemicals (e.g. lime or antimicrobial agents, like for

instance peracetic acid, Kitis, 2004). Such methods contribute often significantly also to other purposes, like stabilisation and conditioning of sludge.

Sludge treatment techniques under research / development include for example sludge minimisation technologies (MicroSludge™, ultrasonic cell lysis, thermal hydrolysis etc.), liquid stabilisation technologies (temperature phased anaerobic digestion, two-phase acid-gas digestion), thickening and dewatering techniques (flotation thickening using digester gas, membrane thickening, inclined screw presses), etc. (Nazareth, 2007)

1.2.3 Composition of treated sludge

The structure and composition of treated sludge is apparently dependent, except of its origin, also on the treatment it has been posed to. Taking into account the great variety of processing options applied in WWTPs, it is easily deduced that sludge characteristics affected by treatment will also be diverse among sludges. This paragraph focuses on the composition of stabilized sludge, i.e. before conditioning and dewatering (the treatments studied during the current work) are applied. A short general discussion on the properties affected by conditioning and dewatering is also included, although this topic is discussed more thoroughly in the Experimental part of the manuscript. Therein, literature data on the individual effects, of the conditioning and dewatering treatment methods studied, on sludge properties are summarized and considered in relation with the results of the work.

As already mentioned, stabilisation is normally applied to combined primary and secondary sludge. Primary sludge is included in higher proportion in the mixture, as it is produced in amounts of 150-250 g/m^3 of wastewater (and has a DM of 3.0 – 4.5%), while the activated sludge corresponds to 50-90 g/m^3 (with 0.3 – 1.0% DM) (Qasim, 1985).

The process of anaerobic digestion occurs in a number of discrete stages. Each stage is dependent on a different bacterial population, with all of the separate bacterial species living together in direct or indirect symbiotic associations [Guijer and Zehnder, 1983]. At the 1st main phase (hydrolysis), destruction of sludge-flocs is observed and EPS is released into the solution. More specifically, it has been found that EPS in activated sludge is constituted of two fractions: one consisting of lectin-like proteins, linked to polysaccharides by calcium and magnesium ions and one being a mixture of proteins, polysaccharides and humic acids, that are “collected” by iron and aluminium. By anaerobic digestion of sludge, iron is actually reduced, thus portion of the associated material is being released (not much is known about the aluminium-associated fraction) (Novak et al., 2003; Novak and Park, 2004). Solubilized macromolecular organic compounds (proteins, carbohydrates, lipids) are broken down step-by-step to form corresponding monomers, like sugars, aminoacids, glycerine and organic acids. These compounds are used as oxygen source by facultative bacteria (2nd phase), and are fermented to organic acids (propionic acid, valeric acid, butyric acid, etc.) and other molecules, like ethanol, CO_2, H_2, and NH_3 (Koppe and Stozek, 1999).

The 3rd phase involves acetogenic bacteria, which transform fermentation products to acetic acid, H_2 and CO_2. The final main phase of anaerobical digestion is driven by methanogenic bacteria, which are specialized in transforming small organic molecules to CH_4 (and CO_2). In the presence of sulphates (SO_4^{2-}), sulphate-reducing bacteria act faster on fermentation products than methanogenic

bacteria, oxidizing them to CO_2, and generating H_2S. The latter react with heavy metal ions, giving rise to insoluble sulphide salts (FeS, CuS, HgS, etc.) (Koppe and Stozek, 1999). Last, but not least, humification of organic matter is an important parallel process taking place during digestion of sludge (Pajączkowska et al., 2003).

Digested sludge has a black colour and usually a dry matter of 5-10%, with 30-60% of it belonging to volatile (organic) solids (Qasim, 1985). In undigested sludges most of the nitrogen is found in an organic form. The digestion process converts rather more than half of the total nitrogen into soluble forms, mainly inorganic ammonium compounds. In general, the liquid part of digested sludge is rich in nutrients. As for phosphorus, only a small fraction is likely to be in the form of organic phosphates, the mineral phosphorus (30-98% of total P) mainly consisting of compounds of iron, aluminium, calcium and magnesium, which abound in most sludges (EPA, 1995; EC, 2001b; EC, 2003). The solid part of digested sludge consists of the remnants that microbes couldn't utilise (or further utilize) plus dead bacterial cells and a variety of mineral components present in this matrix. Colloidal material that was not released into solution and stable organic matter, composed from lignin, chitin, cellulose etc., is a significant part of the organic residue in digested sludge (Novak and Park, 2004; chemie.DE). Of the organic matter of digested sewage sludge, in general less than 10% is extractable by organic solvents. The "insoluble" part consists of macromolecular structure that can be divided into lignin-derived, lipid-derived and nitrogenous compounds. The aliphatic nature of the macromolecular structure and the high content of nitrogenous compounds are characteristics of stabilised domestic sludge (Jarde et al., 2003). "Young" humic and fulvic acids (they appear to be more aromatic and of smaller associates than those of soil) represent on average about 17% of the organic matter of sludge (Riffaldi, 1982). While, in any case, anaerobically digested sludge contains, in comparison with undigested sludge, relatively less alkyl-C and higher amount of oxidized forms of C (Smith et al., 2008). Typical parameters of primary, secondary and digested sludge are presented in Table 3.

Table 3. Typical chemical parameters of fresh and digested sludge (Qasim, 1985).

Chemical parameter	Primary sludge	Activated sludge	Digested sludge
TVS (% DM)	60 -90	60 - 80	30 -60
BOD_5 / TVS	0.5 – 1.1	-	-
COD / TVS	1.2 – 1.6	2.0 – 3.0	-
Total N (% DM)	1.5 – 4.0 %	2.5 – 7.0 %	1.6 – 6.0%
Total P (% DM)	0.8 – 2.8 %	2.0 – 7.0 %	1.4 – 4.0%
pH	5.0 – 6.5	6.5 – 7.5	6.5 – 7.5
Alkalinity (in $CaCO_3$ equivalent, mg/L)	500 - 1500	200 - 500	2500 - 3500

Heavy metals concentrations in digested sludges depend on the degree of the industrial character of treated sewage. They are associated with various components of sludge's matrix (humic and fulvic acids, sulphides, carbonates etc.), depending on their nature and sludge type. Most of toxic metals are organically bound to very small particles (<10μm) of sludge (Chapman, 1986).

Organic pollutants can be categorized according to their fate during anaerobic digestion, which varies considerably according to their properties: i) volatile organic compounds, that may be lost in the biogas, ii) compounds like ethanol and sodium stearate, which are easily degraded, iii) molecules degradable after a lag phase (e.g. pthalic acid esters, 2-chlorophenol), iv) non-degradable molecules (benzene, 2,4-D, dieldrin etc.), v) compounds inhibiting sludge stabilisation at the initial stage (e.g. lindane, napthalene) and vi) compounds inhibiting the digestion process throughout the whole incubation (e.g. pentaclorophenol, 2,4,5-T). Degradation processes during digestion can occur abiotically and/or biotically. Due to the breakdown of precursor molecules, the concentrations of some hazardous substances (e.g. phenols) may substantially increase (Wild and Jones, 1989).

Enzymatic activity in general declines to some extent during anaerobic digestion (Novak et al., 2003). Regarding microbial communities present in fresh digested sludge, Kotzé et al. (1968) found that usually about 95% of the cells is constituted of obligate anaerobic bacteria, with only small populations of aerobic and facultative anaerobic present. The two latter types are actually believed to be introduced via the feed (primary and secondary sludge) to the digesters (Kotzé et al., 1968). Stabilisation generally reduces the number of pathogens in sludge (including bacteria, parasites, protozoa and viruses). Nevertheless, digested sludge can usually still contain some pathogens (Sagik et al., 1979).

Application of a different stabilisation method will as a matter of fact affect the composition of sludge. For example, aerobic, in comparison to anaerobic digestion, will result in a limited release of EPS to the solution, as well as a decline of polysaccharides-degrading-enzymes-activity almost to zero and accordingly an accumulation of polysaccharides (Novak et al., 2003), while aerobic conditions favour the conversion of N species to the NO_3^- form (EPA, 1995). Moreover, fresh aerobically-digested sludge incorporates a mixed and varied ecology, including also some anaerobic and facultative organisms (Al-Malack et al., 2002).

During dewatering, components like soluble nutrients forms and dissolved organic matter are expected to be lost in the removed liquid phase (EC, 2001b). The exact means used for dewatering the sludge will apparently affect the structure / composition of the resulted biosolids. In this sense, factors like centrifugal force, heat or pressure are expected to have their own influence on sludge properties.

The impact of sludge conditioning on sludge's physical, chemical and biological properties may be related with several phenomena. Indicatively, sludge conditioning: a) will considerably increase the concentrations of the chemicals added, including impurities of the latter (e.g. Cd and Cr impurities in ferric chloride, Dentel, 2001), b) may lead to the formation of precipitates (e.g. $CaCO_3$ due to lime addition, or aluminium hydroxides by alum-treatment), which may in turn absorb phosphorus or heavy metals (EPA, 1995), c) lead to the formation of complexes, such as of Fe with proteins, or due to the association of added cationic polymers with colloids of digested sludge, d) may result in losses of volatiles produced due to chemical reactions (NH_3 loss by liming, EPA, 1995) or changes in conditions (organics loss due to the change in pH, also by liming, Ivashechkin et al., 2004), e) will affect the structure of sludge particles, due to the coagulation of sludge material, f) may destruct flocs / lead to the formation of new structural motives (e.g. by the use of physical

conditioning methods, like ultrasonics, thermal treatment or freeze-thawing), g) affect living microbial populations in sludge, etc.

Any other additional treatments applied (disinfection, production of granular sludge, etc.) will clearly affect the structure and composition of sludge as well.

1.3 Management

The management of sewage sludge is a critical - although often overlooked - issue; particularly since a significant increase in its production is anticipated in future, due to improved standards in wastewater treatment worldwide, which result in new installations and extension of the existing facilities. Sludge management practice is an important part of the cycle of materials such as organic matter and nutrients. The selected route should thus intend to maximize sludge's beneficial recycling / reuse capacity, but in a manner protective of the environment and public health. Local circumstances influence significantly decision making about the routes to be followed per region (Spinosa, 2007).

Main recycling / disposal routes applied for sewage sludge are landspreading, incineration and landfilling (EC, 2001b). In the past, dumping in the sea was also a common practice (Chapman, 1986). While other, minor routes are applied per case, such as use in forestry and silviculture, land reclamation and revegetation (EC, 2001b). Some data about the outlets of sludge produced in different parts of the world, as well as the trends and future prospects per case are given in Annex II.

Each of the sludge recycling / disposal options has advantages and disadvantages. Decision-making should optimally go through Life Cycle Assessments. Such studies have been performed in the past for specific regions (EC, 2001b), but they have basically met difficulties in producing clear conclusions, since, although it is more feasible to assess specific benefits and impacts of sludge management options (e.g. environmental impacts on soil pollution, eutrophication of waters, atmospheric pollution etc.), it is problematic to compare different impacts and benefits. Among conclusions derived from these studies, is the negative impact of transport needs for the produced sludge and the necessity to distinguish between sludges of different qualities (EC, 2001b). More discussion towards the sustainability of sludge management can be found in literature sources (for example, LANUV, 2001 and Nthethe, 2007).

New technologies in the area of sludge management, focusing on the production of energy and ashes, which afterwards can be reused, are gasification and plasma pyrolysis (EC, 2005, Fytili and Zabaniotou, 2008).

Moreover, in addition to the above mentioned disposal / reuse options, many processes are developed, through which sludge can be transformed into added value products. Techniques like wet oxidation (EC, 2005), thermal conversion (Nazareth, 2007) and nutrients recovery (Montag et al., 2007), and sludge conversion products such as building materials (cement, concrete, glass aggregates), oils, adsorbents, bioplastics, biosurfactants, polymers, enzymes and biopesticides are examples of new techniques being developed for the re-utilization of sludge (Ben Rebah et al., 2002; Brar et al., 2005).

2. Amendment of sludge on agricultural land

2.1 Benefits

Sludge amendment on land provides from a first point of view an on-going and cost-effective means of managing the waste produced in WWTPs (US-EPA, 1995). It is a measure to prevent waste, by recycling as much as possible the resources, towards a more sustainable society. Except that, sludge amendment has important beneficial aspects for the farmers, while it represents also a contribution to limit the climate change.

Agricultural benefits arising from the use of sludge are related to the presence of nutrients and organic matter contained in sludge. The nitrogen (N) is one of the main factors in favor of its use. Plants can assimilate only mineral nitrogen. Thus, the agricultural value of the sludge is determined by the amount of inorganic N and the aptitude of its organic N to be mineralized. The N content of sludge ranges between 1 and 6% DM, depending apparently from the wastewater type and sludge treatment. Storage of sludge is known to significantly reduce its N content. Sludge origin and treatment affect also the distribution of the different forms of N. The liquid phase of sludge is in general richer in N than the solid phase, and includes compounds that can be easily or directly used by plants. Extrinsic factors (temperature, humidity, pH and texture of the soil, landspreading practice) have also some influence on the availability of N. Losses of N from soil can occur, except of crop uptake, by volatilization (NH_3, NO_x, N_2, mainly after microbiological transformations), leaching of nitrates and organic compounds, and run-off (EC, 2001b; OECD, 2001; EC, 2003).

Sludge-borne phosphorus (P, content 1-4% DM) is of particular interest, as phosphorus is a limited natural resource. Phosphorus is used by the plant amongst other for its growth, the rigidity of its cell walls, and for the development of its root system. As it was mentioned above (paragraph 1.2.3), most of P in digested sludge is present in inorganic forms. In case iron or aluminum salts have been used to flocculate sewage or to condition sludge, P is expected to be present in very insoluble forms, with considerably low availability. Contrary to N, the P content in sewage sludge is not significantly reduced after storage (EC, 2003).

Sludge normally provides only low amounts of the third macronutrient crops need , i.e. potassium (K) (Eberle et al., 1994). The agricultural value of micronutrients crop needs (S, Mg, Ca, etc.) concerning their level in sludge is not extensively documented in the literature (EC, 2003).

Known benefits of organic matter application to soil are the improvement of its physical properties, such as structure (e.g. improvement of the soil bearing strength) and the retention capacity of minerals and water (resulting also in reduced potential of surface runoff and water erosion). Lastly, organic matter is an energy source for micro-organisms living in soil. Therefore

sludge spreading may induce an increase of the soil microbial population and activity, resulting in parallel in increased mineralization capacity. Nevertheless, it has been calculated that the minimum threshold level for detectable effects of sludge additions on soil physical properties (5 t organic matter/ha, corresponding to about 10 t dry solids/ha) is not reached, considering at least the current quantity limitations for agricultural use of sludge implemented in EU Member States (see below) (EC, 2003).

Soil is a very significant carbon pool on earth's surface, already about 3.3 times bigger than the atmospheric pool and 4.5 times bigger than the biotic pool (Lal, 2004). As almost all carbon pools, it actually represents a dynamic equilibrium of soil carbon, with gains and losses. It has been estimated that sludge-amendment has a significant potential to move the equilibrium further to carbon sequestration, i.e. transferring atmospheric CO_2 into long-lived carbon forms and storing it securely so it is not immediately reemitted. It was found for example that soils where manure (which has a similar effect as sewage sludge) was added had soil organic carbon levels 1.34% higher than un-amended soils, and 1.13% higher than soils amended with chemical fertilizers, over a 50-year period. Hence this practice may contribute to a mitigation of greenhouse gas emissions, especially for the next 20-30 years, where drastic emissions reduction will be required, although with a limited potential in a long term view, since carbon sequestration in soil is not permanent (Smith, 2004).

More indirect effects of sludge spreading on carbon emissions and climate change include the reduced erosion (which keeps organic matter in the surface layer), improved water retention (less energy for irrigation), suppressive power (meaning and implying less energy for the production of pesticides), etc. This topic is however very complex, subject to a number of assumptions and considerations that suggest that the conclusions should be used with prudence (EC, 2003). Recently it was found, for instance, that agricultural soil erosion does not contribute to global warming, but is rather a small carbon sink♦ (Van Oost, 2007).

2.2 Criteria for sludge application permission

Sewage sludge spreading in agricultural land is controlled by regulations at different local levels. Herein the requirements set to allow the application of sludge according to the central EU and USA legislation (Council Directive 86/278/EEC and Rule Part 503 of the Code of Federal Regulations respectively), as well as according to two examples of national/state regulations (of Germany – Sewage Sludge Ordinance ["Klärschlammverordnung"] and of Virginia State - Biosolids Use Regulations of the Code of Virginia) are summarized.

2.2.1 Sludge amount / Pollutants and pathogens limits

The application of sewage sludge in agriculture and its respective amount are regulated, in the frame of the Directive 86/278/EEC (CEC, 1986), on the basis of heavy metals and nutrients. As for heavy

♦ According to this study, eroded subsoil during its journey absorbs carbon, which is subsequently buried within the soil in the deposition areas. This fact does not imply that erosion control should not be pursued: controlling erosion has agronomical and environmental benefits.

metals, provision is taken for maximum concentrations in soil, as well for the maximum annual introduction into the soil. The latter (maximum application level per soil area and time) can be implemented by the Member States either by setting, within the national laws, directly limits for the quantity of heavy metals to be introduced into the soil, or alternatively by setting tolerance values for their concentrations in sludge in combination with sludge quantity limits (amount of sludge to be applied per time and area of soil). Maximum concentration values to be incorporated in the national laws are provided in the annex of the Directive. Additionally, the Directive requests, in case of soils with pH below 6, a reduction of the limit values laid by the Member States, if necessary, when regarding the corresponding increased mobility and availability to the crop of heavy metals. As for nutrients, the restriction is expressed in a vague way, stating that the plant needs are taken into account, and that the quality of soil, surface and ground waters is not being impaired.

The Directive is incorporated into the national German law in the frame of "Klärschlammverordnung" or "AbfklärV" (BMJ, 1992). Tolerance values are laid down for the concentrations of heavy metals in soil and in sludge, while a maximum application amount of 5 t. DM per hectare for a period of 3 years is set. Lower limits are set for metals where soil has a pH below 5. In addition, limits are specified for the levels of some organic pollutants (PCB and PCDD/PCDF) in sludge. The nutrients amount added through the sludge application is regulated according to the plants-needs, taking into account the nutrients and organic matter amount of the soil to be amended.

In AbfklärV caution is additionally taken in regard to the effect of sludge on soil pH. In particular, sludge cannot be applied on soils for which a target pH lower or equal to 5 has been set. In the case of pH greater than 5 while the current soil pH is below this value, lime may be added before sludge, under the condition the alkaline components of sludge are taken into account when calculating the amount of lime to be applied.

In USA legislation (CFR, 1999), limits have been adopted concerning the concentrations of trace elements in sludge ("ceiling concentrations") and the cumulative loading amounts for a site per year (also for each trace element). A second set of more stringent limits has also been provided. The latter are called "pollutant concentration limits". Sludge that belongs to that higher quality class can be applied even on soils where the cumulative load has been reached. Nutrients according to Part 503 should be applied at or less than the rate required to supply the N need of the crops, in order to minimize N passing below the roots zone. If P poses a threat to water quality, sludge should be used according to P needs. pH considerations are present also in USA legislation, with sludge amendment being prohibited on soils with pH less than 5.5.

In contrast to EU legislation, Part 503 includes specific limits on pathogen densities in sludge. Two classes of sludge are defined, according to the pathogens content: Class A sludge is characterized by densities of faecal coliforms or *Salmonella* lower than specific limits provided, in combination with one more criteria (the latter being either application of specific advanced treatment – see 2.2.2 and "PFRP" in Table 4, or densities of additional indicators, namely enteric viruses or viable ova of parasitic worms). Class B sludge either presents densities for the above indicator organisms below the corresponding limits (Class B sludge limits) or has been treated by one

of the appropriate treatments mentioned in the regulation ("PRSP", see 2.2.2 and Table 4).

Table 4. Options for reducing pathogens provided by the U.S.A. Part 503 Regulation (CFR, 1999).

A. Processes to Significantly Reduce Pathogens (PSRP)
1. Aerobic digestion - Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20 degrees Celsius and 60 days at 15 degrees Celsius.
2. Air drying - Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of three months. During two of the three months, the ambient average daily temperature is above zero degrees Celsius.
3. Anaerobic digestion - Sewage sludge is treated in the absence of air for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35 to 55 degrees Celsius and 60 days at 20 degrees Celsius.
4. Composting - Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40 degrees Celsius or higher and remains at 40 degrees Celsius or higher for five days. For four hours during the five days, the temperature in the compost pile exceeds 55 degrees Celsius.
5. Lime stabilization - Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 after two hours of contact.
B. Processes to Further Reduce Pathogens (PFRP)
1. Composting - Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the sewage sludge is maintained at 55 degrees Celsius or higher for three days. Using the windrow composting method, the temperature of the sewage sludge is maintained at 55 degrees or higher for 15 days or longer. During the period when the compost is maintained at 55 degrees or higher, there shall be a minimum of five turnings of the windrow.
2. Heat drying - Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content of the sewage sludge to 10 percent or lower. Either the temperature of the sewage sludge particles exceeds 80 degrees Celsius or the wet bulb temperature of the gas in contact with the sewage sludge as the sewage sludge leaves the dryer exceeds 80 degrees Celsius.
3. Heat treatment - Liquid sewage sludge is heated to a temperature of 180 degrees Celsius or higher for 30 minutes.
4. Thermophilic aerobic digestion - Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time of the sewage sludge is 10 days at 55 to 60 degrees Celsius.
5. Beta ray irradiation - Sewage sludge is irradiated with beta rays from an accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20 degrees Celsius).
(6) Gamma ray irradiation - Sewage sludge is irradiated with gamma rays from certain isotopes, such as \60\ Cobalt and \137\ Cesium, at dosages of at least 1.0 megarad at room temperature (ca. 20 deg. Celsius).
7. Pasteurization - The temperature of the sewage sludge is maintained at 70 degrees Celsius or higher for 30 minutes or longer.

Organic chemicals in biosolids are not regulated by Part 503, with the reasoning that a) the chemicals of potential concern have been banned or restricted for use in the United States, b) are no longer manufactured in the United States, c) are present at low concentrations based on data from EPA's 1990 national sludge survey (US-EPA, 1990), or d) because the limit for an organic pollutant

identified in the Part 503 risk assessment is not expected to be exceeded in biosolids that are land applied (US-EPA, 1992). However, restrictions will be imposed to agricultural use if testing of certain toxic organic compounds proves that biosolids contain pollutants at levels that could cause harm to human health or the environment.

More demanding regulations may be set by individual States. In the example of Virginia State (VDH, 1997), further provisions taken are a) the encouraging of an infrequent application of sludge (once every three years) and b) a restriction on the loading rate of biosolids, if they contain appreciable amounts of lime. Virginia State Regulation specifies that soil pH should not exceed 6.5 – 6.8 (depending on the local region), as increased pH may generate micronutrients deficiencies in crops.

2.2.2 Sludge treatment

The EC-Directive requires that sludge shall be treated before being used in agriculture, with the term "treatment" being, as mentioned above (paragraph 1.2.2), vague. It is also stated that use of untreated sludge may be authorized by the Member States, if it is injected or worked into the soil. In AbfklärV it is clarified, that simple dewatering is not considered as "treatment".

Much more specific requirements regarding sludge treatment are set by the U.S.A. regulation. They aim at ensuring reduction of pathogens and of the attraction of biosolids for vectors (e.g. mosquitos). As for the pathogens, two lists of alternative treatments are provided: the Processes to Significantly Reduce Pathogens (PSRP) and the Processes to Further Reduce Pathogens (PFRP) (Table 4). PSPR determined either in the Annex of the Part 503 Regulation or by the State authority provide correspondingly two of the three alternative criteria sets for Class B biosolids (the third being, as mentioned in the previous unit of this paragraph, pathogen densities limits). Accordingly, PFRP comprise two (Annex, State authority regulation) of the six alternative criteria sets for Class A biosolids; for these biosolids however, additional pathogen indicator monitoring is always necessary. For the higher quality Class A biosolids, the rest four alternative criteria consist of two based on supplementary pathogen indicators levels as explained above and two including specific sludge treatments: a) maintaining the temperature at a specific value for a specific time, the exact parameter values depending on the dry matter of sludge or b) combining a raise of pH (above 12, for 12-72 hours), of temperature (above 52^{o}C, at least for 12 hours during pH is above 12), and air drying (at the end of the pH raising period, so that dry matter is at least 50%).

Reduction of the vector attraction potential of biosolids can be succedeed either after or at the same time meeting pathogen reduction requirements. According to Part 503, the reduction (in vector attraction) is satisfactory when one of ten further provided alternative criteria sets are met. These alternatives are presented in Table 5.

Virginia State Regulation does not prescribe any stricter treatments for the sludge before applying on agricultural land.

2.2.3 Site suitability / Timing and method of application

The EU legislation prohibits the application of sewage sludge on a) grassland that is to be grazed or

forage crops to be harvested within a minimal period of 3 weeks, b) soils where fruit or vegetable crops are growing (with the exemption of fruit trees) or, if the latter are to be eaten raw, even if the soil is intended to be cultivated with such crops (in this case application is prohibited for a period of 10 months preceding the harvest of the crops)

Table 5. Options for reducing vector attraction provided by the U.S.A. Part 503 Regulation (CFR, 1999).

Vector attraction reduction criteria (alternatives)
(1) The mass of volatile solids in the sewage sludge shall be reduced by a minimum of 38 percent.
(2) When the 38 percent volatile solids reduction requirement cannot be met for an anaerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge anaerobically in the laboratory in a bench scale unit for 40 additional days at a temperature between 30 and 37 degrees Celsius. When at the end of the 40 days, the volatile solids in the sewage sludge at the beginning of that period is reduced by less than 17 percent, vector attraction reduction is achieved.
(3) When the 38 percent volatile solids reduction requirement cannot be met for an aerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge that has a percent solids of two percent or less aerobically in the laboratory in a bench scale unit for 30 additional days at 20 degrees Celsius. When at the end of the 30 days, the volatile solids in the sewage sludge at the beginning of that period is reduced by less than 15 percent, vector attraction reduction is achieved.
(4) The specific oxygen uptake rate (SOUR) for sewage sludge treated in an aerobic process shall be equal to or less than 1.5 milligrams of oxygen per hour per gram of total solids (dry weight basis) at a temperature of 20 degrees Celsius.
(5) Sewage sludge shall be treated in an aerobic process for 14 days or longer. During that time, the temperature of the sewage sludge shall be higher than 40 degrees Celsius and the average temperature of the sewage sludge shall be higher than 45 degrees Celsius.
(6) The pH of sewage sludge shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for two hours and then at 11.5 or higher for an additional 22 hours.
(7) The percent solids of sewage sludge that does not contain unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 75 percent based on the moisture content and total solids prior to mixing with other materials.
(8) The percent solids of sewage sludge that contains unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 90 percent based on the moisture content and total solids prior to mixing with other materials.
(9), (10) [They regard sludge application practice (see section 2.3)]

According to the German legislation, it is not allowed to apply sludge in land used for the growing of fruit and vegetables, field forage, as well on permanent grasslands, forestry areas and protected zones. Fruit and vegetables cannot be grown on a soil in the year that sludge has been applied, nor the year after. Regarding forage-intended crops, sludge may still be applied only prior to sowing, and only with the precondition to apply deep-turn tillage or incorporate the sludge into the soil.

In regard of the U.S.A. regulation, restrictions (limits and management practice requirements) are placed on land application based on site physical characteristics such as topography, soil permeability, infiltration and drainage patterns, depth to groundwater, and

proximity to surface water. Potentially unsuitable areas for biosolids application include: 1) areas bordered by ponds, lakes, rivers, and streams without appropriate buffer areas, 2) wetlands and marshes, 3) steep areas with sharp relief, 4) undesirable geology (karst, fractured bedrock) if not covered by a sufficiently thick layer of soil, 5) undesirable soil conditions (rocky, shallow), 6) areas of historical or archeological significance, and 7) other environmentally sensitive areas, such as floodplains (Evanlyo, 1999).

According to the Code of Virginia State, particular guidelines / restrictions are provided for any land with slope, erosion potential, subjected to frequent flooding or snow-covered ground, or furthermore within short distance from sensitive areas (springs, dwellings etc.). For example, best management practices (e.g. direct injection into the soil) are to be followed in slope-areas the period between November and March (main rainfall period). Sludge has to be incorporated within 48 hours into the soil in snow-covered ground. If the snow layer is higher than 3 cm, liquid sludge is generally not allowed at all.

2.3 Agricultural practice

2.3.1 Calculating the amount of sludge to be spread

From the reference to the legislation examples above (2.2) it is apparent that the amount of sludge to be spread will depend on the crop nutrient needs, soil nutrients and organic matter, the heavy metals levels in soil and sludge, and the lime content of the sludge. The basic calculation is usually performed on the basis of the nitrogen needs of the crop. In general a sludge amendment that supplies the adequate N will supply excess P and insufficient amount of K (Harrison et al., 2003). For this reason, an upper limit on the basis of P needs can also be determined, in order to avoid transport of excess P to the surrounding environment. Moreover, it may be needed to supply extra K to the agricultural soil, in the form of chemical fertilizer.

The availability of nutrients is an essential parameter that must be taken into account. NH_4^+-N and NO_3^--N are considered directly available to be assimilated by the plants, in contrast with organic N compounds, which can supply N after being mineralized (here it is reminded that liquid sludge has high proportions of inorganic nitrogen, while in dewatered sludge organic N forms predominate). NH_3 losses is a factor also to be taken into consideration (NH_4^+-N may be not completely available, as it is prone to volatilization, especially if sludge is surface-applied and the temperature is above 10^oC). If sludge is incorporated into the soil, most of NH_4^+-N will be adsorbed to the clay in the soil. Ammonium in soil is convertible to nitrate, which can not only be taken up by the crop, but also leach to the groundwater or be subjected to denitrification from soil microorganisms, and thus lost to the atmosphere. Regarding organic N compounds, their mineralization rates depend on the sludge and soil parameters (10-50% of it will be available during the first year after sludge application). The total content of P and K in sludge can be considered as available to the crops (Eberle et al., 1994).

Data are available on the mineralization rates of organic N as a function of sludge treatment and soil characteristics and models exist, to calculate the appropriate amounts of sludge for each

particular case.

The steps that should be followed for calculating the agronomic rate for the land application of sewage sludge, as they are outlined in a leaflet of an educational program of the Agricultural Department of an American University, appear in Table 6.

When limed sludge is applied, it is possible that the amount will be limited by the lime content. This may be the case if the latter is high, as for example in lime-stabilized Class A biosolids (according to the American classification). These biosolids are actually used as liming agents and the organic matter/nutrients are only incidental in terms of the application rate (Forste, 1996).

Typical agronomic rates of sludge in the U.S.A. seem to lie on the range of 5-10 tons DM / ha (0.5-1 kg DM / m^2). Maximum allowable sludge application rates in EU countries range between 1 and 10 tons DM / (ha * year), with the daily application practice on cropland usually not exceeding 2-3 tons / ha / year (0.2-0.3 kg DM / m^2) (EC, 2001a; ECETOC, 2003; Schowanek et al., 2004).

Table 6. Typical procedure for determining the agronomic rate for sludge application (Eberle et al, 1994).

Step 1. Determine the amount of each form of nitrogen (ammonium, nitrate, and organic) in the sludge based on laboratory analysis.	amount of organic nitrogen that will become available due to mineralization of previously applied sludge. It assumes that the organic nitrogen mineralization rate for the second and third years will be about half the first-year rate.
Step 2. Estimate the amount of the sludge ammonium nitrogen that will be retained in the soil, taking into account different volatilization losses based on the method of application.	**Step 6.** Determine the crop nitrogen requirement, varying with type of crop, crop yield, and soil factors.
Step 3. Calculate the amount of organic nitrogen in the sludge that will become available through mineralization for plant use during the current year. The mineralization rate varies with the type of process used in producing the sludge.	**Step 7.** Subtract the existing nitrogen (credits) from the requirement to determine the amount of additional nitrogen needed for production.
Step 4. Calculate the total available nitrogen for the year from the sludge.	**Step 8.** Calculate the agronomic loading rate of sludge based on nitrogen content of sludge and additional nitrogen needed. This also obtains an "approved loading rate" that uses a multiplier to increase the certainty that there will be sufficient nitrogen for optimum crop production.
Step 5. Determine residual available nitrogen in the soil, from soil tests, and nitrogen credits from previous crops. Further adjustment is made by taking into account the	

2.3.2 Timing of sludge spreading

The timing for spreading sludge on land depends on cropping patterns, which vary according to the season across Europe (Schowanek et al., 2004). Most biosolids are applied to fields in the fall and early spring when standing crops are not present (Stehaouver, 1999). Applications in winter are preferably avoided, in order to avoid leaching of nutrients, especially in nitrate sensitive areas. Normally sludge is applied the latest 1-2 days prior to cultivation and sowing. Due to the fact that it is being produced throughout the year, it usually has to be stored (Schowanek et al., 2004).

2.3.4 Methods of spreading

The suitable method for sludge spreading depends basically on its type (liquid – dewatered -

pelletized) and its amount (Fig. 5). Solid sludge is normally applied in box spreaders (the same used for applying commercial fertilizers or manure). Liquid sludge may be applied on surface or injected into the soil. If a large amount of liquid sludge has to be applied, an irrigation system is necessary. Methods used for spreading liquid sludge on the surface of agricultural land are: by farm tractors, tank wagons, special applicator vehicles, and tank trucks. Injection may be performed by either of the following means: tractor-drawn slurry tank wagons with injection shanks or tank trucks fitted with injection shanks. Finally, irrigation may be accomplished by portable or fixed systems, or via flood irrigation (Muse et al., 1991).

Figure 5. Tank wagon for spreading liquid sludge (left, LRRM) and tractor applying dewatered sludge (right, Fox River Watch).

Flood irrigation has the disadvantage of being often connected with problems such as difficulty in achieving uniform sludge application rates, clogging of soil pores, and tendency for the sludge to turn septic. Instead of spreading large amounts of liquid sludge, it is often a more feasible and better practice to supply part of N needs of the crop with commercial fertilizers or apply liquid sludge in low doses. Regarding liquid sludge, in general comparison with dewatered sludge, the first has the advantage of being applied by simpler methodology, but the drawbacks of increasing transport and storage costs (Muse et al., 1991).

After applying the sludge on the surface of agricultural land, it is recommended to incorporate it into the soil within a maximum of 24 or 48 hours, to prevent loss of N via NH_3 volatilization, odour and possible loss of sewage sludge from the site by erosion or surface runoff. More accurately, a tillage operation such as disking, plowing or roto-tilling to a 10-20 cm depth is suggested as a good agricultural practice. Incorporation or injection should not be greater to allow maximum use of nutrients by the crop (Rutgers Cooperative Extension, 2001).

2.4 Risks and exposure pathways

Use of sludge on farmland is associated with both short and long-term benefits and risks. From the aforementioned legislation provisions for a safe recycling of sewage sludge to agricultural soils, many of the risks associated with this practice become evident. A more complete list would include the following risk factors: i) heavy metals, ii) organic pollutants, iii) radionuclides, iv) pathogens, v) endotoxins, vi) macro- and micro-nutrients and vii) nuisance (odour, vector attraction). Under risks are considered to be a) humans, animals and microorganisms which come in direct or indirect contact with the hazardous constituents of sludge, b) surface water systems and groundwater bodies in the

nearby areas and c) the plants growing on the farmland. Exposure to hazardous components may be due to different mechanisms, like 1) direct contact / uptake, 2) off-site transport, via the air (volatilization / wind), or due to the carrying out by water (leaching / surface run-off), or 3) transport and accumulation through the food chain.

Focusing on organic pollutants, which is the main topic of the present work, in the rest of this section the corresponding direct exposure pathways and mechanisms for crops, animals, humans, as well as microorganisms are briefly analyzed. Here it is noted that the vast variety of indirect exposure routes can in general have greater impact to an organism than a direct exposure. In this way, drinking water and vegetable / meat products can be a significant source of pollution for humans, if the former are directly or indirectly connected to sludge-amended soils. The fate (transport, degradation and binding) in the soil environment is the topic of the third chapter of the manuscript. While the exact mechanisms of action and corresponding effects of organic pollutants on organisms' health, which vary widely according to the substance and the organism, are considered to be out of the scope of this introduction.

An organic molecule present in soil can enter vegetation basically through the root system, from the soil solution, but also through its aerial parts (foliage, fruit, stem, bark), in this case from the atmosphere (Paterson et al., 1990). In the first case, the chemical present in the water phase of soil may migrate through the root, enter the xylem and be transported in the sap, eventually reaching the leaves. Via this pathway, a chemical is carried together with the conducted water. Before reaching the xylem, the principal vessel for transferring the water solution upwards to the rest of the plant, a chemical will first encounter endodermis and the Casparian strips. Depending on the interactions taken place there (sorption, binding, metabolism, etc.), it will either be accumulated in the roots or be transported to and/or react with further tissues of the plant. Regarding the uptake from the atmosphere, it may occur through volatilization from soil and then absorption from the aerial plant parts. Furthermore, wet and dry deposition from the atmosphere comprise two additional exposure pathways for the vegetation, though much more indirect ones (Paterson et al., 1990). Physicochemical properties of the organics, environmental parameters and plant characteristics influence all plant uptake processes.

Direct exposure of animals (mammals, birds) and humans to organic pollutants in sludge/soil occurs basically when soil particles are ingested either as attached to their food (e.g. via pasture or unwashed vegetables) or deliberately (geophagia) (Dean and Scott, 2004). The interference with digestive fluids of animals (i.e. gastric and intestinal secretions) can be important, leading to the release of toxic organics from soil particles. As long a chemical has entered the blood circulation, it may be directed to different organs of the organism’s body. Moreover, inhalation of sludge/soil particles aerosols is another possible pathway of exposure. Employees involved in spreading the sludge are prone to such incidents. Finally, dermal exposure can in some cases also comprise an important route, for instance for invertebrates (e.g. earthworms).

Microorganisms differ in their ability to assimilate compounds from the different compartments of soil environment. The fraction of organics contained in the aqueous phase of soil is generally considered to be the most available one. The desorption rate, from the organic (and

inorganic) components of soil, will be a determining parameter for the exposure via this pathway. Besides, some species have developed mechanisms to facilitate desorption from soil organic matter exist, e.g. excretion of surfactants (Tang et al., 1998). Microorganisms species can also overcome the sequestration or binding of organic molecules (Alexander, 2000), for instance via the action of extracellular enzymes. The mechanisms implemented by microorganisms for the assimilation of organic compounds are described with more detail in Chapter 3, as biodegradation is a crucial fate process in soil.

3. Fate of organic pollutants in sludge-amended soils

3.1 The soil environment

With the "sludge" component of the sludge-amended soil having been described in the first chapter of the introduction, this section gives an insight into the soil medium. Soil is a porous medium, where solid, aqueous, and gaseous phase can be discretely, though in intimate interdependence, regarded. It moreover sustains a living population of microorganisms and invertebrates, while plant roots consist also part of the system.

3.1.1 Solid phase

The solid phase of soil consists of inorganic and organic material. Soil particles can be physically classified according to their size into gravel (larger than 2 mm), sand (2 - 0.05 mm), silt (0.05 – 0.002 mm) and clay (small than 0.002 mm). Sand and silt particles affect mainly macroscopic properties of soil (e.g. the presence and distribution of macroscopic pores, and thus the air/water ratio of the system) and the transport of water and chemicals through it. Clay particles have a high surface area and are often involved in physicochemical interactions, for instance with organic compounds. The most common structural units to be found in soil clays are layer silicates. Additionally, iron oxides (hematite *α*-Fe_2O_3, maghemite *β*-Fe_2O_3, goethite *α*-FeOOH etc.) and aluminum oxides (gibbsite $Al(OH)_3$, boehmite *β*-AlOOH) commonly occur in soils. Significant quantities of calcium carbonate or calcium sulfate may be present in soils formed from related materials. Other oxide minerals, like Mn oxides or TiO_2, are less abundant. (Yaron et al., 1996).

Soil organic matter (the non-living portion of soil organic fraction), although being most often a small part of soil solid phase, is of major importance in defining physicochemical and biological properties of the media. It is a heterogeneous mixture of products resulting from microbial and chemical transformation of organic residues (humus) plus undecayed animal and plant tissues. Humus is composed of humic and non-humic substances. Humic substances (50-60% of soil organic matter, Paul, 2007) are characterized by the fact that they are dissimilar to the biopolymers of microorganisms and higher plants (such as aminoacids, carbohydrates, fats, waxes, resins, organic acids). They are high molecular weight, yellow to black colored substances formed by multiple secondary synthesis reactions. They are produced mainly through condensation reactions involving polyphenols and quinones. Humic substances have not a well defined structure or composition, as the number of molecules involved in humification and the ways in which they combine are almost unlimited. Their typical elemental composition is C (50-60%), O (30-35%), H (2-6%), N (2-6%) and S (0-2%) (Yaron et al., 1996).

According to its solubility, soil organic matter can be fractionated to humin (insoluble in alkali), humic acid (soluble in alkali, insoluble in acid) and fulvic acid (soluble in alkali and acid). Furthermore, hymatomelanic acid is considered to be the alcohol-soluble part of humic acid. Characteristic parameters of different humic substances fractions, as presented by Stevenson (1982), are shown in Fig. 6. Essential are the hydrophobic properties of humic acids and humin, which govern their interactions with non ionic solutes. Proposed model structures for humic and fulvic acids are given in Fig. 7 and Fig. 8. At alkaline pH, their acidic groups are charged and these macromolecules adopt an expanded (and more soluble) state, as a result of repulsion forces between the various components (Yaron et al., 1996).

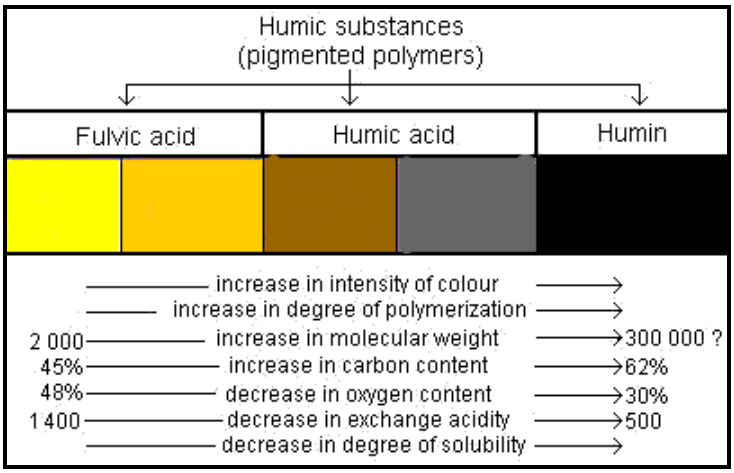


Figure 6. Properties of humic substances (Stevenson, 1982).

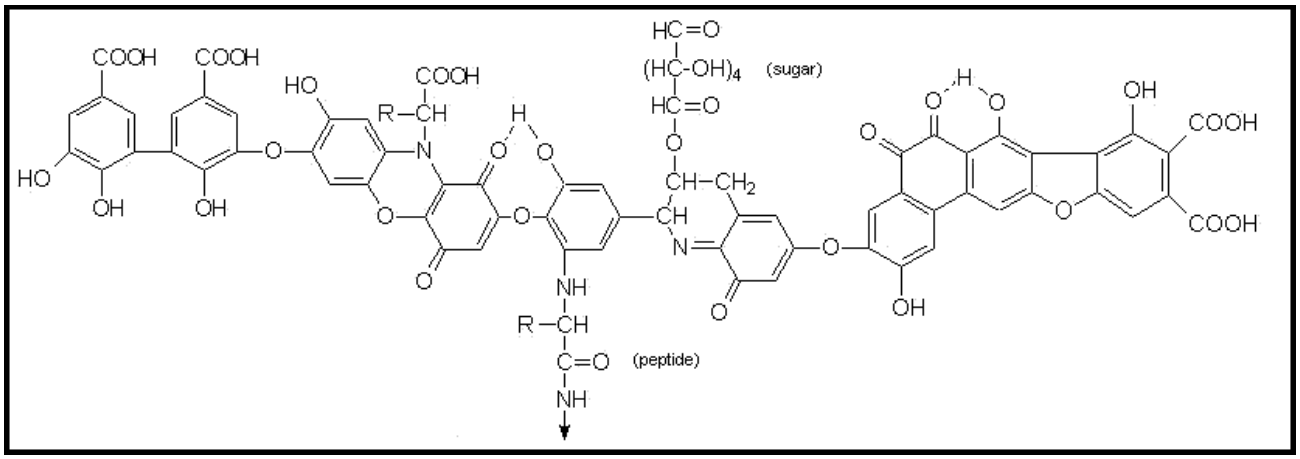


Figure 7. Model structure for humic acid (Stevenson, 1982).

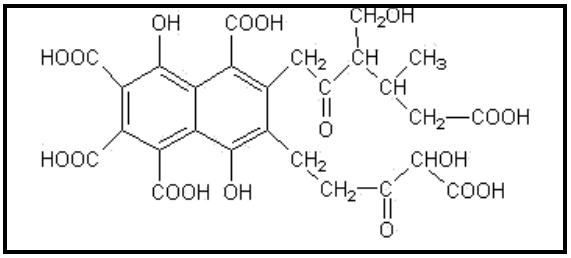


Figure 8. Model structure for fulvic acid (Buffle, 1977).

Interactions among the components of the soil solid phase strongly affect its surface activity (Wolfe et al., 1990). Coating of clay by metal oxides and organic material is a very common characteristic of soil material (Greenland, 1965, Ahlrichs, 1972). Nevertheless, there is evidence that much of clay surface is not covered with organic matter, specifically in the interlayer spaces of smectites (phyllosilicate materials with relatively open structure) (Ahlrichs, 1972). The most extensive interactions between soil solid phase components are those between organic compounds and the clay surface – for natural soils, the most important being those involving humic and fulvic acids. Since organic anions in the latter are normally expelled from negatively charged clays, adsorption is mediated by polyvalent cations, basically Fe^{3+}, Al^{3+} and Ca^{2+} (Yaron et al., 1996). Nevertheless, other mechanisms contribute to the adsorption as well, including protonation, anion exchange, water bridging, ligand exchange, hydrogen bonding, Van der Waals interactions, etc. (Mortland, 1970).

The soil solid surface has a net charge, which is controlled by the presence of relevant functional groups (e.g. M-OH) and properties of the further molecules which are bound to their surface. Hence the soil solid surface is composed by one or more layers of counter or co-ions plus uncharged molecules such as water (Yaron et al., 1996).

3.1.2 Liquid and gaseous phase

In the liquid phase of soil, one could regard separately the near-surface water and the "free" water zone (Yaron et al., 1996). Primary and secondary solvation shells comprise the adsorbed water, which can be considered to extend up to approximately 1.0 nm from the plane of the clay phyllosilicate surface (corresponding to 2-3 layers of water molecules) (Sposito, 1984). In the case of the interlayer water of clay minerals, the ability to be replaced by much less polar solvents is an interesting characteristic (Mamy, 1968; Sposito and Prost, 1982). When describing the near surface water in soil, the affinity of ionized carboxylic groups of the organic matter (but also of other groups, like phenolic, alcoholic, amines, aldehydes, esters etc.) to aqueous solutes must furthermore be mentioned (Yaron et al., 1996).

The composition of the soil solution will strongly depend on the quality of incoming water (rain, irrigation), as well as on the equilibrium with soil solid phase. In the water beyond the thin film attached to the soil surface cations, anions, ionic organic and non-ionic organic compounds can be found, together with colloids. Complexation between trace elements and organic ligands takes place in this phase and influences the equilibrium of chemical substrates between soil phases (Yaron et al., 1996).

Soil porosity and soil moisture determine at each time point the volume of the soil gaseous phase. The latter's composition is normally similar to the atmospheric one. Gases arising from biological activity such as nitrogen oxides are present, although they may react fast with soil components after their generation (Paul and Clark, 1989). Parameters such as the moisture level, which affects the accessibility of soil pores to the atmosphere and also biological activity, or temperature, pressure and salt concentration, affecting the solubility of gases to water, are often

determinant of soil gaseous phase' composition. For instance, the CO_2 level in the soil atmosphere is around 1-2% for well aerated soils, but may reach values up to 10% in heavy soils with high moisture content (Henin, 1976; Yaron et al., 1996).

3.1.3 Soil organisms

Soil biota consists of prokaryotic and eukaryotic organisms. To the first belong bacteria and archaea and to the second fungi, algae (eukaryotic) and fauna species. In general the microbial biomass in soil is to a great extent proportional to the amount of bioavailable organic matter (Nishiyama et al., 2001). Population densities decrease with soil depth, though not extincting in high depths (Yaron et al., 1996).

Bacteria is the most numerous class of microorganisms in soil. Moreover, they are the most diverse in taxonomy and functionality and possess the most efficient dispersal and survival mechanisms. In contrast with eukaryotes, they are characterized by absence of a unit membrane-bound nucleus and, usually, lack of cell organelles. Their size is within the range of several μm (diameter or length) - thus most of them being larger than clays - though with notable exemptions. Because of their small size, they have a high surface area, an attribute connected with their ability to assimilate nutrients and other substrates even when the latter are in low concentrations. They usually carry a negative charge (Yaron et al., 1996; Killham and Prosser, 2007).

Considering their role in soil processes, it is useful to further classify bacteria according to their energy and carbon sources, as well according to their oxygen requirements. Energy can be derived either from light (by phototrophes) or from chemical compounds (by chemotrophes). As source of cell carbon, CO_2 ("lithotrophic" or "autotrophic") or organic compounds ("organotrophic" or "heterotrophic") may be utilized. As some bacteria are able to adapt their physiological operation to the availability of energy sources, this classification is not absolute. Obligate aerobes require oxygen, as a terminal electron acceptor during aerobic respiration. Obligate anaerobes require the absence of molecular oxygen, removing instead hydrogen from organic compounds and dissipating them through reaction with products of carbohydrate breakdown. Facultative bacteria can grow in the presence or absence of oxygen. The important role of microaerophiles (obligate aerobes that grow best at low oxygen tensions), in carrying out many aerobic processes in soil microenvironments in which O_2 levels have been thoroughly decreased by decomposers (or also at wet soils or water saturated soil aggregates), has been recently emphasized (Killham and Prosser, 2007).

The bioactivity of microorganisms is tightly connected to enzymatic reactions. Some of the involved enzymes are "constitutive", being produced routinely by cells (e.g. urease), and others are "adaptive" or "induced", being formed only in the presence of a susceptible substrate (Yaron et al., 1996). The overall enzyme profile of a soil bacterium determines the range of substrates it can use. Both extracellular and intracellular enzymes are involved in biological reactions, with many substrates (e.g. of the size of lignin or cellulose) needing first to become depolymerized out of the cell, before simple building blocks can enter the cell and further utilized. In many cases a suite of enzymes operate for specific substrate transformations (Killham and Prosser, 2007). Extracellular enzymes are often entrapped in organic and inorganic colloids of soil, hence they are not found

associated with the microbial biomass (Yaron et al., 1996).

Bacteria are localized in diverse microenvironments in soil structure: they show attachment to sand grains, they intensively colonize macroaggregates pores (diameter 25 - 100 μm) and they are found also in microaggregate pores (diameter 0.2 – 2.5 μm). However, small necks of pores of soil microaggregates often prevent the entry of organisms. As a result, only a small fraction of small pores can be invaded by bacteria (Yaron et al., 1996).

Many prokaryotes possess the ability of autonomous movement (motility), with the most common mechanism to be by the use flagella. Motility is a rather slow movement probably unable to provide transport between soil pores of different size classes. Nevertheless, bacteria can move in the soil environment through the bulk flow of water or as attached to soil animals or roots. Additionally, alternative motility mechanisms exist, like through an axial filament or by gliding over surfaces (e.g. in cyanobacteria) (Killham and Prosser, 2007).

Regarding the dynamics of soil biomass, the competition of species able to assimilate the same substrate for growth will be controlled by the growth rate of the competing species, which in turn is to a considerable extent controlled by the available concentration of the substrate. Bacteria with higher growth rates at high concentrations of substrate (zymogenous) will predominate in areas such as the rhizosphere, while bacteria adapted better to low concentrations (autochthonous) will be probably found in zones where organics are released in low rates from soil organic matter. In this way the availability of substrates and nutrients determine the diversity of microbial populations. Bacterial diversity will tend to be enhanced due to factors like the facultativeness of bacteria (genetic adaptation, ability to form spores, capacity to cometabolize substrates, etc.) and the ability to form competitive microcolonies (Killham and Prosser, 2007).

Fungi possess in many natural soils the highest biomass, even higher than the sum of the biomass of all other organisms together. This is particularly the case in soils with relatively unfavorable conditions for microbial life, like shortage in nutrients and carbon, low temperature, dryness etc. Their predominance is lost in agronomic soils, nonetheless their roles are still very important. Characteristic are their filamentous form and the release of exudates. Their cell size ranges between 1 and 20 μm, with exemptions also present (Thorn and Lynch, 2007).

A major role in soil environment is connected to their ability to decompose complex substrates of plant origin, through the production of lignocellulose-degrading enzymes. This way they contribute essentially in the release of nutrients from insoluble reserves. Additionally, they possess a major role in aggregating the soil (Thorn and Lynch, 2007).

Considering their interactions with other soil organisms, two characteristic attributes can be mentioned: a) the wide trophic interactions and thus interconnection of different trophic levels, as well as the capacity for symbiosis and parasitic interactions with plants and b) the outcompeting of other organisms in processes such as the decomposition of plant materials. The second attribute relies on characteristic and special abilities of fungi. One of them is the formation of hyphae colonies (myceliums), which can penetrate litter and soil, cover nutrient-lacking and dry spaces and transport nutrients through macroscopic distances (even meters). The ability to secrete antimicrobial compounds significantly contributes to their competing strength (Thorn and Lynch, 2007).

Soil fauna is a diverse group of unicellular and multicellular heterotrophs, with sizes from a few μm up to cm. They assist bacterial and fungal activity and diversity in soils. Among their roles in the soil environment, of particular interest are the transport of microbes to big distances and the therefore assisted colonization, the fragmentation of soil particles and the creation of channels through which for example aeration and water and solutes transport may occur (Coleman and Wall, 2007).

3.2 Fate of organic pollutants in soil: processes and factors

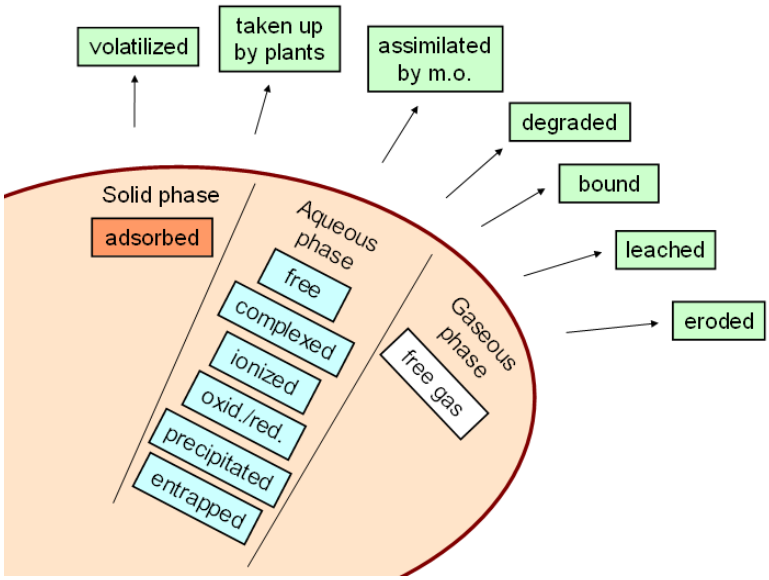


Figure 9. Consideration on different forms (inside the brown cycle) and irreversibly lost fractions (outside) of organic pollutants in soil. "Oxid./red." and "m.o." stand for "oxidized/reduced" and "microorganisms" respectively.

Describing the fate of organic pollutants in soils (or soil-sludge mixtures) is always a challenge, due to the complexity of the matrix, as well as the involvement of numerous interconnected processes. The use of simplified models is thus essential in interpreting experimental or monitoring data, or predicting the behavior of a chemical in soil environments. Such a consideration could include the phases / forms appearing in Fig. 9. At each time point the fate of an organic pollutant would then be described as a distribution of the initially added amount among these categorized fractions (rectangulars).

All forms surrounded by the cycle in Fig. 9 can be considered as residues of the parent pollutant. In contrast, states presented outside the cycle would rather be characterized as "losses" from the soil environment, in the sense that they either comprise part of other environmental compartments indeed (atmosphere, groundwater, organisms not living in soil etc.) or comprise new chemical forms that can practically not be transformed back to the parent molecule. This arbitrary model intends to take into account all possible states of an organic molecule in soil. Therefore, the "adsorbed" or "bound" fractions include not only the fractions attached (by weak forces or strong, chemical bond respectively) to the bulk solid matter of soil, but also the fractions associated to the

suspended particles of the aqueous phase of soil. The solid phase may originate from soil or sludge and be of organic or inorganic nature. Likewise, as “eroded” is regarded every molecule being part of a soil particle that has been leached as suspended to water flowing downwards, taken away by the wind or runoff water, or ingested by animals. Furthermore, “entrapped” is meant being still located in liquid phase, but in positions neither accessible by microorganisms nor to the rest of soil’s aqueous phase (these could be in small spaces tightly surrounded by solid matter or in isolated thin water film in partially dried soil pores).

The distribution among all these states (“fate”) is basically controlled in their interfaces. Definition of the existing interfaces and of the factors that direct the exchange between the participating phases/forms/states is the objective of any fate interpretation or fate prediction study. In this chapter distribution mechanisms are shortly discussed, divided into partition, binding and degradation ones. The first group includes equilibrium processes between soil phases, as well as pathways leading to other environmental compartments and it is discussed in section 3.3. Section 3.4 regards separately binding mechanisms to soil solid organic matter and minerals, because of their significance in soil systems. Finally, section 3.5 is focused on abiotic and biological degradation mechanisms for soil organic pollutants, including the assimilation process by microorganisms. The interfaces involved and the factors (or more determinant factors) influencing the distribution are discussed for each of the divided fate mechanism types, with focus on sludge-amended systems. The factors may include compound properties, compound concentration and localization, and environmental parameters (environmental conditions, composition of aqueous and solid phases of soil and sludge, and microbial mass and quality).

3.3 Partition

A complex network of interactions exists between the phases where an organic pollutant can be found in soil and its surroundings. Herein the interfaces in which each of these phases participates will be briefly analyzed.

Starting with the *volatilized* fraction, it shares an interface with i) the soil’s gas phase, ii) the free and the reduced/oxidized form in the aqueous phase lying on the surface of soil and iii) the adsorbed fraction in the solid phase of soil surface – when there is no water film attached to it. The transport of an organic molecule from soil’s gas phase to the atmosphere is regulated from the diffusion rate of air upwards to the atmosphere, which depends on its temperature, and the mechanical structure of soil, namely rifts that allow air in soil pores to escape. The evaporation from soil surface occurs at two steps. Firstly, molecules partition to the gaseous layer directly above the surface – where there is relatively little air movement – and then the vapor is lost due to partitioning or the wind to the atmosphere (Yaron et al., 1996). Since the first step is practically the same process occurring in the interface between soil’s gas and liquid/solid phases, it is analyzed below, where the latter interfaces are discussed. Factors affecting the second step of volatilization are the wind speed and turbulence in the vicinity of soil surface, the surface roughness and to a smaller extent the temperature of the atmosphere, here regarded only as a factor affecting the mixing rate

of the molecules of bulk atmosphere.

Plant uptake is, as it was mentioned in 2.4, a transport from the aqueous phase of soil, through the roots. A dissolved substance, either in free (ionized/de-ionized and oxidized/reduced) or in complexed form may theoretically be taken up. Moreover, absorption from the gaseous phase of soil by roots could also be possible. According to the literature, there are four mechanisms, with which a chemical can be transported from the aqueous solution of soil to the roots of a plant: a) partition to the roots' solid matter (roots' surface), b) passive uptake through water transpiration, c) active compound specific uptake and d) uptake and transport in oil cells, which are found in oil containing plants like carrots and cress (Ryan et al., 1988). The pathways "c" and "d" apply only for specific cases. For the common root uptake mechanisms "a" and "b", it has been shown (Briggs et al., 1982; Ryan et al., 1988; Duarde-Davidson and Jones, 1996) that:

- compounds with $K_{ow} > 3.0$ will presumably be retained by the root surface
- compounds with K_{ow} between ca. 2.5 and 3.0 will have moderate potential for root uptake and translocation
- compounds with K_{ow} between ca. 1.0 and 2.5 will have high probability for root uptake and translocation
- compounds with K_{ow} between ca. 0.5 and 1.0 will have moderate potential for root uptake and translocation
- compounds with $K_{ow} < 0.5$ will not enter the root system.

It is clear, that depending on the mechanism of root uptake, factors like the concentration in aqueous phase, and the form of the organic chemical in aqueous phase (complexed, ionized etc.) will influence the process, while plant physiology will be expected to play also an important role. The effect of sludge on these processes is in essence posed on the equilibrium between different forms of organics in the aqueous phase and the partition between solid and aqueous phase, processes that are discussed later in this section. Furthermore, since the amended sludge influences the growth of plants, it has an indirect effect on the uptake rates for various chemicals in sludge and soil.

Leaching towards a groundwater horizon depends solely from the downward transport of soil aqueous phase. The bulk aqueous phase (excluding the precipitated and entrapped fractions) has the potential to leach, but the entrapped water, as it was defined above, is not available for leaching. The ratio between water content and water holding capacity (WHC) of the soil will be determinant for the amount that will escape the soil system. Preferential flow of water through soil macropores is also a very important factor, which may add to the leaching of pollutants even those with high affinity to the soil matrix (Flury et al., 1994; Bundt et al., 2001). Here it is reminded that sludge amendment increases the WHC of soil.

Taking into account the pathways connected to the "*eroded*" fraction (see 3.2), the latter will share an interface with the adsorbed and precipitated fractions, as these are actually associated with soil particles. Parameters such as the compactness of soil, water load and leaching ratio, the

wind profile and tillage practices, and on the other side the diameter of suspended particles, will be the determinant factors here. Sludge amendment may have a considerable influence on “erosion” processes. Vinten et al. (1983) found that transport of the strongly sorbed pesticide DDT could be enhanced by sorption to mobile sewage sludge-derived solids. In this way, 18% of DDT applied to a loamy sand reached a depth of 0.09 m. (Beck et al., 1996).

Passing to reversible processes in which the aqueous phase forms participate, which characterize key interfaces for soil processes, one may firstly regard the equilibrium between *ionized* and free molecular forms of an organic pollutant. The pH of soil is obviously the controlling factor of this interface: the acid/base equilibrium will be shifted towards the acid side at low pH and towards the base side at high pH. While the balance between the levels of oxidizing and reducing compounds in soil aqueous phase will control the oxidative state of an organic solute, if the latter can hold such a dynamic equilibrium. The effect of sewage sludge amendment on soil pH is considerable, and can even be dramatic in the case of limed biosolids (appropriate amounts of the latter are usually used to raise the pH of soils to a predetermined target value). On the other hand, oxidation/reduction reactions may be catalyzed by components of soil or sludge matrices.

The formation of *complexes* or chelates between an organic pollutant and a soil solution species is a critical reaction, responsible among others for major shifts in the distribution between liquid and solid phase. The formation of a complex can be considered as a reaction between a Lewis acid and a Lewis base (Yaron et al., 1996). Being the organic molecule of interest a hard (e.g. RNH_2, ROH, CH_3COO^-, R: alkyl group) or soft base (RH, RSH, R_3P), it may form a complex with a Lewis acid (Na^+, K^+, Mg^{2+}, Fe^{3+}, Al^{3+}, CO_2, Fe^{2+}, π-acceptors such as quinones etc.) and vice versa. In fact there are many natural and artificial compounds with strong ability to form complexes or disperse free molecules. Substances belonging to the dissolved organic matter (humic and fulvic acids, root exudates etc.), surfactants, as well as colloidal formations (e.g. colloidal clay metal-oxides) can play this role (Yaron et al., 1996). The forces involved may be electrostatic or covalent bonds, or even weaker interactions. In case the complex formation represents an irreversible reaction, the process appertains to degradation processes, according to the model of Fig.9. Complexing reactions are controlled, except the properties of the central unit and the ligands, by the availability of competing species. Also water molecules can play the role of exchange ligands.

It has been reported that shortly after sludge amendment leaching of lipophilic compounds such as PAHs and PCBs can be enhanced, due to a facilitated transport with sludge derived dissolved organic matter (Dunnivant et al., 1992; Larsen et al., 1992). Sludge dissolved organic matter may include water soluble polysaccharides and fulvic acids (Beck et al., 1996). Likewise, a long-term downwards migration of persistent PAHs was observed at an experimental station by Jones et al. (1989). Such phenomenon was suggested to be due to an association of the organic pollutants with mobile colloids (Beck et al., 1996).

The equilibrium between the dissolved phase of an organic molecule in soil aqueous phase and its *gas phase* (in the headspace of a soil pore) can be described with the help of Henry’s law (Jury et al., 1983; Yates et al., 2002). According to the latter, ”at a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the

partial pressure of that gas in equilibrium with that liquid ($p = k_H \cdot c$, where p is the partial pressure of the solute above the solution, c is the concentration of the solute in the solution, and k_H is the Henry's Law constant)". From this equation it turns up that the amount of a volatile compound partitioned to the gaseous phase will be proportional to the concentration in aqueous phase and also proportional to the headspace volume of a soil pore. Concerning the constant k_H, it depends, apart from the compound under consideration, on the solvent and the temperature. The solvent in soil being water (at this point only the equilibrium with the free form is regarded), the constant will be related to the affinity of the molecule to water (water solubility) and be dependent also from the temperature. According to Thibodeaux (1979), k_H is proportional to the vapor pressure of the pure compound and its molecular mass and inversely proportional to the temperature and water solubility (Ryan et al., 1988). In fact solubility of gases is decreasing with increasing temperature, shifting the equilibrium towards the volatilized fraction in high temperatures. Practically only compounds with a Henry's law coefficient (c_{air} / c_{water}) above 1×10^{-4} are susceptible to volatilization from the soil aqueous phase (Duarte-Davidson and Jones, 1996).

Entrapment and precipitation are so called non-adsorptive retarding mechanisms (Yaron et al., 1996). *Entrapment* of an organic molecule, as it was defined in 1.3.2, may occur following a mechanical disturbance of soil, or due to the drying of a soil pore. Moreover, humic substances swollen because of high moisture may entrap organic solutes after a shrinking following dehydration (van Dijk, 1971; Chassin and Leberre, 1979; Yaron et al., 1996). Finally, liquids immiscible with water may be immobilized in disconnected pockets in soil (Schwille, 1984; Yaron et al., 1996). A possible mechanism for that is in water-wet soil pores firstly being saturated with water. By the removal of water, "blobs" or "ganglia" of residual organic liquid remain behind, not longer being connected to the bulk liquid phase of soil (Yaron et al., 1996).

In the interface representing the *precipitation* process, a compound comes from the aqueous dissolved form into a new solid form in soil. This may happen when the solubility limit is reached – a phenomenon that can often take place at a microscale level. Even below the saturation level, cationic pollutants can possibly be precipitated in the presence of lamellar charged particles with impurities (Yaron et al., 1996). Alternatively, precipitation phenomena may be the result of poor diffusion, a situation similar to the entrapment of non water-miscible organics. In this case, thin layers of immiscible compound may arise in wet soil pores. Precipitation occurs normally homogeneously into existing solid phases. Multi-precipitation, however, can lead to layers-oriented formations of inhomogeneous composition. The presence of successive precipitation layers may interrupt the equilibrium with the dissolved forms in soil aqueous phase.

In the interfaces between other (non free) aqueous forms of organic solutes in soil liquid phase - ionized, reduced/oxidized, complexed or entrapped forms – or between those and the gas phase, similar phenomena occur, like those described above for the free solutes. Of particular interest are however the equilibriums between all aqueous forms (and also the gas phase) and the fraction adsorbed on the soil solid phase. With the description of these interfaces, in the paragraphs below, the brief analysis of partition mechanisms in the soil environment is completed.

As *adsorption* is defined the excess concentration of a chemical substance at the soil solid

interface compared with that in the bulk solution of aqueous or gaseous phase (Yaron et al., 1996). The discrimination between adsorbed and bound residues is based on the reversibility of the process. More specifically, herein bound compounds will be regarded those that have formed covalent bond(s) with components of soil's solid phase. Coming back to adsorption, its definition postulates a higher activation energy for the respective desorption process (than for adsorption).

In the literature, adsorption has been described separately for ionic and non-ionic pollutants. The adsorption of ionic pollutants at soil's inorganic and organic matter is regulated by electrostatic forces. The equilibrium illustrates the balance between this force and the tendency for diffusion into the solution. The ions sorbed to the solid phase are also called exchangeable ions, since they can be replaced by an ion of the same charge. Here it is worth to note that repelling of equal charges is an opposite process of adsorption also occurring in this soil interface (Yaron et al., 1996).

Factors affecting adsorption of ions are related to the solid surface (charge, structural characteristics, charge distribution, area), the solute to be adsorbed (charge and dimensions, which affect also their hydration volume), the concentration of the solute, the composition of the mobile (liquid or gaseous) phase (mainly the pH and the electrolytes content) and environmental parameters (moisture, temperature). The influence of the pH is essential and it is connected to the competition effect of H^+ ions for adsorption on mineral surfaces in soil (clay minerals in soil are normally negatively charged), as well to the surface acidity (Yaron et al., 1996).

Electrostatic adsorption can be also the case for organic pollutants. Cationic organics can actually compete with mineral ions for the same adsorption sites. Here the pH value is a determinant factor. It has been reported that at low pH an organic molecule is much more strongly adsorbed on the soil surface than a mineral ion with the same valence, while at moderate pH the mineral ion adsorption is favored (Yaron et al., 1996). The polarizability and the molecular configuration are important properties of organic compounds participating in such processes.

Interactions responsible for the adsorption of non-ionic pollutants are: i) protonation, ii) water bridging, iii) cation bridging, iv) ligand exchange, v) hydrogen bonding, vi) π-bonds, vii) van der Waals interactions, and viii) hydrophobic sorption. In fact, the cause of most of these interactions is enthalpy-related. In other words, the bonding between surface and the sorbate are stronger than those between water and solute. Different is though the basis of the hydrophobic interactions; hydrophobic sorption occurs due to a large increase in entropy, when a non-polar organic is partitioned out of the polar aqueous phase (that is due to the destruction of the structured water shell surrounding the solvated organic) (Yaron et al., 1996). The leading force for this phenomenon is the stronger interactions between water molecules, which hence expel the organic molecule. Hydrophobic surfaces in soils are essentially present in soil organic matter, but may also be specifically arranged –Si-O-Si bonds at mineral surfaces. Hydrophobic sorption provides the major contribution to sorption of hydrophobic organic pollutants on soil surfaces (Yaron et al., 1996).

Adsorption processes for non-ionic organics are controlled by compound and surface properties, compound concentration (in relation to the adsorption capacity and saturation of the surface), the composition of the aqueous (or gaseous) phase of soil and environmental parameters. In the literature a close relation between the organic carbon partition coefficient (K_{oc}) and the K_{ow} is

reported (Ryan et al., 1988). Mineral adsorption may occur not only in clay, but for example also in amorphous Al and Fe hydroxides and oxides, as the latter have high surface area and proton-donor functional groups. Nevertheless, in general in soils where the clay content is relatively low, pollutant sorption occurs primarily on the organic fraction of the soil (Hamaker and Thompson, 1972; Rao and Davidson, 1980; Ryan et al., 1988). The degree of sorption of the non-ionic organic pollutant is then dependant upon the organic carbon content in the soil. Regarding soil organic matter properties, among which the ratio between humic acids, fulvic acids and humin, the presence of active groups (COOH, OH, C=0, OCH_3, NH_2 etc.) and the surface area are considered critical. The pH affects except the extent of the adsorption also the place it will occur (e.g. clay or humic acids), while the type of association between mineral and organic matter is also important (their association restrains adsorption possibilities) (Yaron et al., 1996).

Among environmental parameters, soil humidity and temperature show significant effects on adsorption phenomena. Soil moisture will at a first site enhance the transportation / diffusion of solutes to the bulk of soil solid phase, but on the other hand water (depending also from the pH value) will be a competitor for adsorption sites. Decrease in moisture will thus facilitate adsorption, but a later increase may again remobilize the adsorbed species. The influence of the temperature factor is to a high extent related to the fact that adsorption is an exothermic process (Yaron et al., 1996).

Two aspects of adsorption that need further to be considered are its rate and a phenomenon called hysteresis. Adsorption rate is controlled by the probability of solute and adsorbent to come in contact. Although the sorption mechanisms for ionic and non-ionic pollutants are different as described above, it can be said that the rate determinant steps is the diffusion of the solute through the hydrated layers of the surface and the hydrated micropore spaces in soil particles rather than the diffusion in the bulk liquid phase. A steady state may be reached in a few seconds, but also in days periods (Yaron et al., 1996).

Hysteresis is observed when the adsorption and the desorption isotherm curves (adsorbed amount as a function of concentration in mobile phase) are not the same (Yaron et al., 1996), i.e. after adsorption takes place, any decrease in the concentration in the aqueous phase doesn't lead to an accordingly high release from the sorbent. This phenomenon may be explained by a diffusion of the sorbate into the organic matter aggregates, which, strictly speaking, is an additional fate process (a type of complexation in the solid phase). The release from the bulk organic matter may either be very or extremely slow, or never occurring. The first case (accompanied by slow release) will be referred in this work as "sequestration" (leading to "sequestrated" residues), while the second case presupposes a chemical transformation / formation of a covalent bond and it will be referred as "binding", accounting accordingly for the "bound" fraction (discussed in 3.4). By oxidizing soil samples (practically their organic matter) Saltzman et al. (1972) observed a significant decrease in the hysteresis effect for parathion, thus showing that this phenomenon is mostly the case for the adsorption into the organic phase of soil. In agreement was the finding by Gaillardon et al. (1972) that desorption of the herbicide terbutryne exhibited hysteresis in the case of humic acid and clay-humic acid mixtures, but not for clay surfaces (montmorillonite) (Yaron et al., 1996). The condensed

glass-like matter of humin has been suggested as a favorable place for sequestration, in contrast with the rubber material associated with humic and fulvic acids, where presumably the faster process of sorption occurs (Xing and Pignatello, 1997; Gevao et al., 2000).

The influence of sewage sludge amendment on the adsorption of organic pollutants in the soil system includes contrasting factors. For example, additional organic matter is supplied, and a greater number of storage pores is produced, but on the other hand the water holding capacity of the system increases and dissolved organic matter is added (the latter especially for liquid sludge). Nevertheless, it has been stated that sludge organic matter induces stronger sorption interactions in comparison with soil organic matter. Increased accumulation of PAHs and PCBs from atmospheric deposition was observed for sludge-amended soils, in comparison with control soils, by Wild et al. (1991) and Alcock et al. (1993) respectively (Beck et al., 1996). Increased sorption capacities of soil-sludge mixtures may also be explained by the significant content of ash that may be present in sewage sludges (due to a process similar to adsorption in active carbon) (Beck et al., 1996). An increase in the sorption of atrazine in liquid-sludge amended soil was also reported by Cells et al. (1998), although in a parallel amendment just with the dissolved organic matter of sludge desorption was increased. On the contrary, other studies report an insignificant effect of sludge amendment on the adsorption of organics in soil (e.g. Hernández-Soriano et al., 2007).

3.4 Binding

Although the (covalently) bound molecules of parent (not degraded) organic pollutants in soil may share an interface with their adsorbed, aqueous (free, ionized, oxidized/reduced, entrapped) and gaseous fractions, in practice a first approach of the solute (and also any necessary enzymes or other catalysts) to the solid face is a prerequisite. Thus the adsorbed fraction of the parent organic molecule is expected to present the most important interface with the bound fraction.

Binding may be induced either chemically, enzymatically (either involving active microorganisms or extracellular enzymes) or photochemically. Theoretically it may occur either at the surface, or in the bulk matter of the solid phase. The second pathway refers to the binding of sequestrated molecules, which after being diffused into the organic matter may be further retained by a chemical bond. The assimilation of an organic chemical by a microorganism can be also responsible for the formation of bound residues, during the humification of the dead mass of the organism. The microbial assimilation process is discussed in 3.5.

A common formation mechanism for the covalent bonds with the humic substances of soil is the oxidative coupling. According to this mechanism, phenols, anilines and other functional groups are linked together after oxidation by an enzyme or chemical agent. The catalyst may be abiotic or biotic, including plant and microbial enzymes, inorganic chemicals and clay, and UV. C-C, C-O, C-N and N-N bonds may be created (Gevao et al., 2000; Dec et al., 2003).

The functional groups and the free radical content of humic substances are critical factors for the humification process (Wais, 1998). Compounds with functionalities similar to the components of humus will tend to form bound residues with soil organic matter. Such groups may however

introduced by microbial catabolic processes. In this matter, for example -NH_2 groups may be added or phenols may be oxidized, before amines or phenolates respectively can be bound to humus (Gevao et al., 2000). Nevertheless, it has been noted that compounds including C=O, quinone and carboxyl-groups tend to form covalent bonds with humic substances without the mediation of microorganisms (Gevao et al., 2000).

The relative importance of microbes-mediated and physicochemical binding of organic xenobiotics in soil has attracted the attention of scientists the last decades. By performing experiments with sterilized and non-sterilized soils, it was generally concluded that non biologically-driven bound residues formation is a process accomplished very rapidly in the first 2 days after the introduction of an organic in the soil matrix. It is very interesting, that this fraction of bound residues is often the major one. On the other hand, biologically-mediated retention increases with time continuously, contributing gradually more and more to the total bound residues (Benoit, 1994). Barriuso et al. (2008) posted that among the environmental factors affecting the formation of bound residues, microbial activity has a direct and significant effect. The ratio between physicochemical and biological binding depends on the nature of sorbate and sorbent (Yaron et al., 1996), as well as on the environmental conditions.

Regarding the localization of bound residues in the soil matrix, in a study by Barriuso et al. (1996) on the non-extractable residues of atrazine in soil during 16 months of incubation, it was found that the largest proportion of total bound residues in the whole soil was in the clay size (0.2-2.0 μm) fraction, which also contained 50% of the total soil organic carbon (Yaron et al., 1996). Moreover, in this study it is reported that the ratio of bound residues to organic carbon content decreased with the particle size, and it was highest in fractions >50 μm, those rich in non-humified organic matter. Two possible explanations were given by the authors. Either the chemical nature of the humified organic matter is connected to a lower binding capacity of the finest size fractions, or fine mineral surfaces are accessible to organic compounds for strong interactions (however non-extractable residues are not necessary due to covalent bonding – entrapment or sequestration can also lead to non-extractable residues). Besides, a considerable variation of the distribution of bound residues with time was noticed.

Release of covalently bound residues is possible only after a decomposition of the recalcitrant polymeric humic substances of soil. Nevertheless, even at this long-term time scale, the release of a bound molecule will presumably not have the same structure; microbial decomposition is combined with hydroxylation and other oxidation reactions. As regards the decomposition of organic matter, for sewage sludge it has been found to be a two-stages-process. For instance, Terry et al. (1979) reported that 26-42% of the organic carbon in municipal sludge added to soil was lost relatively rapidly, whilst 55-80% was resistant to decomposition. Similar experiments by the same authors with synthetic sewage sludge also showed a stage of rapid decomposition over a period of 28 days, whereafter the rate of decomposition was decreased markedly, with 46% of the sludge derived organic carbon being evolved as CO_2 after 336 days of incubation (Terry et al., 1979; Beck et al., 1996). Besides, spreading of biosolids may affect the decomposition rate of soil organic matter: in the same study, a 100% increase of the decomposition rate of soil organic carbon in the presence of

sludge amendment was recorded.

3.5 Degradation

3.5.1 Abiotic degradation

Abiotic degradation is a process to which all fractions of a parent organic compound in soil are subjected. In soil aqueous phase, reactions such as hydrolysis, reduction/oxidation transformation and photolysis may occur. Although this classification is not absolute (a hydrolysis or a photolytic reaction may at the same time be an oxidation), it indicates the species or factor mediating the transformation. During hydrolysis, H^+ or OH^- originating from the dissociation of water attack the organic substrate, breaking an existent bond and forming a new one with the latter. Hydrolysis in soil aqueous phase may be pH-moderated or pH-independent. Redox reactions may be reactions with chemicals present in the aqueous phase of soil which result in an electron transfer, with the organic pollutant being either electron donor or electron acceptor. Abiotic oxidation of organic pollutants occurs in the soil solution under aerobic conditions, while abiotic reduction may occur in water saturated soils (Macardy et al., 1986; Yaron et al., 1996). The top layer of soil (ca. 0.5 cm) can be directly affected by the solar radiation. Light may either excite the pollutant molecule – which thus can undergo further reactions – or any other photoreactive component (sensitizer), which can then react with the pollutant. Electronically excited molecules of natural dissolved organic matter in soil's aqueous phase may react with organic toxicants or alternatively with molecular oxygen; the excited singlet molecular oxygen is a powerful oxidant able to form photo-oxidation products of electron acceptors (Yaron et al., 1996).

The surface of soil solid phase can catalyze abiotic degradation processes. Catalysis can be due to a component of the surface or due to a third adsorbed species. It has been shown that clay surfaces can catalyze many transformations, including hydrolysis, redox reactions, oligo- and polymerization, rearrangements, etc. Not only the type of the clay, but also the type of counter cation plays an important role in such processes (Yaron et al., 1996).

Organic matter has also high potential to catalyze chemical transformation of organic compounds. That has been explained by a) the presence, in the soil organic matter, of many reactive groups, known for their capability of enhancing chemical changes in several families of organic substances, b) the strong reducing capacity of humic substances, and c) the presence of relatively stable free radicals in the fulvic acid and humic acid fractions of soil organic matter (Stevenson, 1982; Yaron et al., 1996). Among others, inducing of processes like hydrolysis, solubilization, and photosynthesis has been reported (Senesi and Chen, 1989).

Except clay surfaces and organic matter, metal oxides possess also catalytic properties. Furthermore, the fact that soil surfaces are charged can initiate many chemical transformations; the electrical field at these surfaces can polarize or even dissociate solutes and solvents, and moreover it produces a concentration gradient of charged substances, which then in concentrated form may successfully react with organic solutes (Wolfe, 1990; Yaron et al., 1996).

Apart from the organic pollutant properties, factors influencing abiotic degradation processes

are environmental parameters (temperature, light, moisture), pollutant concentration, pH of soil's liquid phase and soil surfaces properties (hydration status, exchangeable cations). The correlation between degradation rate and reactant concentration can give information about the reaction type (zero, first, second order, hyperbolic, etc.) (Yaron et al., 1996).

Among other factors, the method of sewage sludge application is expected to influence the decomposition rates of adsorbed organics. For instance, sludge applied on the surface of soil will be more subjected to photolytic reactions (Beck et al., 1996). When regarding abiotic degradation processes in soils in comparison to sludge-amended soils, factors like the partition of pollutants (solid matter of soil / solid matter of sludge / aqueous phase) and the nature and composition of the surfaces and liquids of sludge and soil should be considered. It is noted, that Dolaptsoglou et al. (2007) observed a decrease in the abiotic degradation rate of the herbicide terbuthylazine when spiked in (sterilized) urban sewage sludge amended soils, in comparison with non-amended soils.

3.5.2 Biodegradation

An organic chemical may be degraded by a microorganism for different reasons. Firstly, the chemical may provide source of a limited nutrient, like C, N, S, P, etc. Biodegradation might also serve detoxification purposes. In that case, an organic chemical that exerts a toxic pressure on the cell is modified, in order either to sequestrate it within the cell, or to remove it from the cell. Additionally, biodegradation may be "accidental", meaning that cells degrade other compounds present in the same environment. This "cometabolic transformation" occurs when an organic pollutant resembles a natural substrate, an enzymatic system has broad or relaxed specifity, or when extracellular enzyme systems generate long-armed free radicals to achieve reaction (Van Hamme, 2004).

A chemical transformation by a microorganism can take place out of the cell, on the cell surface, or inside the cell. The first case relates to reactions induced by extracellular enzymes. This mechanism is mainly used by fungi, although some bacteria show also similar behavior. Cell surface agents and membrane-bound enzymes are responsible for the second case. While after the uptake of a chemical, degradation may occur by intracellular enzymes or in vesicles and storage bodies of the cell. Microbes may possess more than one mechanisms for degrading the same substrate (Van Hamme, 2004).

There are many challenges to be overcome by a microorganism for transforming an organic chemical. The proximity, the contact/sorption to the surface and the transport (uptake) are determining for biodegradation processes (Van Hamme, 2004). For an organic chemical it is even possible to be assimilated by a microorganism and later be removed unaltered from the cell (toluene efflux mechanisms exist for example for Pseudomonas, Ramos et al., 1998 as cited in Van Hamme, 2004). The reversibility of microbial assimilation has though not been taken into consideration in the model presented in Fig. 9.

"Chemotaxis" is a term used to describe the directed movement of cells along extracellular gradients of a compound, in order to achieve proximity to it. This requires a directional sensing mechanism, followed by a corresponding change of cell's configuration, leading to motility (cell movement). Sensing normally relies on chemical receptors located along the perimeter of the cell. In

this way, by measuring receptor occupancy in different directions, the cell can steer on the chemical gradient. The underlying directional sensing system acts as a compass favoring for instance pseudopodia formation towards (or away from) the source of the chemical under consideration, and thereby orients cell movement in relation to the extracellular gradient. Rearrangement of the membrane and cytoskeleton to achieve a polarized morphology, and contractile forces generated by myosin motor proteins at the rear of the cell are also mechanisms of response to a signal from a sensing system. Furthermore, chemotaxis in higher organisms may take the form of directional cells growing (this can be the case also for fungi) (Janetopoulos and Firtel, 2008).

In the case of hydrophobic compounds, the contact with the cell may be relied either on its sparing solubility in water (thus being partitioned away from the water phase) or on the contrary, on a pseudosolubilization due to the production of biosurfactants. Nevertheless, conflicting reports exist on the effect of biosurfactants on the bioavailability of organics in the soil environment. An additional contact mechanism comprises the direct adhesion of cells to bulk liquids or solids, which may require cell surface alterations (Van Hamme, 2004). The complex structure of a bacterial cell can behave as either a cation or an anion and react with respectively charged surfaces through ion exchange (Daniels, 1980; Yaron et al., 1996).

Uptake to the inner phase of the cell can be passive or active. A passive transport takes place via diffusion or partitioning equilibrium. Specific active uptake systems exist, by which compounds are uptaken selectively in the presence of compounds with similar partition properties (Van Hamme, 2004).

An organic pollutant may be converted by a soil microorganism to biomass, to a transient or stable metabolite, or to inorganic products (mineralization). Many types of reactions may occur, including a) oxidation, such as hydroxylation, N-dealkylation, ß-oxidation, decarboxylation, ether cleavage, epoxidation, oxidative coupling, aromatic ring cleavage, and sulfoxidation, b) reduction, e.g. of nitro groups or double or triple bonds, sulfoxide reduction, reductive dehalogenation, c) hydrolytic reactions, e.g. ester cleavage or hydrolytic dehalogenation, and d) synthetic reactions, including conjugation, such as methylation and acetylation, or condensation to oligomeric or polymeric compounds. Metabolism may be aerobic (“aerobic respiration”) or anaerobic. A microbially mediated decay in the soil medium may follow zero-order, first-order (typically one or two compartment kinetics) or hyperbolic reaction kinetics (Bollag and Liu, 1990; Yaron et al., 1996).

Factors affecting the biodegradation of a soil organic pollutant are i) the compound properties, ii) the compound bioavailability (influenced by its distribution and its forms), and iii) the compound concentration. Some effects related to the substrate concentration are the toxicity (beyond a specific threshold) and the organism response, i.e. the enzymatic excretion that occurs after a compound reaches a defined level (Van Hamme, 2004). The kinetics of biodegradation are described by the Mechaelis-Menten equation and the Monod equation (Alexander, 1994).

Persistent pollutants are either resistant to biodegradation, are biodegraded at low rates, or leave, after their consumption, low concentrations of residues (Nocentini et al., 2000). Persistence may be due to:

- structural properties of substrates (e.g. polyaromaticity, steric hindrance) / lack of an appropriate enzyme and transporters
- a limited bioavailability of substrate or cofactor
- absence of appropriate microorganisms or groups of microorganisms
- unfavorable conditions (pH, temperature etc.)
- competition with non-degrading microorganisms, for the specific compound

Regarding bioavailability, it is generally believed that desorption from soil's solid surface must precede the biodegradation. Nonetheless, there are examples in the literature where sorbed substrates were degraded directly by attached cells (Park et al., 2001; Van Hamme, 2004).

Sludge has a multiple role regarding the biodegradation processes of organics in the soil environment. Sludge adds available C and nutrients to the soil. In a field incubation by Debosz et al. (2002), the total amount of inorganic N produced due to N mineralization was shown to be increased during the peak of the first 3-4 months after sludge amendment (peak observed for both amended and not amended soil), but its total amount being rapidly decreased and reaching similar values with non-amended soil measured after this period. A stimulation of CO_2 production was also observed in the same study (in dark room incubation), applying however only for the first 2 months after amendment. Enzymes activities increased immediately after sludge amendment and remained high for the 1st month. That applies even for intracellular enzymes, meaning that sludge introduces active microorganisms to the soil system or simply stimulates the growth of soil endogenous species. Moreover, increases have been reported in parameters like soil's biomass (soil microbial cells' total C and N amount), as well amounts of available N and P. In fact the latter is one of the few parameters with a long-term change (Debosz et al., 2002). Nevertheless, Perrin-Ganier et al. (2001) observed no stimulation of the degradation of isoproturon in sludge-amended soil, although addition of N and P in the form of fertilizer acted in this direction. It is interesting that the above mentioned effects of sludge amendment have been found to vary according to the conditions of the experiments (laboratory or field incubation) (Debosz et al., 2002).

In general it is believed that sludge introduces organics-degrading microorganisms and nutrients inducing microbial growth, but it provides also sites for sorption. Thus the total effect on degradation rates will depend on compound characteristics; easily biodegradable compounds will degrade faster after sludge amendment, while when chemical degradation is more important the sorption will play the most important role (Sánchez et al., 2004; actually these considerations assume that the organic is applied on the soil - either sludge-amended or not). Besides, organic toxicants and heavy metals introduced together with the sludge, if in great amounts, may have also effects, which need to be taken into account (Galiulin et al., 1992).

Interactions between soil and sludge microorganisms may further complicate the total effect on pollutants' degradation in amended soils. Some experiments showed that soil and sludge microorganisms both contribute significantly to the degradation of a phthalic ester (Jianlong et al., 1997). In general, when soil and sludge are mixed, there is competition between the species. Which

will survive will depend on many parameters, even on nutrients levels and storage time of the individual samples. More specifically, indigenous organisms in stored biosolids were found to be the less antagonistic to inoculated pathogens the longer the storage time, with the possible explanation that they were less healthy, due to an exhaustion of available nutrients (Sidhu et al., 2001). Smith (1996) reported that over 90% of sludge's pathogen population dies within 30 days of application to soil.

4. Nonylphenol

4.1 Nonylphenol in sewage sludge: origin and concentrations

Nonylphenol (NP) has the general formula $C_6H_4(OH)C_9H_{19}$. It actually corresponds to a large number of isomeric compounds, which vary in the position of the nonyl chain group on the phenol ring and in its degree of branching. The commercially produced NP consists predominantly of 4-NP, with a varied and undefined degree of branching, as the nonyl moiety is formed by polymerization of propylene (ECB, 2002; Ruß et al., 2005).

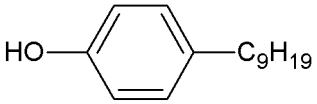


Figure 10. General structure of 4-Nonylphenol.

According to data for the year 1997, 73,500 tons of NP were produced in EU, where the total use was estimated to be 78,500 tons, as the imports were greater than the exports. Always for the same year, 60% of NP was used for the production of Nonylphenol ethoxylates (NPEOs), 37% was utilized in the polymer industry, for the production of resins, plastics, stabilizers etc., and 3% was used by just one company for the production of phenolic oximes, the latter being used as a reagent for the extraction and purification of copper from ore. From these uses, the direct release of NP to wastewater has been estimated to be negligible. The levels of NP found in sewage sludge are actually related to the uses of NPEOs (ECB, 2002).

NPEOs are used as non ionic surfactants for industrial and institutional cleaning, as dispersants to improve the stability of formulations like coatings and adhesives (or even aid the polymerization reactions employed for the production of polymer conditioners for the wastewater and sludge treatment), as detergents and fiber lubricating agents in the textiles industry, and also in the leather industry, for the production of pesticides formulations etc. They are also used in the public domain, in products such as non-agricultural pesticides, vehicle and office cleaning agents, cosmetics, and office products such as correction fluids and inks (ECB, 2002).

It has been estimated that, on a molar base, up to 50% of the NPEOs entering a wastewater treatment plant may end up as NP in the anaerobically digested sludge (Brenner et al., 1987). The transformation seems to occur basically during the anaerobic digestion, with the NPEOs that have reached the sludge to break down to the resilient, under these conditions, NP (Giger et al., 1984; Giger et al., 1987). Noteworthy, NP is not formed as a persistent end product during aerobic

stabilization of sludge (Hernandez-Raquet et al., 2007), while it has been found that NP levels in such sludges are much lower (Brenner et al., 1987).

Many studies have reported levels of NP in sewage sludge. The levels range from non-detectable till values such as 2.5 g/kg (dw basis) for NP and even up to 7.3 g/kg for the sum of NP and NPEOs (Erbardt and Prüeß, 2001; ECB, 2002; Harrison et al., 2006). Levels of several hundreds of mg of substance per kg of dry sludge are not rare. Because of the risk deriving from the release of NP in the environment, voluntary agreements between EU member states and industry have resulted, as for instance that to phase out nonylphenol ethoxylates in all detergent applications by the year 2000 (ECB, 2002). In one study by Jobst (1998), evidence was found that NP concentrations in sludge in Germany have been decreasing, already during the early period of voluntary agreements with industry. Recently, restrictions on the marketing and use of nonylphenols have been included in European Union legislation, in the form of the Directive 2003/53/EC (CEC, 2003).

4.2 Physicochemical properties, hazards and risks

Commercially produced NP is a clear to pale yellow viscous liquid with a slight phenolic odour. It has a molecular mass of 220.34 g/mol. Its pour point is about -8^{o}C. It boils at 290-300^{o}C, being however decomposed already at lower temperatures. Its vapor pressure has been estimated, from extrapolation of existing data, to be ca. 0.3 Pa at 25^{o}C. It is described as sparingly soluble to water, with solubility values reported ranging around 6 mg/L (at 20^{o}C) and being probably dependent on the pH. NP is hydrophobic, with log K_{ow} reported to vary between 3.28 and 4.77, depending presumably on the commercial product tested. Regarding its acidity, although a pK_a value of 4.53 has been reported, it has been commented as presumably wrong, as alkylphenols should theoretically be slightly less acidic than phenol, for which a pK_a of 9.9 is reported. Hence, a value of ca. 10 (or even higher) has been estimated as an expected value (ECB, 2002).

NP has been shown to be highly toxic to aquatic organisms, at low concentrations (Ahel and Giger, 1993; Vazquez-Duhalt et al., 2005). In the frame of the risk assessment performed by the European Union (ECB, 2002), the regional Predicted Environmental Concentration (PEC) for NP was estimated for surface water at 0.6 μg/l, a value which exceeded the Predicted No Effect Concentration (PNEC, 0.33 μg/L)♦. Thus a significant risk was concluded to be caused by emissions to the aquatic environment and a need for risk reduction measures was identified.

Limited terrestrial effects data exist in the literature. The most sensitive species group appears to be the invertebrates. As concentrations due to atmospheric deposition are expected to be negligible due to the amounts released and the atmospheric behaviour of nonylphenol, the calculated soil concentrations, in the risk assessment report by EU, are due to application in sewage sludge (ECB, 2002). A considerable risk for the terrestrial compartment was identified in this report, while secondary poisoning of top predators is also characterized as a considerable risk factor. Risk

♦ this PNEC has been calculated taking into account the most sensitive – in NP - aquatic species studied until now (i.e. the freshwater algae *Scenedesmus subsicatus*) and an additional assessment factor of 10 – to account for uncertainty.

reduction measures connected to some of the NP uses are proposed for these two cases (terrestrial compartment and the case of secondary poisoning pathways).

Apart from environmental effects, human exposure is also an issue related to NP emissions. As key health effects to human health have been identified the acute toxicity, the corrosivity, the repeated dose toxicity, and the reproductive effects. Regarding the latter, observed effects reported include estrogenic activity in vitro and in vivo assays, minor perturbations in the reproductive system of offspring in a multi-generation study, and testicular changes in gavage studies (ECB, 2002). Potential endocrine disrupting properties are connected to its estrogen mimicking properties. NP's structure has similarities to the strong estrogenic hormone estradiol (Porter and Hayden). Studies suggest that nonylphenol can bind to estrogen receptor sites, causing severe hormonal changes in animals. Mutagenicity and carcinogenicity of NP are thought to be low (ECB, 2002). Regarding human health effects, the report study by EU judges, based on the corresponding risk assessment procedure, that no additional risk reduction measures are necessary.

4.3 Fate in sludge-amended soils

As it was mentioned in the preface of the manuscript, this work generally intends to contribute in predicting (and thus also in controlling) the fate of organic chemicals in sludge-amended soils. More specifically, it focuses on the sludge conditioning and dewatering method, as a potentially major factor affecting sludge-pollutants' fate in soil. A literature research revealed that – to the best knowledge of the author - no studies on the role of this factor in soil fate processes have been the case, not only for the model compound chosen (nonylphenol), but actually for any other organic pollutant. On the other hand, the influence of other factors, regulating these processes, has been reported through dozens of publications. Concerning organic pollutants fate in sludge-amended soils, NP is in fact one of the most studied compounds, if not the predominantly studied one, something that can be explained by its detection at very high levels in sewage sludges around the world. Hence its selection as a model compound for the present work serves, among other aspects, also the possibility to roughly assess the significance of the sludge conditioning/dewatering factor, in comparison with other fate influencing factors in the soil-sludge environment.

In this paragraph, firstly the big range of reported values, for rates of transformation and transportation of NP in sludge-amended soils, will be referenced, to illustrate the uncertainty in predicting the behaviour of a chemical in soil. Subsequently, a short overview of studies will be provided, during which the effect of selected parameters on the fate of NP has been recorded.

Nonylphenol's degradation in sludge-amended soils has been reported to occur with rates from 10% / 30 d. (Xia and Jeong, 2004) up to 99% / 30 d. (Mortensen and Kure, 2003). Amended systems with NP residues of 35% of the initial amount after 120 d. of incubation were reported by Hesselsoe et al. (2001), while in the same study under different conditions the residues accounted for only about 1%. In the latter study, mineralization rates of NP in sludge-fertilized soils occurred at rates high up to 70% / 38 d. Nonetheless, NP in experiments by Telscher et al. (2005) was mineralized in rates down to 20% / 135 d. Leaching of NP is reported to be in general low. Trace levels of NP

could be determined in soil layers below a 15 cm soil/sludge mixture with a concentration of 0.56 mg / kg (dw) by Jacobsen et al. (2004). Similarly, NP mobilization from a sludge aggregate didn't exceed 0.6% in 1 cm distance and 0.07% in greater distances, after 120 d. of incubation in the experiments performed by Hesselsoe et al. (2001). Volatilization was insignificant 30 d. after soil amendment in the experiments of Xia and Jeong (2004), while it accounted for losses up to 6% during the incubations performed by Telscher et al. (2005), which lasted for 135 d.

By comparing the conditions of the experiments of different studies, it is often possible to derive assumptions about the role of specific parameters and factors in the differentiation of the environmental fate of a compound. However herein only parameters and factors investigated under the same study (and thus under the same conditions) will be reported. For NP these include: temperature, light, season, plant growth, water saturation, NP type/origin/localization and concentration, soil type, the presence of sludge or other organic residues, nutrients supply, soil/sludge ratio, sludge amendment method, and finally the origin of, and the stabilization technique in which sludge was posed. An overview of the literature in this topic is presented in Table 7.

Table 7. Influence of specific parameters / factors on the fate of NP in sludge-amended soil according to the literature.

Parameter / Factor	Study	Effect
Temperature	*Qiao et al., 2008*	Increase in temperature enhanced the degradation rate of NP
Light	*Xia and Jeong, 2004*	Degradation in dark: 10-15% / 30 d., under simulated sunlight: 30% / 30d.
Season	*Gomez-Rico et al., 2008*	Mean degradation during the first 6 months of incubation (winter and spring): 39% / 51%, further degradation during the next 3 months (summer): 70% / 66% , for two different soils respectively [determined for the sum of NP + NPEOs]
Plant growth	*Mortensen and Kure, 2003*	Rape growth => lower NP residues (26% → 13%, 18% → 8.3% [a]) / 30d. [but for spiked instead of naturally occurring NP => no effect]
	Roberts et al., 2006	Presence of maize had a significant negative impact in the mineralization of NP (10% in comparison with 14% / 23 d.) only in one of two soil types tested.
Water saturation	*Gejlsbjerg et al., 2001*	[40 % WHC → 80% WHC] => lower mineralization rates (63% → 56%, 58% → 44% / 2 months) [note: water was possibly evaporated during incubation]
NP type / origin / localization / concentration	*Mortensen and Kure, 2003*	Much faster degradation for spiked linear NP as "naturally" occurring NP (residues 16% → 1%, 36% → 3% / 30 d.). However NP concentration was higher in the first mixtures (428 μg/kg ~ 129 μg/kg dw)
	Petersen et al., 2003	NP degradation was faster when it was spiked in the soil rather than in sludge
	Roberts et al., 2006	NP mineralization as a proportion of added NP decreased with NP concentration (difference up to 37% in 60 d. for the levels of 1 mg/kg and 1000 mg/kg), but mineralization rates in mg NP /g x h were greatest at the highest NP concentrations (maximum difference: 0.1 against 12 mg /g x h, for the same concentrations).
Soil type	*Gejlsbjerg et al., 2001*	Not significant (for final mineralization after 2 months) (tested: coarse sandy, sandy, more clayey)

	Hesselsoe et al., 2001	Comparing a coarse sandy soil and a sterilized quartz sand, mineralization and binding rates of NP were higher in the latter: Mineralization / 38 d.: 48% → 59%, 58% → 70%, Bound residues / 38 d. (as losses in mass balance): 5% → 18%, 2% → 11%
	Roberts et al., 2006	NP leaching was higher in a sandy than in a loamy soil (0.5% → 4% leached after pouring 20 soil saturation volumes of water)
	Gomez-Rico et al., 2008	The soils with the highest pH and organic matter contents showed the highest NP mineralization rates (% per month): limestone: 0.22% ± 0.08%, marl: 0.24 ± 0.12%, sandstone: 0.11 ± 0.02%
Sludge presence	*Dubroca et al., 2005*	Total mineralization increased (31% → 55% / 96d.) and bound residues decreased (40% → 22% / 96d.) after sludge amendment. [However author contends the opposite effect for the $t_{1/2}$ of the process]
	Telscher et al., 2005	Mineralization and binding increased by low amendments of sludge, but not for higher amounts (1% sludge amendment resulted in increases for mineralization: 20% → 30% and bound residues: 43% → 49%).
	Roberts et al., 2006	NP mineralization was stimulated by the presence of biosolids (μg NP / kg / d: 19 → 27, 3 → 15, 22 → 30)
	Qiao et al., 2008	Sludge amendment inhibited NP degradation in soil
Presence of organic / inorganic amendments	*Roberts et al., 2006*	Presence of glucose, glutamate (artificial root exudates), NPK fertilizer and dead maize roots enhanced the mineralization of NP after 120 d. of incubation, although at the first stage (20 d.) root exudates and nutrients suppressed it (maximum increase: 19% → 37% in 120 d., for combined exudates and nutrients).
Sludge / soil ratio	*Gejlsbjerg et al., 2001*	Not significant difference in final mineralization after 2 months (tested ratios, dw: 1/20, 1/100)
	Telscher et al., 2005	After 135d., mineralization and bound residues formation were the highest for the lowest sludge amendment: Mineralization/Bound residues: 30%/49% for 1% ratio, in comparison with ca. 20%/30% for both ratios of 10% and 50%. A sludge / soil ratio of 10% resulted in comparison with a ratio of 50% to faster degradation (91% ~ 82%) and higher production of a degradation product [4-(3,5-dimethyl-3-heptyl)-2-nitrophenol, 29% ~ 39%]. Volatilization was the lowest for the highest sludge amendment (2% in comparison with 5-6% in the other cases).
Sludge amendment method	*Hesselsoe et al., 2001*	Homogenous mixture: faster mineralization (48-70% / 38d.) in comparison with non homogenous (40-76% / ~120d.)
Sludge origin	*Dubroca et al., 2005*	There was some difference when comparing results for two sludges (one from a plant which receipts urban and industrial and another which receipts only urban wastewater) (mineralized, extractable, bound residues) = (55% → 36%, 5% → 8%, 22% → 45%) (for urban & industrial and urban respectively, after 96 d. of incubation).
Sludge stabilisation method (different plants)	*Hesselsoe et al., 2001*	NP was mineralized slightly faster and formed slightly lower bound residues when spiked in an anaerobically digested sludge (DM: 23%, Organic Matter – OM: 49% of DM) as in an aerobically digested one (DM: 14%, Organic Matter – OM: 68% of DM) which later amended on soil: Mineralization: 48% → 58%, 59% → 70%, Bound residues (as mass balance losses): 5% → 2%, 18% → 11% /

		38 d.
	La Guardia et al., 2001	Leaching potential (test procedure) : Lime: 1.2%, Composts: 1.8-2.3%
	Mortensen and Kure, 2003	Higher residues (NP + bound residues) for compost than aerobic / anaerobic sludge (16% → 30%, 0.9% → 2.6%) / 30d. (however not equal initial concentrations)
	Xia and Jeong, 2004	No significant difference in NP degradation / 30 d. (tested: dewatered in comparison with a dewatered + composted sludge)

[a] Values separated with commas refer to different systems tested (e.g. different soil types, different conditions etc.)

II. MATERIALS AND METHODS

5. Materials and methods

5.1 Synthesis of radioolabeled Nonylphenol

5.1.1 Materials and equipment

The materials used for the synthesis of radiolabeled NP were the following:

- 3,5-Dimethyl-3-heptanol:	99%, ABCR GmbH & Co KG (Karlsruhe, Germany)
- [U-14C]-phenol:	22.2 GBq/mmol, 370 MBq/mL (ethanol solution), radiochemical purity ≥ 97%, Hartmann Analytic Braunschweig, Germany,
- Phenol:	99.5%, Fluka (NeuUlm, Germany)
- Boron trifluoride diethyl etherate:	Sigma-Aldrich (Steinheim, Germany)
- Petroleum ether:	analytical grade, boiling point 60-80^{o}C, Acros Organics (New Jersey, USA)
- Ethanol absolute:	Molecular biology grade, Applichem GmbH (Darmstadt, Germany)
- Acetonitrile HPLC grade:	> 99.9%, Rotisolv HPLC grade, Carl Roth (Karlsruhe, Germany)
- Sodium sulfate:	>99%, Riedel-de Haën (Hanover, Germany)
- Scintillation vials:	Pocto vials (6 mL) and Super Polyethylene vials (20 mL), Packard Bioscience B.V. (Groningen, Netherlands)
- LSC scintillation cocktail:	LumaSafe Plus, Lumac LSC (Groningen, Netherlands)
- HPLC scintillation cocktail:	Quicksafe Flow 2, Zinsser Analytic GmbH (Frankfurt, Germany)

The equipment used was the following:

- Mass balance:	SBA 31, Scaltec Instruments GmbH (Heiligenstadt, Germany)
- Thermostated Water bath / stirring device:	IKA Labortechnik (thermostat: ETS-D4-fuzzy) (Staufen, Germany)
- Rotavapor:	Janke and Kunkel, IKA Labortechnik
- Liquid Scintillation Counter (LSC):	Beckman Coulter LS 6500 (Harbor Boulevard, USA)
- Gas Chromatograph – Mass Spectrometer (GC-MS):	HP 5890 Series II GC coupled with an HP 5971A mass selective detector (Hewlett Packard, Palo Alto, CA, USA)

	with an Electron Ionization (EI) source. The column was an FS-Supreme-5 silica capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 mm (Chromatographie Service, Langerwehe, Germany)
- HPLC with radiodetection:	1100 series (Agilent/Hewlett Packard, Böblingen, Germany), equipped with autosampler, degasser, and diode array detector, and coupled to an online liquid scintillation flow radiodetector, consisted of an LS-pump and a Ramona radiodetection unit (Raytest Isotopenmessgeräte GmbH, Straubenhardt, Germany) with a cell volume of 1300 µL. The column was a Nucleosil C18 reverse phase column (250 mm x 4 mm, Macherey Nagel, Düren, Germany). The pre-column was a 20 mm x 4 mm, with the same material as the main column (Macherey Nagel).
- Water purification system:	Milli-Q Water System (Millipore, Schwalbach/Ts. Germany) with 0.22 µm membrane filter

5.1.2 Methods

Synthesis of [ring-U-14C]-p353-nonylphenol (*NP)

The synthesis is a Friedel-Crafts alkylation reaction (Fig. 11) and it was performed according to Vinken et al. (2002). The apparatus consisted of a 25 mL two-necked reaction flask with a magnet for stirring, a reflux condenser, and a drying tube containing potassium hydroxide. The apparatus was in the beginning dried with a high temperature drier (as water deactivates the catalyst), while the water bath was set at 50^{o}C. After the apparatus cooled down to room temperature, the reactants were added in this sequence: 0.0378 g 3,5-Dimethyl-3-heptanol, 0.499 mL Phenol 25 mg/mL in petroleum ether, and 1.266 mL [U-14C]-phenol solution (concentration ca. 1.1 mg / mL). Afterwards, 199 µL of the catalyst BF_3 were added and the apparatus was brought into the water bath, where it was left under magnetic stirring.

After 55 min, 3 mL of ultrapure water (of 4 ^{o}C) were added into the flask, to stop the reaction, while the apparatus was removed out of the thermostated water bath. The mixture was then remained under stirring for 15 min, after which it was transferred to a separatory funnel, with the help of 3 mL of additionally added solvent. To the separatory funnel 2 x 0.5 mL of petroleum ether for rinsing the reaction flask were also transferred. The aqueous phase was removed and the radioactivity amount contained was determined by LSC (by sampling 20 µL aliquots). After this step, sequential liquid-liquid extractions with 3 mL water were performed, until the radioactivity of the aqueous phase was less than 1% of the radioactivity removed with the first extraction step. The petroleum ether phase was transferred, along with 2 x 1 mL rinsing of the flask, to a conical flask, where it was dried above sodium sulfate for ca. 1 h. The dried organic phase was finally transferred

to a pre-weighted pear-shaped flask and was concentrated until dryness (pressure down to 86 mbar). The flask was weighted again to determine the amount of the produced substance.

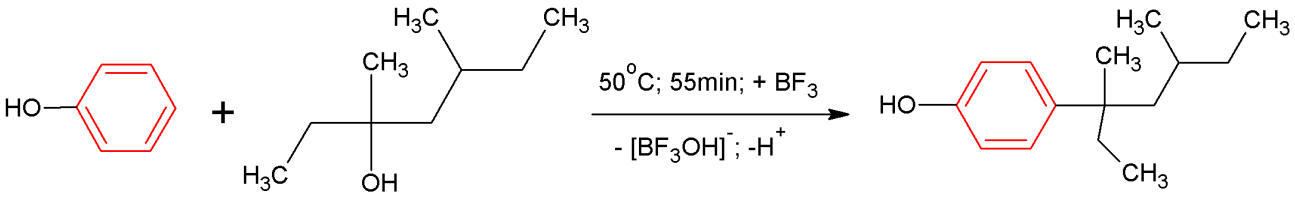


Figure 11. Friedel-Crafts alkylation reaction for the synthesis of [ring-U-14C]-p353-nonylphenol.

Determination of the purity of the produced NP

The (liquid) product of the reaction was diluted with absolute ethanol and respective aliquots were analyzed by HPLC-radiodetection (corresponding to ca. 200000 dpm, i.e. 3.3 kBq) and GC-MS (corresponding to ca. 0.05 μg), to determine its radiochemical and chemical purity accordingly.

Liquid scintillation counting

The aliquots to be measured for radioactivity were transferred to liquid scintillation vials, and mixed with adequate volume of scintillation cocktail LumaSafe (for organic aliquots at least with an equal amount of cocktail, and for aqueous ones at least with 10 times the same amount). The number of disintegrations of radioactive atoms per minute was determined through measuring the produced fluorescent light. Automatic correction for quenching was included (accounting for light adsorbed by the matrix of the measured aliquot), while the chemiluminescence (i.e. the part of the light not corresponding to disintegrations), was always tracked, to ensure that it was less than 1%. The measurements' duration was 3 or 10 min.

HPLC analysis

The aliquots (20 to 100 μL) were eluted with a gradient of water (A) and acetonitrile (B) as follows: 25% B with linear gradient to 90% B during 22 minutes. Finally, isocratic for 5 minutes, before the system returns to its initial conditions (25% B) within 10 minutes, where it was kept for 3 minutes. The UV signals were measured at 280 and 294 nm.

GC-MS analysis

The injector temperature was 250 °C, and the detector temperature was set at 280 °C. The oven program was 5 min at 50 °C and 10 °C/min to 280 °C (5 min). As carrier gas, He was used.

5.2 Preliminary experiments (methods development)

5.2.1 Materials and equipment

The materials used for this part of the work were, apart from those mentioned in 5.1.1, the following:

- Sodium hydroxide: Per analysis, pellets, Riedel-de Haën
- Ferric chloride: >97.0%, anhydrous, Fluka
- Calcium oxide: 98%, Aldrich
- Cationic polymer: NEROLAN CE 679 S, Nerolan Wassertechnik GmbH (Krefeld, Germany), a donation from Soers WWTP
- Tween-80: (Polyoxoethylate-Sorbitan-Monooleat), Aldrich
- Acetic acid: 100%, glacial, anhydrous, pro analysi, Merck (Darmstadt, Germany)
- Organic solvents: Available in raw-quality, were dried and distilled in the lab
- Soil: 2 mm-sieved standard soil, loamy sand (clay: 7.9%, silt: 14.6%, sand: 77.5%), organic C: 2.3%, pH: 5.7, WHC: 48 g/100 g, LUFA Speyer (Speyer, Germany)
- Sludge: Liquid mesophilic-anaerobically digested and thickened sludge (DM: 2.4%), sampled from Soers municipal WWTP in Aachen, Germany, in the morning of 11.07.2005
- Oxidizer LSC cocktail: Carbomax Plus, Canberra-Packard (Frankfurt, Germany)
- Paper cones for combustion: Combusto cone flexible-1000, PerkinElmer (Massachusetts, USA)

The additional (to that mentioned in the previous chapter) equipment used was the following:

- Shaker: 3017, GFL (Burgwedel, Germany)
- Centrifuge: 2K15, Sigma Laborzentrifugen GmbH (Osterrode, Germany)
- Ultrasonics device: Transsonic 460, Elma (Singen, Germany)
- Soxhlet thermostat: PILZ-Heizhauben, Isopad-GmbH (Heidelberg, Germany)
- Syringe: B-BRAUN (Melsungen, Germany)
- Oxidizer: Biological Oxidizer OX500, R.J. Harvey Instrument Corporation (Tappan, USA)
- Drying oven: Salvis KVTS 11, AG Emmrbrücke (Lucerne, Switzerland)
- Vortex: ZX3, VELP Scientifica (Milan, Italy)

5.2.2 Methods

Soil handling

Soil was received with a water content equal to 25% of its WHC. During the period of the present study (about 3 years), it was kept at 4^{o}C, in the plastic bag where it was delivered.

Sludge handling

Sampled sludges were stored at plastic 2 L containers in room temperature for a period up to 17 days, to prevent a fast further precipitation and aggregation of their dry matter.

Combustion

Dry samples up to 0.3 g of the respective solid material were packed in conical cellulose paper and combusted in the Oxidizer for 3 min. The resulting $^{14}CO_2$ was trapped in a scintillation vial containing Carbomax Plus cocktail and subjected to LSC. The recovery of the combustion process was determined by oxidizing paper cones filled with cellulose, spiked with known amounts of standard radioactive solution. Correction according to blank samples was also performed. The final corrected values for the radioactivity obtained after combustion were calculated according to the formula:

$$FV = (MV - B) \times \frac{S}{R - B} \quad ,$$

where FV is the final (corrected) radioactivity value, MV is the measured value, B the average blank value, S the average radioactivity spiked in cellulose containing paper cones, and R the value measured for the spiked cellulose after combustion.

Determination of the dry matter of soil / sludge

DM of soil was determined by placing it in the drying oven at 105°C until constant mass. The DM of sludges was determined either by the same method, or, in some cases of sludges dewatered in the lab, indirectly, on the basis of the mass of water losses during treatment.

5.3 Incubation of sludge-amended soils – homogenous spiking

5.3.1 Materials and equipment

Additional materials used for this experimental set were the following:

- Sludge:	Liquid mesophilic-anaerobically digested and thickened sludge (DM: 2.4%), sampled from Soers municipal WWTP in Aachen, Germany, in the morning of 19.08.2005
- Ethylene glycol:	99.5%, Riedel-de Haën
- Calcium chloride:	Calcium chloride dehydrate, pro analysi, Merck
- Hydrochloric acid:	37%, fuming, Merck
- Centrifuge tubes:	Centrifuge bottle assemblies from polyallomer, Beckman (Palo Alto, USA)
- Paper filters:	MN615, 110mm, Macherey-Nagel (Düren, Germany)

The pumps connected to the flow-through systems described in the experiments below were peristaltic pumps made by Ismatec (Wertheim-Mondfeld, Germany).

5.3.2 Methods

Sludge treatment

Sludge was conditioned using four various treatments, i.e. freeze-thawing (freezing at -21 °C overnight), addition of $FeCl_3$ (15% / DM), addition of $Ca(OH)_2$ and $FeCl_3$ (50% and 15% / DM), and addition of a polyacrylamide-based cationic polymer (1.0% / DM), respectively. The chemicals for conditioning were added in the form of aqueous solutions and correspond to maximal doses applied in practice (Ivashechkin et al., 2004). Using high doses of conditioning agents in this investigative experiment would highlight possible correlations between sludge treatment and NP fate in soil. The aliquots were homogenized by shaking for 30 min at 180 rpm. The four conditioned sludges plus one non-conditioned were dewatered by centrifugation in centrifuge tubes (3,500 g, 60 min).

Organic matter and nutrients determination in sludges and in soil

Organic matter, total N and NH_4^+-N were determined in single aliquots of sludges (liquid and treated, in parallel with the spiked ones) and the soil, by ISA (Institut für Siedlungswasserwirtschaft), according to the following protocols: Volatile Solids (indicator of OM): DIN EN 12880, Total-N: DIN 38409-H 28, NH_4^+-N: DIN 38406-E 5.

Soil amendment and spiking

Three 1.5 g (wet mass, WM) aliquots of each dewatered sludge and non-dewatered sludge (as control) were amended to 13 g soil samples. Sludge aliquots were delivered in a think coil form by a plastic syringe, as described in paragraph 7.3.6. The eighteen sludge-soil systems were spiked with *NP at 0.16 MBq (ca. 10^7 dpm), corresponding to 15 mg/kg of amended soil (on dw basis). Spiking of sludge-amended soil was performed by adding 4 mL aqueous solution spiked with 150 µL of *NP solution in ethanol. Spiking corresponded to an irrigation of the soil until 90% WHC (100% WHC for the liquid sludge). The spiked soil-sludge mixture was homogenized by stirring before incubation.

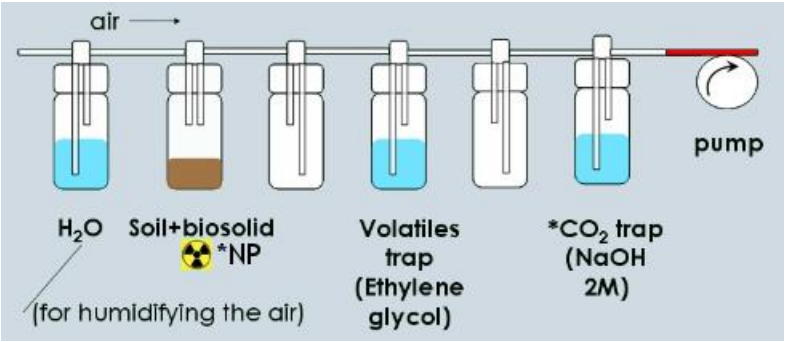


Figure 12. Flow-through system for monitoring the fate of radiolabeled NP in soil-sludge mixtures.

Incubation of sludge-amended soils

The sludge-amended soils were incubated over a period of 95 days at 20 °C in flow-through systems in triplicate series (Fig. 12). During the incubation, the mineralization of labeled NP was determined by trapping the $^{14}CO_2$ in flasks containing 2M NaOH. 0.5 mL aliquots from the NaOH solution were sampled regularly for radioactivity measurement using LSC. Volatilized compounds were trapped in ethylene glycol traps.

Dry matter determination of incubated soils

The dry mass of the wet soil-sludge aliquots after incubation was determined before n-hexane extraction, after evaporating remaining acetone under a stream of N_2. Although acetone dissolves also part of the organic matter, the error induced by determining the dry mass by this method had been measured to be negligible, during preliminary experiments (data not shown).

pH measurement of incubated soils

The pH of the soils in water and $CaCl_2$ 0.21M was measured. At first the collected samples (ca. 1 g soil in ~12 mL glass vials) were centrifuged (850 rpm for 5 min), to allow particles having stacked on the walls to precipitate together with the bulk mass of the sample. Then the necessary amount of water was added (soil : water = 1:5, WM) and the mixture was shaken well and centrifuged, to accelerate the precipitation of soil particles. The pH of a drop of the transparent supernatant was measured by a pH-indicator paper of an appropriate pH range. By adding concentrated $CaCl_2$ solution to the soil-water mixture and repeating the same procedure (mixing and centrifugation), the pH in $CaCl_2$ was afterward determined for the same sample (protocol according to Rayment and Higginson, 1992).

Soil extraction and fractionation

After homogenization, 5 g aliquots from the incubated sludge-amended soils were extracted by a sequential extraction and shaking (180 rpm). The solvents used were acetone (25 mL, 5 min), n-hexane (25 mL, 40 min), methanol (25 mL, 40 min) and NaOH 0.1 M (twice, 25 mL, 40 min). After each extraction step the slurries were centrifuged (3,500 g, 10 min) and the radioactivity was determined in 1 mL from the supernatants using LSC before pooling the organic extracts and alkali solutions separately. Both masses of sampled aliquots and the total mass of the removed supernatants were determined, for calculating the exact extracted amount of radioactivity, respectively. The NaOH extracts were later extracted by n-hexane to obtain the physically bound NP residues. The remaining NaOH phase was further fractionated by acidifying to pH<1 with HCl 3 M to humic acids (HA, precipitated) and fulvic acids (FA, dissolved). The radioactivity of the supernatant containing the fulvic acids fractions was measured by LSC. The extracted soils after sequential extraction were dried before taking samples for determining the radioactive residues bound to humin and minerals. On this stage the soil consisted of two fractions (“humin” and “mineral” fraction, as described in paragraph 7.3.3). The two fractions were separated manually and sampling (0.2-0.3 g) was performed. Catalytic combustion and subsequent LSC measurement were carried out for the determination of the residues bound to these fractions. The recovery of NP from freshly spiked soil-sludge mixtures (otherwise identical to this incubation experiment) was determined in a preliminary test to be 98.8–99.2%.

Another 2 g aliquot from the incubated systems was subjected to a modified method of the U.S. EPA TCLP procedure (as described in paragraph 8.2.4). The leachate extract was later extracted by n-hexane for further analysis.

Extracts processing and analysis

The extracts from soil samples were filtered through Na_2SO_4, concentrated by a Rotary Evaporator and analyzed by HPLC coupled to radiodetection.

Mass balance – Expression of fraction values

Radioactivity values are hereafter given normalized, i.e. expressed in percent of the radioactivity recovered in each system (sum of volatilized, mineralized NP, extractable and bound residues fractions). Data about the total radioactivity values recovered are given in the paragraph 9.2.9.

5.4 Incubation of sludge-amended soils – spiking on sludge

5.4.1 Materials and equipment

The sludge used for this experimental series was liquid mesophilic-anaerobically digested and thickened sludge (DM: 4.8%), sampled from Soers municipal WWTP in Aachen, in the morning of 02.05.06. The sludge was stored in an 1L glass container.

Concentrated polymer solution (the same as used for the experiment described in the previous chapter) was also given by Soers WWTP.

The high performance centrifuge used was a J-21C centrifuge by Beckman (Munich, Germany).

5.4.2 Methods

Spiking and treatment of sewage sludge

Aliquots from the sampled liquid digested and thickened sludge (24 g each) were poured in glass centrifugation tubes and spiked with 230–239 µg of [ring-U-14C]-p353-nonylphenol using approximately 150 µL of a concentrated solution in ethanol. The liquid sludges were then conditioned by means of four different methods. Three chemical treatments (percentage expressed in relation to the dry matter of sludge), i.e. 8% $FeCl_3$, 8% $FeCl_3$ + 26% $Ca(OH)_2$, and 0.5% cationic polymer, and one physical treatment (freeze-thaw: freezing at -21 ^{o}C for 48 h, thawing at room temperature) were used. The chemical conditioning doses correspond to average doses used in WWTPs (Ivashechkin et al., 2004). Mixing with the conditioning agents, which were added in the form of aqueous solutions ($FeCl_3$, 10%; $Ca(OH)_2$, 20% and polymer, 0.7% by weight), was performed under shaking for 30 min at 180 rpm. The conditioned sludges were centrifuged for 60 min at 16,000 g by a high performance centrifuge device. After centrifugation, the radioactivity was determined by means of LSC in 1mL aliquots of the supernatants prior to the removal of the liquid phase. The expected amount of losses of NP through the sludge processing was determined during a preliminary test carried out under strictly identical conditions. The exact amount of NP spiking was based on these results, in order to obtain dewatered sludges containing the same final amount of NP. During an additional preliminary test, the possibility of an early transformation of NP during liming (pH of limed liquid sludge: 11) was investigated by extracting treated sludge and analyzing the extract by HPLC. Each spiking/treatment

protocol was applied in triplicate. Three further sludge samples after spiking were dewatered without any prior conditioning (conditioning control). Finally, three smaller aliquots (2.5 g) were spiked, but not dewatered (dewatering control).

Amending of sludge to soil and incubation

Soil samples (13 g each) were poured in glass tubes, irrigated with 1ml of water and incubated in the dark under thermostated (20 ^{o}C) and continuous aeration (with air saturated in water) conditions in flow-through systems for one week. Afterwards, the radioactive sludges were amended gradually on wet soil samples and the mixtures were homogenized with a spatula. One milliliter of water was added again to each system (except the soils amended with liquid sludge) and homogenization was repeated. The humidity of the soil-sludge mixtures was set to 65% and 73% of their WHC for dewatered and liquid sludge amendments, respectively. At the end, a slight pressure was exerted on the top of the sludge-amended soils, in order to produce similar oxygen penetration profiles for all soils. The amounts of radioactivity lost during the amending procedure (residues on centrifugation tubes, spatula and flattening tool) were determined by using adequate protocols (extraction with ethanol, catalytic combustion and LSC). The soil-sludge mixtures were connected again to the flow-through systems, where they were incubated for 87 d.

Determining the fate of NP in sludge-amended soils

Mineralization of NP: During incubation, the mineralization course of radioactive NP was determined by trapping the evolved CO_2 in flasks containing 50 mL 2 M NaOH. One milliliter aliquots from the NaOH solution were sampled at various time points of incubation for measurement of radioactivity assignable to $^{14}CO_2$ (LSC).

Extractable residues of NP and degradation products: After homogenization, 5 g wet aliquots from the incubated sludge-amended soils were extracted sequentially with acetone (25 mL, 5 min shaking at 180 rpm), n-hexane (25 mL, 40 min shaking), methanol (25 mL, 40 min shaking), NaOH 0.1 M (twice, 25 mL, 40 min shaking), and methanol (25 mL, vortex shaking for 15 s). After each extraction step the slurries were centrifuged (3500 g, 10 min) and the radioactivity was determined in 1 mL of the supernatants using LSC before pooling the organic extracts and alkali solutions separately. The organic extracts were first filtered under vacuum through 15 g of Na_2SO_4 (over glass wool) and then through a paper filter. Afterwards they were concentrated in a rotary evaporator (40 ^{o}C, 100 mbar). The concentration of NP and degradation products was determined in the concentrated extracts using HPLC coupled to a radiodetector. The NaOH extracts were used for the analyses of bound residues (described below).

Leaching potential of NP and degradation products: Two grams of aliquots from the incubated sludge-amended soil were subjected to leaching test described in paragraph 8.2.4. The leachate extract was later extracted by means of liquid–liquid extraction with ethyl acetate (30 mL, 5 min shaking at 80 rpm). After centrifugation (3500 g, 5 min), the two phases were separated. The ethyl acetate phase was further processed using filtration through 15 g Na_2SO_4, concentration in a rotary evaporator, drying under gentle stream of N_2, and resuspension to 300 mL of methanol, followed by

HPLC analyses.

Bound residues: The NaOH extracts were firstly extracted using ethyl acetate (twice, 25 mL, shaking for 5 min at 80 rpm), to obtain the physically bound (entrapped) NP residues. The radioactivity of the organic ethyl acetate phase was measured by means of LSC (1 mL aliquot). The remaining NaOH phase was further fractionated by acidifying to pH < 1 with HCl 3 M, in order to separate precipitated HA from the soluble FA. The radioactivity of the supernatant containing the fulvic acids fractions was measured by LSC. After sequential extraction the soils were air dried before taking samples to determine the radioactive residues bound to humin and minerals. At this stage the soil consisted of two fractions ("humin" and "mineral" fractions, as defined in paragraph 8.2.3), which were separated manually and 0.2–0.3 g aliquots were analyzed by catalytic combustion and subsequent LSC measurement.

Other methods: GC-MS and HPLC analysis were performed as described in paragraph 5.1.2. OM, nutrients and DM were determined according to the protocols described in paragraph 5.3.2.

5.5 Microbial profiling

5.5.1 Materials and equipment

Materials used for the purposes of microbial profiling and not mentioned in the previous sections were the following:

- UltraClean™ Soil DNA Isolation Kit:	MOBIO Laboratories Inc. (Carlsbad, USA)
- FastDNA® SPIN Kit for Soil:	Q-BIOgene (Irvine, USA)
- Red'y'star mix:	Eurogentec (Seraing, Belgium)
- DNA primers U968, L1401:	MWG Biotech (Ebersberg, Germany)
- PCR water:	Molecular biology grade, Eppendorf (Esseling-Berzdorf, Germany)
- Ethidium bromide:	Sigma
- Acrylamide solution:	Stabilized aqueous solution of 40% acrylamide and N,N-Methylenbisacrylamide, Rotiphorese® Gel 40 (37, 5:1)
- TAE buffer:	Applichem
- Deionized formamide:	>99.5%, Roth
- Agarose:	Molecular biology grade, Eurogentec

The following equipment was used:

- Microcentrifuge:	Mikro 20, Hettich (Tuttlingen, Germany)
- PCR cycler:	Mastercycler personal, Eppendorf

- DGGE system: DCode Universal Mutation Detection System, Biorad (California, USA)
- Scanner: ScanMaker i900, Microtek (Husinchu, Taiwan)
- GelCompar software: GelCompar II, Applied Maths (Austin, USA)

5.5.2 Methods

Sampling and storage of soil aliquots

About 1 g of each of the incubated soil-sludge mixtures was sampled, after homogenization, with a metallic spatula, transported to sterilized Eppendorf 1.5 – 2 mL vials, and stored directly at -21°C, until DNA extraction. The spatula was cleaned with a tissue with ethanol before each sampling.

DNA extraction

After trials using the UltraClean™ Soil DNA Isolation Kit, which produced extracts not pure enough to perform successful PCR analysis (due to inhibitor components), the FastDNA® SPIN Kit for Soil was applied. According to the general protocol followed, 0.16 – 0.36 g of thawed soil were first mixed with a matrix containing cell lysis material (ceramic and silica particles plus a DNA stabilizing and nuclease inactivating solution) and buffers enabling sample homogenization and protein solubilization (Modified Tyrode's [MT] buffer plus sodium phosphate buffer). The mixtures were shaken at the maximum vortex speed (for 10 min horizontally and for 10 min vertically). Afterwards (centrifugation and removal of the supernatant were involved between subsequent steps) a protein precipitating solution was added. The protein-free solution was treated with a DNA-binding matrix, on which DNA was later purified by the use of a GENECLEAN® procedure, by means of a salt ethanol washing solution. The purified DNA, after being eluted from the binding matrix, was stored in the form of 50 µL of ultrapure water solution at 4°C for short time, or at -21°C for longer intervals.

According to the manufacturer of the kit, it enables the extraction of genomic DNA from all bacteria (as well as fungi, plants and animals) in a soil community, including historically difficult sources, such as eubacterial spores and endospores, and gram-positive bacteria.

Polymerase Chain Reaction (PCR) amplification

The 16S ribosomal DNA genes are present in all bacteria. These genes contain both conserved regions, which allow primer design for PCR amplification, and variable regions, which can be used to distinguish species from each other (Li et al., 2006). Amplification of 16S rDNA from the purified DNA extract was performed on a PCR system, under a mixture containing: 25 µL Red'y'star mix (containing HotStart activated Taq DNA polymerase [GoldStar DNA polymerase], dNTP 200 µM (final concentration), $MgCl_2$ 1.5 mM (final concentration), 2x buffer and red loading dye), 3 µL of $MgCl_2$ 25 mM, 1 µL of forward primer U968 (AAC GCG AAG AAC CTT AC), 1 µL of reverse primer L1401 (GCG TGT GTA CAA GAC CC), 1 µL of DNA template and 19 µL of PCR water. The DNA template originated from the DNA extracts after a dilution by an optimized factor of 1/500. PCR amplification was performed at 95 °C for 10 min, followed by 35 thermal cycles of 94 °C for 1 min, 54 °C for 1 min, and

72 °C for 1 min, and final single extension at 72 °C for 10 min. The size of the PCR product was visualized by electrophoresis in 1% agarose gels after ethidium bromide staining. The presence of clear bands of approximately 450 bp was verified. Blank samples (i), as well as standard DNA and - where needed - internal DNA standard in soil DNA extracts (ii), were amplified in parallel, as controls for false positive (i) and false negative results (ii).

Denaturing Gradient Gel Electrophoresis (DGGE)

Samples of PCR product (10 μL) were loaded together with 10 μL of green loading dye onto 6% (w/v) polyacrylamide gels in 1 x TAE (Tris-Acetate-EDTA) buffer. The polyacrylamide gels were made with a linear denaturing gradient ranging from 40% denaturant at the top of the gel to 70% denaturant at the bottom (100% denaturant is defined as 7 M urea and 40% (v/v) deionized formamide). The electrophoresis was run at 50 V and 60 °C for 17 h (after a first run at 100 V for ca. 10 min, until the loaded samples migrate to the gel). After electrophoresis, the gels were stained with silver (using the following sequence of solutions: Buffer A: 10% ethanol, 0.5% acetic acid, Buffer B: 0.1% $AgNO_3$, Buffer C: 1.5% NaOH, Buffer D: 0.75% Na_2CO_3).

Clustering of DNA profiles

Clustering of the bacterial DNA profiles was performed according to the statistical algorithm of Ward, and was based on the Pearson similarity coefficient. These statistical methods were applied through the software GelCompar.

5.6 Investigation of the NP local concentration factor

5.6.1 Materials and equipment

The sludge used for this experiment was a liquid mesophilic-anaerobically digested and thickened sludge (DM: 1.8%), sampled from Soers municipal WWTP in Aachen, Germany, in the morning of 01.09.2007. Nevertheless, it was spiked and amended on soils 46 days after its sampling. On that time sludge was characterized by precipitated solids. Before its use it was homogenized by shaking.

5.6.2 Methods

The spiking and amending of sludge and the incubation of the amended soils were performed as described in 5.4, with some modifications, which were as follows in the next paragraphs.

For this experiment, two *NP spiking solutions were prepared. The first one (of low concentration) was prepared by diluting 0.5 mL of a stock solution of 0.69 mg/mL (with a specific radioactivity of 101579 dpm/μg) with 5 mL of absolute ethanol. The second (high concentration) was prepared by diluting 0.5 mL of the same stock solution with 5 mL of a solution of non-radioactive NP of the same concentration (0.69 mg/mL). As a result, the two solutions contained the same radioactivity amount, but the second one had 11 times higher amount of total NP.

Two liquid sludges aliquots of about 25-26 g each (wet mass - WM, ca. 11 times higher amount than this used in the previous incubation) were spiked with 1.65 mL of the low concentration NP

solution and two more with 1.65 mL of the high concentration one. Two sludges had thus the concentration of NP that the LQ sludges of the previous incubation had (per DM) and two sludges contained 11 times lower concentration of NP. The four sludges were amended on an equal amount of soil samples, of 143 g each (again 11 times higher amount than the previous incubation), which had been placed in glass containers with a diameter of 10 cm. The diameter of the containers was chosen so that the height of the soil-sludge mixtures will be the same as in the previous incubation (therein the diameter was 3 cm). Before their incubation, the systems were irrigated with 11 mL of water (previously 1 mL). The incubation lasted for 96 d.

The radioactivity of each system was roughly 10^7 dpm, exactly the same as previously, which allowed a sensitive follow-up of the mineralization process. Concerning soil analysis, an extraction of 11 times higher amounts was not feasible. In the end, approximately 15 g of each wet soil-sludge mixtures were extracted (previously ca. 5 g), using the same solvent volumes as before. During the determination of bound residues, HA, FA, and physically bound radioactivity was not measured separately. Moreover, the leaching potential of the spiked NP was this time not determined.

5.7 Characterization of NP fate mechanisms

5.7.1 Materials and equipment

Additional materials, used for the purposes of this experimental series, were the following:

- Sludge: Liquid mesophilic-anaerobically digested and thickened sludge (DM: 1.8%), sampled from Soers municipal WWTP in Aachen, Germany, in the morning of 01.09.2007. Nevertheless, it was spiked and amended on soils 12 days after its sampling, when also sterilized aliquots were available.
- NP: As synthesized by the author, and described in chapter 6 (specific radioactivity: 101579 dpm/μg)
- Vent filters: Millex-FG vent filter unit (PTFE Membrane), Millipore

The device used for the sterilisation of glassware was a 3870 ELV automatic sterilizer by Tuttnauer (Brenda, The Netherlands).

5.7.2 Methods

The procedures for the spiking, treatment, amendment and incubation of soils were as described in 5.4, with the differentiations mentioned in the next paragraphs.

Aliquots of soil and liquid sludge were placed in glass bottles of 25-50 mL and were sent to the installations of Beta-Gamma-Service GmbH & Co. KG (BGS, Wiehl, Germany), where they were irradiated by γ-radiation at a dose of at least 25 kGy. This method (γ-irradiation) has been found to provide a combination of effective sterilization, absence of toxic residues on soil, and moderate changes on the structure and the composition of soil (McLaren, 1969; McNamara et al., 2003). 103.5 μg of NP were spiked in the form of 150 μL of a concentrated solution of 0.69 mg/mL on sterilized

and non-sterilized liquid sludge aliquots of 2.5 g (to be amended as LQ) and of 40 g (to be centrifuged and then amended as CE). Centrifugation was performed in autoclaved polyallomer centrifuge tubes. The losses of NP during centrifugation were monitored. 13 g soil aliquots (sterilized and non-sterilized) were put on autoclaved glass tubes, before being irrigated with 1 mL of autoclaved ultrapure water and amended with sludges (LQ or CE). The glass tubes had been heated up for ca. 1 min with a high temperature hair dryer and be let to cool down just before bringing the soil samples inside, to ensure avoidance of any late contamination during the time elapsed since autoclavation (drying at 60^{o}C was performed after autoclavation). The homogenized mixtures were further irrigated by 1 mL and 0.3 mL of (autoclaved) water, for soils amended with CE and LQ respectively, i.e. until a humidity level of ca. 60% WHC. The tubes containing the soil-sludge mixtures were connected to the rest of the autoclaved flow-through apparatus. Autoclaved filters were connected to the air inlet. The filters were equipped with a membrane designed for sterile ventilation of autoclaved vessels, to avoid contamination of the systems from the flowing air. The sixteen systems (duplicates of the combinations shown in Table 8) were incubated for 70 d.

Table 8. Composition of the incubated mixtures (S: sterilized, N: non-sterilized, LQ: Liquid, CE: Centrifuged).

Abbreviation	A	B	C	D	E	F	G	H
Soil	***S***	***S***	***N***	***N***	***S***	***S***	***N***	***N***
Sludge	***LQ-S***	***LQ-N***	***LQ-S***	***LQ-N***	***CE-S***	***CE-N***	***CE-S***	***CE-N***

After the extraction of the incubated soils, and during the preparation of extracts, the concentrated extracts before being analyzed by HPLC were centrifuged by a microcentrifuge in Eppendorf vials at 9,168 g for 2 min, to allow insoluble aggregates formed precipitating. The losses of radioactivity during this centrifugation procedure were monitored.

The leaching potential of NP residues in the incubated soils was not determined.

5.8 Differentiation under extrinsic factors

5.8.1 Materials and equipment

Additional materials used included:

- Sludge: Liquid mesophilic-anaerobically digested and thickened sludge (DM: 2.18%), sampled from Soers municipal WWTP in Aachen, Germany.
- NP: NP available in the laboratory (specific radioactivity: 52981 dpm/μg)
- Grass seeds *Poa pratensis*

Regarding the equipment, high pressure sodium lamps were used for the irradiation, while the centrifuge used was the high performance centrifuge mentioned in paragraph 5.4.2.

5.8.2 Methods

The protocol followed was as in 5.4, with some modifications, as mentioned in the following text.

Polymer and freeze-thaw conditioning were applied. The cationic polymer was added to the sludge at a level of 0.85% (per DM, in comparison with 0.5% in the former incubation). The conditioned and dewatered sludges, together with an additional sludge, which was only dewatered, were mixed separately with 13 g of soil (after its irrigation with 1 mL of water) and the grass seeds were added to the mixture (30 seeds per assay). One additional mL of irrigation water was provided to each system (the total water content corresponded to ca. 65% of WHC, as in the former experiment). After the final irrigation, no mixing of the amended soil was performed. The incubation lasted 82 days, during which the light was being turned on for 8 h per day.

Regarding the analytical procedures followed, an additional determination of the radioactivity taken up by grass was performed. The leaves of grass were separated from roots and were ground separately using a homogenizer. The roots were cleaned up with water in order to remove soil particles. Leaves and roots were extracted using a solvent system consisting of dichloromethane/methanol/water (1:2:0.8). The radioactivity of the extracts was determined by LSC. After extraction, grass containing non-extractible residues of NP was combusted to determine the amount of the therein bound residues. Considering soil analysis, no fractionation of the NaOH extracts into physically-bound, HA and FA-bound radioactivity was carried out. Furthermore, no leaching potential test was performed.

III. RESULTS AND DISCUSSION

6. Synopsis of the experiments

The objectives of the current work were a) to identify the correlation between sewage sludge conditioning / dewatering processing and fate of Nonylphenol (as a contaminant of sludge) in sludge-amended soils and b) to provide, if possible, clues about the mechanism driving this correlation. Apart of providing supplementary information on the fate of a major pollutant found in sewage sludges around the world that may be included in future legislation controlling the quality of agricultural soil amendments, Abad et al., 2005), this study employs NP also as a model pollutant; as described in the Preface, in view is a contribution in predicting the fate of organic xenobiotics in the soil-sludge environment, by means of examining an until now not-studied, but nevertheless potentially determinant factor (the sludge conditioning/dewatering treatment).

As the NP commercially available – and also emitted to the environment - consists of numerous isomers, a practical problem in implementing such a study is that one would have to follow the fate of each of these isomers separately. As this setup would be technically very difficult, as well as time-consuming, a compromise was made, which was to focus on the fate of a single isomer of NP. p353-nonylphenol (4-[1-ethyl-1,3-dimethylpentyl] phenol, Fig. 13) was chosen, as being one of the most relevant isomers of the technical mixture of NP in terms of abundance and endocrine effect (Russ et al. 2005; Preuss et al. 2006). In fact, "p353-nonylphenol" includes four stereoisomers (two pairs of enantiomers), as there are two asymmetric centers in the molecule.

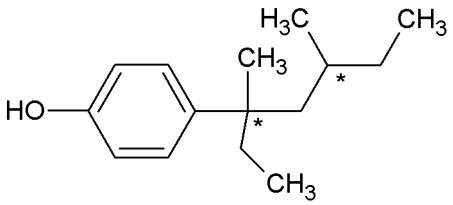


Figure 13. Structure of the selected isomer of 4-Nonylphenol: p353-nonylphenol. The two chiral centers (carbon atoms 3 and 5 of the nonyl chain) are shown with asterisks.

The selected isomer of NP was used in its radiolabeled form, for allowing a follow-up of all fate pathways (including mineralization to CO_2 and formation of bound residues), and facilitating the determination of its residues and the detection of its degradation products in the sludge-soil matrix. At the first experiments, [ring-U-14C]-p353-nonylphenol previously synthesized in the institute was used. New amounts of NP were synthesized by the author later, and were actually used for the last experiments performed, described in chapters 12 and 13. The *synthesis of NP* is described in the next chapter of the experimental part of the manuscript.

Setting NP synthesis aside, the first experimental cycle of the study included *preliminary experiments*, namely the development of methods necessary for the main incubation experiments. In this frame, firstly a procedure for the determination of the extractable residues of NP in soil was developed, as well as a method for evaluating the leaching potential of NP. The second was necessary, as a periodical irrigation or a collection of leachate were not fitting to the experimental setup of incubations (flow-through systems, see chapter 5). Moreover, an adequate spiking technique was developed, to avoid effects to soil microorganisms, for which the common use of organic solvents is often accused. Finally, the simulation of sludge conditioning and dewatering in the lab had to be tested. That's because treating the same precursor sludge was chosen as a considerably better strategy than sampling sludges treated by different methods from different WWTPs: the latter strategy would mean that compared sludges had also different initial composition.

Regarding the *incubations*, whereby NP fate would be investigated, a choice had to be made between water slurry and solid phase systems. In the end solid phase systems were selected as a more appropriate setup, as they resemble better the real soil systems. Nonetheless, one additional dilemma existed and it was related to the spiking of NP: adding the pollutant selectively on sludge would offer the advantage of a more realistic scenario, as the study concerns sludge-born pollutants. On the other hand, as soil and sludge aggregates normally do not form a homogeneous mixture, this strategy could result to a strong dependence of the fate from the – random – localization of sludge aggregates in the incubated soil-sludge mixture, for instance for some systems on the well aerated surface and for other at the bottom of soil columns. The decision was in this case to run two different incubations, one by spiking homogeneously the soil-sludge mixture, and one by spiking only the sludge. This even offers a special advantage: possible effects of sludge treatment on NP degrading microbial populations of the soil-sludge mixture (likely to monitor during both incubations) could be distinguished from effects due to the bioavailability of NP in sludge (possible to monitor only by spiking in sludge). Actually, spiking for the second set of incubations was particularly performed in liquid sludge, i.e. before the application of conditioning and dewatering treatments, for providing an even more realistic scenario. Therein, practical difficulties related to potential losses of NP during treatment and the transportation of sludge to the soil were resolved by applying adequate protocols. The two incubations sets are described in chapters 9 and 10.

The next series of experiments (described in chapter 11) was dedicated to the *microbial profiling* of the incubated soils. The DNA from stored incubated soil samples was extracted, and the bacterial fingerprint of soils fertilized with different sludges was produced by using molecular biology tools. Data regarding microbial populations in combination with information collected from the literature, about the influence sludge conditioning or dewatering may have on microbial populations in sludge, were used at this point, to assess the involvement of such phenomena in the observed correlation between sludge treatment and NP fate in soil.

The assumptions made in view of the results of the experiments carried out emphasized the need for *clarifying the role* of some specific factors, involved in the relation between sludge treatment and soil fate processes. Two experimental setups were therefore designed to answer the corresponding major questions, derived from the evaluation of the obtained results. These additional

incubation experiments are presented in chapters 12 and 13.

The experimental part of this work is completed with a further, but rather different incubation. This time, the influence of some of the sludge treatments studied is investigated in the presence of two additional extrinsic factors: light and plant growing on the amended soil. Results from the *soil-sludge-grass incubations* (chapter 14) could then be used as to preliminary test, whether the phenomena observed, driven by sludge treatment and affecting the fate of organic pollutants in soil, would be the same under the influence of those extrinsic factors. Here it is noticed that the respective experiment took actually place at a former stage of this work, in the frame of collaboration with a colleague (Karolina Nowak), who worked under a different AQUAbase project. With that project mainly targeting on studying the effect of plant growth on organics fate processes in soil, the corresponding experimental setup was designed to serve both studies. Nevertheless, because of the period this experiment was performed, it happened that the selection of sludge treatments did not allow testing the major effect observed later during the herein presented work.

7. Synthesis of radiolabeled Nonylphenol

7.1 Aims

The aim was to obtain adequate amount of the NP isomer *p*353-nonylphenol to perform the incubation experiments planned for the purposes of this study. The substance should be radiolabeled, for tracking the complete mass balance and a more feasible chemical analysis of the involved samples (for parent compound and degradation products). Uniform labeling was chosen with ^{14}C in the aromatic ring.

7.2 Results

The specific radioactivity of NP used in the experiments described in chapters 8, 9, 10 and 14 was determined as 52981 dpm/µg, and its radiochemical purity as 97%.

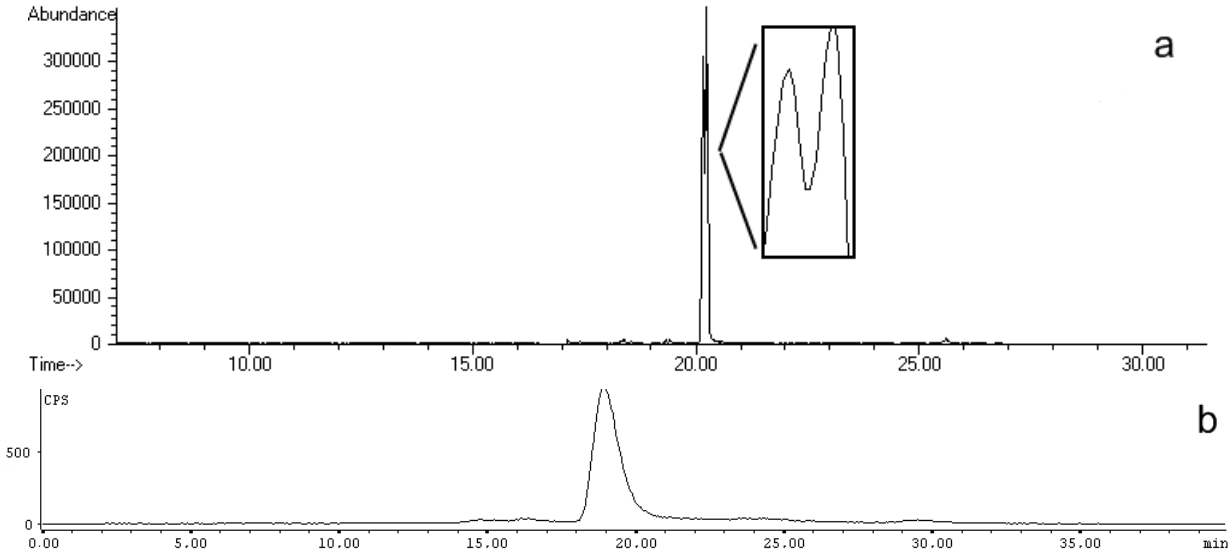


Figure 14. (a) GC-MS chromatogram and **(b)** HPLC-radiodetection chromatogram of the produced NP. In the detail in (a), the double peak, corresponding to diastereomers of the compound, is emphasized.

The product synthesized by the author following the exact protocol provided above was used for the experiments performed lastly, which are described in chapters 12 and 13. Its mass was determined as 13.8 mg and it had a yellowish color. The specific radioactivity was 101579 dpm/µg. Its chemical (Fig. 14a), as well as its radiochemical purity (Fig. 14b) were calculated from the respective chromatograms as 95%. The identity of p-353-Nonylphenol was certified by means of its EI mass spectrum (Fig. 15), which was successfully compared with the mass spectrum reported by Vinken et

al. (2002) for this compound (therein confirmed also by use of IR data).

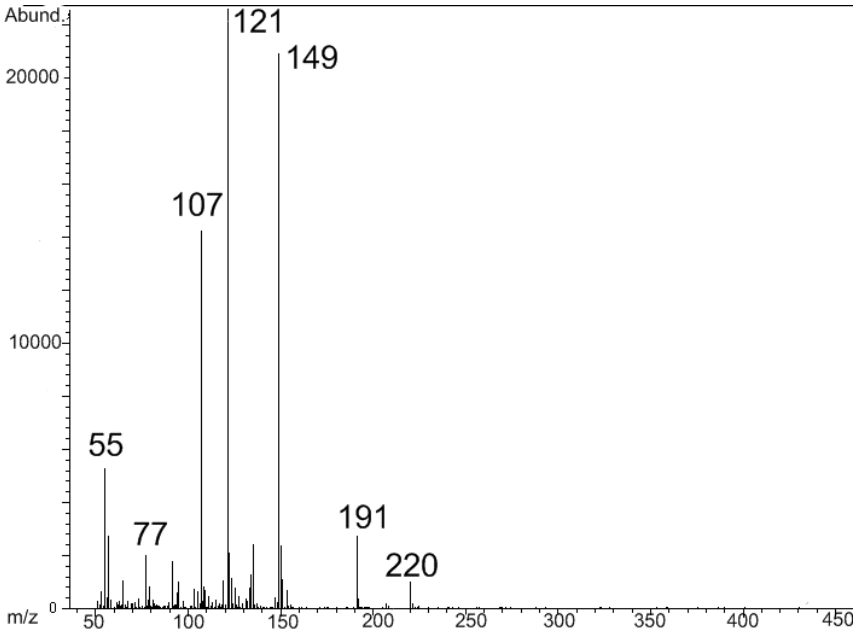


Figure 15. EI-mass spectrum of the produced NP.

The produced NP was diluted to 5 mL absolute ethanol and was stored at 4^{o}C for further use. Its specific radioactivity denotes that on average approximately 1 every 10 molecules contains a labeled carbon atom. For the substance used at the first experiments, about 1 every 20 NP molecules is labeled.

8. Preliminary experiments (methods development)

8.1 Aims

A protocol was developed for the determination of non-(covalently)bound residues of NP in soil-sludge mixtures. The ideal extraction technique should not alter the chemical structure of soil, and, as such, be able to extract as much as possible from the NP present in soil. The developed technique would also be used for the determination of extractable degradation products of NP in incubated sludge-soil mixtures.

Second aim of this experimental series was to establish an indicator for the leaching potential of NP (and degradation products) in soil. Leaching is an important fate pathway in the environment, though not easy to be taken into account in incubation experiments. Therefore such an indicator would be a useful complementary tool, in assessing the potential behavior of the organic pollutant in soils amended with differently treated sludges.

Spiking of a compound in soil is a critical step during any fate evaluation study, although it does not always receive the necessary attention. For the purposes of the herein presented study, and particularly for the incubation to involve spiking of soil-sludge mixtures, an effort was spent in order to develop an adequate, optimized protocol. The ideal spiking method should combine the following advantages: a) no side effects to microbial populations, b) homogenous distribution to the solid matrix, and c) accuracy in the spiked amount.

The last milestone during this preliminary experimental part was the implementation of sewage sludge treatment (conditioning and dewatering) in the lab. Before that, a selection had to be made for the treatment methods to be included, in fact consisting of commonly used techniques around the world.

*8.*2 Methods development

8.2.1 Soil selection

The soil chosen for the present study was a standard loamy sand. Because of its sandy texture, and not high organic matter content (2.3%), it can be considered as one of the representative types of soil, of which the consistence would be improved by sludge amendment. Moreover, its pH (5.7) was suitable for amendment of limed sludge; soils with pH lower than 5 or 5.5 are often strictly regulated for sludge amendment, due to the increased mobility of heavy metals, while a final pH above 6.5 may pose micronutrients deficiencies to the plants (see section 2.2). The selected soil originated from a meadow in Hanhofen (Rheinland-Pfalz, Germany), was sampled in March 2005, and it hadn't

received any fertilization since the year 2000.

8.2.2 Determination of NP extractable residues

As a first step in the search for a successful extraction protocol, a screening of various methods was made. For each extraction method tested, 5 g of soil were brought into 100 mL glass centrifugation tubes and spiked with *NP at ca. 0.75 mg/kg (dw) ♠. Spiking was performed in 5 equal doses of 15.7 mg/L ethanol solution, applied homogeneously on the surface. The spiked soil was then left at room temperature for 1.5 h. Extraction took place on a shaker device at a speed of ca. 60 rpm for predetermined time periods. After each extraction step the slurries were centrifuged (3'500 g, 10min) and the radioactivity was determined in 1 mL of the supernatants using LSC. The total volume of the supernatants was determined by a cylinder. The total radioactivity recovery was compared with the amount of radioactivity spiked, which had been determined by injecting the exact amount of *NP solution to LSC vials at the time of soil spiking procedure.

Table 9. Recoveries of spiked *NP from soil by applying different extraction techniques.

Extraction method	NP Recovery			Total NP recovery
	1st step	2nd step	next steps	
100 mL Tween-80, 10 g/L [3 h → 5 h]	68%	5%	-	73%
50 mL Tween-80, 20 g/L [30min → 1h → 2 h]	53%	5%	3%	61%
50 mL Tween-80, 50 g/L [30min → 1h → 2 h]	52%	4%	2%	58%
1) 3mL water + 0.5 g KCl + 25 mL CH_2Cl_2 [1 h] 2) 25ml CH_3OH [1 h]	42%	29%	-	71%
1) 25 mL n-hexane [1h] 2) 25 mL CH_3OH [1h]	64%	11%	-	75%
1) 25 mL acetone [30 min] 2) drying under N_2, 25 mL CH_3OH [30 min] 3) 25 mL n-hexane [30 min]	63%	6%	0.8%	70%
Soxhlet, ca. 200 mL CH_3OH [19 h]	69%	-	-	69%
1) 25 mL acetone [30 min] 2) drying under N_2, 25 mL Tween-80, 20 g/L [30 min] 3) 25 mL acetone [30min] 4) drying under N_2, 25 mL Tween-80, 20 g/L [30 min]	54%	7%	2%, 0.6%	63%
50 mL Tween-80, 25 g/l, 50°C, magnetic stirring [1 h]	66%	-	-	66%
50 mL acetone (ultrasonication) [1 h]	66%	-	-	66%
1) acetone, 25 mL [40 min] 2) petroleum ether, 25 mL [40 min]	-	-	-	70.2% (67.4 – 74.3%)[a]
1) n-hexane, 25 mL [40 min] 2) CH_3OH, 25 mL [40 min]	-	-	-	74.0% (70.5 – 76.2%)[a]

[a] average and range of triplicate experiment

♠ The concentration value refers to the total spiked NP (with the specific radioactivity given in 7.2) and not only to ^{14}C-NP molecules, as it may be wrongly deducted.

During this screening, methods tested included extraction by organic solvents and aqueous surfactant solutions, while potentially enhancing factors like increased temperature, ultrasonication, and addition of electrolytes (for enhancing the partition from soil liquid phase to organic solvents) were also investigated. Among the techniques tried was Soxhlet extraction, which is traditionally applied for the recovery of extractable residues of organics from soil. The first tests ran as single experiments, in order to select the most promising techniques for further investigation. Relying on these results, two methods were applied in triplicates, as to be compared for their potential to extract the spiked *NP from soil. For the last methods, determination of the non-extracted radioactivity was also performed, and NP recovery values were then calculated as a fraction of the total measured radioactivity (extractable + non-extractable), to correct for random deviations in the spiking amount. The results for some of the screened methods, as well for the two triplicate experiments are shown in Table 9.

After evaluating the extraction results, the method using n-hexane and methanol was considered satisfactory, as it showed the highest recovery (on average 74.0%), while being at the same time also simple and no time-consuming.

At this stage, a further optimization of the selected extraction method was attempted by adding a preliminary extraction step by acetone (25 mL) for 5 min, followed by drying under N_2. The aim of adding this step was twofold:

- to allow the non-polar solvent n-hexane interact directly with soil organic phase: Soil aqueous phase is not mixed with n-hexane, hence it may inhibit NP extraction. Acetone, as a water-miscible solvent, will remove soil humidity.
- to provide the possibility to roughly determine the dry matter of soil after the incubation experiments: Determination by drying *NP-spiked soil aliquots at 105^{o}C was assessed as dangerous for the health, as incontrollable *NP contamination of the air in the laboratory may well occur. While the time-consuming air-drying can anyway not remove all humidity of soil.

A triplicate extraction according to the optimized technique gave when tested an improved total average recovery of 79.6% (range: 76.7 – 81.1%). The established protocol was later tested also in 5 g aliquots of polymer-conditioned, dewatered sludge (DM ~ 15%), spiked at 0.75 mg/kg (wet weight, ww), giving a recovery of 98.2% (97.6 – 99.0%). In these final tests in soil and sludge the bound (covalently) residues were partially fractionated, by involving two steps for extracting humic and fulvic acids (see below).

8.2.3 Fractionation of NP bound residues

An extraction by a NaOH solution (0.1 M) was implemented for the extraction of the humic and fulvic acids bound residues of NP from soil (by this method the majority of such residues can be extracted, Romaris-Hortas et al., 2007). The extraction consisted of two identical steps, each involving 25 mL of aqueous NaOH solution shaken with the soil at ca. 60 rpm for 40 min. Humic and fulvic acids extraction took place after the methanol stage. They were followed by a final methanol extraction

step (1 min, shaken on vortex), to allow a fast drying of the soil (under N_2) before samples for combustion will be taken for the determination of residues bound to the humin and mineral fraction of soil. The extraction results obtained for soil and dewatered sludge (this time not corrected according to the mass balance) are given in Table 10.

Table 10. Recoveries of spiked *NP from soil and dewatered sludge by using the established extraction protocol (averages and ranges of triplicates).

	acetone	n-hexane	Methanol	NaOH 0.1 M	NaOH 0.1 M	methanol	combustion	Mass balance
Soil	67.7% 65.7 – 69.6	1.4% 0.9 – 2.2	7.8% 6.9 – 9.1	3.4% 3.0 – 3.8	2.2% 1.7 – 2.5	1.1% 1.0 – 1.2	12.9% 11.9 – 14.8	96.4% 93.8 – 99.7
Sludge	78.1% 75.5 – 80.3	15.7% 14.4 – 17.1	2.3% 2.0 – 2.6	0.2% 0.0 – 0.5	0.0% 0.0 – 0.0	0.0% 0.0 – 0.0	1.7% 1.0 – 2.4	98.0% 97.0 – 98.8

In the case of soil, and at the stage of sampling aliquots for combustion in the Oxidizer, it was noticed that the dry extracted soil consisted of two concrete fractions, a dark brown elastic layer, and a light colored sandy material. As it was anyway difficult to take representative samples, the two fractions were separated manually, and weighted and sampled accordingly. The results about the mass and the radioactivity bound in each of these fractions in the tested soils are shown in Table 11.

Table 11. Fractionation between "humin" and "mineral" fraction for the extracted *NP-spiked soil.

	Relative mass	Relative radioactivity	*NP Partition coefficient ("c_{humin} / $c_{mineral}$")
Elastic layer	16.3%	93.5%	
("humin" fraction)	(15.9 – 16.8%)	(93.3 – 93.8%)	73.9
Sandy material	83.7%	6.5%	72.0 – 74.9
("mineral" fraction)	(83.2 – 84.1%)	(6.2 – 6.7%)	

From the results it is apparent that more than 90% of bound NP in the freshly spiked and extracted soils is localized in the much lighter elastic layer, hereafter referred as "humin" fraction (with the sandy material referred as "mineral" fraction). Regarding the real nature of the two fractions, the elastic layer is expected to contain the majority of organic matter of soil. In fact the latter represents, according to the supplier, only 2.3% of soil's mass. Therefore it is anticipated that this layer must also contain small particles associated with the organic matter. A comparison with the distribution of particles according to soil's profile could give an indication, that the elastic layer practically contains the particles smaller than 0.02mm (clay plus fine silt), which accounts for 15.5 % ± 2.4% of the specific soil's mass.

8.2.4 Determination of NP leaching potential

The method finally employed for assessing the leaching potential of NP in soil was a modified version of the U.S. Environmental Protection Agency (EPA) Toxicity Characteristic Leaching Procedure (TCLP, SW-846, EPA Method 1311). TCLP is used by the U.S. EPA for the determination of the mobility of

contaminants present in wastes. The method simulates leaching from a landfill under a mismanagement scenario (unlined landfill).

According to the established protocol, extraction was performed using an aqueous solution (40 mL) containing NaOH (0.13 M) and acetic acid (0.19 M) (pH~5) under gentle shaking (80 rpm) for 10 h. The radioactivity contained in 1 mL sample of the extract (after centrifugation, 3,500 g, 10 min) was measured by LSC.

Extraction of 5 g soil samples, spiked with *NP at 0.75 mg/kg (dw) and left in room temperature for 1.5 h, recovered 4.1% (3.8 – 4.5%) of the radiolabeled pollutant. These values have not been corrected according to the mass balance that calculated after determining the non-extracted *NP residues. The mass balance for the leaching test ranged between 95.1% and 100.5%.

8.2.5 Spiking of NP on soil

As a carrier for bringing NP into the soil matrix, water was chosen as more advantageous, in respect of the possible side effects to microbial populations. As water is a much more "soil-friendly" solvent, aqueous spiking solutions can be added in relatively high amounts, thus resulting in a more homogenous distribution of the model pollutant. In the frame of the present study, it was calculated that up to 4 mL of water would be possible to be added to air-dried soils before incubation, with the double role of irrigation and spiking. This technique has however disadvantages. If it is taken into account that for the planned incubation experiments around 10^7 dpm of *NP should be spiked per system (soil-sludge mixture), it is concluded that 188 μg of the substance should be carried in 4 mL of water. The concentration of such a solution would be 47 mg/L, which greatly exceeds the solubility limit for NP in water (ca. 6 mg/L).

A solution that was tried to overcome this problem was to prepare the aqueous spiking shooting solution by injecting a very small volume of concentrated *NP ethanol solution (100 μL of 1500 mg/L *NP) to 4 mL of water. By this means, it was hoped that the amount of ethanol would enhance NP solubility and at the same time, in the form of 2.5% mixture with water, would not significantly effect microbial populations in soil. After preparing a spiking solution as described and sampling aliquots to assess whether it was homogeneous, it was found out that the later the samples were taken, the less their radioactivity (down to 89% of the calculated on the basis of a homogenous solution, 5 min after mixing). As the mentioned technique would not produce accurate spiking of soils, an optimized protocol should additionally include determination of the non-spiked (probably adsorbed to the walls of the glass container or precipitated) *NP.

The exact steps of the optimized method were as follows:

1. mass measurement of the glass vial which will be used for preparing the spiking solution
2. pouring of 4.0 mL water + 100 μL [*NP, 1550 mg/L] into the glass vial
3. sampling 3 X 100 μL control aliquots from the [*NP, 1550 mg/L] solution
4. transferring all aqueous solution to soil by a glass pipette
5. rinsing of the glass vial that contained the aqueous solution and the glass pipette with ~5ml ethanol

6. mass measurement of the glass vial, to give the mass of the added ethanol
7. mass measurement of two LSC vials
8. sampling 2 X ca. 1 mL of rinsing solution to LSC vials and again mass measurement, to give the fraction of rinsing solution counted for radioactivity

A test, where "spiking" was performed directly to LSC vial instead of soil, to measure the exact spiking amount, showed that the method is reliable, producing an error not higher than 1% in the calculated spiking amount. As disadvantage up to 17% of radioactive NP is lost during this procedure, which nevertheless can to a great extent be recovered in the form of rinsing solution.

8.2.6 Sludge treatment methods selection and their simulation in the lab

Commonly applied chemical conditioning methods (ferric chloride, ferric chloride plus lime, cationic polymer) plus a physical one (freeze-thaw conditioning) were selected, for comparing their effect on NP's fate in soil. Chemical conditioning of the sampled liquid mesophilic anaerobically digested sludge was performed in 100 mL glass centrifugation tubes in the form of aqueous solutions, in doses representing the lowest and highest applied in practice (Ivashechkin et al., 2004). The liquid mixtures were homogenized by shaking for 30 min at 180 rpm. For freeze-thawing samples were stored in a freezer, at -21°C, and thawed at room temperature.

Centrifugation was chosen as the technique to implement sludge dewatering, on the basis of the available equipment in the laboratory. Centrifugation is a very commonly applied technique in WWTPs. The effects of the concentration of conditioning agents and of the duration of centrifugation (at 1,850 g) on the dry matter of the produced sludge were investigated, with the results shown in Table 12.

Table 12. Studies on the optimization of dewatering (centrifugation) performance.

Parameter studied	Values compared	Sludge DM (% ww) (average)
Liquid sludge Dry Matter	-	2.4
Centrifugation duration ($FeCl_3$ - 7.5%, 1850g)	5 min	8.7
	15 min	9.4
	30 min	10.1
No conditioning (1850g, 30min)	-	9.9
$FeCl_3$ dose (1850g, 30min)	4% (dw)	9.3
	7.5% (dw)	9.8
	15% (dw)	8.7
Lime / $FeCl_3$ dose (1850g, 30min)	13 / 4% (dw)	13.8
	25 / 7.5% (dw)	14.6
	50 / 15% (dw)	14.5
Polymer dose (1850g, 30min)	0.05% (dw)	10.4
	0.2% (dw)	11.1
	1% (dw)	11.5
Freeze / thaw (1850g, 30min)	-21°C / room temperature	13.8

Optimum conditioning doses were found to be the following: $FeCl_3$: 7.5% (median), Lime ($Ca(OH)_2$) / $FeCl_3$: 25 / 7.5% (median), Polymer: 1% (maximum). However, the differences by using

different doses were not great. Another conclusion was that by treatment in the lab, under the conditions tried, it was not possible to obtain sludge as dry, as it is achieved on Soers WWTP (i.e. 32-35% with a 0.2% dose of polymer).

Table 13. Studies on the optimization of dewatering (centrifugation) performance.

Method studied	Conditioning applied	Sludge DM (% ww) (change, see Table 12)
Maximum centrifugation performance (3500g, 1h)	Polymer – 1%	11.5 → 14.4
Air drying overnight	$FeCl_3$ – 4%	9.3 → 11.0
	$FeCl_3$ – 15%	8.7 → 11.4
Air drying overnight + Filtering	$FeCl_3$ – 4%	9.3 → 12.8
Air drying overnight + Filtering under vacuum	$FeCl_3$ – 15%	8.7 → 11.1

For that reason, further improvements were tested, including higher centrifugation speed and duration, overnight air-drying after centrifugation, and filtration after centrifugation (Table 13). Maximum centrifugation performance improved the dewatering of sludge and it was decided to be applied during the present study. In the contrary, filtration was associated with practical problems, while air drying did not improve dewatering to an adequate extent to compensate for uncertainties related to NP losses and sludge composition (air-dried sludge presented a differently colored surface). Moreover, an interesting observation made was that, after filling the centrifuged sludge in a syringe (as it was tested for the purposes of filtration), the texture of the outcoming sludge appeared homogeneous, while such a handling could be used to easier deliver the desirable amount. Thus this step is included in the sludge treatment described in the next chapter.

9. Incubation of sludge-amended soils – homogenous spiking

9.1 Aims

This set of experiments included the first main incubation with soils amended with differently treated sludges in order to assess the influence that sludge treatment may have on organic chemicals (and particularly NP) fate processes in the soil environment. Spiking NP homogenously on the respective soil-sludge mixtures was implemented, as a simple, although not completely adequate methodology for simulation of sludge-bourn pollutants. Possible effects of sludge treatment on microbial populations of the fertilized soil system were in focus. The factor of availability of NP in sludge matrix was for the moment partially overlooked (NP would at least partially be adsorbed on soil, at the beginning of the experiment).

Bearing in mind the model presented in Fig. 9 (section 3.2), the fate of NP was hereby investigated by monitoring i) its intact [i.e. not chemically transformed] non-bound residues (extractable residues), ii) the volatilized fraction, iii) the degraded fraction, and iv) bound residues. Furthermore, an indicator of the potential of leaching was determined. Carbon dioxide formation due to the mineralization of the aromatic ring of NP was quantified to close the mass balance, to assess the fate of degradation products, and to provide a real time follow-up of the fate processes (apart from the volatilized NP, all the other fractions were checked only at the end of the incubation).

9.2 Results

9.2.1 Dry matter of dewatered sludge

The six sludges amended on soils during this experiment had the dry matter given in Table 14. In this table, also the abbreviations used hereafter to refer to accordingly treated sludge are provided. The DM of soil before irrigation and sludge amendment was 94%.

Table 14. Abbreviations and dry matter of differently treated (plus a not further treated) sludges.

Conditioning	Dewatering	Abbreviation	DM
-	-	**LQ** *("liquid")*	2.4%
-	centrifugation	**CE** *("centrifuged")*	14%
$FeCl_3$	centrifugation	**FE**	12%
$Ca(OH)_2$ + $FeCl_3$	centrifugation	**LM** *("limed")*	21%
cationic polymer	centrifugation	**PO**	15%
freeze-thawing	centrifugation	**FT**	19%

9.2.2 Organic matter and nutrients in soil and sludges

The Volatile Solids (VS), total N and NH_4^+-N of aliquots of parallel to the incubation soils and treated sludges were determined, with the results shown in Table 15 (for the soil's VS, the value of organic matter content provided by the supplier is provided). It is noted, that the samples were prepared a long time after the incubation experiment (the sludge had been stored at 4°C for ca. 1 year after its use for the incubation experiment, before aliquots were taken, treated and measured).

9.2.3 Water loss during incubation

Determination of the humidity of the incubated soils and its comparison with the starting humidity, according to the dry mass of soil and sludges and the water added indicated that no measurable amount of water was lost during incubation. One of the three replicates of soils amended with CE was the only exemption (loss of the 1/3 of the water content), with the corresponding system however being characterized by fate parameters lying just between the two other equal systems. Thus either water loss occurred at one of the last incubation days, or it didn't have any significant influence on the fate of NP.

Table 15. Volatile solids and N content of the soil and differently treated sludges.

Soil / sludge	VS (dw)	Total N (mg/g, ww)	NH_4-N (mg/g, ww)
soil	2.3%	1.4	0.27
LQ	49.1%	1.7	1.0
CE	46.7%	6.1	2.1
FE	47.6%	5.2	2.1
LM	31.6%	4.5	1.9
PO	48.0%	6.6	2.0
FT	48.8%	5.1	2.0

9.2.4 pH of the incubated soils

The pH of the incubated soil-sludge mixtures as it was measured in water and $CaCl_2$ is presented in Table 16. Significantly higher was the value only for the soils amended with LM.

Table 16. pH of sludge-amended soils after the incubation (range of values measured in two of the three replicate systems).

sludge amended	LQ	CE	FE	LM	PO	FT
pH in water	5.0 -5.0	5.0 – 5.1	4.9 – 5.0	6.5 – 6.5	4.9 – 4.9	4.9 – 5.2
pH in $CaCl_2$	5.0 – 5.1	5.1 – 5.3	5.0 – 5.3	5.7 – 6.0	5.1 – 5.3	5.2 – 5.3

9.2.5 Mineralization

The evolution of $^{14}CO_2$ produced by the mineralization of NP phenolic ring is presented in Fig. 16a for the soils amended with six sludges. In general the mineralization was low, not exceeding 6% after 95 days of incubation (Fig. 16b). $FeCl_3$ conditioning did not influence the process. Liming and freeze-thawing of the sludge amended to the soil led to a relative decrease of about 40% in the CO_2

produced from the mineralization of NP, in comparison to non-conditioned sludge amendment. Conditioning with the cationic polymer led also to low rates, especially towards the end of the incubation period. Concerning NP mineralization, amendment with non-dewatered sludge led practically to the same result as when equal wet mass of centrifuged sludge was applied. The variation in the mineralization rates for soils amended with the same type of sludge was not much lower than the deviation among the different types of sludge (Fig. 16b).

9.2.6 Volatilization

The radioactivity in the ethylene glycol traps was very low (0.1-0.4% of applied radioactivity). No significant differences in the occurrence of volatilized compounds were observed among systems amended with the various sludges (data not shown).

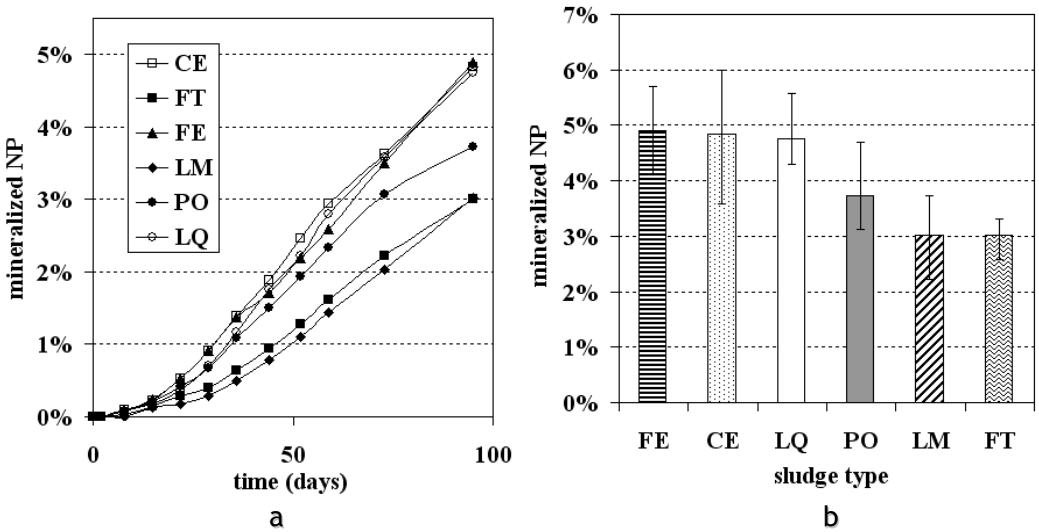


Figure 16. Mineralization of the NP radiolabeled phenolic ring in soil amended with different sludges; (a): evolution during incubation (average of triplicate series) (b): mineralized NP after 95 days of incubation (expressed as % applied NP, average of triplicates, error bars indicate minimum and maximum values obtained). For abbreviations see Table 14.

9.2.7 Extractable NP and degradation products

The radioactivity, which was recovered by extracting the incubated soil-sludge mixtures with organic solvents, was characterized by HPLC analysis. The amounts of NP and its degradation products were determined. After incubation of the eighteen systems 44-87% of the spiked radioactivity was extractable by organic solvents. Depending on the system considered, 73-96% of this radioactivity corresponded to the parent compound. NP was considerably persistent, since extractable NP ranged between 33% and 83% (Fig. 17a). A comparison with mineralization data shows that the amendments which led to relatively diminished mineralization (freeze-thawed and limed sludge), were also characterized by a high amount of unaltered parent compound at the end of the incubation period. Although the data were subject to high variation, a correlation between the high mineralization rates with increased amounts of degradation products (Fig. 17b) was observed, except for polymer conditioned sludge. For the latter, despite a decreased mineralization rate, the amount of NP

available to organic solvents was as low as in soils amended with non-conditioned sludge (Fig. 17a).

9.2.8 Leaching potential

The leaching potential was evaluated by investigating the extractability of NP and degradation products by the applied aqueous solution. At the end of the incubation, the leachate extract contained 3-5% of the radioactivity spiked. In contrast to the organic solvents extract, further HPLC analysis showed that only 47-66% of the radioactivity was attributed to NP. In most of the cases an average of 2.5% of the parent compound spiked was available to the aqueous phase after three months (Fig. 18a). However, it was observed that NP leaching potential from soil amended with limed sludge was higher than for other treatments. On the contrary, less NP could be mobilized from the systems where polymer conditioned sludge was used. For the latter, the amount of radioactivity corresponding to NP metabolites in the leachate-extract was also the lowest (Fig. 18b).

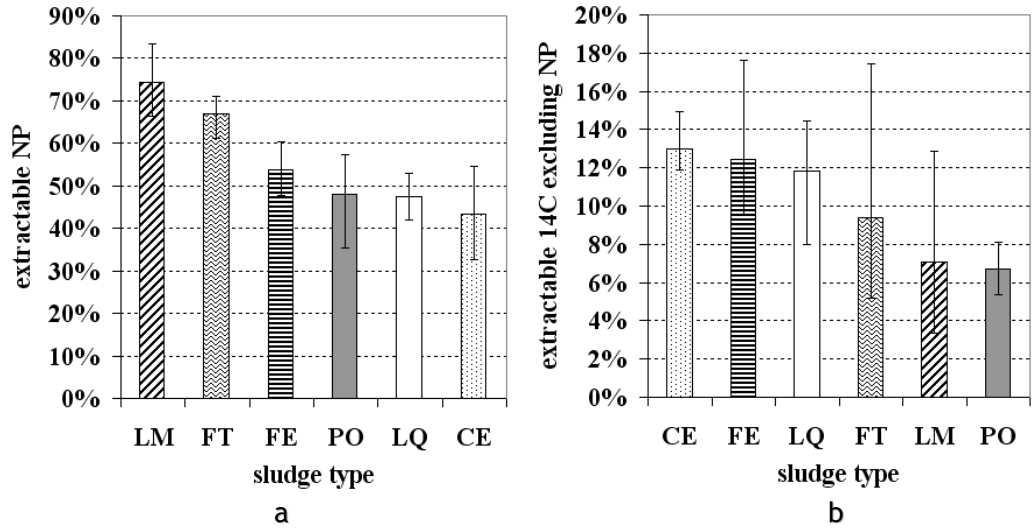


Figure 17. Solvents extractable residues of NP (a) and rest of extractable radioactivity (b) after 95 days of incubation, expressed in % of applied NP, in soil amended with different sludges.

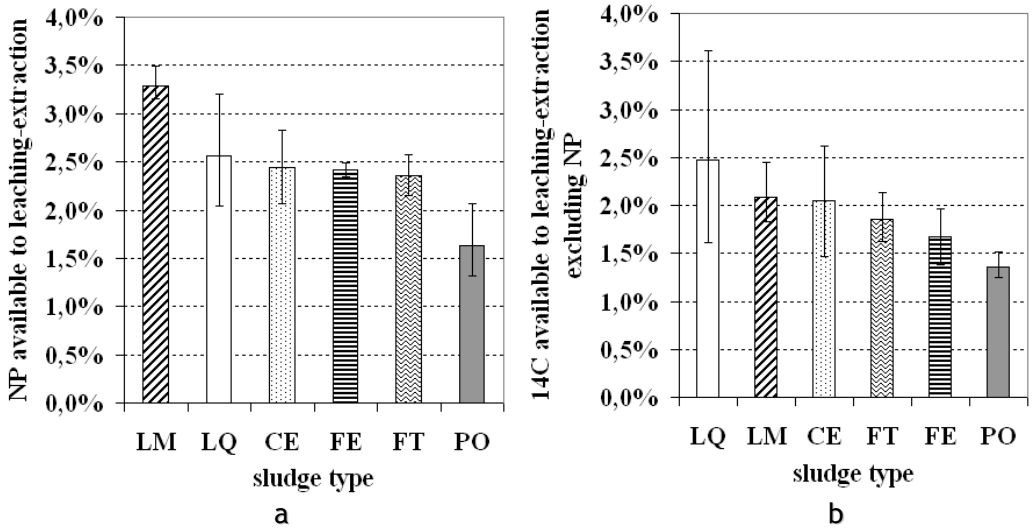


Figure 18. NP (a) and rest of radioactivity (b) amount extracted through leaching-simulation extraction after 95 days of incubation, expressed as % of applied NP, in soil amended with different sludges.

9.2.9 Mass balance

A synopsis of the distribution of radioactivity among all the fractions determined for the sludge-amended soils after the end of the incubation period is given in Table 17. Data related to the leaching potential test are referred separately at the end of the table, as they originate from a second soil sample and therefore are not part of the same mass balance. As already mentioned in section 8.2, radioactivity fractions are given normalized, on the basis of the total radioactivity recovered by the respective soil samples. That is emphasized once more by the sum of radioactivity in Table 17, which is always 100%. In fact, total recoveries ranged for the eighteen sequentially extracted samples between 88.3% and 101.2% (average 93.7%), and for the eighteen samples subjected to the leaching test between 89.3% and 112.6% (average 99.3%). The variations of the radioactivity recovery were presumably due to the inhomogeneity of the soil-sludge mixtures. Deviations are assumed to having occurred basically due to the sampling procedures, i.e. from the incubated soils (as instead of extracting the complete soil amount, different portions were devoted to the two extraction protocols, pH measurement, storage for later microbial profiling, and back-up samples - in case of sample loss during extraction or analysis), but the same or even more importantly, from the dry extracted soils, for the purpose of combustion of the humin and the mineral fractions.

Table 17. Distribution of spiked radioactivity between different fractions, after the incubation of soils amended with different sludges (averages and ranges of triplicate experiments). Percentages refer to the total radioactivity recovery from the soil sample.

	CE	FE	PO	FT	LM	LQ
Mineralized	5% 4 – 6	5% 4 – 6	4% 3 – 5	3% 3 – 3	3% 2 – 4	5% 4 – 6
Volatilized	0.2% 0.1 – 0.2	0.2% 0.1 – 0.4	0.2% 0.2 - 0.2	0.1% 0.1 – 0.1	0.2% 0.1 – 0.3	0.1% 0.1 – 0.2
Extractable NP	43% 33 – 55	54% 48 – 61	48% 36 – 58	65% 55 – 71	75% 67 - 84	48% 42 - 53
Rest extractable	13% 12 - 15	13% 10 - 18	7% 5 - 8	9% 5 - 18	7% 3 - 13	12% 8 - 15
Physically bound	1% 1 - 2	1% 1 - 1	1% 1 - 1	1% 1 -1	1% 1 - 1	1% 1 - 2
HA-bound	6% 4 - 7	6% 6 - 6	7% 5 - 9	3% 3 - 4	2% 1 - 2	10% 8 - 13
FA-bound	5% 4 - 6	5% 5 - 6	6% 5 - 6	4% 4 - 5	3% 3 - 4	6% 6 – 7
Humin-bound	22% 15 - 30	13% 12 - 14	24% 18 - 33	12% 12 - 12	8% 4 - 10	15% 14 - 15
Mineral-bound	0.9% 0.7 – 1.2	0.7% 0.6 – 1.0	1.2% 1.1 – 1.4	0.6% 0.4 – 0.8	0.5% 0.4 – 0.6	1.2% 0.9 – 1.5
2nd methanol extraction	2% 1 - 4	2% 1 - 3	2% 2 – 3	1% 0 - 2	1% 1 - 1	2% 1 – 3
Sum	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**
Potentially leachable NP	*2.4%* *2.1 - 2.8*	*2.4%* *2.3 - 2.5*	*1.6%* *1.3 - 2.1*	*2.4%* *2.1 - 2.6*	*3.3%* *3.1 - 3.5*	*2.6%* *2.0 - 3.2*
Rest potentially leachable	*1.8%* *1.5 - 2.1*	*1.7%* *1.4 - 2.0*	*1.4%* *1.2 - 1.5*	*1.9%* *1.6 - 2.1*	*2.1%* *1.8 - 2.5*	*2.5%* *1.6 - 3.6*

9.3 Discussion

9.3.1 General fate of NP

NP was very persistent in the soil-sludge systems studied, being degraded only partially after three months of incubation. In the literature much higher degradation and mineralization rates are often reported. The difference can be attributed to the use, in the current study, of a single branched isomer of NP. When linear 4-NP was spiked at comparable levels to soil-sludge mixtures at similar ratio and water content, about 50% mineralization was observed after two months (Gejlsbjerg et al., 2001). Linear NP spiked was completely degraded after one month, while significant portion of the naturally occurring branched NP was detectable under the same conditions (Mortensen and Kure, 2003). Concerning leaching, the results are in agreement with literature data (La Guardia et al., 2001) and characteristic of the hydrophobicity of NP (log K_{ow} = 3.3 - 4.8, ECB, 2002).

9.3.2 Variation in parallel incubations

Considering the parallels conducted with the same type of sludge, the variation in fate, although not very high, was comparable to the deviations among the sludges. One possible explanation is the heterogeneity of distribution of spiked NP between soil and sludge organic matter. The sludge aggregates size, which was not constant due to the low dewaterability of sludge under the experimental conditions (12-21% DM for the obtained biosolids, see Table 14) may have played a role as well.

9.3.3 Effect of freeze-thawing (on NP fate in soil)

Soils amended with freeze-thawed sludge were characterized, in comparison with soils amended with non-conditioned sludge (CE), by a lower degree of NP degradation and mineralization (on average 6% less for both pathways together) and also a low degree of bound residues formation (by 16%). That resulted in ca. 22% higher extractable residues of the parent compound.

The persistence of NP can theoretically be attributed to a number of mechanisms, related either to the absence of degrading/binding factors (sludge microorganisms, soil microorganisms, chemical components of sludge including extracellular enzymes, oxygen), or to a low availability of the pollutant to these. At this point, direct evidence about microbial populations and enzymes/ reacting chemicals in different soil systems is not available. Regarding availability related phenomena, the actual availability of NP in sludge may have been overlooked in this experiment (see section 9.1). However since NP after spiking must have been adsorbed not only to soil (which is the same for all systems), but presumably to surfaces of sludge aggregates as well, these phenomena may have played also a role in fate processes. For instance, if NP was more strongly sorbed in the organic matter of FT than of CE, it could be less available for degradation by microbes. Or, if the size of the aggregates of FT was smaller (and thus the surface higher) than those of CE, a bigger portion of NP may had been localized after spiking at sludge matter, hence being more available to sludge consortia and less to soil populations. Some availability evidences are interestingly present.

They can be derived from the ratio between “leached” and extractable (not bound) residues

specifically for NP (not for total radioactivity), which then serves as an indicator of the availability of NP to the aqueous phase of soils (Table 18). The observation is that NP leaching potential for soil amended with freeze-thawed sludge was similar to that observed when soil was amended with non-conditioned sludge (CE), although non-bound NP residues were lower in the latter case. That indicates a lower availability of NP sorbed to freeze-thawed sludge (as soil was the same in the two cases). A possible reason for this phenomenon can be the larger dewatering of FT (19% instead of 14% DM, for CE), leading to a more condensed organic matter.

Nevertheless, other phenomena, including for instance sludge bacteria, cannot be excluded. Sludge biota is expected to be adapted to the presence of NP, so their presence and amount may be important for NP degradation in the environment of the amended soil as well. Freezing may have a negative impact on the endogenous microbial population of sludge, and thus affecting NP degradation processes in soil/sludge mixture.

Information about the organic matter and N content of soils amended with different sludges, calculated on the basis of soil and sludges analysis, are provided in Table 19. A comparison between soils amended with CE and FT does not lead to any particular observation, apart from a slightly higher amount of OM in the latter.

Table 18. Ratio between water-extractable and organic solvents – extractable NP from incubated soils amended with different sludges.

CE	FE	PO	FT	LM	LQ
5.2 - 6.3%	4.1 – 4.9%	2.9 - 3.7%	3.4 - 3.6%	3.8 - 5.3%	4.9 - 6.0%

Table 19. Organic matter and N content of soils amended with differently treated sludges, calculated from the properties of soil and sludges. The first numbers correspond to contributions from the soil.

Soil amended with:	LQ	CE	FE	LM	PO	FT
Organic Matter (mg)	279 + 18	279 + 100	279 + 84	279 + 100	279 + 105	279 + 138
Total N (mg)	18 + 3	18 + 9	18 + 8	18 + 7	18 + 10	18 + 8
NH_4^+-N (mg)	3.5 + 1.5	3.5 + 3.2	3.5 + 3.2	3.5 + 2.9	3.5 + 3.0	3.5 + 3.0

9.3.3 Effect of liming

NP persistence was also high in soils amended with LM. In comparison with the control soils (amended with CE) 9% less of the initially spiked amount resulted in degraded/mineralized products and 23% less as bound residues. This resulted in ca. 32% higher intact NP residues in the systems.

A slightly lower availability of NP to the aqueous phase of soils amended with limed sludge, relatively to non-conditioned sludge (Table 18), is also present, as in FT, although the difference here is less significant. Nevertheless, the persistence of NP in the case of LM was higher than in FT. Thus an effect of lime to factors such as microbial populations may be present, either of sludge, or those of soil after amendment - if the latter play a significant role in NP degradation or binding. It is noted that the pH during sludge liming was 8.5 and that of limed sludge amended soil was 6.5.

The slightly higher leaching potential observed for NP in LM–amended soils can be attributed either to the high amount of NP residues, or to the higher pH of the respective biosolids, slightly

fostering deprotonation of the model pollutant.

A slightly lower amount of N was present in soils amended with LM, in comparison to the control soils (CE amendment) (Table 19). Nevertheless this fact is not expected to be correlated with the effects observed on the fate of NP.

9.3.4 Effect of cationic polymer addition

Following a treatment of sludge with cationic polymer, pathways leading to degradation products and CO_2 were less efficient than soils amended with control sludge, but the formation of bound residues appeared to be slightly enhanced (Table 17). Therefore, polymer-conditioning had no significant effect on the persistence of NP.

Here it is interesting to note that, as presented by Table 18, NP was considerably unavailable to the water phase of PO-amended soils. Thus an assumption can be made, that polymer conditioning lowered the availability of NP to water, as freeze-thawing and liming did, but in the polymer case an additional factor existed, which provided an alternative NP "elimination" mechanism, i.e. an enhanced covalent binding mechanism.

The general leaching potential of NP and degradation products where polymer used was slightly lowered.

9.3.5 Effect of ferric chloride conditioning

A lower tendency for the formation of bound residues, specifically to the humin fraction, could be observed in ferric chloride treated sludge, leading slightly to increased residues of NP. However, this effect was statistically not significant.

9.3.6 Effect of dewatering (centrifugation)

Fractionation of the bound residues in the soils amended with liquid sludge suggested that the type of binding was different than in the case of centrifuged sludge. More specifically, although the radioactivity of bound residues was the same in the cases of LQ and CE, the radioactivity in FA and HA was significantly higher (and lower in the humin fraction) in the case of liquid sludge application. This effect must not necessarily be connected to the properties of sludge organic matter. It can also result from a different distribution of spiked NP between the organic matter of soil and sludge (as dewatered sludges were amended in aggregates).

Here it should be noted that soils amended with LQ were characterized by lower contents of organic matter (-20%) and of N (-20%), in comparison to soils amended with dewatered sludge (CE) however with no influence on the fate of NP. This may mean i) either that these two parameters are not affecting the NP fate processes, ii) or that they have an opposite effect on NP persistence (e.g. OM enhances sorption, but N enhances microbial growth), iii) or even that other additional critical factors exist in LQ or CE, able to counterbalance this influence.

10. Incubation of sludge-amended soils – spiking on sludge

10.1 Aims

The second incubation series performed during this work served three main purposes:

i) to investigate the correlation between sludge treatment and NP fate in sludge-amended soils under conditions simulating better the real systems. This was accomplished by spiking NP on sludge before sludge was conditioned or dewatered. Practical problems had to be solved to implement this strategy, associated with the non-predictable losses of NP during sludge treatment (the sludges should ideally contain NP at the same level at the beginning of incubation), as well with the transport of radioactive sludge to the soil without significant or uncounted losses.

A better simulation meant also the improvement of other parameters, including the dewatering efficiency of sludge (in practice it is higher than that achieved during the previous setup), the dosing of conditioning agents (hereby average instead of maximum doses applied in practice were used), and the humidity of incubated soils (the scenario of the first experiment, with water corresponding to 90-100% of the WHC was not the most representative).

Moreover, the comparison between different sludge amendments was not made on the basis of equal wet masses, but on the basis of equal starting masses of sludge before treatment. Nevertheless, this couldn't apply for the liquid sludge, as the humidity of the resulting soil would be far more than its WHC (slurry). In practice, since more liquid sludge is needed to cover the same nitrogen needs of crops, the sludge is applied either in doses or in combination with chemical fertilizers. Thus, the scenario of this experimental series can be considered realistic, in the sense that it represents the fate of NP in one of the applied LQ sludge doses, except the fact that the addition of fertilizers may affect the fate of NP. While another disadvantage of the new experimental setting is that the level of NP in the matrix of spiked sludges would be different in liquid and dewatered sludges. In the previous experiment NP was spiked on soil, and spiked levels per wet mass of soil-sludge mixture were the same.

ii) to provide more evidence about the mechanism behind any measurable influence of sludge treatment on soil fate processes. This could be partially achieved by comparing the results obtained after applying two different spiking strategies.

iii) to reduce the variation between parallel experiments – in case that was due to experimental deficiencies – so that any correlation will be more clearly emphasized and investigated. Apart from

the fact that spiking on the final solid mixture without controlling the localization of inserted NP could be highly responsible for the variation of the results of the previous incubation, one additional parameter was better controlled during the new series: the radioactive soil-sludge mixtures were exerted a slight pressure, on the top, in order to produce similar oxygen penetration profiles for all systems. Furthermore, the amount of sludge amendment was increased (sludge : soil ratio from 1:60 to 1:10 for dewatered and from 1:400 to 1:100 for liquid sludge (dw basis), with the new ratios being to some extent exaggerated in comparison with the usual practice), while small improvements were applied to the analytical procedures; for this latter reason, the complete protocols are described again in this chapter.

10.2 Results

10.2.1 Fate of NP during sludge treatment

The losses of NP due to partitioning to the water phase that was removed after centrifugation, as well as the dry matter content of the dewatered sludges are summarized in Table 20. Only small amounts of NP were eliminated during sludge dewatering treatment, i.e. maximum of 1.3% in the case of limed sludge. The biosolids produced had a dry matter content similar to that of sludges produced in WWTPs. HPLC analysis of an extract of the limed sludge, in the frame of preliminary experiments, showed that no chemical transformation of NP could take place under these conditions (data not shown). On the basis of data collected during the sludge preparation, sludge treatment and soil amendment processes, the dewatered sludges were loaded with radiolabeled NP at 170–250 mg/kg (DM) and amended at a sludge to soil ratio of approximately 1:10 (on DM basis). For the liquid sludges the level of spiked NP was 1850–1900 mg/kg (DM), while the respective mixture ratio was approximately 1:100 (for the relevance of comparing liquid and dewatered sludge amendments at this incubation experiment, see section 9.1).

10.2.2 Organic matter and nutrients in soil and sludges

The Volatile Solids (VS), total N and NH_4^+-N of aliquots of parallel to the incubation soils and treated sludges were determined, with the results shown in Table 21. The sludge aliquots used for treatment and subsequent determination of OM and nutrients parameters were the last available from the sampled sludge. The amounts of some of the treated sludges were unfortunately proved to be not enough for the determination of all desirable parameters.

10.2.3 Water loss during incubation

Determination of the humidity of the incubated soils and its comparison with the starting humidity, according to the dry mass of soil and sludges and the water added indicated that no measurable amount of water was lost during incubation over the period of 87 days.

10.2.4 Mineralization of NP

The mineralization of NP in sludge-amended soils is presented in Fig. 19. The amount of mineralized

NP after 87 d of incubation was very low, not exceeding 7% of the spiked amount in any of the amended soils. Significant differences between the various treatment methods were observed. The decomposition process was slow when sludge had been freeze-thawed and negligible (only 0.7% had been transformed to $^{14}CO_2$) in the soil amended with limed sludge. The lag-period lasted ca. 15 d for all dewatered sludges, but only 5 d for the liquid sludge, in the matrix of which NP exhibited also the highest mineralization rate.

Table 20. Losses of water [1] and radiolabeled NP during sludge treatment

Sludge	Dry matter [2]	NP losses
LQ	4.8%	-
CE	22% (21 – 23%)	0.75% (0.7 – 0.8%)
FT	25% (24 – 27%)	0.45% (0.4 – 0.5%)
FE	30% (26 – 31%)	0.44% (0.4 – 0.5%)
LM	39% (36 – 41%)	1.31% (1.3 – 1.4%)
PO	28% (27 – 29%)	0.56% (0.5 – 0.6%)

[1] based on the dry matter of the treated sludges (w/w)

[2] for dewatered sludges calculated indirectly, based on the mass of water losses (average and range of three parallel experiments)

Table 21. Volatile solids and N content of the soil and differently treated sludges (ND: Not determined).

Soil / sludge	VS (dw)	Total N (mg/g, ww)	NH_4-N (mg/g, ww)
soil	2.3%	1.4	0.27
LQ	46.9%	1.7	0.76
CE	45.1%	6.9	2.0
FE	ND	7.0	2.0
LM	ND	10	2.0
PO	44.1%	10	2.7
FT	ND	11	3.5

10.2.5 NP extractable by organic solvents

Analysis of the soils amended with dewatered sludges revealed that 87 d after spiking most of NP was intact and extractable with organic solvents (Fig. 20a). Particularly the NP levels in the soils amended with freeze-thawed sludge were slightly higher than in soils where differently treated dewatered sludges were applied (73–79% of the applied amount, in comparison with 65–73% in the 12 systems corresponding to the rest cases). When liquid sludge was used, the amount of NP that could be recovered was remarkably lower, never exceeding 21%. The deviations observed between the parallel experiments were in general low.

10.2.6 Leaching potential of NP

The risk from leaching appeared to be fairly low. The highest amount of mobile NP was observed in limed sludge (Fig. 20b). Nevertheless, the amount transported to the aqueous phase in this case still remained below 2% of the applied compound. Polymer conditioning of sludge resulted in a slight decrease of the total amount of mobile NP after 87 d of incubation. The latter was even lower in

soils amended with liquid sludge (0.4% of applied NP).

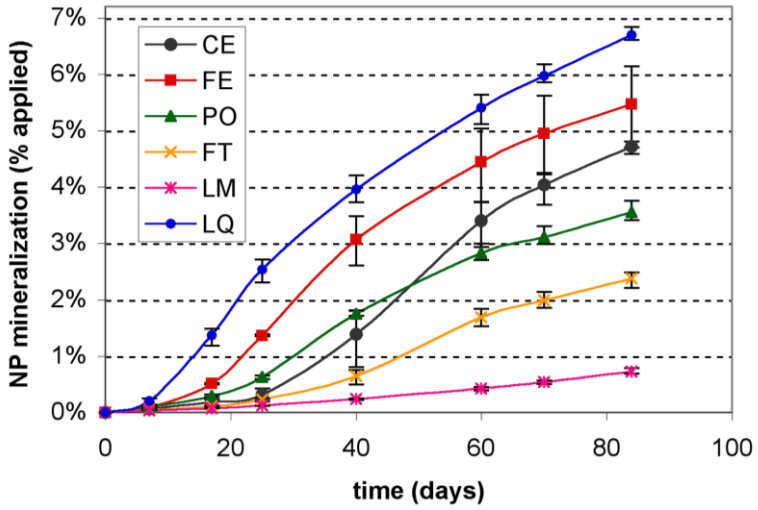


Figure 19. Mineralization of the phenolic ring of NP in soils amended with different sludges (averages and ranges of triplicate series).

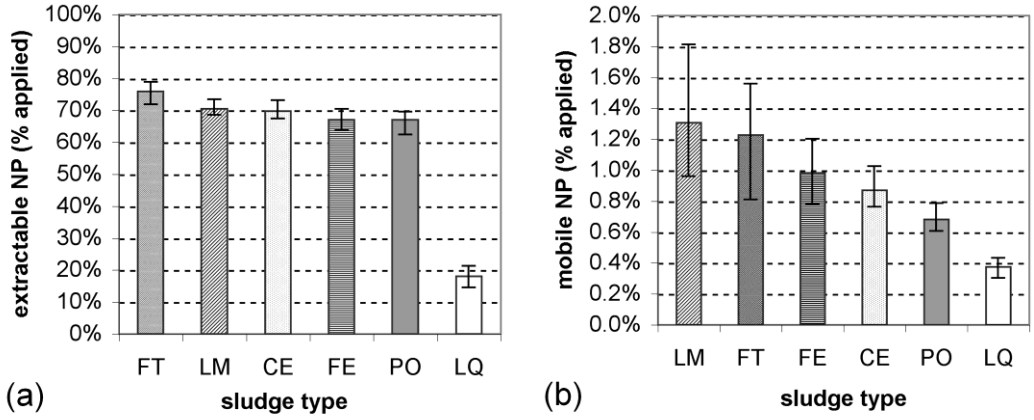


Figure 20. Levels of NP extractable by organic solvents (a) and by the leaching-simulation procedure (b) in the incubated soils (averages and ranges of triplicate series).

10.2.7 NP degradation products

The combined organic phases obtained from sequential extraction were analyzed by means of HPLC. The radioactivity representing NP was subtracted from the total radioactivity of the concentrates, with the resulting values being represented in Fig. 21a. Remaining radioactivity assignable to NP degradation products was much higher in the soils where NP residues were very low, i.e. in soils amended with non-dewatered sludge.

Radioactivity not corresponding to the parent compound but to its degradation products was also quantified in the leaching-simulation extracts. As it appears in Fig. 21b, the soils treated with liquid sludge did not display a high potential for leaching of metabolites/transformation products, although these compounds were present in high amounts in these soils (Fig. 21a). Additionally, it is noteworthy that in some cases the total amount of leachable radioactivity which was not assignable

to the parent compound appeared to be even higher than the extractable amount of the latter (Figs. 21b, 20b). Thus degradation of NP may have occurred during the storage of the aqueous leaching-test extracts and before their analysis.

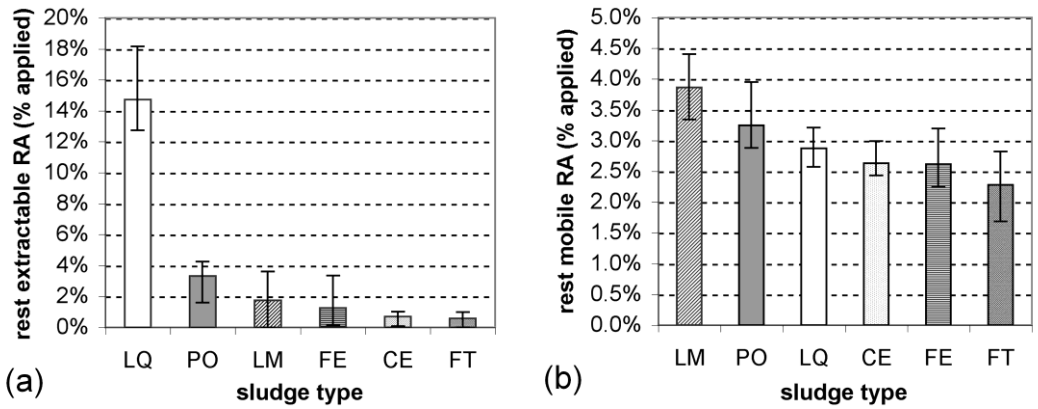


Figure 21. Radioactivity (RA) corresponding to degradation products of NP, extracted by organic solvents (a) and by the leaching-simulation procedure (b) from the incubated soils (averages and ranges of triplicate series).

Fig. 22 shows characteristic chromatograms from the analysis of residues extracted from the sludge-amended soils by organic solvents. A peak corresponding to a polar degradation product of NP could be observed in chromatograms related to liquid sludge-amended soils, where degradation of NP was considerably more advanced (Fig. 22a). An additional degradation product of NP was detected, that occurred only when the cationic polymer had been used as sludge-conditioner (Fig. 22c). The respective compound eluted about 2.5 min after the parent compound and represented, 87 d after spiking, an amount equal to 2–3.5% of the applied radioactivity.

Although present in trace amounts, an effort was made to obtain qualitative information about the degradation product detected in PO-amended soils. The extracts from the soils amended with PO or with CE (the latter as a negative control) were applied on preparative HPLC and the relevant fractions were collected, and analyzed using GC/MS (Fig. 23). Although not any significant difference was observed in Full Scan, or even Selected Ion Current (SIC) chromatograms for ions occurring in the mass spectrum of NP, a peak present only in the polymer-related extracts was detected in SIC chromatograms of ions corresponding to a reported degradation product of NP, the 4-(3,5-dimethyl-3-heptyl)-2-nitrophenol (Telscher et al., 2005). All major ions previously reported for 4-(3,5-dimethyl-3-heptyl)-2-nitrophenol accounted in the mass spectrum corresponding to the peak present in extracts obtained from soils amended with polymer conditioned sludge.

10.2.8 Distribution of extractable and bound radioactivity

Before providing the total distribution of radioactivity in the incubated soils of this experimental setup, the distributions of extractable and bound residues will be herein shortly presented.

The extractable residues were divided into those extractable by acetone, n-hexane and methanol during the sequential extraction procedure. The fractionation data were examined after

normalization relative to the total amount of extractable residues. The normalized distribution was very similar for soils amended with different sludges (83–88% extractable by acetone, 2–7% by n-hexane and 7–12% by methanol, data not shown).

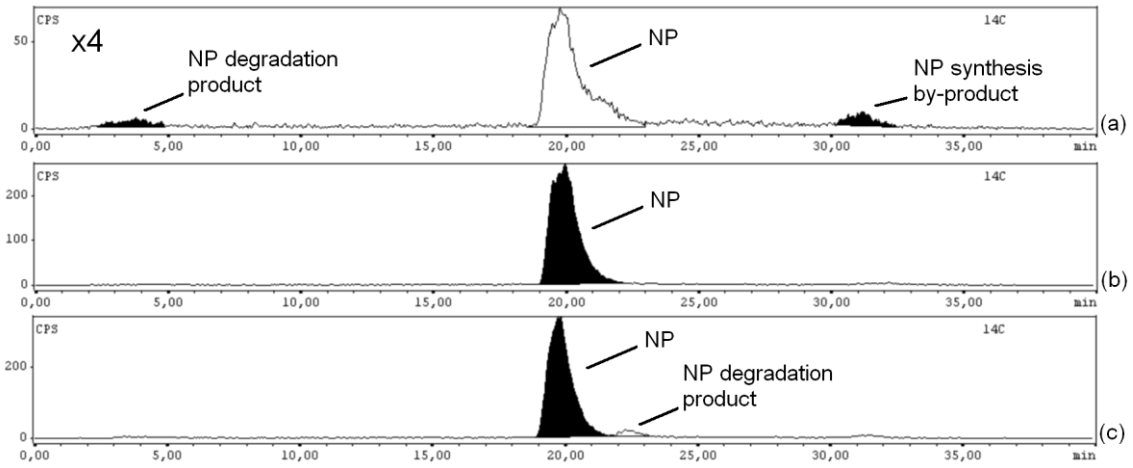


Figure 22. Radioactivity Chromatograms of HPLC-radiodetection for the organic extracts of soils amended with LQ (a), CE (b) and PO (c) after 87 d incubation. The peak at retention time 20 min corresponds to NP. The peak at 31 min corresponds to a by-product of NP synthesis.

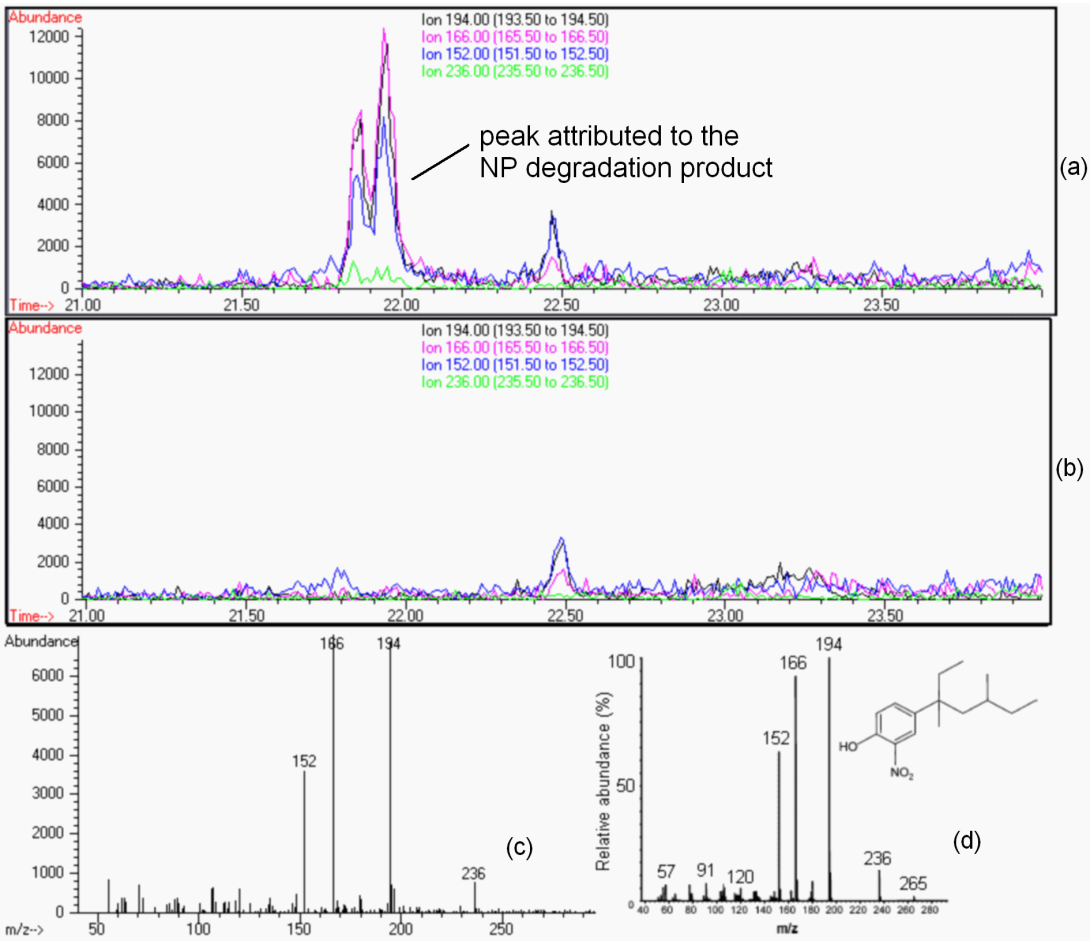


Figure 23. Part of the GC/MS(SIC) chromatograms of the HPLC effluent fractions with Rt=22-23 min, for extracts of soils amended with PO (a) and CE (b). EI-MS of the peak attributed to NP degradation process (c) and comparison with the MS of 4-(3,5-dimethyl-3-heptyl)-2-nitrophenol (d, Telscher et al., 2005) are shown below.

The bound residues were particularly high in LQ systems (57–65%), while not significantly different between CE, FE, PO and LM (22–28%) and slightly lower in the case of FT (18–24%). In contrast with the extractable residues, the influence of the sludge treatment on the normalized distribution of the non-extractable radioactivity was significant (Fig. 24). Binding of NP and possible transformation products seemed to occur preferentially in the humin fraction for all soil-sludge mixtures. Nevertheless, highest binding to the humin was observed for soils amended with limed and liquid sludges. An influence of the various sludge treatments on the distribution of spiked radioactivity between the other fractions of bound residues after incubation was also observed.

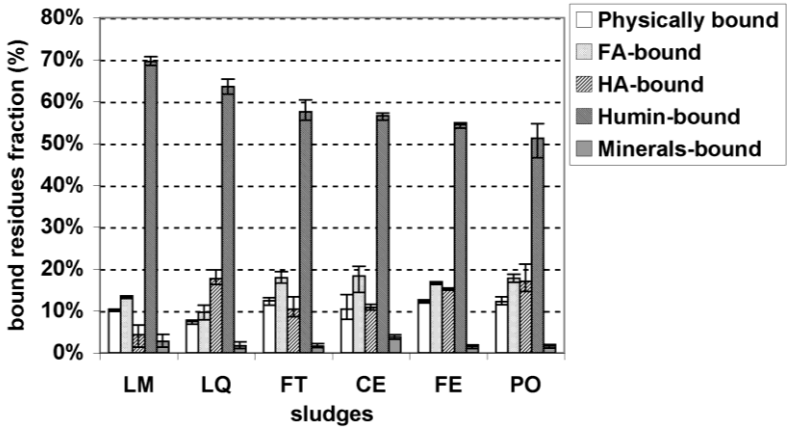


Figure 24. Distribution of the bound residues in sludge-amended soils after 87 d incubation. The values (averages and ranges of triplicate series) represent fractions of the total bound radioactivity per system.

10.2.9 Mass balance

The distribution of the radioactivity in the incubated soils is presented in Table 22. As already mentioned in section 5.3 and the paragraph 9.2.9, radioactivity fractions are given normalized, on the basis of the total radioactivity recovered by the respective soil samples. In fact, total recoveries ranged for the eighteen sequentially extracted samples between 86.0% and 111.0% (average 100.1%), and for the eighteen samples subjected to the leaching test between 85.1% and 129.0% (average 100.3%).

10.3 Discussion

10.3.1 Elimination of NP during sludge dewatering

Hydrophobic compounds are expected to have a high affinity to sludge organic matter. Ivashechkin et al. (2004) calculated for Bisphenol A (BPA) a partition coefficient, K_d, of 122–201 L/ kg (K_d = Cs/Cw, Cs, concentration in sludge, Cw: concentration in the liquid phase) for a sludge with the same content of organic matter as in the present study. Based on the NP losses in the supernatant after centrifugation of the spiked sludges a K_d value of ca. 3000 L/kg can be derived for NP (Table 20, CE). This difference reflects the higher partition coefficient for organic carbon Koc of nonylphenol (log Koc = 4.0–5.6 for NP instead of 2.5–3.2 for BPA, Ivashechkin et al., 2004), which is also shown by the

low leaching potential observed in the sludge-amended soils.

The desorption effect of liming for compounds with pK_a > 10 has been predicted by Ivashechkin et al. (2004). The phenomenon, observed for BPA, had been attributed to the deprotonation of those compounds in pH values > 10 achieved by liming. The pK_a of BPA has been measured as 10.3, a similar value to that expected for NP (see section 4.2). The elimination of NP from limed sludge, in the current study, was increased. Nevertheless, the desorption was very low (only 1.3% of the applied amount) in comparison with the BPA desorption (80%) reported at similar conditions. Nevertheless, elimination data indicate a slightly higher affinity of NP to the water phase of LM.

Table 22. Distribution of spiked radioactivity between different fractions, after the incubation of soils amended with different sludges (averages and ranges of triplicate experiments). ND: Not determined

	CE	FE	PO	FT	LM	LQ
Mineralized	5% 5 - 5	5% 5 - 6	4% 3 - 4	2% 2 - 2	1% 1 - 1	7% 7 - 7
Volatilized	ND	ND	ND	ND	ND	ND
Extractable NP	70% 66 - 73	67% 65 - 70	67% 65 - 70	76% 73 - 79	70% 66 - 73	18% 15 - 21
Rest extractable	1% 0 - 1	1% 0 - 3	3% 2 - 4	1% 0 - 1	2% 0 - 4	15% 13 – 18
Physically bound	3% 2 - 3	3% 3 - 3	3% 3 - 3	3% 2 - 4	3% 3 - 3	5% 4 – 5
HA-bound	3% 2 - 4	4% 3 - 4	4% 4 - 5	2% 2 - 2	1% 1 - 1	11% 9 – 13
FA-bound	5% 4 - 5	4% 4 - 5	5% 4 - 5	4% 3 - 4	4% 3 - 4	6% 6 – 6
Humin-bound	14% 12 - 16	14% 14 - 15	13% 13 - 14	12% 11 - 14	19% 17 - 21	39% 34 – 44
Mineral-bound	0.9% 0.7 – 1.2	0.4% 0.3 – 0.6	0.4% 0.3 – 0.6	0.4% 0.2 – 0.5	0.8% 0.6 – 0.9	1.0% 0.7 – 1.2
2nd methanol extraction	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Sum	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**
Potentially leachable NP	*0.9%* *0.8 - 1.0*	*1.0%* *0.8 - 1.2*	*0.7%* *0.6 - 0.8*	*1.2%* *0.8 - 1.6*	*1.3%* *1.0 - 1.8*	*0.4%* *0.3 - 0.4*
Rest potentially leachable	*2.6%* *2.4 - 3.0*	*2.6%* *2.2 - 3.2*	*3.3%* *2.9 - 4.1*	*2.3%* *1.7 - 2.8*	*3.9%* *3.4 - 4.4*	*2.9%* *2.6 - 3.2*

[a] this extraction step was performed indeed, though the extract was this time combined with the other organic extracts, which were analyzed for NP and rest extractable radioactivity

10.3.2 Effect of dewatering by centrifugation (on NP fate in soil)

In discussing the possible mechanism behind the results of this incubation experiment (Table 22), fate-leading factors (soil and sludge biota, sludge chemical and enzymatic components, oxygen) and availability to those should be regarded, as also done in the previous chapter. Evidences are again offered from information about the availability of residual NP in the aqueous phase (Table 23) and about the organic matter and N content of the various soils (Table 24).

By comparing the fate of NP in soils amended with CE and LQ, the effect of sludge dewatering can be assessed. In this experiment, the fraction of NP that was degraded / mineralized during the

incubation was by 16% lower in soils amended with CE. Moreover, bound residues fraction was by 36% lower. As a result, an additional amount of 52% of the spiked NP remained intact and extractable in soils amended with centrifuged sludge (70% in comparison to 18%).

Table 23. Ratio between water-extractable and organic solvents – extractable NP from incubated soils amended with different sludges.

CE	FE	PO	FT	LM	LQ
1.1 - 1.4%	1.2 – 1.8%	0.9 - 1.2%	1.0 - 2.1%	1.3 - 2.8%	2.0 - 2.2%

Table 24. Organic matter and N content of soils amended with differently treated sludges, calculated from the properties of soil and sludges. The first numbers correspond to contributions from the soil.

Soil amended with:	LQ	CE	FE	LM	PO	FT
Organic Matter (mg)	277 + 56	277 + 514	277 + 433 [a]	277 + 385 [a]	277 + 481	277 + 509 [a]
Total N (mg)	18 + 4	18 + 35	18 + 29	18 + 40	18 + 41	18 + 44
NH_4^+-N (mg)	4 + 2	4 + 10	4 + 8	4 + 8	4 + 11	4 + 14

[a] In these cases, the missing data on VS were arbitrary calculated on the basis of the influence that sludge treatment had during the previous incubation experiment

Table 23 shows that the availability of NP in the aqueous phase of soil was significantly higher in the soils where degradation and binding rates were higher (those amended with LQ). This consideration becomes more important, if the fate of NP in the two systems is compared with the corresponding fate when the pollutant was spiked on soil (chapter 9). Thereby, no considerable difference in fate was observed, while noteworthy the aqueous availabilities of NP were statistically the same in soils amended with CE and LQ.

The apparently low availability of NP in soils treated with CE may be due to a sequestration or entrapment of NP molecules in remote sites of the solid produced after centrifugation, as NP was here spiked before the dewatering process takes place. Another factor contributing to the low accessibility of NP may be the amount of OM presence. The same amount of NP that in CE was distributed in 514 mg of OM, in LQ it was distributed in only 56 mg of OM (Table 24). The high amount of NP concentration in the dry matter in LQ may lead to a saturation of primary adsorption sites and hence localization in external, more accessible sites in the sludge matter, enhancing the degradation rates of the pollutant.

A further hypothesis, about the cause of the low availability of NP in centrifuged sludge, is that the presence of dissolved organic matter in LQ may solubilize NP, thus favoring its partition to the aqueous phase in soil. Increased bound residues in the HA fractions of these systems may be related to an increased partitioning to those substances - in the soil environment - which are partly removed with the supernatant during centrifugation.

Regarding microbial populations, and speaking about nutrients, the N content was clearly lower in soils amended with liquid sludge (Table 24), denoting from a first sight that nutrients deficiency in soils amended with CE was not the case. Nevertheless, an increased availability of nutrients in liquid sludge (see paragraph 2.3.1) may have been the case and it can not be excluded as

a potential contributing mechanism. Such differences (in nutrients availability or generally microbial populations), if present here, were then probably also present in soils incubated in the previous experiment, where no effect on NP fate was observed. However, as the amount of sludge spiked in this second incubation was higher, the contribution in OM and nutrients was also higher, and thus this factor may have been more dominant here.

Finally, alternative causes, like for instance a lower degree of oxygen penetration through the solid sludge, are not to be excluded. Hesselsøe et al. (2001) showed that O_2 penetration into sludge aggregates is an important factor for nonylphenol degradation, observing lower diffusion of O_2 and significantly lower degradation in bigger sludge aggregates. The prolonged lag-period observed for CE (in comparison with LQ - amended soils) can be correlated with a slower diffusion of O_2 into CE aggregates.

10.3.3 Effect of freeze-thaw conditioning

A comparison between soils amended with FT and CE reveals for the first a moderately lower extent of degradation/mineralization (3% instead of 6% of spiked NP) and of binding (21% instead of 26%). The resulting small difference in persistence (for FT, 76% instead of 70% NP residues) is significant, although not comparable to the extent of the effect of dewatering. Freeze-thawing obviously mostly affected mineralization (Table 22, Fig. 19).

The indicators regarding the availability in water and the OM and N content don't show any considerable differences between freeze-thawed and non-conditioned sludge (Tables 23, 24). Freeze-thawing in the previous experiment had a bigger effect on NP fate. In point of fact, the availability of NP to water (ratio of extractable by water to extractable by organic solvents) was therein significantly lower in FT-amended soils as well. So, the impact on availability discussed in the previous chapter seems not to be significant under more realistic conditions, where NP is localized specifically on the sludge matrix. Even though low availability could be expected from literature-based data, according to which freeze-thawing in comparison with other conditioning processes is known to produce less permeable sludge flocs with short pores, and smooth pore surface (Martel, 2000; Chung et al., 2003; Chu and Lee, 2004).

At this point it is difficult to attribute the moderate effect of sludge freeze-thawing to a specific factor. As the most important clue for the moment, the significantly lower mineralization of NP in both incubation experiments must be noted.

10.3.4 Effect of lime conditioning

Liming of sludge significant decreased the mineralization of NP (1% instead of 5% when no conditioning treatment was applied), with a concomitant significant increase in the formation of bound residues in the humin fraction (19% instead of 14%). The consequence was that no difference could be detected in the amount of persisting (extractable) NP in the respective soil systems.

No conclusions can be exported from the information that Tables 23 and 24 provide (when comparing LM and CE). Any attempt for assuming responsible mechanisms can at this point only rely in a comparison between the fates of NP in the two subsequent incubation experiments. By doing

this, an agreement can be found in the significant lower mineralization rates in limed sludges, but a controversy in the formation of bound residues. A reduced formation of all types of bound residues was observed in the former experiment; a decline in residues bound to HA, FA and the mineral fraction was observed during the new incubation, but a clear increase of those associated to the humin fraction was the case. Thus, summarizing, the change of methodology had an effect basically on the formation of bound residues in the humin fraction of sludge-soil mixtures.

What was different during the last incubation was the localization and probable sequestration of NP (the latter due to the centrifugation after spiking on liquid sludge), the $Ca(OH)_2$ amount (in total 0.28 g instead of 0.16 g previously / per sludge DM 26% instead of 50% previously) and also the humidity of the soils (relatively lower at the new experiment) - while the sludge was also different. The effect on mineralization rates should thus not be connected to any of these parameters and therefore may be due to an effect of liming on decomposing microbial consortia of sludge or soil. As for the binding to the humin fraction, according to de Oliveira Pimenta and Kilduff (2006) oxidative coupling of phenols is favored at high pH. But according to the same study, the surfaces properties seem to play a more important role in irreversible phenol adsorption. Subsequently, an assumption that can be made is that either the organic matter of sludge favored, in the second incubation, an irreversible adsorption of NP, or the increased pH favored oxidative coupling of NP in sludge matrix.

As also observed in the previous incubation experiment, the new data show a tendency for increased leaching potential from soils amended with limed sludge. This fact could be attributed to the high pH of the respective biosolids, fostering deprotonation of the model pollutant.

10.3.4 Effect of polymer conditioning

Prior conditioning of the amended sludge with cationic polymer didn't result to any significant difference in the sum of degraded and mineralized portion of NP after the new incubation, neither to any difference in the amount of formed bound residues. Nonetheless, two significant effects were still observed: a) a significantly lower mineralization toward longer incubation times and b) a significantly higher amount of extractable degradation products, basically due to the presence of a nitrophenol derivative, which was not detected in any other system.

Between soils amended with CE and PO no considerable differences in the aqueous availability of NP or the amount of organic matter or N could be found (Tables 23, 24). The phenomenon of progressively lower mineralization rates was interestingly present also during the first incubation. However the nitrophenol was not detected before in this work.

Chu et al. (2003) observed a decrease in methane production in prolonged stages of the anaerobic digestion process for activated sludge that was beforehand flocculated with a cationic polymer. In that study, microphotographic data revealed greater floc size and denser structures for the polymer-conditioned sludge. It is possible that a similar effect of polymer conditioning occurred in the current study. At the first stages of the incubation of amended soils the microorganisms would degrade the loose surface of the sludge flocs with adsorbed NP, but reaching the denser cores of the sludge aggregates, the process and the mineralization rate slows down.

Formation of the nitrophenol derivative may be a result of the decomposition of the polymer

agent based on polyacrylamide. The polymer is readily biodegradable in the environment (NEROLAN Wassertechnik GmbH, 2005), while it produces nitrogen oxides after thermal decomposition. Here it must be noted, that the possibility of an artifact, i.e. the formation of the nitrophenol during handling of the soil extracts by rotary-evaporation can be excluded on the basis of respective tests performed in the frame of the current study (data not shown).

Telscher et al. (2005) also detected 4-(3,5-dimethyl-3-heptyl)-2-nitrophenol in incubated soil - sewage sludge mixtures containing NP. In that study, sampled sewage sludge was already dewatered and originated from the same WWTP as the sludge used in the present study. In this WWTP, NEROLAN CE 679 S polymer is applied for conditioning the digested sludge before mechanical dewatering by centrifugation. This fact supports the assumption that in the study of Telscher et al. (2005) the formation of the nitro-substituted nonylphenol was driven by the presence of the specific polymer in the sewage sludge and not by any other chemical or biological component of the studied systems.

NP leaching potential in PO-amended soils was this time not considerably lower than in CE-amended soils, although a tendency for lower values could still be reported (Table 22).

10.3.5 Effect of ferric chloride conditioning

Chemical conditioning of sludge by $FeCl_3$ in doses normally used in practice did not affect any parameter of the fate of NP in sludge-amended soils. A shorter lag-period in mineralization was the only difference observed. The presence already in sludge of Fe in high concentration could theoretically explain the absence of significant effects of this process. Nevertheless, in Soers WWTP during primary sedimentation, phosphorus is eliminated through aluminum (and not iron) salts. Thus the level of Fe in the digested sludge is expected to be considerably lower than the amounts of conditioning agent added for the purpose of the experiments (8% DM).

11. Microbial profiling

11.1 Aims

After the experiments described in the previous chapters, a picture of the correlation between sludge conditioning and dewatering treatment processes and the fate of NP in sludge-amended soils has been obtained. Additionally, a number of indirect evidences on the mechanism behind this correlation have been extracted from the collected data. Biological processes posses in general an important role in dynamics governing chemical compounds behavior in the soil environment. Effects of sludge treatment on microbial populations of sludge-soil mixtures may have contributed to a considerable extent in the total impact on NP fate. Acquiring direct evidence on microbial populations involved in NP degradation and binding was the target of the experimental series described in this chapter.

The general methodology was based on producing bacterial DNA profiles of incubated soil-sludge mixtures, which are representative of the bacterial populations inhabiting them. As however a vast amount of different species was expected to occur in each soil system, an effort was spent to identify those species (or more accurately DNA bands) involved in NP degradation or binding. For that reason, spiked and non-spiked soil-sludge mixtures were incubated in parallel with the second incubation experiment (chapter 10), with the purpose of identifying species present in increased numbers in the former ones. Later, the DNA profiles of the eighteen systems of soils amended with different sludges would be compared.

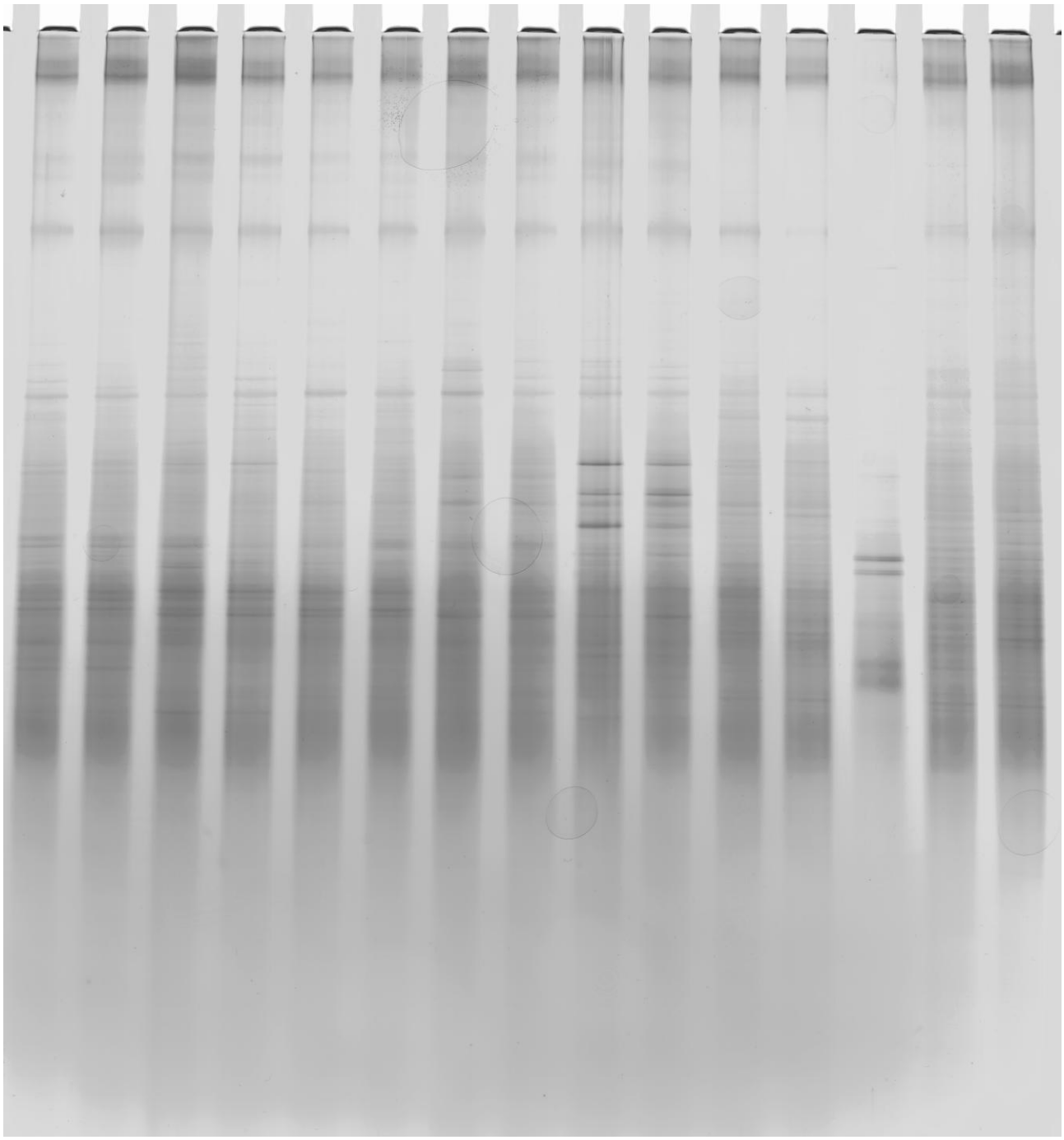
At the end of the chapter, data from the microbial profiling are assessed together with data from the relevant literature, to make an assessment about the role of microbial factors in the mechanism behind the observed correlation between sludge treatment and NP fate.

11.2 Results

11.2.1 DNA profiles of spiked and non-spiked soil-sludge mixtures

In Fig. 25, the bacterial DNA profiles of soils amended with liquid sludge either spiked with NP (LQ-1, LQ-2), or not (LQ'-1, LQ'-2) can be seen (the two profiles correspond to two samples obtained from a single incubated system for each case). A procedure was followed using protocols and algorithms of the software GelCompar, to optimize the ability to distinguish different bands, to remove noise / background signals, and to compare the profiles. In the end, no significant conclusions could be derived about bands being characteristic of NP-spiked systems. Moreover, all efforts to perform

DGGE in narrower denaturant grades resulted in unclear gels.



CE-1 CE-2 FE-1 FE-2 PO-1 PO-2 FT-1 FT-2 LM-1 LM-2 LQ-1 LQ-2 SO LQ'-1 LQ'-2

Figure 25. 16S rDNA profiling after DGGE analysis for soils incubated after amended with different sludges plus of a non-incubated soil sample ("SO"). LQ' stands for soil amended with liquid sludge, but not spiked with NP.

11.2.2 DNA profiles of soils amended with different sludges

The comparison between soils incubated after amendment with differently treated and not treated sludges was not performed on the basis of specific "NP-related" bands, as bands corresponding to species favored in the presence of spiked NP could not be detected. The presence of multiple bands made it difficult to analyze the profiles individually and produce databases with bands present or

absent in each one. Therefore the comparison was based on a correlation regarding the profiles as simple diagrams of intensity versus the denaturant gradient. The profiles were compared according to the Pearson correlation coefficient. Ward algorithm was used to produce a clustering analysis, grouping the profiles according to how well they were correlated to each other. The result of this analysis are shown in Fig. 26.

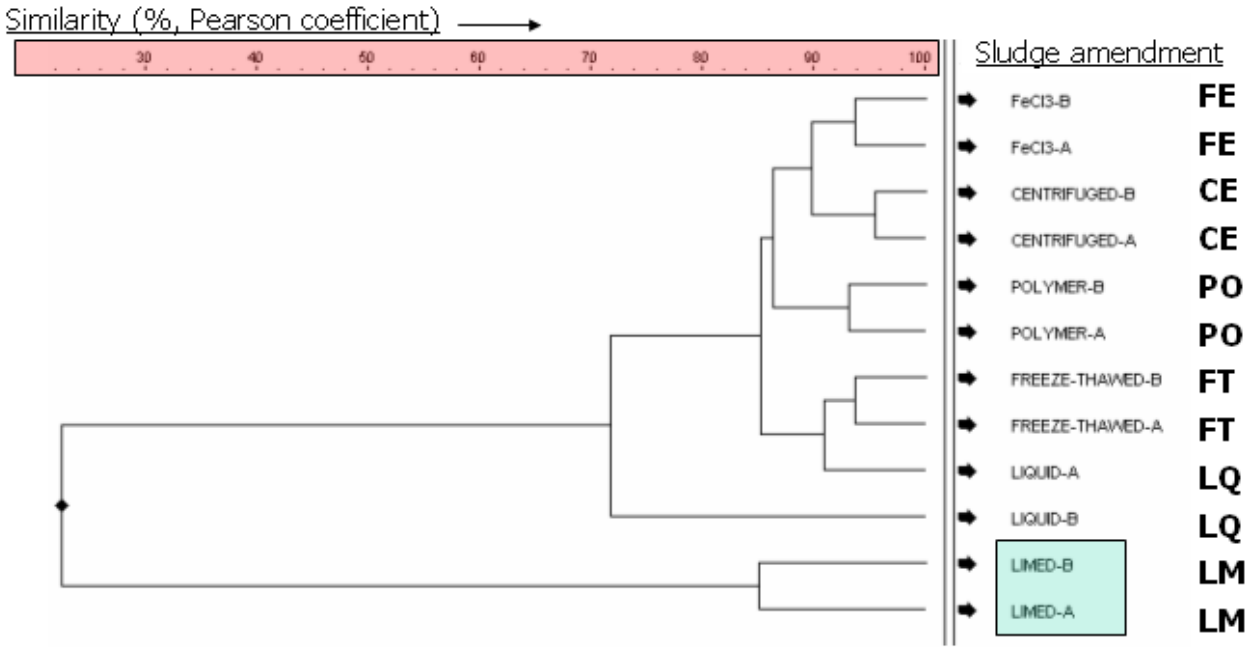


Figure 26. Clustering analysis of the 16S bacterial DNA profiles for the soil-sludge mixtures incubated (spiking on liquid sludge before treatment).

First of all, the analysis indicated a good correlation between samples obtained from the same soil-sludge mixtures (duplicates). Furthermore, a dissimilarity in the distribution of species populations in the bacterial biomass was denoted for soils amended with limed sludge. While the correlation coefficient was measured to be in the range of 85-90% for all other systems (apart from one of the duplicate samples of soil amended with LQ, probably because of noise presence in the upper part of the specific profile), their correlation with soils amended with LM gave a coefficient of only 22%. A particular visual characteristic of the profiles of soils mixed with limed sludge was the presence of three to four strong bands in the middle of the gels.

11.3 Discussion

11.3.1 Data from microbial profiling

The contribution of the microbial profiling in clarifying the mechanism of sludge treatment influence, on the fate of NP in sludge amended soils, was not considerable. Fingerprints of species with special roles regarding NP degradation were not identified. The reason may be the presence already in the sampled sludge of amounts of NP, thus not differentiating significantly the chemical environment of the biomass in sludges spiked and not-spiked with the organic molecule. Alternatively, the microbial

populations distribution may not change considerably in the presence of NP, either because the latter represents a very small fraction of the chemical matrix, or because NP is not readily available in the soil-sludge matrix. Besides, it is possible that "magnifying" small areas of the DNA profiles, by performing DGGE on narrower denaturant gradients, would be necessary to detect any differentiations due to NP spiking. While, finally, the involvement of other microbial types, as fungi, in processes affecting NP's fate in soil, may have been the case.

Nevertheless, a general picture of the bacterial populations in the soils incubated during the second experimental series was obtained, providing an indication that in the case of limed sludge amendment, the predominating bacterial species were different than in all other cases compared. That could theoretically even underlie a direct or indirect influence on NP fate processes. For instance, those species may compete with consortia involved in NP-mineralization, or produce enzymes enhancing NP binding to the humin fraction of soil-sludge mixtures (see paragraph 10.3.4). Still, such assumptions are very hypothetical without existing evidence.

11.3.2 Data from the literature: sludge treatment effects on microorganisms

Literature provides evidence that bacterial populations can rapidly increase after *centrifugation* of digested sludges (Qi et al., 2007). In addition, no significant changes in the proportions of different bacterial groups during sludge dewatering have been observed (Vilanova and Blanch, 2005). In view of these data, a negative impact of centrifugation on NP-degrading species - or species involved in NP-binding processes - does not seem to be expected. Nevertheless, it should not be forgotten that the amendment of liquid sludge may correspond to an increased amount of dissolved organic matter and nutrients, which may favor the growth of species in the soil environment.

Positive evidences on effects of the treatments of *liming* and *freeze-thawing* on microbial populations of sewage sludge have been reported. As for liming, it is known that it adversely affects microbial populations in sludge (Leclerc and Brouzes, 1973) and can be used as an efficient disinfection method. Freeze-thawing has been reported to reduce the number of pathogens of sludge significantly, depending on the strain or the type of bacteria and the freeze-thawing conditions (Chu et al., 1999). That can be either due to osmotic effects (predominating in slow cooling) or due to intracellular freezing (fast cooling) (Sanin et al., 1994).

12. Investigation of the NP local concentration factor

12.1 Aims

Under the changed, more realistic conditions of the second incubation experiment, the largest difference on NP fate in soil was observed between the soils amended with liquid and dewatered sludge. In the first case, NP was degraded to a larger extent and bound to the solid matrix, while in the second case most of NP remained intact and extractable after a period of 3 months. One possible reason might be the fact that, in liquid sludge, the same absolute amount of NP was spiked on a 10-fold lower amount of organic matter.

The experiment described in this chapter was performed in order to clarify whether a lower NP local concentration in liquid sludge would increase the persistence of the pollutant in soil. For this purpose, liquid sludge samples were spiked at two different levels: the higher level of NP corresponded to LQ sludges of the 2nd incubation experiment, while the lower one (by a factor of ca. 11) to the concentration in the DM of the centrifuged sludges of the same experiment. The problem that had to be solved before running such an experiment was that a ten times lower spiking of radioactive NP would generate sensitivity problems when counting the mineralization or analyzing the incubated sludge-soil mixtures.

12.2 Results

12.2.1 Water loss during incubation

The losses were calculated to account for an amount of 10 to 18% of the water present in the systems. However, as the method used for water content determination was by the use of acetone extraction (see paragraph 8.2.2), the calculated water loss may be an artifact: since three times larger mass-aliquots of soil were extracted, the organic matter coextracted by acetone may have given a significant error in the estimation of the water content of the soil-sludge mixtures.

12.2.2 Mineralization of NP

As it can be seen in Fig. 27, the mineralization rate of NP (% of spiked NP per time) in the soil amended with sludge spiked at the lower level was significantly higher (on average 12.3% instead of 9.6% after 95 days).

12.2.3 Mass balance

The distribution of radioactivity among different fractions was examined, as during the previous

experiments. The complete results are shown in Table 25. NP was degraded / bound thoroughly during the incubation period, with only 3-5% to remain intact and extractable afterwards. Extractable NP residues were found to be slightly lower in the case of low level NP spiking, a result being in agreement also with the comparison of the two mineralization curves. In general the fate of NP in the two different types of systems was very similar.

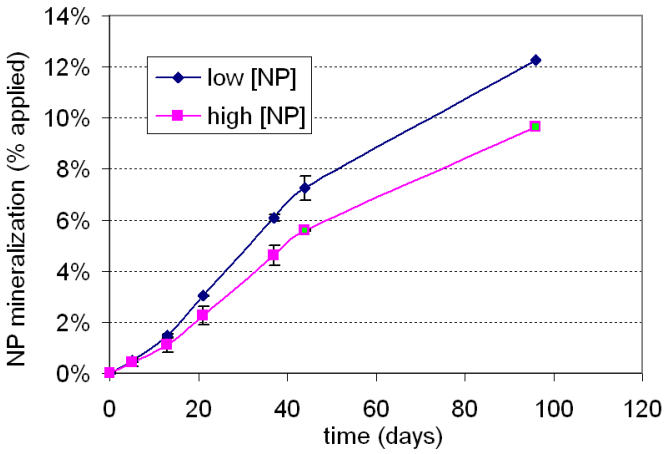


Figure 27. Mineralization of the phenolic ring of NP in soils amended with liquid sludges of different concentrations (see text above; averages and ranges of duplicate series are presented).

Table 25. Distribution of radioactivity between different fractions, after the incubation of soils amended with liquid sludges spiked at low and high levels of NP (averages and ranges of duplicate experiments).

	LQ, low [NP]	LQ, high [NP]
Mineralized	12.3% 11.8 - 12.7	9.6% 9.6 - 9.7
Volatilized	ND	ND
Extractable NP	3.3% [a]	4.7% 4.6 - 4.8
Rest extractable	16.4% [a]	21.3% 21.0 - 21.6
Physically bound		
HA-bound	30.3% 28.1 - 32.4	26.9% 26.8 - 26.9
FA-bound		
Humin-bound	31.9% 31.0 - 33.0	32.2% 31.8 - 32.6
Mineral-bound	5.9% 5.1 - 6.7	5.4% 5.3 - 5.4
Sum	**100.0%**	**100.0%**

[a] due to the loss of one of the two extracts, HPLC analysis could was not carried out

12.3 Discussion

The results showed that, although in reality a liquid sludge amendment will introduce a lower amount of NP to the soil, the fate of the pollutant is expected to be more or less the same as if NP would be introduced at much higher levels (and thus much higher concentrations per DM). Actually, the results showed even a slightly faster degradation/mineralization and binding of NP when at low levels, although exactly the opposite would be expected. It had been anticipated, that higher loading

of NP in sludge would result in a saturation of sorption sites and a subsequent increase of the availability of the pollutant. In conclusion, the high local concentration of NP was not the responsible factor for its low persistence in soils amended with liquid sludges, in comparison with dewatered sludges.

Here it is worth to be noted that a calculation of the rates of degradation or mineralization of NP in [chemical amount per time], instead of [portion of spiked amount per time] would give a completely different result: the absolute amount of NP degraded was almost ten times higher when NP was spiked at a ten times higher concentration. Nevertheless, in the frame of the present work, the fate is being assessed in the form of distribution of spiked pollutant. And, anyway, it seems that, at least for the pollutant and solid matrix tested, such a description is more robust.

The obtained results are in agreement with the study of Roberts et al. (2006), whereby an increase in the concentration of NP from 1 to 10 mg/kg in sludge-amended soil lowered its mineralization rate from 30% to 20% in 60 d (in the current study the two concentrations were 1.4 and 15 mg/kg).

A last remark is that, in the incubation described in this chapter, the persistence of NP was in general lower than observed during the previous incubations. The reason may lie behind the composition of the newly sampled sludge, or behind the fact that the sludge spiked was not fresh, but rather characterized by a preceded natural aggregation / precipitation of solid particles.

13. Characterization of NP fate mechanisms

13.1 Aims

As already discussed, the influence of sludge treatment on NP fate in sludge-amended soil can be due to effects on soil microbiota, sludge microbiota, sludge enzymes or chemical ingredients which react with NP, oxygen penetration, or on the availability of NP on any of these transformation factors. The formulation of correct scenarios requires knowledge about the actual processes involved in NP transformation. Is NP transformation in sludge-amended soils actually driven by biotic or abiotic processes? Do the microorganisms involved originate from sludge or from soil? Answering these questions was the first aim of the herein described experimental series.

For illustrating NP fate processes, *NP-spiked soil-sludge mixtures were incubated as during the experiment described in chapter 10, but with all possible combinations of sterilized and non-sterilized components (soil and sludge). Incubations were carried out for simply centrifuged (CE) and for liquid sludges (LQ), as i) these two cases were considered to represent quite different soil systems and ii) the most considerable difference in NP fate was observed between these two sludge amendments, i.e. due to the dewatering by centrifugation. Anyway, CE served as a control sludge, and therefore variations on NP fate in soils amended with conditioned sludges could be afterwards explained on the basis of the processes involved therein (by CE-amended soils).

A fractionation of the radioactivity of the incubated soils as also performed before would serve the second aim of the current experimental series; that is to define which fate pathway (e.g. degradation, mineralization, binding on various solid fractions) is regulated by which factor, in the control systems (CE, LQ). Such a correlation would allow to interpret the results obtained during all previous incubations of this work in a more secure way, and to extract more essential information from the data collected thereby.

13.2 Results

13.2.1 Sludge treatment and NP losses

The composition of and the abbreviations used for the incubated systems are presented in Table 26.

Table 26. Composition of the incubated mixtures (S: sterilized, N: non-sterilized, LQ: Liquid, CE: Centrifuged).

Abbreviation	A	B	C	D	E	F	G	H
Soil	***S***	***S***	***N***	***N***	***S***	***S***	***N***	***N***
Sludge	***LQ-S***	***LQ-N***	***LQ-S***	***LQ-N***	***CE-S***	***CE-N***	***CE-S***	***CE-N***

The DM of the sludges of the current experiment and the losses associated with their treatment are summarized in Table 27. Sterilized liquid sludge had a better dewaterability. The losses of NP to the supernatant during centrifugation were not significantly different for sterilized and non-sterilized sludges. Although relatively low, they were 3-4 times higher than those during centrifugation for the second main incubation experiment (chapter 10).

Table 27. DM and NP losses associated with the treatment of sludges amended on soils (averages and ranges of similar samples).

Sludge	DM	NP losses
LQ	1.8%	-
	(no replicate measurements)	
CE-N	17.4%	2.64%
	16.7 - 18.5%	2.43 - 2.87%
CE-S	21.8%	2.44%
	21.7 - 21.9%	2.31 - 2.63%

13.2.2 Water loss during incubation

The humidity content of the soils after incubation was determined to be not significantly different than after their irrigation and amendment.

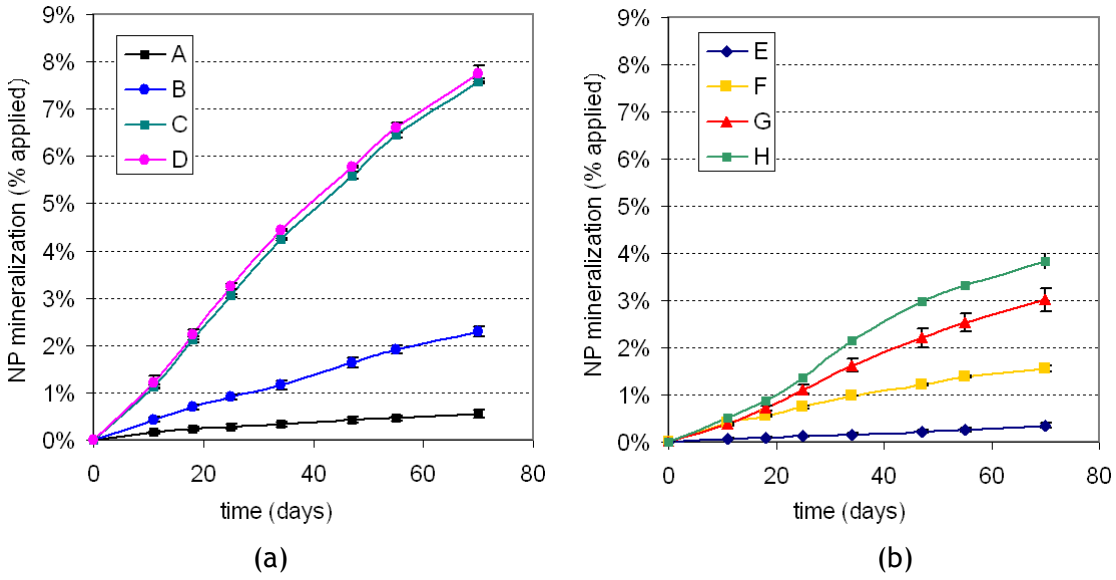


Figure 28. Mineralization of the phenolic ring of NP in soils amended with liquid (a) and centrifuged sludges (b), in different combinations of sterilized and non-sterilized probes (for abbreviations see Table 26; averages and ranges of duplicate series are presented).

13.2.3 Mineralization of NP

The mineralization of the phenolic ring of the spiked NP during the incubation of the various mixtures of sterilized and non-sterilized sludges and soils is shown in Fig. 28. As for the liquid sludge amendment (Fig. 28a), NP was mineralized noticeably faster in the mixtures with non-sterilized soil (about 8% in 70 days). In the same period, only 2% of the aromatic ring was transformed to CO_2 in the

mixtures of sterilized soil and non-sterilized sludge, while less than 0.7% was mineralized when both matrices had been sterilized.

Decomposition rates were in general lower when centrifuged sludge had been added to soil (Fig. 28b). Again mixtures with non-sterilized soil gave the highest mineralization rates for NP (3-4% in 70 d.). Slightly less than half of this was the total rate for the systems where only soil had been sterilized. Mineralization in mixtures of both irradiated soil and dewatered sludge was negligible (never exceeding 0.4%).

13.2.4 Extractable NP and degradation products

Residues of intact and extractable NP after the incubation period were determined to be in the ranges appearing in Fig. 29. When sludge was amended in liquid form (Fig. 29a), NP was generally less persistent as when it was applied in solid form (Fig. 29b). In the mixtures of soil and LQ, extractable NP residues were very low when soil had not been sterilized (C, D). The highest NP residues were observed for the mixture of sterilized soil and sludge (A, 70% of the spiked NP). In the case of CE, NP transformation was the fastest in the presence of both biota (of soil and sludge, mixture H). Nevertheless, the rate of the process was not considerably higher than in mixtures where one of the components had been sterilized (F, G). At last, less than 20% of spiked NP had been transformed in mixtures of sterilized soil and sterilized CE (E), 70 days after soil amendment.

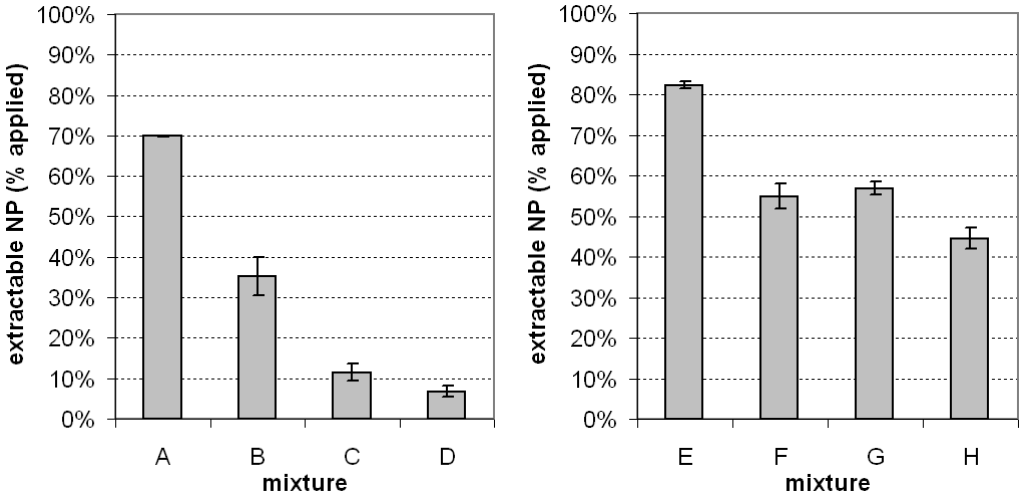


Figure 29. Levels of NP extractable by organic solvents in the incubated mixtures, of soil and liquid (a) or soil and centrifuged sludge (b) (for abbreviations see Table 26; averages and ranges of duplicate series are presented).

Extractable radioactivity that could not be characterized as NP was negligible for mixtures of sterilized aliquots of both soil and sludge (LQ and CE). When microbial populations from any of two matrices were not exposed to γ-radiation, extractable degradation products of NP – containing carbons of its aromatic ring - could be detected, at a range of 12% to 21% of spiked radioactivity (Fig. 30).

An interesting observation made regarding the organic extracts was the presence of two chromatographic peaks apart from the peak of NP. The first one, eluting after NP, was characterized by the retention time of the nitro-phenol mentioned in chapter 10 and was detected in all extracts of mixtures where at least one of the components (soil, sludge) was not-sterilized (Fig. 31a). The second one eluted at about 12 min (with NP eluting at ca. 20 min) and was remarkably present only in the other cases, i.e. in extracts of mixtures of sterilized soil and sludge (Fig. 31b), even if in negligible amounts.

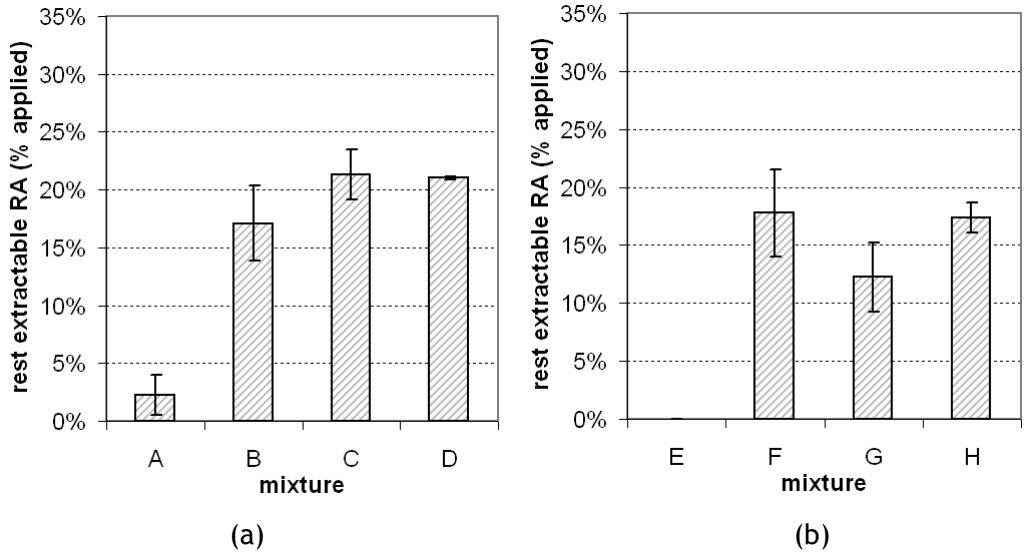


Figure 30. Radioactivity (RA) corresponding to degradation products of NP, extracted by organic solvents, for soils incubated with liquid (a) and centrifuged sludge (b) (averages and ranges of duplicate series).

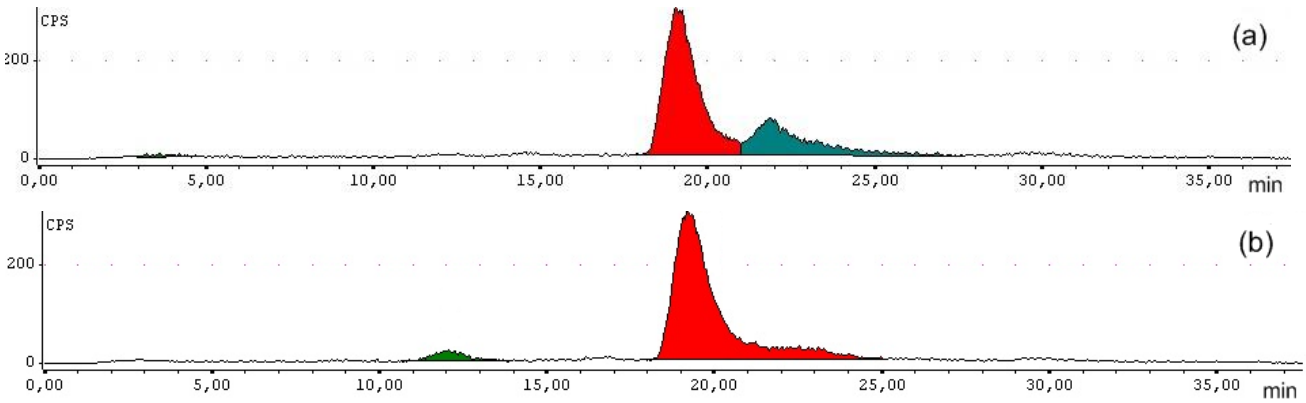


Figure 31. Radioactivity Chromatograms of HPLC-radio detection for the organic extracts of not completely irradiated (a) and completely irradiated (b) mixtures. The peak at retention time 20 min corresponds to NP. Peaks in green correspond to degradation products.

Preparative HPLC followed by fraction concentration and GC-MS analysis pointed out that the peak eluting after NP was the nitro-derivative detected previously in soils amended with polymer-conditioned sludges indeed. As for the peak occurring only when biota from both soil and sludge were practically absent at the beginning of the incubation, the same procedure was followed, but then the

corresponding peak in the GC-MS chromatograms had to be identified.

Characterization of the degradation product in sterilized mixture

In the presence of heavy background from soil and sludge extractable matter, peaks at the level of NP or its degradation products can be detected, in GC-MS, only in extracted ion chromatograms (i.e. not in Full Scan mode). In the case of the nitro-derivative, the relevant report for its occurrence, in the literature, actually assisted the process of identifying the correct GC peak significantly, by providing the respective mass ions. But, in the case of the compound eluting in HPLC before NP, no candidate compounds / spectra were available. Nevertheless, an empirical technique was developed, to assist detecting candidate NP-degradation products in GC-MS chromatograms.

As it was noted before (chapter 6, section 7.2), p353-NP consists of stereoisomers and as a result gives a double peak in GC-MS. Degradation products which reserve the chiral centers would be expected to give also double peaks; actually the nitro-derivative gave a double peak indeed (Fig. 23). For the new compound, with the assumption that the chiral centers in its molecule are intact, a search was performed, limited in double peaks. In particular, the search was focused on those double peaks with the second part slightly higher, as for NP and the nitro-derivative. The investigation was performed for all chromatograms of extracted ions with masses of 150 till 200 (i.e. for 51 chromatograms) as a compromise, assuming that at least one such fragment ion will be present in the MS of the unknown compound. The retention times and the mass spectra of all candidate peaks were noted and sought in the control extracted ion chromatograms (originating from the same HPLC fractions of extracts not containing detectable amounts of this compound). In the end, only one peak fulfilled the criteria and considered to be the peak corresponding to the degradation product of Fig. 31b (Fig. 32).

For evaluating the effectiveness of the method, developed for identifying GC-peaks of NP-degradation products carrying the chiral centers of the parent compound, it was tested for the already identified nitro-metabolite peak. Application of the method in that case led finally to the same conclusion, that there was no other similar double peak absent from the control samples.

According to the MS (Fig. 32), the new compound has presumably a molecular mass of 234 (NP: 220). Its spectrum has many similarities with the spectrum of NP, with almost all ions up to the m/z of 149 being present in both spectra. The relative abundances of these ions are also very similar, apart from the fact that the ion with m/z 121 has considerably lower abundance in the spectrum of the newly identified compound. Focusing on the high mass ions, loss of a water molecule is a very characteristic attribute of the MS of the new compound, following the loss of 29 mass units ($234^{+\cdot} \rightarrow 205^{+}$), and giving an abundant peak at m/z 187.

Loss of water after electron ionization can normally occur in the cases of alcohols, aldehydes and ketones (McLafferty, 1980). Considering the difference between the molecular mass of NP and the probable mass of its degradation product, in combination with the fact that their spectra are very similar, the new compound must be an aldehyde or a ketone. Furthermore, on the basis of the interpretation of the MS of NP (*p*353-NP) by Moeder et al. (2006), the peaks at m/z 107, 121 and 149 must correspond to structures including the phenolic ring, with the latter including also the first

carbon of the nonyl-chain and its two side chains (Fig. 33). Oxidation of the phenolic ring would lead to ring cleavage, thus this possibility can be excluded. Moreover, oxidation of any of the carbons present in the ion with m/z 149 would probably have affected the fragmentation pathway leading to its formation: 149 seems, according to MS/MS data from Moeder et al., to mainly originate directly from the molecular ion. Presence of a carbonyl group in this moiety would result in two subsequent fragmentations, for the oxygen of CO, and the five carbons chain respectively. 149 remains the base ion in the MS of the degradation product of NP, proposing that oxidation has not occurred in any of the carbons close to the phenolic ring.

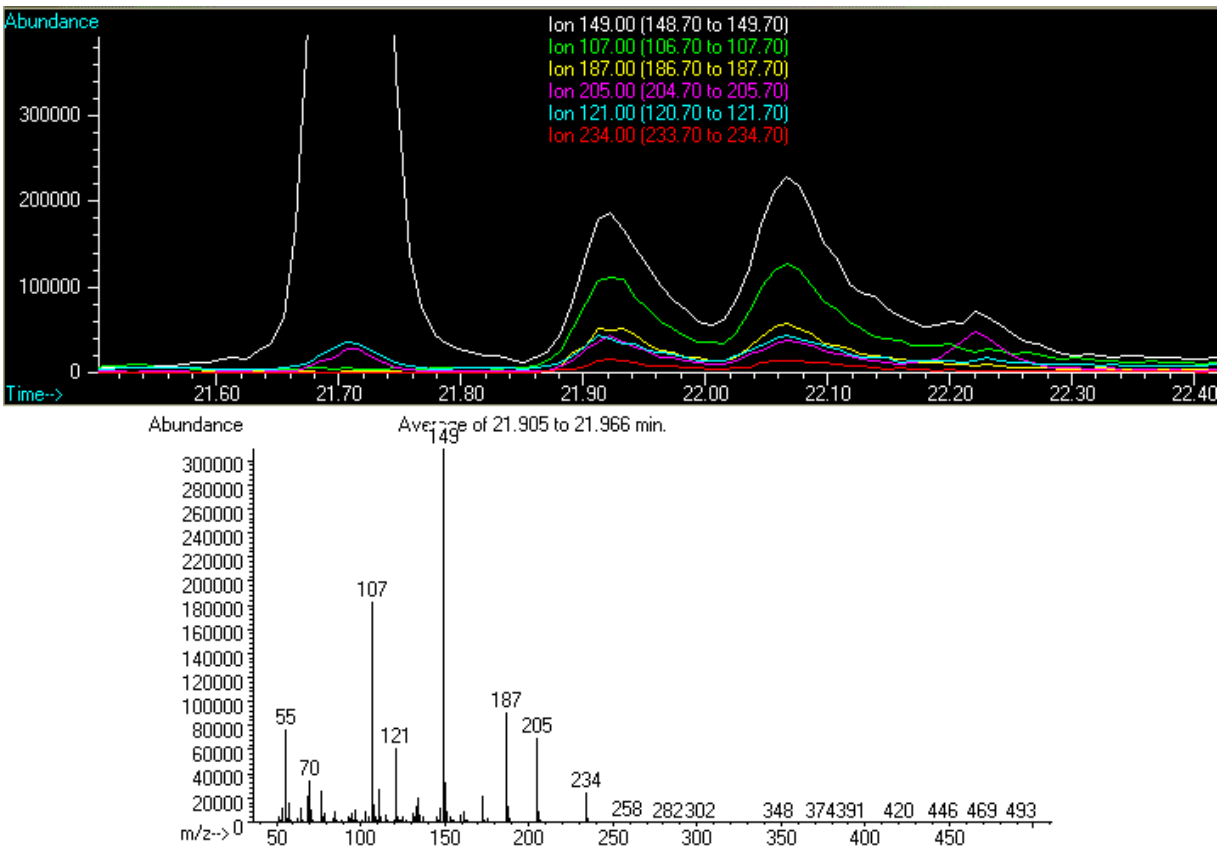


Figure 32. Double GC-MS peak (on the right of the chromatographic window shown) and corresponding mass spectrum for the compound attributed to the degradation product of NP in mixtures of sterilized soil and sludge.

The positions on the nonyl-chain where oxidation can occur include principally all primary and secondary carbons, i.e. seven carbons. The loss of water prerequisites rearrangement(s), during which two hydrogen atoms are attached to the carbonyl oxygen. The donation of H atoms from C atoms of the nonyl-chain may be favored for four of the seven candidate carbons, where temporary rings including four carbons can form (Haib and Stahl, 1990), three of which are distant from the aromatic ring. These positions, where oxidation seems more probable to have occurred, according to the data provided by the mass spectrum, are indicated in the structure of Fig. 34.

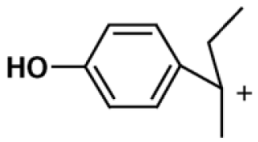


Figure 33. Structure of the ion at m/z 149 in the mass spectrum of *p*353-NP, according to Moeder et al., 2006. Ions at m/z 107 and a small proportion of the ions at m/z 121 presumably result from further fragmentation of this ion (Moeder et al., 2006).

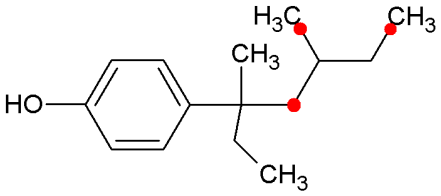


Figure 34. Positions where oxidation of NP is more probable to have occurred, according to MS-data obtained.

13.2.5 Mass balance

The complete mass balance of *NP in the different systems after the incubation period is summarized in Table 28.

Table 28. Distribution of spiked radioactivity between different fractions, after the incubation of different soil-sludge mixtures (averages and ranges of duplicate experiments).

	A	B	C	D	E	F	G	H
Mineralized	0.6% 0.5 - 0.6	2.3% 2.2 - 2.4	7.6% 7.5 - 7.6	7.7% 7.6 - 7.9	0.3% 0.3 - 0.4	1.6% 1.5 - 1.6	3.0% 2.8 - 3.3	3.8% 3.7 - 4.0
Volatilized	ND	ND	ND	ND	ND	ND	ND	ND
Extractable NP	70% 70 - 70	35% 31 - 40	12% 9 - 14	7% 5 - 8	82% 81 - 83	55% 52 - 58	57% 55 - 59	45% 42 - 47
Rest extractable	2% 1 - 4	17% 14 - 20	21% 19 - 23	21% 21 - 21	0% 0 - 0	18% 14 - 22	12% 9 - 15	17% 16 - 19
Physically bound	2% 2 - 3	4% 4 - 4	4% 4 - 5	5% 4 - 5	2% 1 - 2	2% 1 - 2	2% 2 - 2	2% 2 - 2
HA-bound	5% 5 - 5	10% 10 - 11	14% 14 - 14	15% 15 - 15	1% 1 - 1	3% 3 - 3	3% 3 - 3	4% 4 - 4
FA-bound	5% 4 - 5	8% 8 - 8	12% 11 - 12	11% 11 - 11	3% 2 - 3	5% 4 - 5	7% 7 - 7	7% 7 - 7
Humin-bound	10% 10 - 11	18% 16 - 21	24% 23 - 25	28% 25 - 31	10% 10 - 10	15% 15 - 16	14% 13 - 15	19% 18 - 20
Mineral-bound	3% 3 - 3	2% 2 - 3	3% 2 - 3	3% 3 - 3	1% 1 - 1	1% 1 - 1	1% 1 - 1	1% 1 - 1
2nd methanol extraction	2% 1 - 3	3% 1 - 4	3% 2 - 4	3% 1 - 5	1% 1 - 1	0% 0 - 1	1% 1 - 1	1% 1 - 1
Sum	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

As for the soils amended with liquid sludge, non-extractable residues were the highest in the cases where no ingredient or only sludge had been sterilized (61% and 57% on average, respectively), relatively reduced when only soil had been irradiated (43%) and the lowest in completely irradiated mixtures (25%). Bound residues were detected in lower values in the cases of centrifuged sludge amendments; nevertheless the sequence of decreasing bound residues was similar. The highest amount was determined for the non-sterilized mixtures (33%), the lowest for the mixtures containing

sterilized solids (17%) and intermediate values were obtained for the mixtures of sterilized and non-sterilized solids (25% and 27% on average, for sterilized soil and sterilized CE respectively).

13.3 Discussion

13.3.1 NP transformation in sludge-amended soils: abiotic or biological process?

Transformation of NP in incubated soils is the result of two processes: degradation/mineralization and binding. These two pathways are expected in practice not to be independent, as not only NP, but also its degradation products can be bound to the soil matrix (while bound residues can also be mineralized, although theoretically at a much slower rate than more available transformation products). Still, it can be helpful to distinguish between two transformation paths: one leading to extractable degradation products and CO_2, and a second one leading to bound residues, either by binding reactions acting directly on NP, or acting on its degradation products.

The process of transformation of NP into extractable degradation products and CO_2, under the experimental conditions of the current study, was proven to be almost exclusively biological. In sterilized mixtures this process resulted to negligible amounts of products. This was the case for both liquid and centrifuged sludge amendments.

On the other side, binding in sterilized mixtures occurred to a degree of 40% (for LQ) to 50% (for CE) of the amount observed in non-sterilized samples (comparison of A and D for LQ, and E and H for CE, Table 28, paragraph 13.2.5). This may be due to the enzymes present in the systems, which according to the literature can persist under similar γ-irradiation doses, at least to some extent (McNamara et al., 2003). Nonetheless, other components can also act in a catalytic way, like for instance clay surfaces. It has been suggested that the humification process in soil is driven by chemical rather than enzyme reactions (Paul and Clark, 1996 as cited by Grogan and Matthews, 2001). As it was reported in section 3.4, abiotic binding is normally a very rapid process, implemented during the first few days after the entrance of an organic in soil, while microbially-initiated binding contributes rather in a long-term (Benoit, 1994).

13.3.2 NP biotransformation: role of sludge and soil biota

Focusing on the soil systems amended with liquid sludge, the same sum of (extractable) degradation products and CO_2 that accounted in non-sterilized mixtures could be achieved in systems where sludge had been sterilized (ca. 28.7% of the spiked NP, see Table 28). Nevertheless, the sum of these two radioactivity fractions was only 19.3% when soil had been sterilized instead. Thus soils microorganisms seem to play the major role in these processes. A range for the possible contribution of soil species in non-sterilized mixtures in the current experiment is difficult to be calculated, as synergistic effects may be present. Nonetheless, if it is assumed that such phenomena were not significant, the contribution of soil species theoretically cannot be less than 33%, but it can be as high as 100%. The lowest estimate (33%) represents only the amount that is not possible to be carried out in the absence of soil microorganisms [(28.7% - 19.3%) / 28.7%]. The highest accounts for the case that the more competitive soil microorganisms completely outnumber sludge strains in the soil-sludge

mixture. This theoretical range is big, as it includes the uncertainty about the interactions between soil and sludge species in the mixed matrix. Nevertheless, it still indicates that soil biota have presumably the major role.

Interestingly, sludge microorganisms appear to be more involved in degradation and mineralization than soil species, when sludge is amended in dewatered form. By performing similar calculations as just above, it can be concluded that sludge species are responsible for an amount of 28 to 94% of the degraded and decomposed NP in soils amended with CE. This difference between the biodegradation processes in soils amended with LQ and CE may be attributed to the fact that NP located in solid sludge aggregates is more remote to soil organisms than if located in the organic matter of small liquid sludge particles. Nevertheless, in view of the high variation in the radioactivity fraction representing extractable degradation products in soils amended with CE in the last experiment (Table 28, systems F and G), this conclusion may be not significant.

As it was seen in paragraph 13.3.1, binding of NP, 70 days after its entrance in the soil system, is only partially due to biological processes. Comparison of systems containing only one biologically active component could help further fractionating the latter, between soil and sludge-originating species driven processes. Binding of NP in LQ systems promoted by biological processes (ca. 60%) was concluded to be contributed to 30-53% by soil biota and 7-30% by sludge biota, respectively. Accordingly, when CE was added, the roles of species from the two sources must have been practically equally important for NP binding.

13.3.3 Identifying indicators of abiotic, soil-biological and sludge-biological NP transformation

The second aim of the current experimental series was to identify / develop indicators that can specifically point to sludge activity, soil activity or chemical / enzymatic potential for reaction with NP, in order to interpret the data collected during the previous incubations. This can be done by investigating the data of Table 28 for fractions of radioactivity that are associated to the presence of any of these factors. For this purpose, the systems D and H, which involve the combined effect of all factors, will be ignored, and the systems representing abiotic transformation factors (A, E), sludge activity plus abiotic factors (B, F) and soil activity plus abiotic factors (C, G) will be compared. Here it should be noted that any such indicators will in fact be associated not only with the respective fate-leading factors, but also with the availability of NP to them.

The parameter that is most closely associated to physicochemical (abiotic) processes, according to the Table 28, is the amount of radioactivity bound to the mineral fraction of soil. This amount did not increase at all in the presence of sludge and/or soil biota.

Radioactivity fractions exclusively associated either with the activity of sludge species or soil biota could unfortunately not be found. Nevertheless, the highest (proportionally) and most significant difference between systems hosted by soil and sludge microorganisms could be observed in the fraction of mineralized (ring) carbon. In particular, the fraction of mineralized NP was found to be the most increased one in the presence of soil microbial activity. Similarly, the radioactivity bound to fulvic acids appeared to be more associated with soil biota than with sludge biomass.

Fractions of radioactivity more enriched in the presence of living biomass from sludge (than

from soil) were in fact not detected. The only weak indication for such a fraction was associated with the amount of extractable degradation products in the case of CE. This fraction was the only one determined to be higher when sludge instead of soil microorganisms were involved, but the difference was actually not greater than the range of the observed variation in parallels.

13.3.4 Effect of centrifugation

The last incubation experiment was to directly compare the fate of NP in soils amended with LQ and CE, in the presence or absence of microbial populations from sludge or soil or of both. The use of indicators such as those developed in the above paragraph is thus not necessary here. That facilitates the assessment about the driving mechanism behind the effect of centrifugation significantly.

It is possible to roughly quantify the effect that centrifugation had separately on the transformation by soil microorganisms, by sludge microorganisms, and by chemical / enzymatic reactions (Table 29). In order to do this, firstly the contribution of each of these processes, in NP transformation, in soils amended with non-treated sludge (LQ) must be estimated. However, to allow such calculations, several simplifying assumptions have to be made. In these systems, 93% of the NP spiked had been transformed after 70 days (non-sterilized mixture). 30% transformation was observed in completely sterilized mixtures. If abiotic transformation is assumed to happen independently and in parallel with the biological processes (following zero-order kinetics), it can be said that in normal systems 30% of the total 93% was degraded due to abiotic processes. The role of soil and sludge biota in the transformation of the residual 63% can be estimated by the relative strength of these species to transform NP when they were present solely in soil-sludge mixtures. Here it is assumed that no synergistic or antagonistic effects are present and therefore the participation of each of the species in NP transformation depends solely on their transformation rates. It can be concluded that the soil had a dominant role in NP transformation: 88% in comparison with 65% for sludge species (Table 28, systems C and B respectively). Thus, on the base of the above arbirtrary assumption, 63% of biotransformation can be divided accordingly to 36% due to soil biota and to 27% due to sludge biota.

Table 29. Arbitrary estimation of the contribution of different processes on the NP transformation in (non-sterilized) soils amended with LQ and CE.

	NP transformed in 70 d.			**Centrifugation effect**
Transformation factor	**Soil + LQ**	**Soil + CE**	**Difference [relative contribution]**	**Relative difference**
All factors together	93%	55%	-38% [100%]	-41%
Soil microorganisms	*36%*	*18%*	*-18%* ***[50%]***	***-50%***
Sludge microorganisms	*27%*	*19%*	*-8%* ***[20%]***	***-30%***
Chemical / enzymatic potential	*30%*	*18%*	*-12%* ***[30%]***	***-40%***

Similarly, an estimation of the amount of NP transformed by each process in soils amended with treated sludge (CE) can be made. Finally, the effect that centrifugation had on each of the transformation rates can be derived. The respective data are presented in Table 29. The “relative contribution” data of the fourth column of the Table depict that 20% of the centrifugation effect was

due to a decrease in the rate of transformation by sludge microorganisms, 50% by soil biota and 30% due to a decrease in abiotic transformation rates. While the last column can be translated as "50% of the transformation driven by soil microorganisms in liquid sludge amended soil didn't take place when sludge has been centrifuged, etc."

According to these data, centrifugation seems to affect all processes involved in NP transformation. This information is elucidating the general mechanism, on which the effect of this sludge treatment technique is based. Before the respective discussion, the possible mechanisms related to the centrifugation effect, according to the data presented in the previous chapters, will be summarized:

1. physical entrapment of NP in remote sites of sludge aggregates
2. lower partition in the aqueous phase due to DOM losses from sludge during centrifugation
3. less O_2 penetration through the sludge aggregates
4. less *available* nutrients in centrifuged sludge (due to losses in the supernatant)
5. elimination of microorganisms / impact on soil microorganisms (low probability)
6. less amount of reacting chemicals, enzymes or other catalysts in centrifuged sludge (or alternatively blocking of soil catalysts, e.g. clay, by sludge organic matter).

Moreover, the discussion should include one more possible mechanism, on the basis of the fact derived by the experiment of this chapter, that soil microorganisms contribute significantly in NP transformation, and presumably even more than sludge endogenous species (13.3.2):

7. remote localization of NP in regard to soil biota, in centrifuged sludge aggregates

In view of the data of Table 29, the hypothesis "6" cannot have played an important role, but rather being responsible for maximum 30% of the increased persistence of NP (fourth column of the Table). The last hypothesis ("7") can similarly contribute only to a limited extent, as the total effect was only partially due to the biological activity of the soil matrix (last column). The probability of a negative impact of sludge centrifugation on sludge or soil species populations had been already evaluated to be low, on the base of data from DGGE and the literature. The significant contribution of abiotic processes in the sludge treatment effect, which was shown here, is one more indication that this phenomenon cannot explain the observed effect to a great extent indeed.

The hypotheses about the amount of available nutrients could explain the persistence of NP in biotransformation from soil and sludge organisms, which was found to account for roughly 70% of the observed effect (fourth column of Table 29). Nevertheless, it would not be enough to explain the complete effect that sludge centrifugation had on NP fate in amended soils. The same applies for the case of DOM (hypothesis "2"), except if abiotic binding occurs solely in the interface between the solid and aqeuous phase of soil.

An effect on all transformation processes is more probable to depict a phenomenon like physical entrapment or slow penetration of O_2. NP in centrifuged sludge aggregates may be

entrapped in remote sites, such as in compact structures of condensed matter, not easily accessible by microorganisms and oxygen. Lack of oxygen in the direct environment of NP could have not only refrained aerobic biodegradation by soil and sludge species, but in the same time have affected enzymatic reactions involving (directly or indirectly) molecular oxygen. Of course in the case that abiotic transformation does not involve oxygen, absence of some reacting chemicals or enzymes in centrifuged sludge must be taken into account as well.

13.3.5 Effect of sludge conditioning

Freeze-thawing

The results described in chapter 10 showed that freeze-thawing of sludge resulted in a statistically significant reduction in the mineralization rate of NP in amended soil and an additional decrease in the amount of formed bound residues. The first conclusion is that an effect on biological processes is present. Moreover, a look at the mineral fraction of soil shows a slight decrease after freeze-thawing, indicating a possible effect on abiotic processes as well. Returning to the effect on biological processes, and to the associated parameter (mineralization rate), it is interesting to note that the decomposition rate of NP in soils amended with CE after 70 days during the incubation described in chapter 10 (3.7 – 4.3%) was practically the same as in the last one, described herein (3.7 – 4.0%) (Figures 19, 28b). By the last incubation, a sterilization of sludge before amending resulted in a decrease of mineralization rate to 2.8 - 3.3%. However, the mineralized fraction of NP in soils amended with FT in the former incubation was, after 70 days, even less, 1.9 – 2.1%. Therefore a potential effect of freeze-thawing on sludge endogenous consortia doesn't appear as an adequate explanation.

The decrease in the availability of NP in freeze-thawed sludge seems a more possible mechanism, as it could solely better explain a ubiquitous effect on NP-transformation processes.

Liming

In the former experiment liming of sludge had led to very low mineralization rates and a concomitant increase in the amount of residues bound to the humin fraction of soil (resulting to an equal amount of NP transformation as in the case of non-conditioned sludge). A similar comparison on the amount of mineralized NP after 70 days by the two incubation experiments, like in the paragraph above, drives to the conclusion that such low decomposition rates can occur only if both sludge and soil microbial transformation are almost completely ceased, as in mixtures of sterilized soil and sterilized sludge. This may mean that lime has a very negative impact on consortia necessary to decompose NP, both of sludge and soil or that surviving consortia don't have access to either NP or its degradation products.

The second assumption must rather not be connected to a compact structure of limed sludge, as the availability of extractable NP in LM had been found not to be lower than in FT. It could however be connected to a phenomenon of favoured enzymatic binding (of NP or degradation products) under high pH; this case was discussed also in paragraph 10.3.4., in order to interpret the increase in the amount of humin-bound residues.

The fraction of radioactivity bound to the mineral fraction was unaffected after applying lime-conditioning. Thus there is no indication about effects on abiotic processes.

Polymer-conditioning

In the incubation described in chapter 10, polymer-conditioning of sludge had led to a moderate decrease in mineralization after prolonged incubation periods, and an increase in the amount of extractable degradation products due to the formation of a nitro-product. The knowledge gained during the last incubation experiment, about the processes driving NP transformation in sludge-amended soils, doesn't offer much in interpreting the first observation. Mineralization was already expected to be connected to biological processes. A gradual decrease of its rates, to the extent occurred, can be due to any factor including effects on sludge and soil populations or a decreased availability of NP (the latter explanation was adopted until now, see 10.3.4).

Unexpectedly however the last incubation, although not including conditioning of the sludges, resulted in the formation of the nitro-product. This firstly means that for its formation the presence of the specific acrylamide polymer (or any conditioning polymer) is not a prerequisite. Thus the assumption made seems to be wrong. The fact that the specific product was not formed in the mixtures of sterilized soils and sludges, but in all other combinations/mixtures, provides the information that biological processes are involved in its formation and that both soil and sludge-originating species are able to perform the necessary activity. The fact that microbial life is involved doesn't exclude additional involvement of chemical factors, e.g. the amount of nitrogen present in a particular form.

An attempt to retrieve some information about the formation of the nitro-product can be made at this point by comparing its level in the mixtures of the last experiment. These data are presented in Table 30. What can be seen is that the levels of the product follow the levels of NP (presented again in the same Table for assistance), i.e. higher levels of NP are found together with higher levels of the biodegradation product. That simply says that the nitro-product may be susceptible to degradation or binding, as NP, with those processes depending on the microbial activity present in each soil.

Table 30. Levels of the nitro-aromatic degradation product of NP (as ranges in duplicate experiments) in different mixtures of sterilized and non-sterilized soils and sludges. Levels of intact NP (averages) are presented for comparison. All levels are expressed as fractions of the initially spiked NP.

	A	B	C	D	E	F	G	H
NO_2-product	-	*5 - 8%*	*4 - 4%*	*2 - 3%*	-	*9 - 14%*	*7 - 10%*	*7 - 8%*
NO_2-product (2)[a]	-	*2 - 4%*	*2 - 2%*	*0.2 - 0.8%*	-	*7%*	*5%*	*5%*
NP	70%	35%	12%	7%	82%	55%	57%	45%

[a] after alternative integration, not including the tailing of the chromatographic peak (regarded as part of the NP peak, see Fig. 31a)

A second attempt can focus in answering the question "What was in? common between the mixtures of the last experiment and the mixtures containing polymer-conditioned sludge in the former one?" One could assume that the nitrogen content of the sludge should be related to the

phenomenon; nevertheless, in the former incubation, except polymer-conditioned sludge, also freeze-thawed and limed sludges were found to have slightly increased total nitrogen content (Table 24). Thus this assumption would be valid only if the added polymer formulation provided a great amount of *readily available* N (e.g. after its biological decomposition in soil), not obtainable from the sludge used before, but obtainable from the new sludge. The new sludge may have had more available N in its matrix, either because of the different origin (different sampling date), or because of the 12-days of storage intervened before its use – although storing of sludge is known to significantly decrease its N-content (see 2.1).

Finally, the possibility that the polymer-conditioned sludge had been handled in a different way than all other sludges during the former experiment was evaluated. A view on the exact protocol followed when setting up the former incubation showed two differentiations indeed. First of all, conditioning with polymer was chronically the last applied, with an actual delay of 4 days in comparison with the conditioning / dewatering of other sludges (Table 31). That is in accordance with the increased storage time of the sludge used in the last experiment. The second differentiation was, as it can be seen in the same Table, that in contrast to other sludges, sludge conditioned with polymer had been amended on soil in the same day that it was dewatered, i.e. the dewatered sludge was not stored for considerable time before being mixed with the soil. Here it is noted that this was a characteristic of the last experiment as well.

Table 31. Timing followed in preparing the incubation experiment described in chapter 9 (S: spiking, D: conditioning + dewatering, A: amendment).

date (in May) / Sludge	02	03	04	05	06	07	08
FT	S		D	A			
FE			S D	A			
LM			S D	A			
CE			S D		A		
LQ					S A		
PO							S D A

13.3.6 NP degradation product in sterilized mixtures

The aldehyde or ketone detected only in mixtures where both soil and amendment had been γ-irradiated may be due to trace amounts of microbial activity. The amounts of mineralized and degraded fractions were negligible, but still detectable in the respective soils, denoting the presence of very low amounts of microbes. Such organisms may originate either from contaminations or from spores survived the sterilization treatment. Still the involvement of extracellular enzymes from the soil matrix cannot be excluded. Abiotic oxidation of NP in soil would be expected to be more significant in the presence of light (e.g. due to photocatalytical properties of soil components, Mansour et al., 1999), which however was absent during the incubation performed.

14. Differentiation under extrinsic factors

14.1 Aims

An incubation similar to that of chapter 10 (spiking of NP in liquid sludge before treatment) was performed in the presence of light and plant material growing on the soil. This experiment served the purpose of determining whether the effects that sludge treatment had on NP fate in sludge-amended soils would be in general different under the extrinsic influence of additional factors. Moreover, this experiment, by being a more complete simulation of natural systems, could provide an insight into the influence (of sludge treatment) on additional fate processes, i.e. plant uptake and abiotic photodegradation (although the latter was not monitored separately from biological degradation processes).

The respective incubation was performed at an earlier time point, and particularly in the middle of the experiments described in chapter 10, as it was part of cooperation in the frame of a short-term AQUAbase project. For that reason, the sludge treatments selected to be investigated (freeze-thaw and polymer-conditioning) did not include the one having the largest effect on NP fate (centrifugation). Simply centrifuged sludge was applied as control sludge, but no liquid sludge amendments were performed for deducing the effect of the centrifugation treatment.

14.2 Results

14.2.1 Dry matter, organic matter and nutrients in soil and sludges

The DM of treated sludges were calculated indirectly, on the basis of the amount of water removed as supernatant after centrifugation. VS, total N and NH_4^+-N were determined in aliquots parallel to the ones used for the incubation. The results are shown in Table 32.

Table 32. Dry matter, Volatile solids, and N content of the soil and differently treated sludges.

Soil / sludge	DM	VS (dw)	Total N (mg/g, ww)	NH_4^+-N (mg/g, ww)
soil	92.5%	2.3%	1.4	0.27
CE	17.9% (17.7 – 18.2)	54.3%	7.7	2.1
PO	18.0% (17.3 – 18.9)	52.1%	8.2	2.1
FT	21.9% (21.3 – 22.3)	50.6%	9.9	3.1

14.2.2 Water loss during incubation

The soil-sludge mixtures were found to having lost 10% to 20% of their water content after incubation. In parallel mixtures incubated without grass seeds (data not shown), the amended soils

had in contrary gained 15-30% of additional water. The latter soils were covered by moss at the end of the incubation, in contrast with the soils planted with grass seeds. The loss of water of the soils regarded in the current section can partially be due to the fact that before their analysis they were removed out of the incubation tubes and into porcelain plates for some minutes, in order to separate them from the grass leaves and roots.

14.2.3 Mineralization of NP

As it can be seen in Fig. 35, mineralization of the aromatic ring after 72 days of incubation represented on average 2.6% of the spiked amount for all soils, independently of the type of sludge they were amended with. At the initial stages of the experiment, mineralization of the radioactive carbon was faster in soils amended with centrifuged sludge, with the lowest rates being observed for those amended with freeze-thawed sludge.

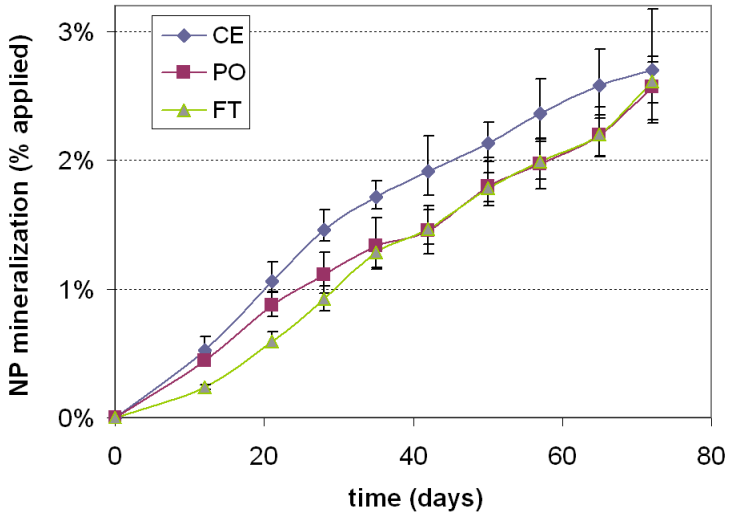


Figure 35. Mineralization of the phenolic ring of NP in soils amended with different sludges: centrifuged (CE), polymer-conditioned (PO) and freeze-thawed (FT) (averages and ranges of triplicate series).

14.2.4 Extractable NP and degradation products

The analysis of the soil extracts did not produce reliable results. Post-incubation, post-extraction degradation of NP was strongly suspected after observing a substantial gradual decrease in the concentration of the parent compound during the sequence of the samples analyzed by HPLC. Although the amount of moss in the systems seeded with grass was not great, the concentrated extracts were green-colored. It is anticipated that photosensitive compounds like chlorophyll acted in a catalytic way in the concentrated extracts, in the presence of light, leading to a rapid transformation of NP in the vials in the autosampler rack. For that reason, in the next only total radioactivity values are given for the extractable fraction.

14.2.5 Mass balance

The distribution of radioactivity in the different fractions of the soil-grass systems after 82 days of incubation is presented in Table 33. The amount of radioactivity taken up by grass was low, never

exceeding 0.7% of the spiked amount. Slightly (but significantly) lower was the uptake for soils amended with non-conditioned sludge. For all systems, the extractable amount of radioactivity represented the most abundant fraction, however without significant differences between soils amended with different sludges. When conditioning polymer was used, the amount of residues bound to humin and minerals fractions was slightly higher than in the other systems. Finally, only small differences were observed in the fraction extractable by NaOH (sum of residues bound to HA and FA).

Table 33. Distribution of spiked radioactivity between different fractions, after 82 days of incubation of soils amended with different sludges and seeded with *Poa pratensis* (averages and ranges of triplicate experiments).

	CE	PO	FT
Mineralized	2.7% 2.2 – 3.0	3.0% 2.8 – 3.2	2.9% 2.7 – 3.0
Volatilized	ND	ND	ND
Taken up by grass	0.28% 0.27 – 0.30	0.58% 0.45 – 0.68	0.50% 0.38 – 0.60
Extractable NP	56%	46%	49%
Rest extractable	51 - 61	46 - 47	46 – 54
Physically bound			
HA-bound	17%	15%	20%
FA-bound	16 - 17	12 - 24	19 - 23
Humin-bound	24%	35%	27%
Mineral-bound	20 - 28	32 - 37	23 - 30
2nd methanol extraction	ND [a]	ND [a]	ND [a]
Sum	**100.0%**	**100.0%**	**100.0%**

[a] this extraction step was performed indeed, though the extract was combined with the other organic extracts

14.3 Discussion

14.3.1 Uptake by grass

The radioactivity located at plant compartments by the end of incubation may theoretically have been transferred there either through volatilization from soil or through translocation via the roots. A comparison with the radioactivity trapped in ethylene glycol traps during the first incubation experiment (homogenous spiking, chapter 9) indicates that the pathway involving the roots must have at least contributed to the uptake by *Poa pratensis*; except if the results obtained herein for grass uptake contain contamination of roots by attached soil particles. In any case, the uptake was very low. That is presumably connected to the high hydrophobicity of NP, which thus is not readily available to the aqueous phase of the soil – as it was seen in the previous experiments during the leaching potential tests.

14.3.1 Sludge treatment effect on NP fate: interference from extrinsic factors

A comparative view on the mineralization curves obtained during the two incubations (Fig. 36) revealed that in the new experiment no detectable lag phase was observed for the NP decomposition process in any soil. A further observation is that the initial mineralization rates in the last

experiment are actually in agreement with the effects detected in the previous incubation: lower rate for polymer-conditioned sludge, even lower for freeze-thawed sludge. As the incubation proceeds, however, there is a trend balancing to the effect of sludge treatment. This is viewed in the form of progressively decreased rates for centrifuged sludge and increased rates for freeze-thawed, thus arriving to a 3% of mineralization at the end of incubation for both treatments (Fig. 35).

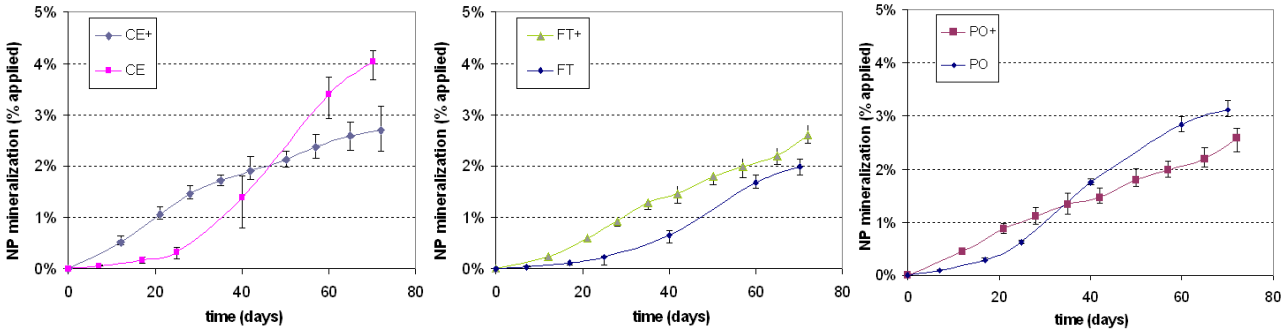


Figure 36. Mineralization of the phenolic ring of NP in soils amended with different sludges [centrifuged (CE), polymer-conditioned (PO) and freeze-thawed (FT)] during the two incubation experiments (without the cross from the experiment described in chapter 10, with the cross in the presence of light and grass). Averages and ranges of triplicate series are shown.

This shows a significant interference of the extrinsic factors with the sludge treatment factor. Several assumptions can be made about the origin of this interference. First of all, regarding the influence on the lag phase of NP decomposition, the presence of light is apparently expected to give rise to the growth of phototrophic organisms. Phototrophic growth means a substantial increase in the energy input to the system, and thus also in the metabolic activity in the soil, which can explain the absence / shortening of the lag period in NP metabolism.

A mechanism adequate to explain a decrease in NP decomposition rate in sludge amended with centrifuged sludge with a concomitant increase for the freeze-thawed sludge amendment can be proposed: In prolonged incubation times the exudates and secretions from plant roots – which are absent during the first stage – stimulate the microbial activity in the rhizosphere. In concurrence with the growth of phototrophic life in soil, this leads to a substantial increase in the amount and the diversity of the biomass. As a result, more even less available (i.e. in freeze-thawed sludge) organic matter can be assimilated, while any effects of sludge treatment on soil or sludge microorganisms are balanced. Nevertheless, the different soil ecology shifts the fate of the assimilated labeled carbon to a lower mineralization rate and to increased fractions of new microbial biomass or bound residues.

Here it is noted that the bound residues in the soil systems were found to be significantly increased in the new experiment, independently of the type sludge applied. Moreover, regarding the possibility the newly sampled sludge to possess properties opposing the sludge treatment effect, it is believed not to be the case, as in the four previous experiments (corresponding to three different sludges) centrifuged sludge amendments in soil were always combined with typical high mineralization rates for NP.

Roberts et al. (2006) detected a significant impact of growing maize in NP mineralization rates in one of two types of soils tested (amended with sludge). For the respective soil, in the presence of plant and after 23 days of incubation, CO_2 accounted for 10% instead of 14% of the spiked compound.

IV. CONCLUSIONS

15. Conclusions

15.1 Answers to the initial questions

Summary of the findings

This study was conducted primarily to establish the correlation between sewage sludge conditioning / dewatering processing and the fate of NP in sludge-amended soils. During the experimental part, effort was made in particular to analyze this correlation under specific, partially controlled conditions (i.e. in the dark, under constant temperature and soil humidity, and with all other handling details mentioned in the respective paragraphs, including soil-sludge ratio, sludge aggregates size, amendment method etc.). Thus the findings that will be summarized here cover this particular aspect. At the end of this paragraph a real case scenario will be set, and an assessment of NP fate will be made on the basis of the above findings, using among other additional information also the results of the "soil-grass" experiment.

Under the conditions tested, the experiments indicated:

- a major impact of sludge centrifugation, leading to a considerably increased persistence of NP in soil
- a moderate impact of freeze-thaw conditioning, leading to a moderate increase on the persistence of NP in soil
- a minor effect of sludge liming, enhancing the formation of bound residues in soil with a concomitant lower mineralization rate of NP, as well as a slight increase in NP leaching potential (no significant effect on overall persistence)
- a minor effect of conditioning by an acrylamide-based cationic polymer, expressed as lower mineralization rates for prolonged incubation periods, the occurrence of a nitro-addition product of NP, as well as a slightly lower leaching potential of NP (no significant effect on overall persistence)
- a negligible impact of ferric chloride conditioning on the fate of NP in soil.

From a quantitative point of view, the major effect, corresponding to sludge dewatering, was an increase in intact NP, from 18% to 70% after 3 months, or from 7% to 45% in 2.5 months, in other incubation (percentage values always represent fractions of the spiked NP). Application of freeze-thawing in combination with centrifugation "protected" even more NP, increasing the intact residues from 70% to 76%, 3 months after spiking / sludge amendment. Minor effects in the balance between bound and mineralized fractions due to the liming effect were of the magnitude of 3-4% of the

(spiked) NP content, when common conditioning doses were used. Whilst of similar extent was the amount of the nitro-product occurred in favour of respectively lower mineralization rates in the case that polymer conditioning was applied. None of the commonly applied treatments accelerated NP degradation in soil. Effects on leaching potential (of polymer and lime-conditioning) were only of the magnitude of 0.2 – 0.3%, applying to a general leaching potential of about 1%, for the non-polar molecule of NP.

The multiplicity of processes and factors involved in NP degradation, decomposition and binding in the complex soil environment, especially in the presence of sewage sludge, was a significant obstacle in interpreting the macroscopic observations in the fate of the model organic compound. Nevertheless, several indicators and a number of specially designed experiments contributed to an assessment about the most probable mechanisms.

The increased persistence of NP in centrifuged sludge is believed to be due to an entrapment in remote sites / compact structures of the "condensed" sludge aggregates, not easily accessible by soil and sludge microorganisms, and where oxygen cannot easily penetrate either. Decreased availability of the NP located in the less permeable flocs of freeze-thawed sludge was similarly proposed as the most probable mechanism behind the moderate increase of NP persistence in soils amended with this type of sludge.

By addition of lime, the increased pH (primarily of sludge, but also of the amended soil) was assumed to be a factor favouring a) enzymatic binding of oxidation products of NP to the soil or sludge humic substances – thus hindering further oxidation to CO_2 – as well as b) a slightly higher degree of deprotonation of NP – hence increasing its mobility towards the aqueous phase and groundwater bodies. Moreover, the biological profile of soils amended with limed sludge appeared to be to an appreciable extent different than all other soils tested. A severe effect on soil and sludge populations involved in NP transformation processes was underlined as an equally alternative – or complementary - hypothesis for the lime-induced effects.

Large flocs with dense cores might be the parameter responsible for the gradually declining mineralization rates for NP located in sludge conditioned with a cationic polymer. The fraction of NP that is sorbed in these cores is probably less available to water, and thus less amenable to leaching. Finally, use of chemicals "familiar" to the sludge matrix, such as $FeCl_3$, seems not to affect significantly the chemistry or biology of sludge.

As for the NP degradation products, sludge treatment proved to be able to affect their formation, i.e. in the case that polymer-conditioning led to the occurrence of a nitro-substituted compound. Nevertheless, this may have been due to coincidental facts, regarding the handling of the particular sludge in the respective experiments, as it was indicated later by the occurrence of the same compound in the absence of polymer. Thus effects of sludge treatment on the degradation pathway of NP on soil should not taken into account before a clear interpretation of the herein observations is made.

Hypothetical scenario

In an attempt to apply the above findings in a hypothetical realistic scenario, the following situation

is considered: the construction of a municipal WWTP is planned, and the application of the produced sludge on nearby grassland areas has been valued as the best management practice. The specific grasslands are often used for grazing. The sludge is not expected to have high levels of heavy metals, as there isn't any industrial contribution to the treated wastewater. Mesophilic anaerobic digestion has been already selected as the technique to be implemented for the stabilization step and it is regarded to eliminating pathogens to a sufficient extent, according to the active legislation (note: that is actually the case according to the active EU legislation, but not according to proposed legislation). The main concern though regards the levels of organic pollutants, and in particular NP, as NPEOs have been found to be present at high levels in the regional wastewater.

The managers working on the WWTP plan consider various sludge treatment options following sludge digestion. To ensure the liability of the planned sludge recycling application, the levels of NP in the final biosolids should not exceed the threshold set by local legislation. The local legislation provides additionally a limit for the total amount of NP in soil – in accordance to current legislation regarding heavy metals in sludge and the soil to be amended. Thus the amount of NP in the treated sludge, and also the further fate of it will play an important role in the decision-making procedure; the second (NP fate) will determine to which extent periodic subsequent applications can be made on the same land, as part of the long-term planning for the WWTP. In addition, local community organizations exert pressure for further measures to protect grazing animals and human health from NP.

As it was seen during the experiments described in the previous chapters, NP levels per dry matter of sludge are not readily affected by the tested conditioning and dewatering treatments. The amount of sludge to be amended will be basically calculated according to its N content and the expected rates for N transformation processes. The latter depend to some extent from the sludge treatment, but they can be considered here to be similar for differently treated sludges. Hence the amount of NP introduced to grassland will be proportional to the dry matter of applied sludge.

Two major alternatives present are the application of sludge in liquid and in dewatered form. In fact, two sub-scenarios can be evaluated. In the first one, the needs of the grassland in N are low, possible to be covered by applicable amounts of liquid sludge. In this case, the alternative of dewatered sludge amendment will be characterized by an equal, or slightly increased amount of sludge dry matter, based on the assumption that sludge dewatering does not lead to considerable losses of N. Thus the NP introduced to the soil will be similar in the cases of liquid and centrifuged (conditioned or not) sludge. Nevertheless, the pollutant is expected to be much more persistent if sludge has been centrifuged. Furthermore, different conditioning treatments will not have great effect on the persistence of NP in soil (especially in the presence of light and grass). The option for liming the sludge in the discussed scenario will actually be probably avoided, as limed sludge is used basically as liming agent, i.e. to increase the pH of the soil. Option to be avoided is also the lack of conditioning, as the resulting biosolids in this case haven't satisfactory properties (DM content). In the above it has anticipated that the influence that light and plant material growth had on the sludge conditioning effect on NP fate will not diminish also the effects observed in dark for sludge dewatering by centrifugation.

In the second sub-scenario, more N is needed to be supplied, and thus, if in liquid form, chemical fertilizers should be supplementary provided. Whilst if in solid form, 5 to 10 times more dry matter of sludge will probably be applied. That means that 5 to 10 times more NP will be introduced into the soil, which, additionally, will be considerably more persistent than in liquid sludge. It is noted that, according to the EU legislation, sludge must be applied to grasslands used for grazing at least 3 weeks before grazing. In time periods of this magnitude (or even periods of a few months), most of NP introduced as part of centrifuged sludge is expected to remain intact (untransformed and extractable by organic solvents). In contrast, periods of 2-3 months will presumably be adequate for an almost complete elimination of NP from soil amended with liquid sludge, into bound residues, as well as a smaller amount of degradation products and CO_2.

The above discussion proposes that liquid sludge amendment will provide a means for significantly reducing risks associated to the presence of NP in sludge to be amended on soil. Nonetheless, in a real scenario several factors might interfere with the effect of sludge treatment. These uncertainties suggest the need for further studies, which is the topic of the last section of this chapter.

15.2 Practical outcomes

Factors determinant to the fate of organics in sludge-amended soils

The data produced during the current study suggested the physicochemical properties of sludge as more determinant to the fate of organic pollutants in soil, in comparison with its biological profile. The size and compactness of sludge flocs are believed to be essential in determining the accessibility of degrading microorganisms to the therein located organic pollutants, as well as the rates of oxygen penetration into the sludge matrix.

Soil-borne, sludge-borne microbial activity, and abiotic factors involved all in the transformation of the model compound in sludge-soil mixtures. In the environment of dewatered sludge aggregates these processes seemed to have an equal contribution to the elimination of the parent compound. In soils mixed with liquid sludge containing the organic pollutant, the differences in the roles of these three factors were again no big, but the role of soil biota was somehow upgraded. If focusing on complete decomposition of the model organic into CO_2, then the role of soil consortia was highlighted as an essential one – probably because the diversity of soil biomass is greater than that of digested sewage sludge.

For non-polar compounds like Nonylphenol, the potential for leaching to groundwater bodies or uptake by plants is expected to be very low, as it was demonstrated by measurements for the model pollutant. Actually the judgment made regarding the critical properties of sludge must rather be adopted for this type of compounds, which are mostly not readily available to the aqueous phase of soil. In the same sense, the picture gained about the origin and outcomes of transformation processes in sludge-amended soil might be related to the presence of the phenolic unit in the model compound; as such structural units are particularly susceptible to enzymatic reactions with humic substances (thus enhancing abiotic binding reactions and in general the formation of bound residues

to the soil or sludge bulk organic matter).

As for the sludge treatment as a factor influencing the fate of organics in amended soils, dewatering appears to be a critical process. Comparison of the effect it had in the present study with the effect reported in the literature for other factors, on the fate of the same compound (soil type, sludge/soil ratio, sludge stabilization method, by Gejlsberg et al., 2001, Hesselsoe et al., 2001, Mortensen and kure, 2003, and Xia and Jeong, 2004 - see also Table 7) points out this fact.

Pathogens elimination vs. persistence of organic toxicants

It must be emphasized that the role of soil microorganisms in the transformation of Nonylphenol in sludge-amended soils was critical, in spite i) the fact that the particular soil had not been amended with sewage sludge, at least since the last 7 years, thus it was not expected to carry microbes adapted to this anthropogenic compound and ii) that the pollutant was located in the sludge matrix, i.e. in a place remote to soil microorganisms. This ability of soil consortia is possibly the main reason behind the importance of physicochemical instead of biological attributes of sludge in sludge-pollutants fate processes. Moreover, this observation suggests that advanced treatments that may be used to ensure the safety of sludge in regard to pathogenic microorganisms will not have drawbacks on the persistence of sludge organic pollutants in the soil environment.

Liming of sludge: an advantageous technique?

Apart from contributing to the hygienization of sewage sludge, liming before dewatering has shown to remove significant portions of organic pollutants that are at least slightly acidic (as for instance the endocrine disruptor Bisphenol A) to the water phase, which can then be adequately treated. In the current study it was shown that drawbacks on the persistence of the residual organics in the soil environment are not to be expected, despite from the negative impact liming has on endogenous and some times even exogenous microbial populations. Although mineralization of remaining organics was negligible in respectively amended soils, the formation of bound residues was enhanced, thus balancing the biological persistence of Nonylphenol. At least this was the fact for the particular compound tested. So, some advantageous aspects of this conditioning technique can be highlighted, if sludge is to be applied in the dewatered form. Nevertheless, the pH of limed sludge limits the range of sites where it is applicable.

On the other hand, the efficiency of liming in eliminating Nonylphenol – an apparently evenly acidic molecule as Bisphenol A - from liquid sludge during dewatering was very poor. It seems that there is a limitation in the applicability of liming as a mitigation technique for the levels of acidic organics. And this limitation appears to lie on the partition coefficient to organic carbon (Koc threshold).

Concentration of organic pollutants in sludge: also a critical fate-affecting factor?

The efficiency of the system soil-liquid sludge in eliminating (degrading and covalently binding) the model organic pollutant was only slightly affected (actually increased) when spiking (of the pollutant) was performed at one order of magnitude lower concentration. It is anticipated that the

concentration of non-polar organic pollutants in sludge isn't a critical factor in fate processes in the soil environment. That is a positive element as regards the extrapolation of the results of the current study to situations characterized by different pollution-levels.

Mitigation of risks associated to the presence of organic pollutants in sewage sludge

In view of the herein presented work was to one side the prediction of the fate of organic pollutants in sludge-amended soils, and to the other side controlling the fate, i.e. mitigating the associated risks to human health and the environment. Centrifugation of digested sludge appeared to entrap NP in remote sites of the compact flocs aggregates produced. An alternative dewatering technique, such as bed drying, would be expected not to have the same effect. In general, any technique that relies on the removal of water rather than the flocculation of solids will very possibly leave the residual organic pollutants more available to oxygen and degrading microbial species. Thus after the contact of that sludge will soil, a faster elimination is anticipated, in comparison with a centrifuged sludge. In that sense, conditioning techniques followed by alternative dewatering treatments may still have significant influence on the fate of organic chemicals in fertilized soils.

Nevertheless, if centrifugation still is the dewatering method of choice, for any reasons like dewatering efficiency, land-area needs etc., any attempt to remove hazardous compounds should take place as long as sludge is in liquid form. Sludge treatment aims at a compact and chemically stable product, something resisting fast elimination rates for any of its ingredients. Any techniques that may be used for this purpose must however manage to balance the losses of palatable ingredients, such as for example nutrients. In an alternative scenario, transformation of available organic matter of liquid sludge into inert, humus-like material could be targeted. Again diligence should be shown in shunning any detrimental effects on the beneficial value of biosolids (e.g. availability and form of nutrients).

15.3 General considerations for future studies

Regarding the methodology used in the present study for following the fate of NP, it is interesting to note that in general the mineralization rates gave the most invariant values between parallel experiments, and the statistically most significant differences between different systems. The reason behind may lie on the fact that these rates represented the whole mass of the incubated systems and not aliquots from them – as was the case for the extraction and fractionation procedures. Particularly since a further sampling was necessary for the determination of the bound residues, the values of which were also used for the normalization according to the total recovery for each system. Soil aliquots for extraction were used instead of the whole soil mass to enable acquisition of multiple data about the incubated systems: extractability by organic solvents, extractability by water, pH, and microbial profile. This methodology was advantageous from the point of view of determining these parameters in the same soil system and not in parallel ones. Though there were drawbacks related to the homogeneity of soil and the adequacy of sampling.

About the general methodology used for interpreting the effect of sludge treatment on NP

fate in soil, a set of macroscopic parameters were assessed for systems simulating real ones (mineralization, degradation, volatilization, plant uptake, binding, for soils amended with different sludges), and it was attempted to connect them with specific parameters either determined in the same systems (availability to the aqueous phase, microbial profiling, nutrients content, water content of sludge), or monitored during especially designed experiments under different, but more controlled conditions (concentration of the pollutant, localization of the pollutant, soil biomass, sludge biomass). This experimental set gave a number of evidences about critical phenomena and parameters determining the fate in sludge-amended soil. As a next step, the exact role of the suspected determinant parameters (size and compactness of sludge flocs, oxygen penetration, etc.) in the fate of organic chemicals could be defined in targeted experiments, with simpler systems and under strictly controlled conditions. In this way data can be produced, that can feed models predicting the fate of pollutants in soil-sludge mixtures. The process should be repeated for model compounds from different chemical classes. Then it will only remain to produce a database about how various sludge treatments or extrinsic factors affect these determinant parameters.

Extrinsic factors to be considered (in other words factors bringing uncertainty to any efforts to extrapolate the herein presented results in different situations) are the light, the growth of plant material, natural freeze-thaw cycles, natural temperature and humidity cycles, sludge dewatering efficiency (DM of sludge), size of sludge aggregates, storage time, and in general timing of sludge treatment and application procedures.

As a last methodological remark, special attention should be given on the spiking strategy. Spiking of artificial pollutants should be optimally performed in such a way, to produce an experimental system which simulates as well as possible the natural one. For example, in the case the effect of sludge conditioning and dewatering is assessed, spiking of the model compound should optimally be performed before sludge stabilization or even in the wastewater before its treatment, since adsorption to wastewater components may influence the compound’s fate during further sludge treatment.

Although the fate of organic pollutants in soils treated with sewage sludge will affect the impact the specific recycling practice has on human health and the environment, it comprises only one dimension of the problem. When decisions are to be made about the best available sludge treatment, a holistic approach has apparently to be followed, taking into account all related beneficial, risk-related and economical factors.

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ANNEX I

Sewage sludge treatment applied around the world

1. Europe (CEC, 2006) ♦

In **Austria**, treatment technologies are different in different regions. In Salzburg, about 34% of sewage sludge (by dry mass) is treated by aerobic and anaerobic stabilisation, adding conditioners (lime and polymers), dewatering, drying and composting. In Vorarlberg, drying and composting are obligatory. The Carinthia region uses drying composting, soilification, sanitation to treat sludge. In Styria, aerobic and anaerobic stabilisation, mechanical dewatering, composting, soilification, drying, calcification are used. Sewage sludge is also watered down before being spread as wet sludge or compost. In Lower Austria, the major methods applied to sludge treatment include aerobic and anaerobic stabilisation (unheated separated, simultaneous, mesophilic or thermophilic), lime dewatering, composting, soilification. In Upper Austria, various types of aerobic and anaerobic stabilisation are used as well as lime dewatering and polymer dewatering.

In the Walloon Region of **Belgium** the methods applied included digestion, aerobic stabilisation, mechanical drying, thermal drying or conditioning with lime or polyelectrolytes. In the Flemish Region the following technologies were employed: aerobic stabilisation, mesophilic anaerobic stabilisation, cold fermentation, thermal drying, and lime stabilisation.

In the **Czech Republic**, aerobic stabilisation is used for approximately 3% of sludge, while anaerobic stabilisation is applied to the remaining part of sludge (unheated approximately 5%, heated approximately 79%).

In **Denmark** the following technologies are employed for treating sludge: stabilization (anaerobic stabilisation by fermentation in heated digester or treatment in a bioreactor; aerobic stabilisation by sludge aeration and composting under conditions where the temperature is not controlled; chemical treatment by addition of lime or slaked lime), controlled composting (composting with daily measurement of temperature so that all material is subject to a temperature of 55°C as a minimum for two weeks), and controlled sanitisation (treatment in reactor which ensures a temperature of 70°C as a minimum for one hour).

♦ data refer to the period 2001-2003

In **Finland** sludge undergoes anaerobic digestion, aerobic digestion, is stabilised by lime conditioning, or composted.

In **France** sludge is subject to prolonged aeration, aerobic or anaerobic stabilisation, lime conditioning, composting, or thermal drying.

The technology used in **Germany** to treat sludge involves a combination of processes, e.g. anaerobic stabilisation with subsequent liming. Sludge is stabilised in particular by means of the following methods: anaerobic (septic tanks), aerobic (oxygenation ditches, long-term treatment) and other (chemical stabilisation by adding lime and other chemicals, thermal stabilisation).

In **Greece** only small quantities of sludge have been used in agriculture so far. Research programmes concerning the treatment of sludge and its use in agriculture continue to be conducted in various areas of the country. Methods for sludge treatments are being examined in these research programmes.

In **Hungary**, biological, chemical, heat treatment, or storage for at least 6 months are applied to sludge.

In **Ireland** sludge is (i) dewatered on filter tables to a solids content of 18%, followed by storage for 3 months prior to application, subject to (ii) anaerobic digestion, (iii) thermal drying, (iv) thermophilic aerobic digestion, and (v) lime stabilisation.

In **Italy** the most common treatments are aerobic digestion (including composting), anaerobic digestion, mechanical dewatering, thermal drying, chemical treatment with alkali. Aerobic digestion is normally carried out on small sized plants up to 50,000 population equivalent (p.e.), while anaerobic digestion is for plants bigger than 50,000 p.e. Composting or co-composting installations which compost solid municipal and other biodegradable wastes treat only a small fraction of sludge (approx. 5%). In addition to aerobic and anaerobic stabilisation, treatment plants carry out mechanical dewatering of sludge by means of drying beds, centrifuging or belt pressing (filter pressing is used in some large plants). Hygienisation and conditioning processes performed away from treatment plants include chemical treatment (with lime or ammonia), physical treatment (drying, pasteurisation) and biological treatment (composting).

In **Luxembourg** sludge is digested and then conditioned with lime or iron salts. Mechanical devices are used for dewatering. Polyelectrolytes are added to sludge which is not conditioned with lime in order to facilitate dewatering.

In the **Netherlands**, sewage sludge must be treated by biological, chemical or thermal processes, by long-term storage or any other suitable method which eliminates most of the pathogenic organisms in

the sludge. Treatment plants are free to choose any treatment method under condition that it brings about the required results.

In **Portugal** the technologies employed are drying beds (drainage on sand beds and evaporation of humidity), thickening, mechanical dehydration (band filters, filter presses, vacuum filters or centrifugal machines) and various stabilisation processes.

In **Spain** anaerobic digestion, long-term storage and composting are the most widely used techniques.

In **Sweden** the following techniques are used: thickening (gravity thickening, flotation), stabilisation (anaerobic, aerobic, lime), conditioning, dewatering (centrifuge, filter belt press, air drying), thermal drying and composting.

In **Slovenia**, sludge is mostly treated by dewatering, stabilisation and conditioning. The most commonly applied treatment methods in Slovakia include anaerobic stabilisation (used by 57% of waste water treatment plants), anaerobic digestion in Imhoff tanks (15% of treatment plants), aerobic stabilisation (34% of treatment plants). Some other treatment methods are also applied, e.g. chemical stabilisation.

In the **United Kingdom** the technologies employed are mesophilic anaerobic digestion, thermophilic aerobic digestion, composting, lime stabilisation, liquid storage, dewatering and storage, thermal drying.

2. USA (US-EPA, 1999)

Major methods of *stabilization* include alkali (lime) stabilization, anaerobic digestion, aerobic digestion, composting, and/or heat drying. Two or more of these processes are often used to treat sludge. Anaerobic digestion is one of the most widely used stabilization practices, especially in larger treatment works (where there is methane recovery potential). Aerobic digestion is commonly used by smaller WWTPs and often is accomplished using WWTPs lagoons containing aeration equipment. High temperature operation (>55^{o}C) for aerobic digestion is becoming more popular (the resulting biosolids have fewer pathogens and higher solids content). As for the composting, the following methodologies are applied: windrow composting, aerated static piles, in-vessel composting.

Dewatering processes include air drying, vacuum filters, plate-and-frame filters, centrifuges, and belt filter presses. Prior to dewatering, biosolids are usually conditioned and thickened.

3. Developing countries (Jimenez et al., 2004)

Many wastewater treatment plants do not include sludge management into their treatment schemes.

In many cases the sludge is applied without treatment or just air dried – the reasons are economical: the sludge management represents the 50% of the total wastewater treatment cost.

ANNEX II

Sewage sludge management around the world

1. Europe

Regarding **European Union** members and the period 1996-1998, agricultural use was the principal outlet for sewage sludge, accounting for about 2.7 million t DM, representing about 38 % of total sludge production. Landfilling was the second major route, representing 37% of total sludge production. Incineration accounted for about 9% of sludge produced in the Member States. Nonetheless, countries presented very diverse profiles: agricultural use counted for about a few percents in Netherlands, up to 70% in Luxembourg. In addition, as these data were collected before the ban on the disposal to sea on December 31st, 1998, a part of the sludge produced in UK and Ireland has since been redirected to other routes. In the case of Ireland, the sludge that was disposed to sea was later directed to agricultural use. Moreover, relevant data is differently integrated to national statistics. Land reclamation and silviculture are often considered as agricultural use, or integrated to other uses, without being precisely documented. Other uses in Member States covered: composting and revegetation (Austria), disposal to sea when allowed (Spain), composting (Netherlands), discharge to the surface waters (Portugal) and use in cement kilns (Denmark) (EC, 2001).

Agricultural use and landfilling were the two major outlets for sewage sludge in **European Union Accession Countries** by that time, accounting for about 250,000 and 400,000 t DM, i.e. respectively 31% and 50% of total sludge production. Disposal to sea was the only disposal route in Malta, as agricultural landscape and types of culture (horticulture and fruit production) made the use in agriculture difficult. In Slovakia, other routes were the use of sludge in silviculture, green areas and land reclamation. Incineration, being a rather expensive technology was not being used widespread: it was only performed in Czech Republic, corresponding to 1 % of the sludge (EC, 2001).

According to Tsagarakis (1999), 80% of the sludge in **Europe** is being disposed of via landfill, whereas use in agriculture and use in forestry only concerns 6% and 4%, respectively, of sludge. The remaining 10 % is disposed of within the WWTP.

The plans (EC, 2001) until 2005 were, for France, UK, Luxembourg, Germany and the Netherlands to further develop incineration. Agricultural use of sewage sludge was expected to increase in Ireland, Finland, UK and Portugal. It should concern about 55% of the sludge produced in the European Union, whereas landfilling should concern about 19% and incineration 23%.

In a more recent report of the European Commission (CEC, 2006), the percentage of sludge applied in agriculture in some of the member states of European Union are summarized. These data,

corresponding to the year 2003, are presented in Figure A1, along with the total amounts amended on land. Complementary to this graph it is mentioned that in UK (missing from the graph) the highest amount of sludge was applied on land (820,000 t, DM), representing 61% of the total amount produced in this country in 2003.

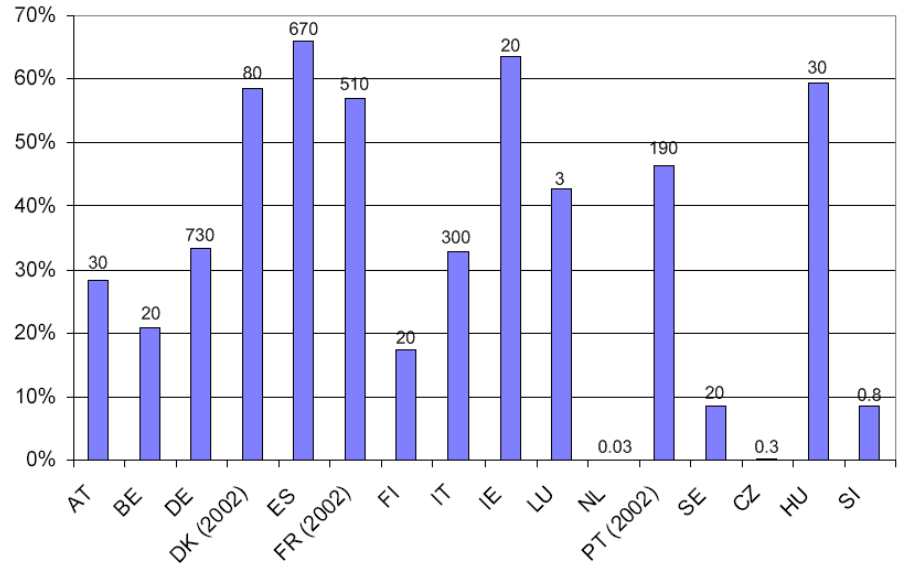

Figure A1. Percentage and absolute amounts of sludge (thousand tons, DM) used in agriculture in some Member States in 2003 (AT: Austria, BE: Belgium, DE: Germany, DK: Denmark, ES: Spain, FR: France, FI: Finland, IT: Italy, IE: Ireland, LU: Luxembourg, NL: Netherlands, PT: Portugal, SE: Sweden, CZ: Czech Republic, HU: Hungary, SI: Slovenia) (CEC, 2006).

Data on the actual evolution of sludge management in the years between 1998 and 2005 for the case of North Rhine-Westphalia, the most populated Federal State in Germany, appear in Figure A2. As it can be seen, the part of municipal sludge used for agricultural purposes was indeed decreased from 33% in 2000 to 22% in 2005, while in the same period the incineration proportion climbed to 64% from 39% in 2000 (LANUV-NRW).

Regarding current developments, in **Western Europe** great attention is now being given to the recycling of all biodegradable wastes within the context of a sustainable soil policy. Revision of the EU Directive 86/278/EEC has not been completed yet, since it will rely on a basis legislation for soil protection, which is still under development (Spinosa, 2007). According to Spinosa, agricultural application seems to remain a major option for many more years, although declining public acceptance and more stringent limits are becoming a limiting factor. While landfilling will presumably be reduced, due to methane emissions, mainly contributing to greenhouse effects. Hence, some areas will change their sludge disposal route from landfilling to landspreading, others from landspreading to incineration and some others will leave out one step , going directly from landfilling to incineration (Spinosa, 2007).

In **Eastern Europe**, cheap methods of sludge disposal prevail. Due to legislation changes (especially in countries joined in the EU), a decrease of landfilling is expected, and a slow increase in the market share of incineration (probably much slower than in Western European countries). The

agricultural use, with more strictly controlled quality, seems to continue to be a sustainable solution in this region (Spinosa, 2007).

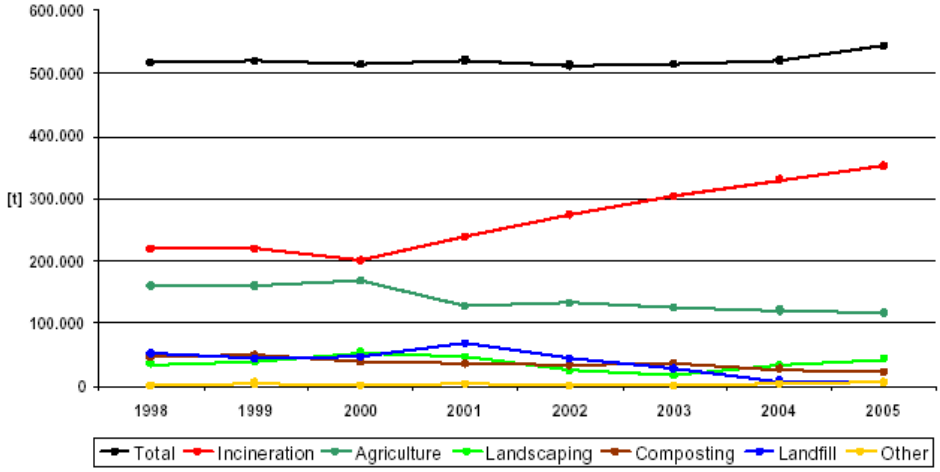

Figure A2. Sewage sludge from municipal waste water treatment plants in North Rhine-Westphalia, 1998 – 2005 (LANUV-NRW, sludge amounts correspond to dry matter).

2. North America

In **Canada**, approximately 389,000 t of biosolids (DM) are produced per year. About 43% of these are applied to land, 47% are incinerated and 4% are sent to landfill, with the remainder used in land reclamation and other uses. Land application has been increasing in recent years as many municipalities move away form incineration and landfill disposal due to environmental concerns with these processes (Apedaile, 2001, data correspond to the year 2000).

In **USA**, in 1998 6,9 million tons (DM) of biosolids were generated. 41% of that amount was amended on land, 22% was incinerated, 17% was handled by landfilling / surface disposal, 12% was composted, 7% followed other beneficial uses and about 1% followed other disposal routes (US-EPA, 1999).

The yearly amount of sludge produced in USA was expected to increase from 7.1 million tons in 2000 to 8.2 million tons in 2010. Due to regulations encouraging the beneficial use of biosolids, as well as the improved perception of the public on sludge recycling practice (because of related scientific research results and marketing efforts – although in some areas acceptance problems persist), land application is expected to increase. On the other hand, restrictions posed from the regulations, on incineration, surface disposal, and landfilling (Part 503 Biosolids Rule, Part 258 Landfill Rule) will probably have a negative influence on the application of these practices. Nevertheless, decreases in landfill costs are causing, in some municipalities, shifts towards landfilling and reductions in biosolids recycling. The projections for the year 2010 include a 48% for land application, 19% for incineration and 10% for landfilling (US-EPA, 1999).

3. Latin America and the Caribbean

With the effort focused on sanitation aspects (water supply, sewerage and wastewater treatment), sludge management has received small attention in this part of the world. In some cases, legislation has been adapted from industrialized countries without adapting it to the local situations (Spinosa, 2007). In Brazil, the available data refer to a 50% of produced sludge being disposed in landfills, a 15% applied to agricultural fields and the rest following unknown routes (Andreoli et al., 2007, data correspond to 2001).

4. Asia

In **Japan** the use in agriculture is minor and is decreasing. Composting seems to be an alternative option, though there are obstacles, namely strict regulations and farmers acceptance. Japan invests in advanced technologies, such as incineration and other thermal solidification processes and use of the final products in cement, bricks, gravels etc. However, energy cost problems have to be solved (Spinosa, 2007).

In **South Korea** most of sewage sludge is dumped into the ocean. However, this practice is to be ceased in 2008. Current laws prohibit the use of sludge as fertilizer in agricultural land. Successful trials on alternative uses have been recently performed, regarding use as feedstock for earthworms and use of mixtures containing sludge against erosion on sloping cut ground (Spinosa, 2007).

It seems that there are practically no regulations on sludge treatment and disposal in **China**. Many wastewater treatment plants are being built and the amount of sludge is expected to increase, with agriculture pinpointed as favoured end outlet (Spinosa, 2007).

In **Vietnam**, only 1 WWTP is in operation, 6 more are planned and the produced sludge is currently lagooned. In **Singapore** almost all sludge is landfilled on an off-shore island site. In **Malaysia** it is planned to replace lagooning with landfilling and land reclamation in the next years. **Taiwan** is following a policy of total reuse by 2010, from landfilling to a mix of composting for horticulture and co-incineration with municipal solid waste with ash reuse (Spinosa, 2007).

5. Africa

The effort in Africa has been concentrated on basic sanitation aspects. When present, on-site sanitation systems (septic tanks, bucket latrines, pit latrines) are the rule, with the faecal sludge management consisted of on-site storage or integrated pond systems, many of which have never been de-sludged. Co-treatment with activated sludge is also used, especially in South Africa, where a great numbers of WWTPs are in operation (Spinosa, 2007).

In **South Africa**, sludge from WWTPs is mostly disposed as direct land application or stockpiling on-site. Other uses, including composting, use in golf courses, cultivation of instant lawn and use as bulking agent have been also referred (Snyman, 2007).

Faecal sludge from on-site sanitation systems is disposed of into the urban and peri-urban environment and used in agriculture or aquaculture or discharged indiscriminately into lanes, drainage ditches, onto open urban spaces and into inland waters, estuaries and the sea, causing serious health impacts, water pollution and eye and nose sores (Strauss and Montangero, 2002;

Snyman, 2007).

6. Australasia

In **Australia**, beneficial recycling is applied on sludge from major cities, where over 50% of the population is residing. **New Zealand** is heavily relying on landfilling, but with a lot of research activity on beneficial outlets around biggest cities (Spinosa, 2007).

Summary

The objectives of the current work were a) to identify the correlation between sewage sludge conditioning / dewatering processing and fate of a major sludge organic pollutant (Nonylphenol, NP) in sludge-amended soils and b) to provide, if possible, clues about the mechanism driving this correlation. In view was a contribution to predicting and controlling the fate of organic xenobiotics in the soil-sludge environment, by examining an until now not adequately studied, but nevertheless potentially determinant factor, i.e. the sludge treatment.

The fate of a radiolabeled isomer of NP was compared in soils amended with sludges which had been treated by different conditioning and dewatering methods in the lab. Under the conditions tested, the experiments indicated:

- a major impact of sludge centrifugation, leading to a considerably increased persistence of NP in soil
- a moderate impact of freeze-thaw conditioning, leading to a moderate increase of the persistence of NP in soil
- a minor effect of sludge liming, enhancing the formation of bound residues in soil with a concomitant lower mineralization rate of NP, as well as a slight increase in NP leaching potential
- a minor effect of conditioning by a cationic acrylamide-based polymer, expressed as lower mineralization rates for prolonged incubation periods, as well as a slightly lower leaching potential of NP
- a negligible impact of ferric chloride conditioning on the fate of NP in soil.

For interpreting the effect of sludge treatment on NP fate in soil, it was attempted to link its transformation rates with specific parameters, either determined in the same systems (availability of NP to the aqueous phase, nutrients content, water content of sludge, profile of bacterial populations), or controlled during especially designed experiments (concentration of the pollutant in sludge, localization of the pollutant, soil or sludge biomass). Moreover, related data from the literature were co-evaluated.

The increased persistence of NP in centrifuged sludge was attributed to its entrapment in remote sites / compact structures of the condensed matter in sludge aggregates, where it is not easily accessible to soil and sludge microorganisms, and where oxygen cannot easily penetrate either. Decreased availability of the NP located in the less permeable flocs of freeze-thawed sludge was similarly proposed as the most probable mechanism explaining the moderate increase of NP persistence in soils amended with this type of sludge.

By addition of lime, the increased pH (primarily of sludge, but also of the amended soil) was

assumed to be a factor favouring a) binding of oxidation products of NP to the soil or sludge humic substances – thus hindering further oxidation to CO_2 – as well as b) a slightly higher degree of deprotonation of NP – hence increasing its mobility towards the aqueous phase and groundwater bodies. A effect on soil and sludge populations involved in NP transformation processes was underlined as an equally alternative – or complementary - hypothesis for the lime-induced effects.

Large flocs with dense cores appealed as a potential fact responsible for the declining mineralization rates for NP in sludge conditioned with a cationic polymer. The fraction of NP sorbed in these cores was probably less available to water, and thus less amenable to leaching.

The results highlighted the physicochemical properties of sludge (e.g. size and compactness of sludge flocs) as more determinant to the fate of non-polar organic pollutants in soil, in comparison to its biological profile. As a consequence, application of advanced treatment techniques for the hygienization of sludge is not expected to have considerable drawbacks regarding the biological degradation of hydrophobic pollutants in the soil environment.

The results furthermore propose sludge dewatering as one determinant process for the fate of organics in amended soils. Liquid sludge amendment showed to provide a means for significantly reducing risks associated to the persistence of NP and probably other pollutants in these systems, although in a real scenario extrinsic factors might interfere with the effect of sludge treatment. For mitigating the respective risks associated with the application of dewatered sludge, use of dewatering techniques relying on the removal of water rather than the flocculation of solids (e.g. bed drying) or an elimination of its pollutants whilst in liquid form (e.g. by bioaugmentation) were proposed.

Finally, when decisions are to be made about the best available sludge treatment technique, a holistic approach has to be followed, taking into account, apart from organic pollutants related aspects, all beneficial, risk-related and economical factors associated with the recycling of sludge to the terrestrial environment.

Zusammenfassung

Ziel dieser Arbeit war a) die Bestimmung der Korrelation zwischen der Konditionierung / Entwässerung von Klärschlamm und dem Verbleib eines organischen Hauptschadstoffs (Nonylphenol, NP) in Klärschlam-gedüngten Böden und b) die Identifizierung des dafür verantwortlichen Mechanismus. Dabei sollte ein Beitrag zur Vorhersagbarkeit und Steuerbarkeit des Verbleibs organischer Fremdstoffe in Klärschlamm-Boden-Systemen geleistet werden. Im Fokus der Untersuchungen stand ein bisher nicht angemessen studierter, doch möglicherweise bestimmender Faktor - die Klärschlamm-Behandlung.

Der Verbleib eines radiomarkierten Isomers von NP in mit Klärschlämmen gedüngten Böden wurde untersucht. Dabei wurden die Klärschlämme zuvor im Labor durch verschiedene Konditionierungs- und Entwässerungs-Methoden behandelt. Bei den untersuchten Parametern zeigten sich unter anderem folgende Effekte:

- die Klärschlamm-Zentrifugation führte zu einer beträchtlich erhöhten Persistenz des NPs im Boden
- eine Behandlung des Klärschlamms durch Gefrierauftauen führte zu einer moderaten Erhöhung der NP-Persistenz im Boden
- eine Klärschlamm-Kalkung hatte eine geringe Wirkung in Form einer erhöhten Bildung von gebundenen Rückständen im Boden, einer verkleinerten Mineralizationsrate, sowie einer leichten Erhöhung des Leaching-Potenzials
- die Konditionierung des Schlamms durch ein kationisches, Akrylamid-basiertes Polymer hatte eine geringe Wirkung, die sich in Form einer niedrigeren Mineralizationsrate bei langen Inkubationszeiten, sowie in einer geringen Herabsetzung des Leaching-Potenzials zeigte
- eine $FeCl_3$-Konditionierung hatte keinen bedeutenden Effekt auf den Verbleib des NPs im Boden.

Zur Interpretation des Einflusses der Klärschlammbehandlung auf den Verbleib von NP im Boden wurden die Transformationsraten der Chemikalie mit Parametern in Verbindung gesetzt, die entweder im selben Experiment direkt (Verfügbarkeit von NP in der wässrigen Phase, Nährstoff- und Wassergehalt des Schlamms, Populationsprofil der Bakterien im Boden), oder durch begleitende analytische Experimente (Konzentration des Schadstoffs im Klärschlamm, Lokalisierung des Schadstoffs, NP-Transformations-Potential der Boden- bzw. Klärschlamm-Biomasse) bestimmt wurden. Zusätzlich wurden die Ergebnisse der Experimente mit betreffenden Daten aus der wissenschaftlichen Literatur verglichen.

Die erhöhte Persistenz des NPs im zentrifugierten Klärschlamm wurde auf dessen Einschluss in oberflächenferne Lagen und kompakte, 3-dimensionale Strukturen der komprimierten Schlamm-Aggregate zurückgeführt, der weder für die Boden- und Schlamm-Mikroorganismen, noch für

Sauerstoff zugänglich ist. In ähnlicher Weise wurde die reduzierte NP-Verfügbarkeit in den weniger permeablen Flocken des gefrier-aufgetauten Klärschlamms als Ursache der moderaten Erhöhung der Persistenz von NP in mit solchem Klärschlamm gedüngten Böden angenommen.

Bei der Kalkung des Schlamms wurde der erhöhte pH-Wert (nicht lediglich des Klärschlamms, aber auch des gedüngten Bodens) als ein Faktor angenommen, der a) die Bindung von Metaboliten des NPs an boden- oder schlammbürtige Huminstoffe unterstützt – und in dieser Weise die komplette Mineralisation zu CO_2 behindert – sowie b) die Deprotonierung von NP zur Folge hat – wodurch dessen Mobilität in der wässrigen Phase des Bodens und im Grundwasser erhöht wird. Zusätzlich (oder alternativ) wurde ein Effekt der Kalkung auf die für den Ausbau des NPs verantwortlichen Spezies (entweder im Schlamm oder im Boden) angenommen.

In mit kationischem Polymer konditioniertem Klärschlamm scheinen große Flocken mit kompakten Kernen bei der Verringerung der Mineralizarionsraten von NP eine Rolle zu spielen. Das in diesen Flocken lokalisierte NP ist möglicherweiser weniger zugänglich für Wasser und somit auch für Leaching.

Die Ergebnisse der vorliegenden Arbeit heben die physikochemischen Eigenschaften des Klärschlamms (z.B. Größe und Kompaktheit der Schlamm-Floken) als bedeutender für den Verbleib von unpolaren organischen Schadstoffen in Boden hervor, im Vergleich zu dessen biologischem Profil. Infolgedessen ist voraussichtlich durch die Anwendung von weitergehenden Behandlungsmethoden zur Hygienisierung des Klärschlamms nicht mit signifikanten Nachteilen bezüglich der Persistenz von hydrophoben Schadstoffen gegen biologischen Abbau im Boden zu rechnen.

Darüberhinaus konnte die Klärschlammentwässerung durch die Ergebnisse der vorliegenden Arbeit als ein bestimmender Prozess für den Verbleib von organischen Stoffen in gedüngten Böden identifiziert werden. Düngung mit flüssigem Schlamm zeigte sich in den Experimenten als ein möglicher Weg zur Reduzierung des Persistenzrisikos von NP und anderen Schadstoffen in diesen Systemen, obwohl in der Praxis auch externe Faktoren den Einfluss der Schlammbehandlung beeinträchtigen könnten. Wird der Schlamm in entwässerter Form auf Boden aufgebracht, so können vermutlich die ensprechenden Risiken durch einen Ersatz der Zentrifugation durch alternative Techniken reduziert werden. Hierbei sind Entwässerungstechniken zu nennen, die anstatt auf Flockenbildung auf der Entfernung von Wasser basieren (z.B. Trockenbett), oder auch eine Elimination der Schadstoffe im noch flüssigen Schlamm, d.h. vor der Trocknung (z.B. Bioaugmentation).

Bei der Wahl der besten verfügbaren Technik für die Klärschlammbehandlung sollten neben der Problematik der organischen Schadstoffe auch alle weiteren vorteilhaften, sicherheitsrelavanten und ökonomischen Faktoren in Betracht berücksichtigt werden, die für das Recycling von Klärschlamm in der terrestrischen Umwelt relevant sind.

Lebenslauf

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