Sorption and leaching of glyphosate on partly sealed urban areas

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Preface

This work was conducted within the research training group (Graduiertenkolleg) 780/II “Perspectives on Urban Ecology II” (speaker: Prof. Dr. W. Endlicher, HU Berlin) funded by the German Research Foundation (Deutsche Forschungsgemeinschaft – DFG). This interdisciplinary research training group with PhD projects in social and natural sciences just started its third three years period and was initiated in 2002 by three Berlin universities (Humboldt Universität, Technische Universität, Freie Universität) and two research institutes (Leibniz-Institut für Gewässerökologie und Binnenfischerei, Leibniz-Institut für Regionalentwicklung und Strukturforschung).


Cohort 2 (2005 – 2008): Perspectives on Urban Ecology II - Shrinking cities: structural changes as opportunities for the development of urban nature and enhanced environmental quality for city dwellers


The first three years period (2002 – 2005) of this research training group focused on the more general perspectives of urban ecology in large cities like Berlin. The aim of the second cohort (2005 – 2008) was 1) to develop further perspectives of general urban ecology particularly on the basis of the first group’s work and 2) to focus on urban ecology issues in shrinking cities (e.g. urban vacant lots).

My project focused on point 1) as my project continues the work started by the forerunner project from Dr. Thomas Nehls who investigated the properties of partly sealed urban soils and the sorption of heavy metals thereto (Nehls 2007). While his project focused on water fluxes in paved urban soils and heavy metal sorption thereto, my project deals with the sorption and leaching of an organic compound (the pesticide glyphosate) in paved urban soils.

Additionally to my disciplinary work an interdisciplinary research project (chapter five of this work) within the research training group was conducted together with Valeska Contardo-Jara from the Leibniz-Institut für Gewässerökologie und Binnenfischerei (Leibnitz-Institute of Freshwater Ecology and Inland Fisheries) in Berlin.
Abstract

The motivation for this work was the application of the herbicide glyphosate for weed control on pavements in Berlin by the Berlin public cleansing service (Berliner Stadtreinigungsbetriebe). In contrast to numerous studies concerning the fate of glyphosate in agricultural soils, studies regarding the leaching in urban areas are missing. As the construction material for pavements, e.g. coarse sand, has lower sorption capacities for glyphosate than agricultural soils, the aim of this study was to assess the risk of glyphosate leaching into deeper layers after its application on partly sealed pavements.

Therefore the fate of glyphosate was investigated on four methodological levels after a basic characterisation of the seam material:

1. sorption batch experiments with seam material from pavements in Berlin,
2. outdoor experiment with partly sealed lysimeters,
3. column experiments with material from a lysimeter and from a pavement,
4. ecotoxicological experiments with the worm *Lumbriculus variegatus*.

On partly sealed areas the seam material, the material between the paving stones, is the only infiltration pathway and therefore plays an important role concerning the discharge of pollutants into the soil and the groundwater, respectively. It was shown that the upper 0-1 cm layer possesses clearly different physico-chemical properties (i.a. increased contents of organic carbon, dithionite and oxalate extractable iron and aluminium, heavy metals and black carbon) than the lower 1-5 cm layer due to urban depositions like particulate matter, soot, abrasions from tires, foliage and animal faeces. With the batch experiments a significant higher sorption capacity of the 0-1 cm layer compared to the 1-5 cm layer was determined. Thus, the urban deposition increases the sorption capacity of the seam material for glyphosate and acts like a filter. However, no significant correlation between the studied parameters and the sorption capacity of the upper seam material was found.

Outdoor experiments with partly sealed lysimeters, which were filled according to the construction guidelines for pavements in Germany and covered with different common surface coverings [pavement flagstones (S95), paving stones (S50), loamy sand (L)], were conducted to study the glyphosate transport under conditions close to reality. 24 hours after the application of a 10% Roundup Ultra solution with the roller wiper Rotofix the areas were artificially irrigated. Unexpected high concentrations of glyphosate were detected in the leachate of the partly sealed lysimeters S95 and S50. A Maximum concentration of \(> 1000 \mu g L^{-1}\) was measured in the leachate of lysimeter S95. In total almost 60% of the...
amount applied was found as glyphosate equivalent (sum of glyphosate and degradation product AMPA) in the leachate of lysimeter S95. Moreover, with 10% of the amount applied a high discharge of glyphosate and AMPA was as well observed in the other partly sealed lysimeter (S50).

The transport of glyphosate via preferential flow could be demonstrated with tracer experiments (bromide + brilliant blue). The partly cover of the surface of lysimeters S95 and S50 with paving stones caused a reduced flow cross section of only 50%. Additionally the transport of glyphosate via matrix flow was assumed.

For the investigation of glyphosate transport in pavement material under controlled laboratory conditions, especially for testing the assumption of glyphosate transport via matrix flow, column experiments with $^{14}$C-labelled glyphosate were conducted with realistic application and infiltration rates. Therefore four stainless steel columns were filled up to depth of 11 cm with

a) material from the lysimeter experiment (L) and

b) material from a pavement (P).

During the 117 days of the column experiment no glyphosate equivalent breakthrough was observed in the leachate of columns P, filled with material from a pavement. This can be attributed to the high sorption coefficient ($K_f$) of the material ($K_f = 26.3 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$) compared to the coarse sand from the lysimeter ($K_f = 5.1 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$). By contrast about 33% of the amount applied was leached during the whole experiment from columns L, filled with the coarse sand of the lysimeters. Maximum concentrations of $> 400 \mu \text{g L}^{-1}$ were measured in their leachate. The assumption of glyphosate transport via matrix flow proved true for the coarse sand as preferential flow could be excluded due to the bromide flow behaviour. The breakthrough curves were fitted with the numerical transport model HYDRUS 1-D. Best fits were obtained with a non-linear two-site sorption model resulting in $K_f \leq 1 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$. The $K_f$ calculated from the column breakthrough curves for the coarse sand is an order of magnitude lower than the one derived from the batch experiments.

For the ecotoxicological investigations sublethal concentrations of $0.05 - 5 \text{ mg L}^{-1}$ were tested over four days on the worm *Lumbriculus variegatus* in a system without sediment. The bioaccumulation and the effects on biotransformation- and antioxidant enzymes of glyphosate and its formulation Roundup Ultra were tested. The study shows that glyphosate accumulates in the organisms despite its hydrophilic character and thereby causes an elevation of biotransformation (sGST) and antioxidant enzymes (SOD). The reaction was clearly higher in the animals exposed to Roundup Ultra, indicating that the formulation Roundup Ultra is due to it’s additives of more ecotoxicological relevance than the glyphosate itself.
This study shows that the leaching of glyphosate on partly sealed areas can not be ruled out: On the one hand due to preferential flow paths under partly sealed areas, but as well due to low sorption capacities of material used for the construction of pavements, which can lead to relocation via matrix flow. Thus, the use of glyphosate in urban areas should be reconsidered.
Zusammenfassung


Dazu wurde nach einer grundlegenden Charakterisierung des Fugenmaterials das Verhalten von Glyphosat auf vier methodischen Ebenen untersucht:

1. Sorptionsbatchexperimente mit Fugenmaterial von Berliner Gehwegen,
2. ein Freilandversuch mit teilversiegelten Lysimetern,
3. Säulenversuche mit Material des Lysimeters und Fugenmaterial eines Gehwegs,
4. ökotoxikologische Versuche mit dem Wurm Lumbriculus variegatus.

Da auf teilversiegelten Flächen das Fugenmaterial, das Material zwischen den Pflastersteinen, teilweise der einzige Versickerungspfad ist, spielt es eine entscheidende Rolle beim Eintrag von Schadstoffen in den Boden bzw. das Grundwasser. Es konnte gezeigt werden, dass die obere 0-1 cm Schicht aufgrund von urbaner Deposition (z.B. Feinstäube, Ruß, Bremsenabrieb, Laub, Kot) gegenüber der unteren 1-5 cm Schicht deutlich veränderte physiko-chemische Eigenschaften (u.a. erhöhte Gehalte an organischem Kohlenstoff, dithionit- und oxalat-extrahierbarem Eisen, Schwermetallen und Black Carbon) aufweist. Mit Hilfe der Batchversuche konnte eine signifikant höhere Sorptionskapazität der 0-1 cm Schicht gegenüber der 1-5 cm Schicht für Glyphosat nachgewiesen werden. Die städtische Deposition erhöht somit die Sorptionskapazität des Fugenmaterials für Glyphosat und wirkt wie ein Filter, allerdings konnte kein signifikanter Zusammenhang zwischen den untersuchten Parametern und der Sorptionskapazität des oberen Fugenmaterials festgestellt werden.

Um die Verlagerung von Glyphosat unter realitätsnahen Bedingungen zu untersuchen, wurden Freilandversuche mit teilversiegelten Lysimetern durchgeführt, die entsprechend den Vorschriften für den Wegebau in Deutschland befüllt und mit unterschiedlichen, im kommunalen Bereich üblichen Belägen [Gehwegplatten (S95), Kleinsteinpflaster (S50), wassergebundene Wegdecke (L)] bedeckt waren. 24 Stunden nach der Applikation einer 10%igen Roundup Ultra-Lösung mit dem Walzentreichgerät Rotofix wurden die Lysimeter
künstlich beregnet. Im Sickerwasser der teilversiegelten Lysimeter S95 und S50 wurden unerwartet hohe Konzentrationen von Glyphosat gemessen. Eine Höchstkonzentration von > 1000 µg L\(^{-1}\) wurde im Sickerwasser von Lysimeter S95 beobachtet. Insgesamt wurden fast 60% der aufgetragenen Menge an Glyphosat als Wirkstoffäquivalent (Summe Glyphosat und Abbauprodukt AMPA) im Sickerwasser dieses Lysimeters wiedergefunden. Auch in dem zweiten teilversiegelten Lysimeter (S50) war mit insgesamt 10% der applizierten Menge ein hoher Austrag an Glyphosat und des Abbauprodukts AMPA zu verzeichnen.

Aufgrund der durchgeführten Tracerversuche (Bromid + Brilliant Blue) konnte eine Verlagerung des Herbizids durch präferentielles Fließen nachgewiesen werden. Wegen der Teilversiegelung der Oberfläche mit Pflastersteinen nahmen nur 50% der Bodenmatrix der Lysimeter S50 und S95 am Fließgeschehen teil. Zusätzlich zu der Verlagerung über präferentiell Fluss wurde eine Verlagerung über Matrixfluss vermutet.

Zur Untersuchung der Verlagerung von Glyphosat im Gehwegmaterial unter kontrollierten Laborbedingungen, insbesondere zur Überprüfung der im Lysimeterversuch vermuteten Verlagerung von Glyphosat durch Matrixfluss, wurden Säulenversuche mit \(^{14}\)C-markiertem Glyphosat mit realistischer Applikations- und Beregnungsrate durchgeführt. Dazu wurden vier Edelstahlsäulen 11 cm hoch mit

a) Material des Lysimeterversuchs (L) und
b) Material von einem Gehweg (P) befüllt.

Während der 117 Tage des Säulenversuchs wurde in den mit Gehwegmaterial befüllten Säulen P kein Durchbruch von Glyphosat oder seines Abbauproduktes ins Sickerwasser beobachtet. Dies ist auf den, im Gegensatz zum Grobsand der Lysimeter, hohen Sorptionskoeffizienten (\(K_f\)) des Materials (\(K_f = 26,3\) mg\(^{1-n}\) L\(^n\) kg\(^{-1}\)) zurückzuführen. Im Gegensatz dazu wurden über den gesamten Versuchszeitraum in den mit dem Grobsand der Lysimeter (\(K_f = 5,1\) mg\(^{1-n}\) L\(^n\) kg\(^{-1}\)) befüllten Säulen L ca. 33% der applizierten Menge als Wirkstoffäquivalent mit dem Sickerwasser ausgetragen. Im Sickerwasser wurden Höchstkonzentrationen von > 400 µg L\(^{-1}\) gemessen. Da ein präferentielles Fließen aufgrund des Bromid-Fließverhaltens ausgeschlossen werden konnte, bestätigte sich die Vermutung, dass ein Austrag von Glyphosat und dessen Abbauprodukten im Grobsand auch über Matrixfluss stattfindet. Die Durchbruchskurven wurden mit dem numerischen Transportmodell HYDRUS-1D angepasst. Die beste Anpassung wurde bei einem Modell mit zwei Domänen und nicht-linearer Sorption, mit einem \(K_f \leq 1\) mg\(^{1-n}\) L\(^n\) kg\(^{-1}\), erreicht. Der für den Grobsand aus den Durchbruchskurven der Säulenversuche errechnete \(K_f\) Wert ist damit eine Größenordnung kleiner als der im Batchexperiment bestimmte.

Die ökotoxikologischen Untersuchungen wurden in einem sedimentlosen System mit dem Wurm _Lumbricus variegatus_ mit sublethalen Konzentrationen von 0,05 – 5 mg L\(^{-1}\) über vier Tage durchgeführt. Dabei wurde die Bioakkumulation und die Effekte von Glyphosat und
seiner Formulierung Roundup Ultra auf Biotransformations- und Antioxidationsenzyme getestet. Sie zeigen, dass Glyphosat trotz seines hydrophilen Charakters in den Organismen akkumuliert und dadurch eine erhöhte Aktivität von Antioxidations- (SOD) und Biotransformations-Enzymen (sGST) bewirkt. Die Reaktion der Organismen war für die reine Glyphosat Substanz geringer als für die Formulierung Roundup Ultra, was darauf hindeutet, dass die Formulierung Roundup Ultra aufgrund ihrer Zusatzstoffe eine größere ökotoxikologische Relevanz aufweist als Glyphosat selber.

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\(^{14}\)C radioactive labelled carbon  
AAS atomic absorption spectrophotometer  
\(\text{Al}_{\text{dit}}\) dithionite extractable aluminium  
\(\text{Al}_{\text{ox}}\) oxalate extractable aluminium  
AMPA amino-methylphosphonic acid  
ANOVA analysis of variance  
BB Brilliant Blue  
BET Brunauer-Emmet-Teller  
BC black carbon  
BCF bioconcentration factor  
BTC breakthrough curve  
CAT catalase  
CDE convection-dispersion equation  
CEC\(_{\text{eff}}\) effective cation exchange capacity  
ChE cholinesterase  
\(C_{\text{org}}\) organic carbon content  
\(C_{\text{tot}}\) total carbon content  
\(C/N\) ration of carbon to nitrogen  
\(\text{DT}_{50}\) half-life time  
dw dry weight  
EC\(_{50}\) effective concentration, where 50% of tested organisms are effected by tested compound  
FC field capacity  
\(\text{Fe}_{\text{dit}}\) dithionite extractable iron  
\(\text{Fe}_{\text{ox}}\) oxalate extractable iron  
fw fresh weight  
GST glutathione S-transferase  
HPLC high performance liquid chromatography  
IC\(_{50}\) inhibitory concentration, where biological or biochemical functions of 50% of tested organisms are inhibited by tested compound
ICP  inductively coupled plasma spectrometer
Ksat  saturated hydraulic conductivity
LC50  lethal concentration, where 50% of tested organisms are killed by exposure to tested compound
LSC  liquid scintillation counting
NOEC  no observed effect concentration
N$_{tot}$  total nitrogen content
mGST  membrane bound glutathione S-transferase
MPC  maximum permissible concentration
OECD  Organisation for Economic Co-operation and Development
OM  organic matter
PAH  polycyclic aromatic hydrocarbon
POEA  polyoxyethylene amine
P$_{tot}$  total phosphorus content
ROS  reactive oxygen species
sGST  soluble glutathione S-transferase
SOD  superoxide dismutase
SOM  soil organic matter
SSA  soil surface area
SSQ  sum of squares
UD  urban dust

Sample abbreviations
OS  sampling site Oberschöneweide
SS  sampling site Siemensstadt
GS  sampling site Großer Stern
SHA  sampling site Schönhauser Allee
FBS  sampling site Fehrbelliner Straße
AWD  sampling site Am Weidendamm

L  lysimeter covered with loamy sand
S50  lysimeter 50% sealed with paving stones
S95  lysimeter 95% sealed with pavement flagstones

L1  column filled with coarse sand from lysimeter experiment (replicate 1)
L2  column filled with coarse sand from lysimeter experiment (replicate 2)
P1  column filled with pavement material from sampling site OS (replicate 1)
P2  column filled with pavement material from sampling site OS (replicate 2)
1 General introduction

This study deals with the sorption and leaching of the herbicide glyphosate after application on partly sealed urban areas.

The motivation for this study was the application of the herbicide glyphosate for weed control on pavements in Berlin by the Berlin public cleansing service (Berliner Stadtreinigungsbetriebe). While the fate and behaviour of glyphosate in agricultural soils is well investigated (Borggaard and Gimsing 2008), its fate after application to urban soils is unknown. Thus, this study contributes to the assessment of pesticide use in urban areas.

Urban soils

Urban soils are known to differ clearly in their properties from agricultural soils due to the anthropogenic influence (e.g. many artefacts, alkaline pH, high content of technical organic carbon, high contamination, high bulk density, low soil moisture, high soil temperature, young age), but are rarely investigated (Lehmann and Stahr 2007). In 2007 more than 50% of the world’s population lived in urban areas and thus urban soils (soils affected by urbanisation) are of increasing importance for urban ecology and the well-being of the city dwellers. Pedological research started to examine anthropogenic urban soils in the 1970s (Maechling et al. 1974; Blume 1975; Blume and Runge 1978; Fanning et al. 1978). At the beginning, research mostly focused on the classification of urban soils. In the 1980s and until the beginning of the 1990s the pollution of urban soils with organic and inorganic contaminants was in the focus of most studies (Thornton 1991; Lux 1993; Radtke et al. 1997). Thereafter the research on anthropogenic urban soils broadened: The BMBF (Federal Ministry of Education and Research) research project “Evaluation of Urban Soils” (Bewertung anthropogener Stadtböden) from 1993 to 1996 included work on chemical, physical and biological properties of anthropogenic urban soils and involved the main centres of german urban soil research: the soil science institutes of the universities of Kiel, Essen, Hohenheim, Halle, Rostock and Berlin. Main results are presented in Blume and Schleuß (1997).

From 2001 to 2007 the DFG research group Interurban (Water and Organic Matter in Anthropogenic Soils: Dynamics and Processes) studied the dynamics of water and materials at urban locations while giving special consideration to spatial heterogeneity, organic soil
substance, and soil-biological transformation processes. Seven subprojects investigated (1) the spatial heterogeneity and its interaction with sub-processes, (2) the influence of biotic and abiotic factors on the characteristics of the organic substance, (3) the recording possibilities of water- and material transport from heterogeneous urban locations and appliance to an area and recorded (4) the interaction between biotic and abiotic factors and their participation in the dynamics of the release of pollutants (Reemtsma et al. 2003; Braun et al. 2006; Taumer et al. 2006; Schaumann et al. 2007).

**Seam material**

Wessolek and Facklam (1997) started to study physical and chemical site characteristics (e.g. texture, heavy metal content, infiltration rates) of partly sealed urban soils – the seam material. Furthermore, they started to investigate the hydrology of partly sealed urban areas in collaboration with the Berliner Wasserbetriebe on lysimeters with different surface coverings. Seam material, the soil between the paving stones on pavements, is an anthropogenic influenced artificial urban soil. The upper layer of the coarse sand between the paving stones, which is used as building material for pavements, enriches over the time with urban dirt like foliage, cigarette butts, food residues, animal faeces, oil and urban dust containing e.g. soot, abrasions from tires, brakes and asphalt. This results in a different composition and thus different properties of the upper seam material layer (Wessolek and Facklam 1997; Nehls 2007). This is of particular interest as seam material takes on soil functions as filtering, buffering and groundwater recharge in urban areas with a high degree of sealing (up to >35%) (Wessolek 2008). As seam material is the only infiltration pathway on partly sealed pavement systems, it plays an important role concerning the discharge of pollutants into the soil and the groundwater. Partly sealed pavement systems are constructed with retention-weak materials to assure a high hydraulic conductivity (a rapid penetration of water) of the pavement bed and therewith prevent damages of the pavement system, e.g. by frost or flooding. Measurements of the infiltration rates of different paved surfaces show high infiltration rates (36 up to 180 mm h\(^{-1}\)) with a great variability between the different surface coverings as well as between different locations on the same paved area (Illgen et al. 2007). Illgen et al. (2007) showed that the infiltration rates decrease due to compaction and with increasing age of the seam material due to clogging effects caused by the accumulation of fine material in the upper layer of the seam material. A point-wise decrease of infiltration rates by factor 10 or even 100 compared to new built pavements was observed. Nevertheless Flöter (2006) found that the infiltration rate on an 8 years old paving is still comparably high. He observed that paving with 3 mm seams and 5% seam material (95% paving stones) infiltrates up to 80% of the rainfall if the rainfall intensity is low to moderate. Only heavy rainfall events cause high runoff rates.

Aside from the desired low retention for infiltrating water of the material (e.g. coarse sand) used for the construction of pavement systems, however, this material mostly has low carbon
contents and low adsorption capacities for organic or inorganic pollutants (Spliid et al. 2004). Consequently, there is minimal opportunity for sorption and/or degradation of pollutants like herbicides (Ramwell et al. 2002), while the potential for herbicide removal to surface water bodies by runoff or for herbicide leaching into the soil or even the groundwater is high. However, Nehls (2007) observed a positive filter function of the upper layer of the seam material, which is enriched by urban deposition, for heavy metals. This might apply as well for other pollutants because the organic carbon content of the upper seam material layer is increased (Nehls 2007) and organic carbon is known to influence the sorption of lots of substances.

**Weed control on pavements**

Alongside with the important role for the water infiltration of partly sealed areas, the seam material is a habitat for plants. Especially pavements with a high percentage of seam material like mosaic pavements, which are quite common in Berlin, are prone to be partly covered by weeds. However, as the main function of pavements is to provide a stable, secure, dry and even ground for pedestrians, weeds on pavements are often unwanted for safety or aesthetic reasons (presence of weeds on pavements tends to indicate a city in decline) and thus controlled by the responsible public authority. Thereto different chemical and non-chemical (thermal and mechanical) methods are available and in use (Hansen et al. 2004; Kempenaar and Spijker 2004; Rask and Kristoffersen 2007). As the use of non-chemical methods is more time-consuming and hence more expensive, most competent public authorities, like the Berlin public cleansing service (Berliner Stadtreinigungsbetriebe), prefer to use the application of the herbicide glyphosate (mainly the formulation Roundup Ultra) for weed control on pavements as a cost-effective chemical method. In Germany the use of glyphosate on hard surfaces like pavements and paved driveways, courtyards or squares, is forbidden. Thus, users have to grant exemption at the competent authority of the federal states (Bundesländer) before the use of glyphosate on hard surfaces according to § 6, 3 of the German Plant Protection Law (Pflanzenschutzgesetz) (Bundesgesetzblatt 1998). The authorities can regulate what kind of application technique has to be used. In Berlin the only allowed technique is the application of Roundup Ultra via the risk-reducing roller wiper Rotofix. In contrast to spray application, this machine coats the herbicide via a roll specifically onto the weeds, whereby a direct soil contamination should be avoided. Additionally, the principles of Good Professional Practice (Gute fachliche Praxis) (BMELV 2005), e.g. no application if rain is likely, have to be respected for every glyphosate application. However, in other countries (e.g. Finland, Latvia) no specific regulations for the use of glyphosate on pavements and roadsides exist, whereas some countries, e.g. The Netherlands and Denmark, have started programs for weed control on hard surfaces aiming to reduce and phase-out herbicide use within urban areas (Kempenaar and Spijker 2004; Kristoffersen et al. 2004). This is particularly reasonable as the use of herbicides in urban areas leads to different environmental issues compared with agricultural
use, due to the before mentioned minimal opportunity for sorption of herbicides (and the small infiltration areas). Water quality monitoring studies have demonstrated that a disproportionate contamination of waters by non-agricultural herbicide use exists (Kristoffersen et al. 2008). Several studies showed that the urban use of the herbicide glyphosate contributes to surface water contaminations with the substance and its degradation products (Skark et al. 2004; Kolpin et al. 2006; Byer et al. 2008).

**Glyphosate**

The herbicide glyphosate is - due to its non-selectivity and its comparably good environmental properties - frequently used for chemical weed control in urban areas. Because of its distinct tendency to adsorb to soil constituents, its fast microbial degradation and its low toxicity, the risk of surface or ground water contamination is generally assumed to be low (Vereecken 2005). Nevertheless a wide contamination of surface and some ground water resources with glyphosate and its main degradation product amino-methylphosphonic acid (AMPA) has been reported (Feng et al. 1990; Newton et al. 1994; Skark et al. 1998; Grunewald et al. 2001; BMU 2004; Kiefer and Sturm 2008; Peruzzo et al. 2008). Furthermore, ecotoxicological studies showed negative effects of glyphosate and its formulation Roundup Ultra on non-target organisms in sublethal concentrations (Glusczak et al. 2007; Costa et al. 2008).

As glyphosate is the most widely used herbicide in agriculture worldwide, whose use rapidly increased within the last ten years due to the introduction of genetically modified glyphosate-resistant crops in the USA and South America (Kiely et al. 2004; Scribner et al. 2007), numerous studies investigating the fate of glyphosate in agricultural systems exist. By contrast, investigations regarding the fate of glyphosate and its degradation products in urban areas, e.g. on pavements, are rare (Strange-Hansen et al. 2004; Spanoghe et al. 2005). As above mentioned studies concerning the transport and fate of glyphosate in agricultural systems (with comparably high retention capacities for glyphosate) already showed that the use of glyphosate can be problematic under certain circumstances, it is of high importance to gain more information about the fate of glyphosate in urban systems, like partly sealed pavements, with low retention capacities for glyphosate and increased runoff and infiltration rates.

**Objectives**

Hence, the aim of this study was to assess the risk of glyphosate leaching into deeper layers of the pavement bed after its application on partly sealed pavements in urban areas and to verify if observed leaching concentrations may have an impact on non-target organism.

Thereto the pavement seam material was fundamentally characterised and the behaviour of glyphosate thereon was investigated on four levels:
a) batch sorption experiments  
b) column experiments  
c) lysimeter experiments  
d) ecotoxicological experiments with in b) and c) detected leaching concentrations

This thesis consists of six chapters. In chapter II of this work the seam material is characterised. Besides basic physico-chemical properties of the seam material and microscopic investigations (organic petrography), the sorption capacity of seam material for the herbicide glyphosate is determined with batch sorption experiments in order to see if the upper seam material layer acts like a filter for glyphosate, preventing or reducing the leaching into lower layers of the pavement bed in case of low glyphosate sorption capacities in the lower layer. In chapters III and IV the transport of glyphosate on two different levels is described. In chapter III the results from an outdoor lysimeter experiment with glyphosate are presented, which aimed to find out, whether a risk of glyphosate leaching into lower layers of the pavement bed exists, after its application on partly sealed pavements with the roller wiper Rotofix. In chapter IV the results of glyphosate leaching through packed laboratory columns is presented. The column experiments aimed to see whether leaching of glyphosate via matrix flow occurs in materials with low sorption capacity used for the construction of pavements. Chapter V is about the ecotoxicological impact of glyphosate and its formulation Roundup Ultra on the sediment dwelling worm *Lumbriculus variegatus*. This study should find out if a sediment living organism is negatively influenced by an exposition to glyphosate and/or the formulation Roundup Ultra in surface waters. This is an interdisciplinary project which was conducted, analysed and written together with Valeska Contardo-Jara from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Department of Inland Fisheries, Biochemical Regulation). This part of the work is already published (Contardo-Jara et al. 2009).

Each of these chapters can stand alone and has its own introduction and conclusions. This structure was chosen to permit readers to read a chapter on its own, because they are often only interested in single aspects of the whole work.
2 Properties of pavement seam material and sorption of glyphosate to seam material

2.1 Characterisation of pavement seam material

2.1.1 Introduction

Over the years the sandy construction material in the first centimetre changes to darker colours due to the deposition of urban dirt like foliage, cigarette butts, food residues, animal faeces, oil, urban dust containing e.g. soot, abrasions from tires, brakes, asphalt and so on. Wessolek and Facklam (1997) and Nehls (2007) found clearly different properties of the upper seam material layer compared to the original construction sand and attributed those to the different composition of organic matter (OM) due to the contamination with anthropogenic forms of organic material like black carbon (BC).

In this part of the second chapter seam material is characterised regarding its composition and its main physico-chemical properties. Samples from two depths (0-1 cm and 1-5 cm) were collected on 6 pavements covered with cobblestones within Berlin. The aim was to get information about the special composition and the variability of the anthropogenic influenced seam material.

The studied parameters were selected with the aim to characterise the seam material and to possibly find explanations for the sorption behaviour of the herbicide glyphosate in the seam material (2.2). Additionally to main parameters (such as soil texture, pH, $C_{\text{org}}$ content), the content of oxalate and dithionite extractable iron and aluminium, the content of heavy metals and the specific surface area of the seam material were analysed. Furthermore, the organic particles of two sampling sites were classified by a microscopic method (organic petrography). These results were compared with the type of the sampling site to see whether relationships exist.
2.1.2 Materials and methods

2.1.2.1 Sampling sites and sample collection

Samples of seam material were collected on six different pavements adjacent to roads within Berlin in two depths after an earlier pre-sampling of 13 sites. Sampling sites were chosen considering different traffic densities and different land uses (housing area and industrial area) (Tab. 2.1). Samples were taken from the following locations: “Laufener Straße” (OS) in Oberschöneweide, junction of “Rohrdamm” and “Motardstraße” (SS) in Siemensstadt, roundabout “Großer Stern” (GS) in Tiergarten, “Schönhauser Allee” (SHA) in Prenzlauer Berg, “Fehrbelliner Straße” (FBS) in Mitte, “Am Weidendamm” (AWD) a street on the bank of the river Spree in Mitte. Furthermore, seam material samples from three sampling sites collected and analysed by Thomas Nehls (2007) were included into Tab. 2.1. Samples B1 and B1a were taken at “Monbijouplatz” in Mitte, B2 and B2a were taken in the same street but different location as AWD “Am Weidendamm” and B4 and B7 were taken at “Großer Stern” like samples GS but from different locations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Street name</th>
<th>District</th>
<th>Traffic (2005) [d⁻¹]</th>
<th>Type of site</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS 0-1 cm</td>
<td>Laufener Straße</td>
<td>Oberschöneweide</td>
<td>&lt; 5000</td>
<td>industrial site</td>
</tr>
<tr>
<td>OS 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 0-1 cm</td>
<td>Rohrdamm / Motardstraße</td>
<td>Siemensstadt</td>
<td>22208</td>
<td>industrial site</td>
</tr>
<tr>
<td>SS 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS 0-1 cm</td>
<td>Großer Stern (roundabout)</td>
<td>Tiergarten</td>
<td>52696</td>
<td>high traffic density</td>
</tr>
<tr>
<td>GS 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHA 0-1 cm</td>
<td>Schönhauser Allee</td>
<td>Prenzlauer Berg</td>
<td>25031</td>
<td>high traffic density</td>
</tr>
<tr>
<td>SHA 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS 0-1 cm</td>
<td>Fehrbelliner Straße</td>
<td>Mitte</td>
<td>&lt; 5000</td>
<td>housing area</td>
</tr>
<tr>
<td>FBS 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AWD 0-1 cm</td>
<td>Am Weidendamm</td>
<td>Mitte</td>
<td>&lt; 5000</td>
<td>ship traffic</td>
</tr>
<tr>
<td>AWD 1-5 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples from Nehls (2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 0-1 cm</td>
</tr>
<tr>
<td>B1a 1-5 cm</td>
</tr>
<tr>
<td>B2 0-1 cm</td>
</tr>
<tr>
<td>B2a 1-5 cm</td>
</tr>
<tr>
<td>B7 0-1 cm</td>
</tr>
<tr>
<td>B4a 1-5 cm</td>
</tr>
</tbody>
</table>

At each location samples were taken between cobblestones of approximately 6 × 6 cm on pavements. Samples were taken from the material between the pavement stones – the seam
material. They were separated in the upper darker 0-1cm layer and the lighter 1-5cm layer (Fig. 2.1). The fresh seam material was sieved (< 2mm), air-dried and stored at ambient temperature until further processing.

![Image of seam material](image)

**Fig. 2.1: Seam material.**

### 2.1.2.2 Determination of basic physico-chemical soil properties

The following physico-chemical soil properties of the seam material were determined (Tab. 2.2): pH was determined in duplicate with a combined glass/calomel electrode in 0.01 M CaCl$_2$ using a solution/soil ratio of 2.5:1. The particle size distribution was determined according to ISO 11277 (1998a). $C_{tot}$, $N_{tot}$ and C/N were determined with a C/N analyzer (Elementar, Vario EL III, Hanau, Germany) after homogenisation and grounding of samples. For $C_{org}$ determination samples were treated with HCl to remove carbonates and subsequently measured with the C/N analyzer. Heavy metal contents and nutrients were determined after digestion with HNO$_3$ using pressure vessel technique (Ruppert 1995) with ICP (Thermo Scientific, Iris, MA, USA). The CEC$_{eff}$ was determined according to ISO 11260 (1994) and Mg was measured at 278.8 nm with ICP (Thermo Scientific, Iris, MA, USA). The oxalate extractable Al and Fe was determined according to DIN 19684-6 (1997) and dithionite extractable Al and Fe according to Mehra and Jackson (1960) and measured with AAS (Perkin Elmer 1100B, Waltham, MA, USA).

### 2.1.2.3 Soil surface measurements

Water vapour adsorption isotherms were measured with a gravimetric method in a vacuum chamber at a temperature of 20 ± 0.1°C. Soil samples were dried in a vacuum chamber with concentrated sulphuric acid until constancy of weights prior to adsorption measurements. Aliquots of 3 g were exposed to five stepwise rising relative water vapour pressures,
Properties of Seam Material

$p/p_0$ (with $p$ = equilibrium vapour pressure in the chamber, $p_0$ = saturated vapour pressure at the temperature T), ranging from 0.05 to 0.35 which were controlled by sulphuric acid of stepwise decreasing concentrations. The samples were equilibrated with each water vapour pressure until constancy of weights. All adsorption measurements were repeated three times.

The amount of adsorbed water at a given $p/p_0$ was calculated as the difference of the weight of the humid sample (after equilibration for 48 h at given $p/p_0$) and the dry sample (after drying for 24 h at 105°C).

The specific soil surface area (SSA) was estimated from the water vapour adsorption isotherms using the Brunauer-Emmet-Teller (BET) method (Brunauer et al. 1938). In a first step the monolayer capacity ($N_m$) was obtained from the linear regression analysis of the experimental data using the equation

$$\frac{x}{N(1-x)} = \frac{1}{C_{BET} N_m} + \frac{(C_{BET} - 1)}{C_{BET} N_m} x$$

where $C_{BET}$ is the BET constant and $N$ is the amount adsorbed water vapour per g of an adsorbent.

Thereafter the specific surface area was calculated from the dependence

$$SSA = N_m \times M^{-1} \times L \times \omega$$

where $L$ is the Avogadro number [$6.02 \times 10^{23}$ mol$^{-1}$], $M$ is the molecular weight of gas or vapour [g mol$^{-1}$] and $\omega$ is the molecule cross-sectional area of a single adsorbate molecule ($1.08 \times 10^{-19}$ m$^2$ for water).

These measurements and calculations were conducted in the Institute of Agrophysics of the Polish Academy of Sciences in Lublin by Prof. Dr. Z. Sokolowska and her colleagues.

Organic Petrography

Samples from two sites in each depth (OS 0-1 cm, OS 1-5 cm, GS 0-1 cm, GS 1-5 cm) were analysed by organic petrography. The seam material samples were treated with HCl and HF in order to remove the mineral fraction. Following samples, which are concentrates of organic particles, were embedded in an epoxy resin and polished. Microscopic investigations were carried out with a Leitz DMRX-MPVSP microscope photometer. For characterisation of the organic matter incident white light as well as UV + violet-light illumination (fluorescence mode) with immersion objectives under oil (magnification 200-500x) were used.

To describe and classify the different organic particles the classification system for macerals from Taylor et al. (1998) and the one proposed by Ligouis et al. (2005) were followed. The quantitative estimation of maceral volume fractions was determined by a point counting.
method used in coal petrography (Taylor et al. 1998) where at least 500 points were counted in each sample. The contributions of various constituents in the sample was expressed as volume percent of total OM. To get more information from the petrographic analysis, soot particles emitted from traffic and from oil combustion were counted separately from the char particles produced by coal combustion.

The organic petrography analysis was done by Dr. Bertrand Ligouis from the Laboratories for Applied Organic Petrology (LAOP) Tübingen, Germany.

2.1.2.5 Statistical evaluation of data

Paired T-Tests were performed to detect statistically significant differences in measured parameters between the 0-1 cm layer and the 1-5 cm layer. Correlations between two measured parameters were analysed by linear regression analysis (SigmaStat 2.03, SPSS Inc, 1997). Furthermore, a twostep cluster analysis including following parameters (CEC$_{eff}$, C$_{org}$, C$_{tot}$, SSA, oxalate and dithionite extractable Al + Fe, clay %) was carried out (SPSS 2002).

Linear regression analysis (SigmaStat 2.03, SPSS Inc, 1997) was performed to detect whether dependencies of the effective CEC and the SSA from the C$_{org}$ content exist.
2.1.3 Results and discussion

2.1.3.1 Basic properties of seam material

The pH of the seam material samples ranged from 5.9 to 6.8 (Tab. 2.2). No significant difference between the top (0-1 cm) and the underlying (1-5 cm) layer was found. The slightly higher pH of the samples taken and analysed by Nehls (2007) might be due to a slightly different pH determination with a different solution/soil ratio and were therefore not included.

The \( C_{\text{tot}} \) and the \( C_{\text{org}} \) contents of the seam material varied in the top layer from 11.6 to 25 g kg\(^{-1}\) and 10.5 to 23.1 g kg\(^{-1}\), respectively. They were significantly lower (\( p \leq 0.001 \)) in the 1-5 cm layer varying from 1.4 to 6.7 g kg\(^{-1}\) and 0.2 to 4.4 g kg\(^{-1}\), respectively. Also the effective CEC, the content of oxalate and dithionite extractable Al and Fe, the SSA, the percentage of clay and silt and the contents of heavy metals (e.g. Pb, Cu, Zn) were significantly higher (\( p \leq 0.001 \)) in the 0-1 cm layer.

A performed two-step cluster analysis including ten measured parameters (2.1.2.5) grouped all top samples to cluster number one and all samples from the 1-5 cm layer to cluster number two, showing that a sample can be assigned to one of the two layers due to its properties. Thus, the composition of the upper, clearly darker 0-1 cm layer of the seam material is changed by urban deposition. These changes can be attributed to the continuously deposition and accumulation of anthropogenic contaminations like dust, soot, rubber and organic matter (containing e.g. heavy metals) which are typical in the urban environment. Comparing the seam material from the 0-1 cm layer with the 1-5 cm layer, the 1-5 cm layer was regarded as only slightly changed original inbuilt sandy construction material. The transport of deposited material and contaminants into the 1-5 cm layer can not be excluded, but seems to happen only in a small fraction as the \( C_{\text{org}} \) content of this layer is much lower. The fine deposited material might causes clogging of pores resulting in a concentration of the material in the 0-1 cm layer. A significant higher content of finer particles (clay and silt) in the upper layer supports this theory (Fig. 2.2).
## 2.1 Characterisation of Pavement Seam Material

### Tab. 2.2: Physico-chemical properties of seam material.

<table>
<thead>
<tr>
<th>Sample</th>
<th>particle size distribution [%]</th>
<th>pH†</th>
<th>$C_{\text{ad}}$ [g kg$^{-1}$]</th>
<th>$C_{\text{eff}}$ [g kg$^{-1}$]</th>
<th>C/N</th>
<th>CEC$^{-\frac{1}{2}}$ [cmol(+)/kg]</th>
<th>SSA [m² g$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>clay</td>
<td>silt</td>
<td>sand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td>0-1 cm</td>
<td>2.2</td>
<td>10</td>
<td>87.8</td>
<td>6.7</td>
<td>15.8</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>1</td>
<td>3.6</td>
<td>95.4</td>
<td>6.7</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>SS</td>
<td>0-1 cm</td>
<td>2.8</td>
<td>14</td>
<td>83.2</td>
<td>6.0</td>
<td>11.6</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>1.5</td>
<td>3.5</td>
<td>95.1</td>
<td>5.9</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>GS</td>
<td>0-1 cm</td>
<td>6.4</td>
<td>20</td>
<td>73.6</td>
<td>6.5</td>
<td>23.5</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>2.7</td>
<td>9.6</td>
<td>87.6</td>
<td>6.5</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td>SHA</td>
<td>0-1 cm</td>
<td>3.4</td>
<td>9.6</td>
<td>87.0</td>
<td>6.6</td>
<td>22.6</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>1.6</td>
<td>1.9</td>
<td>96.5</td>
<td>6.8</td>
<td>6.6</td>
<td>2.7</td>
</tr>
<tr>
<td>FBS</td>
<td>0-1 cm</td>
<td>2.2</td>
<td>12.4</td>
<td>87.6</td>
<td>6.6</td>
<td>5.0</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>0</td>
<td>2.7</td>
<td>97.3</td>
<td>6.6</td>
<td>6.7</td>
<td>1.7</td>
</tr>
<tr>
<td>AWD</td>
<td>0-1 cm</td>
<td>0.7</td>
<td>10.7</td>
<td>88.5</td>
<td>6.6</td>
<td>25.0</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>0</td>
<td>3.9</td>
<td>96.1</td>
<td>6.6</td>
<td>1.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Samples from Nehls (2007)

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH†</th>
<th>$C_{\text{ad}}$ [g kg$^{-1}$]</th>
<th>$C_{\text{eff}}$ [g kg$^{-1}$]</th>
<th>C/N</th>
<th>CEC$^{-\frac{1}{2}}$ [cmol(+)/kg]</th>
<th>SSA [m² g$^{-1}$]</th>
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<td>B1</td>
<td>0-1 cm</td>
<td>1.8</td>
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<td></td>
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<td>B2</td>
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<td>3.6</td>
<td>96</td>
<td>7</td>
<td>1.8</td>
</tr>
<tr>
<td>B7</td>
<td>0-1 cm</td>
<td>2.3</td>
<td>13.9</td>
<td>83.7</td>
<td>7.2</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>1.6</td>
<td>3</td>
<td>95.8</td>
<td>7.1</td>
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<table>
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<tr>
<th>Sample</th>
<th>Fe di$^a$ [g kg$^{-1}$]</th>
<th>Fe ox$^b$ [g kg$^{-1}$]</th>
<th>Al di$^a$ [g kg$^{-1}$]</th>
<th>Al ox$^b$ [g kg$^{-1}$]</th>
<th>Pb [mg kg$^{-1}$]</th>
<th>Cu [mg kg$^{-1}$]</th>
<th>Zn [mg kg$^{-1}$]</th>
<th>Cd [mg kg$^{-1}$]</th>
<th>Cr [mg kg$^{-1}$]</th>
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<td>OS</td>
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<td>0.6</td>
<td>0.8</td>
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<td>76</td>
<td>323</td>
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<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>14</td>
<td>14</td>
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<tr>
<td>SS</td>
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<td>0.4</td>
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<td>0.2</td>
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<tr>
<td>GS</td>
<td>0-1 cm</td>
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<td>134</td>
<td>57</td>
<td>165</td>
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<td>0.5</td>
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<td>SHA</td>
<td>0-1 cm</td>
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<td>0.5</td>
<td>0.7</td>
<td>138</td>
<td>87</td>
<td>242</td>
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<td>0.1</td>
<td>0.1</td>
<td>20</td>
<td>10</td>
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<td>FBS</td>
<td>0-1 cm</td>
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<td>2.0</td>
<td>0.6</td>
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<td>284</td>
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<td>1-5 cm</td>
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<td>0.4</td>
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<td>0.1</td>
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<td>71</td>
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<td>0-1 cm</td>
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<td>4.1</td>
<td>0.3</td>
<td>0.8</td>
<td>82</td>
<td>54</td>
<td>244</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>0.8</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>10</td>
<td>6</td>
<td>22</td>
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</table>

Samples from Nehls (2007)

<table>
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<tr>
<th>Sample</th>
<th>Fe di$^a$ [g kg$^{-1}$]</th>
<th>Fe ox$^b$ [g kg$^{-1}$]</th>
<th>Al di$^a$ [g kg$^{-1}$]</th>
<th>Al ox$^b$ [g kg$^{-1}$]</th>
<th>Pb [mg kg$^{-1}$]</th>
<th>Cu [mg kg$^{-1}$]</th>
<th>Zn [mg kg$^{-1}$]</th>
<th>Cd [mg kg$^{-1}$]</th>
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<td>B1</td>
<td>0-1 cm</td>
<td>5.7</td>
<td>4.3</td>
<td>0.4</td>
<td>0.7</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>3.0</td>
<td>0.9</td>
<td>0.2</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B2</td>
<td>0-1 cm</td>
<td>6.4</td>
<td>6.5</td>
<td>0.5</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>0.7</td>
<td>0.6</td>
<td>0.1</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B7</td>
<td>0-1 cm</td>
<td>4.5</td>
<td>4.3</td>
<td>0.5</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1-5 cm</td>
<td>1.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Sand*</td>
<td>0.5</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*a dithionite extractable, b oxalate extractable

† 0.01 M CaCl$_2$, ‡ effective cation exchange capacity, *pure sand used for BC experiments (2.2.2.5)
The CEC$_{eff}$ of both layers is with values ranging from 0 to 6.3 cmol(+) kg$^{-1}$ (mean 2.6 cmol kg$^{-1}$) relatively low compared to values for arable German sandy soils with 12 to 14 cmol(+) kg$^{-1}$ (Renger 1965). A linear regression analysis showed a strong and highly significant influence of the C$_{org}$ on the CEC$_{eff}$ for the samples of both layers (F = 37.3; P = < 0.001; r$^2$ = 0.7). Nehls et al. (2008) found a good correlation between the potential CEC and the OM content for the top seam material layer, but a low contribution of the upper seam materials C$_{org}$ to the potential CEC and explained it with a low specific surface area and a low surface charge density of the seam material. In contrast to their findings, the goodness of fit for a linear regression analysis between the CEC$_{eff}$ and the content of OM only for samples from the 0-1 cm layer was only r$^2$ = 0.21 and no significant influence of the C$_{org}$ on the CEC$_{eff}$ was observed (F = 1.9; P = 0.22) (Fig. 2.3). However, in the 1-5 cm layer, a strong and significant dependency of the effective CEC from the content of C$_{org}$ was found with:

$$\text{CEC}_{eff} \text{ [cmol(+) kg}^{-1}] = 0.59 \cdot \text{C}_{org} \text{ [g kg}^{-1}] - 0.14 \ (r^2 = 0.7; \text{F} = 16.2; \text{P} = 0.005) \ (\text{Fig. 2.3}).$$

Fig. 2.2: Soil texture of top and underlying seam material layers.

Fig. 2.3: Dependency of CEC$_{eff}$ from C$_{org}$ content in 0-1 cm layer (●) and 1-5 cm layer (○).
2.1 CHARACTERISATION OF PAVEMENT SEAM MATERIAL

The $C_{\text{org}}$ of the 1-5 cm layer contributes with 590 cmol(+)$\cdot$kg$^{-1}$ C to its effective CEC. This is similar to values of up to 680 cmol(+)$\cdot$kg$^{-1}$ C found for Berlin sandy forest soils with similar $C_{\text{org}}$ contents (Wilczynski et al. 1993). Furthermore, no dependency of the soil surface area (SSA) from the $C_{\text{org}}$ content was found in the 0-1 cm layer ($r^2 = 0.18; F < 1; P = 0.404$), but a strong and significant dependency in the 1-5 cm layer:

\[
\text{SSA} \left[ \text{m}^2 \text{g}^{-1} \right] = 1.346 \cdot C_{\text{org}} \left[ \text{g kg}^{-1} \right] + 1.2 \left( r^2 = 0.89; F = 31.9; P = 0.005 \right)
\]

with a contribution of its $C_{\text{org}}$ to the SSA similar to those found by Wilczynski et al. (1993).

These findings show that the 1-5 cm layer is in contrast to the upper 0-1 cm layer not strongly influenced by the urban deposition and that its OM has similar properties as the one of non-urban sandy soils. Besides it indicates that other constituents than humus, e.g. black carbon (BC), must play a major role in the composition of the OM of the top layer or that a different parameters than OM influence the SSA and $\text{CEC}_{\text{eff}}$. The analysis of samples from both depth of sampling sites OS and GS by organic petrography (2.1.3.2) showed that up to 36% (mean 24%) of the OM consists of BC (including char, charcoal and soot). This is in accordance with investigations by Nehls (2007) who found BC accounting for up to one third of the OM in the 0-1 cm layer.

2.1.3.2 Organic petrography

For the organic petrography study two sampling sites were selected. One is the industrial influenced sampling site OS, which is located in Oberschöneweide, one of the formerly very important industrial areas in Germany. The other one is the traffic influenced sampling site GS at the roundabout “Großer Stern” in the middle of Berlins biggest park “Tiergarten” (210 ha). From both sampling sites the 0-1 cm layer and the 1-5 cm layer were analysed.

The various groups of OM identified by organic petrographic analysis are shown in Tab. 2.3. Photographs of selected OM particles are presented in Fig. 2.4. However, as only two sampling sites were analysed by organic petrography the results can not be generalised for all seam material but is helpful for getting an idea of the composition of the OM of the seam material.

Three major OM groups can be distinguished (Ligouis et al. 2005). (1) A recent organic matter group that consists of woody phytoclasts, humic gels, seed coatings, suberized tissues, fungal phytoclasts and pollen and spores. (2) A fossil organic matter group that consists primarily of pollen, spores, algae, amorphous organic matter and bitumen. (3) An anthropogenic OM group which includes particles of raw brown coal, hard coal, charcoal, brown-coal and hard-coal coke, char, soot and coal- and petroleum derived fluorescent materials (tar, oils, lubricants, asphalt etc.). All particles were classified according to their morphology and optical properties.
### Tab. 2.3: Organic matter composition (petrographic analysis).

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th></th>
<th>GS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 cm</td>
<td>1-5 cm</td>
<td>0-1 cm</td>
<td>1-5 cm</td>
</tr>
<tr>
<td>Recent organic matter</td>
<td>38.3</td>
<td>22.2</td>
<td>18.2</td>
<td>19.4</td>
</tr>
<tr>
<td>Raw brown coal</td>
<td>23.4</td>
<td>28.5</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Hard coal</td>
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<td>8.0</td>
<td>9.3</td>
</tr>
<tr>
<td>Charcoal</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Coke</td>
<td>14.6</td>
<td>10.6</td>
<td>37.8</td>
<td>20.1</td>
</tr>
<tr>
<td>Residues of coal hydrogenation (Other carbons)</td>
<td>0.4</td>
<td>x</td>
<td>2.7</td>
<td>8.1</td>
</tr>
<tr>
<td>Char</td>
<td>4.0</td>
<td>1.6</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Soot</td>
<td>4.0</td>
<td>21.2</td>
<td>24.7</td>
<td>29.5</td>
</tr>
<tr>
<td>Coal- and petroleum- derived fluorescent materials</td>
<td>7.6</td>
<td>2.2</td>
<td>1.5</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Total anthropogenic particles</strong></td>
<td><strong>61.7</strong></td>
<td><strong>77.8</strong></td>
<td><strong>81.8</strong></td>
<td><strong>80.6</strong></td>
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</tbody>
</table>

*Fig. 2.4: Photomicrographs showing different carbonaceous particles identified by organic petrography techniques in seam material samples. First row (sample OS 0-1 cm): general view (left); gelified phytoclast (middle); brown coal matrix (right). Second row (sample OS 1-5 cm): general view (left); sub-bituminous coal (middle); soot (right). Third row (sample GS 0-1 cm): general view (left); coke with unfused and fused inclusions (middle); soot (right). Fourth row (sample GS 1-5 cm): general view (left); low reflecting charcoal (middle); coal tar pitch (right). All photomicrographs taken with white reflected light and oil immersion.*
The second group of particles, the fossil organic matter group, was not present in any of the seam material samples. As this group of fossil organic matter is typical for ancient sediments, it was not expected to be found in seam material.

About 62 to 82% of the OM present in the seam material was of anthropogenic origin (group 3). The total content of OM was higher in the 0-1 cm layer (as determined before). The similar OM composition in both layers suggests that the OM fraction present in the 1-5 cm layer originates from the 0-1 cm layer and was washed out into the 1-5 cm layer. Nevertheless some fractions of anthropogenic OM in the upper layer are increased in comparison with the lower layer and might be the reason for the different behaviour of the upper layers OM mentioned in section 2.1.3.1.

The higher content of recent OM in the sample OS 0-1cm (38%) can be attributed to the higher content of gelified woody phytoclast (23%) in that sample compared to the other three samples (8-11%) and therefore is the reason for the lower percentage of anthropogenic particles in this sample. This can be explained with the location of this sampling site close to a tree, whereas on sampling site GS trees are at least 10 m away. Due to a high microbial activity (own analysis) (Wessolek 2001; Staikos 2005) in the 0-1 cm layer most of the humic detritus is quickly degraded and only parts of it reaches the 1-5 cm layer.

Comparing the OM composition of the two sampling sites, the eight times higher percentage of raw brown coal for sampling site OS in both layers compared to site GS stands out. As site OS is situated within an former industrial area, in the immediate vicinity of the former power station “Kabelwerk Oberspree” built by the AEG concern in 1895 and in use until 1933, the high content of raw brown coal can be attributed to the delivery and use of brown coal for the power plant and other factories in Oberschöneweide.

Regarding BC as the sum of char, charcoal and soot (Goldberg 1985) between 14 and 45% of the anthropogenic originated OM consists of BC. Remarkable is the high percentage of soot (21 – 30%) in all samples except for OS 0-1 cm (4%) (Tab. 2.3). Soot is formed through incomplete combustion of hydrocarbons and therefore mainly traffic related. As sampling site GS is situated on a pavement adjacent to a roundabout with a high traffic density, the high percentage of soot on the anthropogenic OM is for sample OS 0-1 cm only 6% whereas it is > 27% for the other samples. The low content of soot in the upper 0-1 cm layer might be attributed to the low traffic density in the dead-end road where the sampling site is situated. However, the occurrence of a higher content of soot in the lower 1-5 cm layer is not in line with the latter explanation. Due to the former highly industrial character of the area it would be expected that the traffic volume, especially with heavy good vehicles, was high due to frequent delivery of stocks, before the German reunification caused
close-downs of resident factories. Therefore a similarly percentage of soot in the upper layer would have been expected.

Another interesting detail in the composition of the OM of the seam material is the high content of coke in the material from sampling site GS. In both layers the fraction of coke of the total anthropogenic OM is clearly higher (44 and 24%) compared to the sampling site OS (15 and 9%). Especially in the 0-1 cm layer coke dominates the anthropogenic OM with 44% (soot 25%). This is surprising as sampling site GS at the roundabout “Großer Stern” was thought to be mainly influenced by its high traffic density as it is surrounded by a 210 ha park “Tiergarten”. As coke is used as combustible for coal-burning stoves in houses but mainly in industrial furnaces (e.g. for steel production) it was not expected to be found in big amounts in the seam material of a traffic influenced site with no housing or industrial area close-by wherefrom airborne particles of coke could come from. A hypothetical explanation could be an accident with a truck carrying coke or a regularly transport of coke with trucks via the “Großer Stern”. As coke is also present in the 1-5 cm layer the incidence might have happened a while ago so that coke particles could be washed into the lower layer.

For this part (2.1) and part 2.2 (Sorption of glyphosate to seam material) a combined chapter “Conclusions and outlook” follows in 2.3.
2.2 Sorption of glyphosate to seam material

2.2.1 Introduction

The second part of chapter 2 addresses the sorption of the herbicide glyphosate to the previously characterised seam material.

The worldwide frequently used herbicide glyphosate, the active ingredient in commercial products like Roundup, is not only used in agriculture but also for weed control on partly sealed urban areas, e.g. in Berlin. The sorption capacity of the seam material for glyphosate was investigated, as in partly sealed urban areas seam material is the only infiltration pathway for water, which does not runoff and reaches canalisation, and therefore plays an important role concerning the discharge of pollutants into the soil and the groundwater.

Glyphosate is a systemic, broad-spectrum, post-emergence herbicide which is used in agriculture, horticulture, forestry, aquaculture and urban areas to control broadleaf weeds, grasses and brush. Its most important chemical properties are given in Tab. 2.4. Glyphosate is an amphoteric compound with several $pK_a$ values. Due to its polarity it is highly soluble in water and almost insoluble in organic solvents. Glyphosate affects the plants by inhibiting the production of essential aromatic amino acids through disrupting the shikimic acid metabolic pathway (Giesy et al. 2000). The degradation of glyphosate in soil is mainly determined by microbial processes. Half-life values in soils range from a few days up to several months and even years. The main degradation product of glyphosate is amino-methylphosphonic acid (AMPA).

Glyphosate has a distinct tendency to adsorb to soil constitutes. It’s adsorption to soil is usually described by the Freundlich sorption isotherm. However large variations in sorption capacity of soils are reported in literature with Freundlich sorption coefficients ($K_f$) ranging from 0.6 to 500000 mg\(^1\text{nL}^{-1}\text{kg}^{-1}$ (Vereecken 2005). In contrast to almost all other pesticides, which are mainly sorbed by soil organic matter (SOM) because of their domination by apolar groups, i.e. aliphatic or aromatic carbon, glyphosate is due to it’s three polar functional groups (carboxyl, amino and phosphonate groups) mainly sorbed by soil minerals. Soil organic matter only seems to have an indirect effect by blocking sorption sites. However, C\(_{\text{org}}\) content and clay content have previously been correlated with the ability of soils to adsorb glyphosate (Glass 1987; Piccolo et al. 1996). Besides the mineral phase, the pH strongly influences the sorption of glyphosate as it governs its electrical charge (McConnel and Hossner 1985; de Jonge and de Jonge 1999; Gimsing et al. 2004a).
Due to its non-selectivity glyphosate is used for weed control in urban areas where weeds are unwanted for aesthetic or safety reasons like roadsides, pavements, courtyards or railways. In Germany the spraying of glyphosate on hard surfaces like pavements has been forbidden in 2003. The municipalities have to grant exemption at the competent authority (e.g. Pflanzenschutzamt) for the use of glyphosate in urban areas. The authorities have to check if the requested application (e.g. on a pavement of a street) is in accordance with § 3 of the German Pest Management Utilization Regulation (Bundesgesetzblatt 2003) and if a special approval can be granted for the use of glyphosate via the risk-reducing roller wiper Rotofix (3.2.4.2) or the wick wiper (German Plant Protection Law § 6 (Bundesgesetzblatt 1998)). Additionally, Good Professional Practice (BMELV 2005) such as no application if rain is likely, have to be respected. However, in other countries no restrictions for the use of glyphosate on pavements and roadsides exist.

In order to see if the upper seam material layer can act like a filter for glyphosate preventing the leaching into lower layers of the pavement bed, as it was observed for heavy metals by Nehls (2007), the sorption capacity of the seam material was investigated via batch sorption experiments. With the help of regression analyses between the in part 2.1 determined parameters of the seam material and its sorption capacity, it was attempted to find components playing an important role for the adsorption of glyphosate in seam material.

This is the first research project that focuses on the sorption of glyphosate in sandy construction materials of pavements and the influence of the deposition in the urban environment thereto. This is of importance as > 30% of Berlin partly sealed and the Berlin
public cleansing service (Berliner Stadtreinigungsbetriebe) prefers the chemical weed control with the roller wiper Rotofix instead of non-chemical methods, which are more time-consuming and thus more expensive. Furthermore, other cities orientate themselves on the practices in Berlin due to several research projects of the Federal Research Centre for Cultivated Plants (JKI) together with the Plant Protection Office (Pflanzenschutzamt) Berlin concerning the use of glyphosate for weed control in urban areas.

2.2 Materials and methods

2.2.1 Seam material

The seam material used for the batch sorption experiments is described and characterised in detail in part 2.1 (Tab. 2.1; Tab. 2.2; Tab. 2.3).

2.2.2 Chemicals

Glyphosate (N-(phosphonomethyl)glycine) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) with 98 ± 0.5% certified purity. The Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) used contains the monoisopropylamine salt of glyphosate (N-phosphonomethyl)-glycine) (360 g L\(^{-1}\)) and surfactants of undisclosed chemical composition and concentration. Roundup Ultra stock solution was prepared by diluting the Roundup Ultra solution with MilliQ water to 0.1% solution (392 µg ml\(^{-1}\)). \(^{14}\)C-labelled glyphosate ([phosphonomethylene-\(^{14}\)C]glyphosate) was obtained from Monsanto (St. Louis, MO, USA) with 98.8% radiochemical and 99% chemical purity. Its specific radioactivity was 1.71 GBq mmol\(^{-1}\). Stock solutions for each concentration of the batch sorption experiments were prepared by mixing MilliQ water with \(^{14}\)C-labelled glyphosate solution and non-labelled glyphosate or Roundup Ultra solution, respectively. The glyphosate concentration of the spiking solutions ranged from 0.04 to 40 µg ml\(^{-1}\).

2.2.3 Batch sorption experiments

Batch sorption experiments with the seam material were conducted according to the OECD Guideline 106 (2000) with \(^{14}\)C-labelled glyphosate. Studied mass ratios of glyphosate in soil were 0.02, 0.2, 2, 5 and 20 mg kg\(^{-1}\) with a soil/solution ratio of 1:10. Glyphosate concentrations per vessel ranged from 0.002 to 2 µg ml\(^{-1}\). Additionally to experiments with pure glyphosate solutions, adsorption isotherms experiments with Roundup Ultra solution were conducted with two soils (OS, SS), where the unlabeled glyphosate was replaced by glyphosate of the formulated product.
Sorption kinetics were analysed by altering the contact time at a constant concentration of 0.5 µg ml\(^{-1}\) (5 mg kg\(^{-1}\)) per vessel for determination of an appropriate shaking time for the sorption isotherm experiments. This concentration was chosen as it corresponds to an application rate of 5 L ha\(^{-1}\) Roundup Ultra which is in the middle of the recommended application rates for non-arable land and agriculture (2 to 10 L ha\(^{-1}\)). They were shaken for 2, 4, 6, 8, 15, 24 and 48 hours, respectively. For two soils (OS, SS) shorter equilibration times of 5, 23, 30 and 60 min, respectively, were additionally tested. Samples were equilibrated and processed as described below.

For the adsorption isotherm experiments with the five selected glyphosate concentrations 2 g of dry soil were mixed with 19 ml of 0.01 M CaCl\(_2\) solution 24 hours before the start of the sorption experiment for pre-equilibration. They were placed on an overhead shaker over night. The following day, 1 ml of glyphosate solution containing \(^{14}\)C-labelled glyphosate was added and samples were shaken overhead for 24 hours at 20 rpm.

After centrifuging the samples for 25 minutes at 2000 RCF, 2 ml of the supernatant were added to 10 ml of liquid scintillation cocktail (Luma Safe Plus, Lumac-LSC, Groningen, The Netherlands). The samples were measured by liquid scintillation counting (LSC, LS 6500, Beckman, Fullerton, CA, USA). The amount adsorbed was determined by subtracting the actual solution concentration from the initial solution concentration. Concentrations of glyphosate are reported as equivalent of parent compound calculated from the counted radioactivity. The measured radioactivity was corrected for background and quench by external standards. The limit of quantification of the LSC method was 0.8 Bq per sample. For each concentration three replicates were prepared. Blanks without soil were prepared to verify that sorption to glass vessels could be excluded. All experiments were carried out at ambient temperature of 20ºC (+/- 2).

Mass balance checks were performed with some samples by analyzing the \(^{14}\)C activity not only in the solution but as well in the soil. Soil samples were dried (24h, 105°C), ground and mixed to obtain homogeneously samples. Aliquots of soil samples were combusted (at 900°C for 3 min) in an oxidizer (Ox 500, Zinsser, Frankfurt, Germany) and the evolved \(^{14}\)CO\(_2\) was trapped in a scintillation cocktail (Oxysolve C-400, Zinsser, Frankfurt, Germany). Radioactivity was determined by liquid scintillation counting.

### 2.2.2.4 Analysis of pH dependency

The pH dependency of the glyphosate sorption was investigated with the batch technique as described above at a constant concentration of 0.5 µg ml\(^{-1}\) per vessel. The experiments were conducted with the seam material of the OS 0-1 cm layer and with the pure sand which was used for the sorption experiments with BC (2.2.2.5).
The pH of the samples was adjusted via addition of varying amounts of 0.25N NaOH and 0.1N HCl, respectively, before pre-equilibration. The percentage of sorbed glyphosate was related to the pH measured at the end of the experiment. For every treatment three replicates were prepared.

2.2.2.5 Sorption experiments with black carbon and urban dust

Sorption isotherm experiments with a pure sand with varying amounts of black carbon (BC) and urban dust (UD) were conducted as described above (2.2.2.3). The sand was glowed at 550°C for 24 h to remove possible organic contamination before use. The content of oxalate and dithionite extractable aluminium and iron in the sand were with 0.5 g kg⁻¹ dithionite extractable iron and < 0.1 g kg⁻¹ dithionite extractable aluminium very low (Tab. 2.2). For the black carbon (BC) experiments mixtures with 5%, 0.5% and 0.05% of BC, respectively, were prepared. Each mixture was produced in triplicate by mixing 1.9 g, 1.99 g and 1.999 g sand, respectively, with the corresponding amount of BC. The BC “Corax® N 220” was obtained from Evonik Degussa (Essen, Germany). This product consists solely of Carbon Black (CAS No. 1333-86-4) and is normally used in the rubber producing and rubber processing industry as a filler.

For the experiments with urban dust mixtures with 50%, 10% and 1% of urban dust, respectively, were prepared in triplicate. The urban dust was collected by Höke (2003) on a pavement in Oberhausen, Germany (sampling site 59.1). The pH of the material is 8.2, the C_{tot} content is 48 g kg⁻¹ and the C_{org} content 38 g kg⁻¹ (Hoeke 2003).

2.2.2.6 Parameter estimation

Sorption parameters for the adsorption isotherm experiments were fit with the linear sorption isotherm (2.3) and the empirical Freundlich equation (2.4):

\[ C_s = K_d \cdot C_w \]  \hspace{1cm} (2.3)

\[ C_s = K_f \cdot C_w^n \]  \hspace{1cm} (2.4)

where \( C_s \) is the concentration in the soil [mg kg⁻¹], \( K_d \) is the linear sorption coefficient, \( K_f \) is the Freundlich sorption coefficient [mg⁻¹ L^n kg⁻¹], \( C_w \) is the concentration in the solution after 24 hours [mg L⁻¹] and \( n \) is an empirical coefficient (Freundlich exponent) indicating the non-linear dependence of the sorption rate on the solution concentration. The Langmuir and BET isotherms were not considered as they postulate uniform sorption sites, which can not be expected for heterogeneously seam material samples.
Additionally, the retardation factor \( R \) for non-linear sorption was calculated with

\[
R = 1 + \frac{\rho}{\theta} n \cdot K_f \cdot C_w^{n-1}
\]  

(2.5)

where \( \rho \) is the bulk density [g cm\(^{-3}\)] and \( \theta \) the volumetric water content [m\(^3\) m\(^{-3}\)] of the soil. For \( n \neq 1 \), the retardation factor \( R \) is depending on the concentration in the solution. \( R \) describes the retardation of glyphosate transport compared to water transport in soil.

### 2.2.2.7 Statistical evaluation of data

A Paired T-Tests (SigmaStat 1997) was performed to detect a statistically significant difference in the determined sorption coefficient \( K_f \) between the 0-1cm layer and the 1-5 cm layer.

Linear and multiple linear regression analysis was performed to detect whether a dependency of the sorption coefficient \( K_f \) on one of parameters described in part 2.1 exists (\( P = 0.05 \)).

Additionally to linear regression every single parameter was fitted with logarithmic, invers, quadratic, cubic, power, sigmoidal, growth, exponential and logistic functions to the \( K_f \) values (SPSS 2002).

### 2.2.3 Results and discussion

#### 2.2.3.1 Batch sorption experiments

The sorption kinetic of the seam material was studied to determine an appropriate shaking time for the sorption isotherm experiments. Equilibration times of up to only 5 min (plus 30 min centrifugation) showed a rapid sorption of glyphosate to the seam material followed by a much slower sorption rate (Fig. 2.5). This two-phase process is often described for pesticide sorption to soils (Smith et al. 2003). Fast surface processes are followed by slower diffusion processes into and out of micropores of OM, minerals and soil aggregates (Pignatello and Xing 1996). Glyphosate degrades quickly (Nomura and Hilton 1977; Rueppel et al. 1977; Strange-Hansen et al. 2004) and sorption appeared to be essentially constant after 24 hours, therefore 24 hours were chosen as shaking time for the sorption isotherm experiments. Within this time no formation of the degradation product AMPA was observed.
The sorption isotherms were well described by the Freundlich equation (Fig. 2.6). Sorption coefficients $K_f$ for the upper 0-1 cm layer ranged from 25.8 to 108.7 mg\textsuperscript{1-n} L\textsuperscript{n} kg\textsuperscript{-1} (mean 51.5 mg\textsuperscript{1-n} L\textsuperscript{n} kg\textsuperscript{-1}) and from 11.1 to 51.5 mg\textsuperscript{1-n} L\textsuperscript{n} kg\textsuperscript{-1} (mean 23.5 mg\textsuperscript{1-n} L\textsuperscript{n} kg\textsuperscript{-1}) for the 1-5 cm layer. Sorption experiments with the formulated product Roundup Ultra were additionally conducted for sampling sites OS and GS but showed no significant differences compared to the sorption of pure glyphosate therein. Thus, the formulation seems not to influence the sorption of glyphosate to seam material. Sorption coefficients $K_f$, Freundlich exponents $n$ and the coefficient of determination $r^2$ for sorption isotherms from all samples are shown in Tab. 2.5. The Freundlich exponents $n$ are similar for all samples for both depths but not identical.
Therefore additionally the retardation factor $R$ was calculated with $\rho = 1.5 \text{ g cm}^{-3}$, $C_w = 0.1 \text{ mg L}^{-1}$ and $\theta = 0.3 \text{ m}^3 \text{ m}^{-3}$ for comparison reasons (Tab. 2.5).

**Tab. 2.5: Sorption parameters of glyphosate in seam material.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$K_f$ ([\text{mg}^{-1} \text{nL}^n \text{kg}^{-1}])</th>
<th>$n$</th>
<th>$r^2$</th>
<th>$R_{Gly}^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS 0-1 cm</td>
<td>25.8</td>
<td>0.91</td>
<td>0.99</td>
<td>145</td>
</tr>
<tr>
<td>OS 1-5 cm</td>
<td>11.5</td>
<td>0.88</td>
<td>0.99</td>
<td>67</td>
</tr>
<tr>
<td>SS 0-1 cm</td>
<td>108.7</td>
<td>0.90</td>
<td>0.99</td>
<td>614</td>
</tr>
<tr>
<td>SS 1-5 cm</td>
<td>47.2</td>
<td>0.87</td>
<td>0.99</td>
<td>280</td>
</tr>
<tr>
<td>GS 0-1 cm</td>
<td>56.0</td>
<td>0.90</td>
<td>0.99</td>
<td>316</td>
</tr>
<tr>
<td>GS 1-5 cm</td>
<td>51.5</td>
<td>0.88</td>
<td>0.99</td>
<td>298</td>
</tr>
<tr>
<td>SHA 0-1 cm</td>
<td>45.3</td>
<td>0.95</td>
<td>0.99</td>
<td>243</td>
</tr>
<tr>
<td>SHA 1-5 cm</td>
<td>20.7</td>
<td>0.92</td>
<td>0.99</td>
<td>116</td>
</tr>
<tr>
<td>FBS 0-1 cm</td>
<td>45.3</td>
<td>0.96</td>
<td>0.99</td>
<td>240</td>
</tr>
<tr>
<td>FBS 1-5 cm</td>
<td>14.7</td>
<td>0.91</td>
<td>0.99</td>
<td>83</td>
</tr>
<tr>
<td>AWD 0-1 cm</td>
<td>42.2</td>
<td>0.92</td>
<td>0.99</td>
<td>234</td>
</tr>
<tr>
<td>AWD 1-5 cm</td>
<td>17.1</td>
<td>0.89</td>
<td>0.99</td>
<td>99</td>
</tr>
<tr>
<td>B1 0-1 cm</td>
<td>55.2</td>
<td>1.10</td>
<td>0.99</td>
<td>241</td>
</tr>
<tr>
<td>B1a 1-5 cm</td>
<td>18.9</td>
<td>1.03</td>
<td>0.98</td>
<td>92</td>
</tr>
<tr>
<td>B2 0-1 cm</td>
<td>56.2</td>
<td>1.11</td>
<td>0.99</td>
<td>244</td>
</tr>
<tr>
<td>B2a 1-5 cm</td>
<td>18.7</td>
<td>1.04</td>
<td>0.99</td>
<td>89</td>
</tr>
<tr>
<td>B7 0-1 cm</td>
<td>28.6</td>
<td>1.04</td>
<td>0.99</td>
<td>137</td>
</tr>
<tr>
<td>B4a 1-5 cm</td>
<td>11.1</td>
<td>1.01</td>
<td>0.99</td>
<td>56</td>
</tr>
</tbody>
</table>

$^a\rho_{B}=1.5 \text{ g cm}^{-3}, C_w=0.1 \text{ mg L}^{-1}, \theta=0.3 \text{ m}^3 \text{ m}^{-3}$

Determined $K_f$ are within the wide range of values mentioned in the literature but values of the 1-5 cm layer range at the lower end of reported values (Hance 1976; Giesy et al. 2000; Vereecken 2005). Strange-Hansen et al. (2004) determined linear sorption coefficients ($K_d$) ranging from 62 to 164 L kg$^{-1}$ in gravelly materials. The $K_f$ of the top seam material were significantly higher ($p \leq 0.001$) than in the 1-5 cm layer indicating that the urban deposition increases the sorption capacity of the seam material. A higher sorption capacity of the upper seam material layer was as well observed by Nehls (2007) for the heavy metals Pb and Cd in batch sorption experiments. He found a good linear correlation between the $K_f$ of Pb and the $C_{org}$ contents of the seam material samples from the upper 0-1 cm layer. This was not the case for glyphosate sorption. For the 0-1 cm layer no significant dependency of the sorption capacity from the $C_{org}$ content was found ($F = 1.8; P = 0.222; r^2 = 0.2$).

Comparing the $K_f$ of the whole sample collective ($n = 18$) with each single measured parameter ($C_{tot}$, $C/N$, $\text{CEC}_{eff}$, SSA, pH, clay content, $P_{tot}$, dithionite and oxalate extractable Al and Fe), a significant influence ($P < 0.05$) of all parameters, except $\text{CEC}_{eff}$ and SSA, on the sorption capacity of the seam material was found but the goodness of fit was with $r^2 < 0.4$ quite low (Tab. 2.6).
The dithionite and oxalate extractable iron fractions showed the highest influence with $F = 9.7$ and $F = 9.4$, respectively. Dividing the samples into two groups (0-1 cm & 1-5 cm; n = 9) showed different results: There was no significant influence of the tested parameters on the $K_f$ in the 0-1 cm layer. Only the pH has an almost significant influence ($P = 0.069$) on the sorption capacity (Tab. 2.6). However, the 1-5 cm layer, which consists of the material which is only slightly influenced by the urban deposition, showed stronger dependencies between the sorption coefficient of glyphosate and the measured parameters (Tab. 2.6). A significant influence of the content of oxalate extractable aluminium ($F = 12.4; P = 0.01$), dithionite extractable aluminium ($F = 9.7; P = 0.017$), oxalate extractable iron ($F = 10.1; P = 0.016$) and pH ($F = 6.7; P = 0.036$) onto the sorption of glyphosate was found. The goodness of fit ($r^2$) was for the aluminium and iron fractions > 0.58. To improve the goodness of fit of the linear regressions, multiple linear regressions with the soil parameters which had a significant influence on the sorption of glyphosate were conducted. The parameters oxalate extractable aluminium and iron and pH were found to be the most important factors for explaining the variations in the glyphosate adsorption. As the correlation between oxalate extractable aluminium and iron is too high only one of these two parameters is used in the model equation (Tab. 2.7):

Tab. 2.7: Multiple linear regression equations.

<table>
<thead>
<tr>
<th>$K_f, 0-1$ cm (n = 9)</th>
<th>$r^2$</th>
<th>F</th>
<th>P</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_f$</td>
<td>0.64</td>
<td>0.58</td>
<td>0.59</td>
<td>0.03</td>
</tr>
<tr>
<td>$F$</td>
<td>12.4</td>
<td>9.7</td>
<td>10.1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$P$</td>
<td>0.465</td>
<td>0.489</td>
<td>0.633</td>
<td>0.95</td>
</tr>
<tr>
<td>$K_f$</td>
<td>0.01</td>
<td>0.017</td>
<td>0.016</td>
<td>0.668</td>
</tr>
<tr>
<td>$F$</td>
<td>6.7</td>
<td>5.1</td>
<td>3.5</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$P$</td>
<td>0.036</td>
<td>0.036</td>
<td>0.036</td>
<td>0.036</td>
</tr>
<tr>
<td>$K_f$</td>
<td>0.42</td>
<td>0.33</td>
<td>0.33</td>
<td>0.09</td>
</tr>
<tr>
<td>$F$</td>
<td>1.8</td>
<td>1.3</td>
<td>1.3</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$P$</td>
<td>0.292</td>
<td>0.292</td>
<td>0.292</td>
<td>0.292</td>
</tr>
</tbody>
</table>

Tab. 2.6: Coefficient of determination, F and P values of a linear regression analysis between $K_f$ and soil parameters.

<table>
<thead>
<tr>
<th>$K_f$</th>
<th>$r^2$</th>
<th>F</th>
<th>P</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_f$ all samples (n = 18)</td>
<td>0.29</td>
<td>0.33</td>
<td>0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>$F$</td>
<td>6.6</td>
<td>7.7</td>
<td>9.4</td>
<td>9.7</td>
</tr>
<tr>
<td>$P$</td>
<td>0.021</td>
<td>0.013</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>$K_f, 0-1$ cm (n = 9)</td>
<td>0.08</td>
<td>0.07</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>$F$</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$P$</td>
<td>0.467</td>
<td>0.489</td>
<td>0.633</td>
<td>0.95</td>
</tr>
<tr>
<td>$K_f, 1-5$ cm (n = 9)</td>
<td>0.64</td>
<td>0.58</td>
<td>0.59</td>
<td>0.03</td>
</tr>
<tr>
<td>$F$</td>
<td>12.4</td>
<td>9.7</td>
<td>10.1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>$P$</td>
<td>0.01</td>
<td>0.017</td>
<td>0.016</td>
<td>0.668</td>
</tr>
</tbody>
</table>

$^\dagger$oxalate extractable, $^\ddagger$dithionite extractable, $^§$effective cation exchange capacity
For the 0-1 cm layer no significant influence of a combination of two tested parameters was found. The combination of pH and oxalate extractable aluminium or iron has a high influence ($F > 25$) on the sorption of glyphosate to seam material of the 1-5 cm layer with a goodness of fit of $r^2 > 0.9$. The importance of iron and aluminium oxides and pH for adsorption of glyphosate has previously been reported in literature (McConnell and Hossner 1985; Piccolo et al. 1994; de Jonge and de Jonge 1999; Morillo et al. 2000; Gimsing and Borggaard 2002; Gimsing et al. 2004a). Due to its three polar functional groups (carboxyl, amino and phosphonate groups) glyphosate is not mainly sorbed by soil organic matter, but is strongly sorbed by soil minerals. As glyphosate is a polyprotic acid, it forms mono- and divalent anions with high affinity for trivalent cations as $\text{Al}^{3+}$ and $\text{Fe}^{3+}$ within the pH range of 4-8 which is found in most soils (Borggaard and Gimsing 2008).

The fact that the sorption capacity for glyphosate in the upper 0-1 cm layer is clearly increased compared to the 1-5 cm layer without finding any significant influences of tested parameters like pH and iron and aluminium oxides as found in agricultural soils and in the 1-5 cm layer, confirms findings of the first part of this chapter (2.1.3.1) showing a different composition of the upper 0-1 cm layer due to urban deposition. The higher complexity of the anthropogenic influenced top seam material layer might cause multiple and non-linear relationships of several parameters influencing the sorption capacity of glyphosate. The findings in part 2.1.3.1 (no linear relationship of effective CEC and SSA from the $C_{\text{org}}$ content) support this assumption.

A few non-linear approaches with only one parameter were tested and saturation functions like power functions increase the significance for the whole sample pool ($n = 18$) but not for the single layers. This is consistent with the finding of a linear relationship in the 1-5 cm layer, the layer with the low $K_f$ and low contents of iron, aluminium, $C_{\text{org}}$, etc. and the missing linear relationship in the 0-1 cm layer with the higher $K_f$ values and contents of the substances.

Nevertheless, more complex non-linear approaches with several parameters on the basis of theoretical concepts and hypothesis will have to be tested, to be able to account for combination effects of several parameters on the sorption of glyphosate. Therefore the pool of samples has to be enlarged and the number of measured parameters has to be increased, to strengthen the findings of relationships between parameters and the sorption capacity for glyphosate as the heterogeneity of seam material is high.
2.2.3.2 Influence of pH

Further studies concerning the influence of pH on the adsorption of glyphosate were conducted with material from the OS 0-1 cm layer and the pure glowed sand used for the experiments with black carbon and urban dust (2.2.2.5).

![Graph showing pH dependency of glyphosate sorption to pure sand and seam material from sampling site OS.](image)

Fig. 2.7: pH dependency of glyphosate sorption to pure sand and seam material from sampling site OS.

Fig. 2.7 shows the results of this experiment. In both materials the adsorption of glyphosate highly depends on pH and decreases with increasing pH as observed in previous studies (McConnell and Hossner 1985; de Jonge and de Jonge 1999; Gimsing et al. 2004a). The significant dependency of the adsorbed amount of glyphosate on the pH can be described with following models (Tab. 2.8):

<table>
<thead>
<tr>
<th>Material</th>
<th>$Gly_{\text{ads}} ,[%] = a \cdot pH + b$</th>
<th>F</th>
<th>P</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>$Gly_{\text{ads}} ,[%] = -15.4 \cdot pH + 183.9$</td>
<td>698.1</td>
<td>&lt; 0.001</td>
<td>0.98</td>
</tr>
<tr>
<td>Sand</td>
<td>$Gly_{\text{ads}} ,[%] = -74.6 \cdot pH + 576.8$</td>
<td>58.7</td>
<td>0.002</td>
<td>0.94</td>
</tr>
</tbody>
</table>

The gradient of the regression line for the sand is steeper than the one for the OS material. Thus, a stronger decrease of the amount of adsorbed glyphosate with each increasing pH unit occurs for the sand. The strong pH-dependency of glyphosate adsorption results from an increasing attraction between the soil surfaces and glyphosate at decreasing pH due to an increasing positive charge on the soil surfaces that more than outweighs the decreasing negative charge of the glyphosate molecules (Gimsing et al. 2004a).
2.2.3.3 Influence of black carbon and urban dust

As up to one third of the OM of the upper seam material layer consists of BC (2.1.3, Nehls 2007) and because BC is as well known to play an important role in the sorptive uptake of organic pollutants such as PAH (Karapanagioti et al. 2000; Kleineidam et al. 2002; Cornelissen et al. 2005; Koelmans et al. 2006; Nehls 2007), further studies concerning the sorption of glyphosate to BC and urban dust were conducted. Even though the OM content was not found to play a major role in the sorption of glyphosate in agricultural soils (Borggaard and Gimsing 2008) no investigations are known regarding the influence of BC onto the sorption of glyphosate.

The results of the batch sorption experiments with a sand, low in aluminium and iron oxides, and different contents of BC and UD, respectively, are shown in Tab. 2.9. No correlation between the amount of added BC or UD and the sorption coefficient $K_f$ were found. Surprisingly the pure sand had the highest $K_f$ value for glyphosate (Tab. 2.9).

<table>
<thead>
<tr>
<th></th>
<th>$K_f$ [mgL$^{-1}$nL$^{-1}$kg$^{-1}$]</th>
<th>$n$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>28.5</td>
<td>0.84</td>
<td>6.4</td>
</tr>
<tr>
<td>BC 0.05%</td>
<td>22.4</td>
<td>0.82</td>
<td>6.7</td>
</tr>
<tr>
<td>BC 0.5%</td>
<td>19.9</td>
<td>0.79</td>
<td>6.7</td>
</tr>
<tr>
<td>BC 5%</td>
<td>19.6</td>
<td>0.84</td>
<td>6.8</td>
</tr>
<tr>
<td>UD 1%</td>
<td>6.5</td>
<td>0.9</td>
<td>7.1</td>
</tr>
<tr>
<td>UD 10%</td>
<td>6.3</td>
<td>0.92</td>
<td>7.4</td>
</tr>
<tr>
<td>UD 50%</td>
<td>14.0</td>
<td>0.91</td>
<td>7.7</td>
</tr>
</tbody>
</table>

A regression analysis with the $K_f$ against the pH revealed that the pH dominates the sorption behaviour of glyphosate and the tested concentrations of BC (0.05%, 0.5%, 5%) and did not influence the sorption. The measured $K_f$ values are in accordance with the results of the previously described pH dependency experiment with the same sand (2.2.3.2) (Fig. 2.8). The same applies for the sand containing 1% and 10% urban dust. Whereas the $K_f$ value of the sample consisting to 50% of urban dust is not in line with the pH regression line. Thus, in case of replacing 50% of the sand with urban dust, a different parameter exceeds the influence of the pH on the adsorption of glyphosate. As the tested concentration of 5% BC was ten times higher than the amounts of BC found in the upper layer of seam material by Nehls (2007), BC seems not to dominate the adsorption of glyphosate to seam material and soils. However, the here tested BC, called carbon black, is only one of lots of different possible BC components and it can not be ruled out that for example soot has a different influence on the adsorption of glyphosate than carbon black.
2.3 Conclusions and outlook

The continuously deposition of various materials present in the urban environment, mainly of anthropogenic origin like dust, soot and rubber, results in a changed composition of the upper layer of the pavement seam material. Due to many factors influencing the amount and the kind of deposition, the heterogeneity of the seam material composition within a city is very high. Therefore a bigger pool of seam material samples would be necessary to be able to detect coherences between composition and site parameters like traffic density or land use. Nevertheless the differences between the upper 0-1 cm layer and the lower 1-5 cm layer are clear enough to assign samples to one or the other group. The petrographic analyses showed a strong anthropogenic influence on the seam materials OM as it was found by Nehls (2007) as well. Thus, the upper seam material behaves different than natural soils and acts due to its increased sorption capacity as a filter for pollutants like glyphosate. Further investigations have to assess, if the lower influence of the seam material OM onto the CEC and SSA is due to changes in the composition of the OM itself (e.g. influence of BC) or if other parameters than OM govern the CEC and SSA and therewith the sorption capacity of the seam material in general.

For the glyphosate sorption in seam material linear dependencies on dithionite and oxalate extractable iron and aluminium and the pH were found for the 1-5 cm layer. Further statistical analyses in combination with theoretical concepts have to show if multiple non-linear...
approaches detect more significant dependencies for the whole sampling pool. Therefore an enlarged sampling pool and the determination of additionally parameters are crucial.

Whether the increased sorption capacity of the only 1 cm layer of aged seam material prevents the leaching of glyphosate into lower layers of the pavement bed with low sorption capacities or if other transport processes like preferential flow dominate the displacement of glyphosate in a pavement system, was investigated in column (chapter IV) and lysimeter experiments (chapter III). Furthermore, the $K_f$ values determined with the batch sorption experiments were compared to the results of the column experiments.
3 Sorption and leaching of glyphosate on partly sealed areas – a lysimeter study

3.1 Introduction

The non-selective, post-emergence herbicide glyphosate [N-(phosphonomethyl)glycine] is one of the most widely used pesticides: It is used for weed control in agriculture, forestry, aquaculture and urban areas and is one of the most sold herbicides (Woodburn 2000). It is usually formulated as isopropylamine salt and in case of the most common formulation Roundup (Monsanto Co, St. Louis, MO, USA) combined with the surfactant polyoxyethylene amine (POEA) (Giesy et al. 2000).

Due to its non-selectivity it is used for weed control in urban areas where weeds are unwanted for safety or aesthetic reasons like roadsides, pavements, courtyards or railways. In Germany, the municipalities have to grant exemption for pesticide use in urban areas at the competent authority. The authorities have to check if the requested application is forbidden according to § 3 of the German Pest Management Utilization Regulation (Bundesgesetzblatt 2003). Since 2003 the spraying of glyphosate has been forbidden in Germany and the application of glyphosate on hard surfaces is only allowed with the risk-reducing roller wiper Rotofix or the wick wiper (German Plant Protection Law §6 (Bundesgesetzblatt 1998)). Additionally, the principles of Good Professional Practice (BMELV 2005) such as no application if rain is likely, have to be taken into account. In other countries no restrictions for the use of glyphosate on pavements and roadsides are imposed. Some countries, e.g., The Netherlands and Denmark have started programs for weed control on hard surfaces aiming to reduce and phase-out herbicide use within urban areas (Kempenaar and Spijker 2004; Kristoffersen et al. 2004).

Due to its distinct tendency to adsorb to soil constituents with $K_d$ values up to 900 L kg$^{-1}$ and fast microbial degradation, the risk of surface or ground water contamination with glyphosate is generally assumed to be low (Rueppel et al. 1977; de Jonge et al. 2001; Vereecken 2005). Nevertheless, recent studies demonstrated that glyphosate sorption and degradation in soil are very variable and depend on soil properties like pH, iron and aluminium oxides, soil structure, application time, and microbial activity (de Jonge and de Jonge 1999; Borggaard and Gimsing 2008). Furthermore, leaching of glyphosate and its degradation product AMPA
(amino-methylphosphonic acid) was observed up to 1 m depth in field studies suggesting a risk for aquatic environments (Kjaer et al. 2005; Landry et al. 2005; Stadlbauer and Fank 2005). Moreover, glyphosate and AMPA are present in surface waters worldwide in considerable concentrations, e.g., up to 2.2 µg L⁻¹ in US rivers (Feng et al. 1990; Newton et al. 1994; Skark et al. 1998; Kolpin et al. 2006; Peruzzo et al. 2008). Due to its high polarity and water-solubility the analysis of glyphosate in water samples is rather time consuming and therefore, glyphosate and AMPA are despite its extensive worldwide use not a common parameter in routine water quality monitoring programmes.

Glyphosate used in urban areas contributes to glyphosate and AMPA concentrations in streams (Skark et al. 2004; Kolpin et al. 2006; Byer et al. 2008). Even if the amounts of glyphosate used in urban areas are very low compared to the amounts used in agriculture, its environmental impact may be greater. The materials of urban surfaces are less sorptive than topsoils that contain organic matter and microorganisms. The material between and under the paving stones etc. consists of coarse sand or gravel with low contents of clay, iron and aluminium oxides, and generally has a high pH. Furthermore, runoff from railway tracks, roadsides and partly-sealed pavements is more likely and runoff volumes are larger than in agricultural systems (Skark et al. 1998; Ramwell et al. 2002; Huang et al. 2004; Spanoghe et al. 2005).

This study was conducted on the basis of two previous experiments with glyphosate in 2004 and 2005 on the same lysimeters (Schmidt and Boas 2006). Thereby additionally tracer experiments, improved application measurements and a higher sampling resolution were applied.

The aim of this study was to assess the risk of glyphosate leaching into deeper layers of the pavement bed after its application on partly sealed pavements in urban areas. The experimental set-up of this study with low depth lysimeters and surface runoff exclusion enhanced pollutant transport to the outflow of the lysimeters. Thus, if we observed negligible amounts of glyphosate and AMPA in the leachate of our experiments, glyphosate and AMPA leaching could be regarded as highly unlikely under natural conditions on paved areas.
3.2 Materials and methods

3.2.1 Chemicals

The Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) used contains the monoisopropylamine salt of glyphosate (N-(phosphonomethyl)-glycine) \(360 \text{ g L}^{-1}\) and surfactants of undisclosed chemical composition and concentration (e.g., polyoxyethylene amine (POEA)). For the field experiment Roundup Ultra was diluted with tap water to a 10% solution \((36 \text{ g L}^{-1})\) including 5% rinsing agent (Palmolive).

\(^{14}\text{C}\)-radiolabelled glyphosate \(\left[^{14}\text{C}\right]\text{glyphosate}\) was obtained from Monsanto (St. Louis, MO, USA) with 98.8% radiochemical and 99% chemical purity. Its specific radioactivity was 1.71 GBq mmol\(^{-1}\). Stock solutions for batch equilibrium experiments were prepared by mixing \(^{14}\text{C}\)-labelled glyphosate and Roundup Ultra solution. The \(^{14}\text{C}\)-activity of the spiking solutions ranged from 17 – 62 Bq ml\(^{-1}\).

For the water tracers, used at the beginning and the end of the lysimeter experiment, respectively, potassium bromide solution with 400 mg L\(^{-1}\) bromide and a brilliant-blue solution with 1 g L\(^{-1}\) were prepared.

3.2.2 Soil properties

At the end of the field experiment the following physico-chemical soil properties of the filling material of the lysimeters were determined using standard methods: pH (CaCl\(_2\)), particle size distribution, C\(_{\text{org}}\) content, water retention characteristics (pF-values), saturated hydraulic conductivity (K\(_{\text{sat}}\)), gravimetric and volumetric water content, effective cation exchange capacity (CEC\(_{\text{eff}}\)), content of dithionite and oxalate extractable Fe and Al (Tab. 3.1). Water retention characteristics were determined according to ISO 11274 (1998b), saturated hydraulic conductivity according to DIN 19683-9 (1998), effective CEC according to ISO 11260 (1994), oxalate extractable Al and Fe according to DIN 19684-6 (1997) and dithionite extractable Al and Fe according to Mehra and Jackson (Mehra and Jackson 1960). Pore volumes of the lysimeters were calculated from the volumetric water contents at approximately field capacity of each layer at the end of the experiment.
Tab. 3.1: Physical and chemical characteristics of the lysimeter fill material.

<table>
<thead>
<tr>
<th></th>
<th>Coarse sand</th>
<th>Seam Material</th>
<th>Loamy sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse Materials (&gt; 2mm) [%]</td>
<td>24</td>
<td>24</td>
<td>0.7</td>
</tr>
<tr>
<td>Sand [%]</td>
<td>99.1</td>
<td>92</td>
<td>74.9</td>
</tr>
<tr>
<td>Silt [%]</td>
<td>0.3</td>
<td>4.5</td>
<td>17.3</td>
</tr>
<tr>
<td>Clay [%]</td>
<td>0.5</td>
<td>3.5</td>
<td>7.8</td>
</tr>
<tr>
<td>pH (CaCl$_2$)</td>
<td>6.5</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td>C$_{org}$ [g kg$^{-1}$]</td>
<td>0.2</td>
<td>10.2</td>
<td>4.7</td>
</tr>
<tr>
<td>CEC$_{eff}$ [cmol kg$^{-1}$]</td>
<td>&lt; 2</td>
<td>4.8</td>
<td>6</td>
</tr>
<tr>
<td>Fe$_{dit}$ [g kg$^{-1}$]</td>
<td>0.4</td>
<td>1.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Fe$_{ox}$ [g kg$^{-1}$]</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Al$_{dit}$ [g kg$^{-1}$]</td>
<td>&lt; 0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Ksat [cm d$^{-1}$]</td>
<td>1235</td>
<td>-</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>FC (pF 1.8) [Vol %]</td>
<td>5.5</td>
<td>-</td>
<td>19.7</td>
</tr>
</tbody>
</table>

CEC$_{eff}$: effective cation exchange capacity; Fe$_{dit}$ and Al$_{dit}$: dithionite extractable Fe and Al; Fe$_{ox}$: oxalate extractable Fe; Ksat: saturated hydraulic conductivity; FC: field capacity.

3.2.3 Batch sorption experiments

Batch sorption experiments with the lysimeter filling material (loamy sand, coarse sand and the seam material, which is the coarse sand between the paving stones and pavement flagstones with deposited urban dust) were conducted according to the OECD Guideline 106 (2000) with $^{14}$C-labelled glyphosate. Studied mass ratios of glyphosate in soil were 0.2, 2, 5 and 20 mg kg$^{-1}$ with a soil/solution ratio of 1:10.

24 hours before the start of the sorption experiment 2 g of dry soil were mixed with 19 ml of 0.01 M CaCl$_2$ solution to pre-equilibrate the soils and placed on an overhead shaker over night. On the next day, 1 ml of Roundup solution containing $^{14}$C-labelled glyphosate was added and samples were shaken overhead for 24 hours at 20 rpm. This relatively short equilibrium period was chosen because glyphosate degrades quickly (Nomura and Hilton 1977; Rueppel et al. 1977; Strange-Hansen et al. 2004), and in our preliminary tests adsorption was essentially constant after 24 h.

After centrifuging the samples for 25 minutes at 2000 RCF, 2 ml of the supernatant were added to 10 ml of liquid scintillation cocktail (Luma Safe Plus, Lumac-LSC, Groningen, The Netherlands). The samples were measured by liquid scintillation counting (LSC, LS 6500, Beckman, Fullerton, CA, USA). The amount adsorbed was determined by subtracting the solution concentration from the initial concentration. Concentrations of glyphosate are reported as equivalent of parent compound calculated from the counted radioactivity. For each
concentration three replicates were prepared. All experiments were carried out at ambient temperature of 20°C (+/- 2).

Sorption parameters were estimated from the Freundlich equation:

\[ C_s = K_f \cdot C_w^n \]  

(3.1)

where \( C_s \) is the concentration in the soil [mg kg\(^{-1}\)], \( K_f \) is the Freundlich sorption coefficient [mg\(^{1-n}\) L\(^n\) kg\(^{-1}\)], \( C_w \) is the concentration in the solution after 24 hours [mg L\(^{-1}\)] and \( n \) is a linearity factor (Freundlich exponent). Additionally, the retardation factor \( R \) for non-linear sorption was calculated with

\[ R = 1 + \frac{\rho}{\theta} n \cdot K_f \cdot C_w^{n-1} \]  

(3.2)

where \( \rho \) is the bulk density [g cm\(^{-3}\)] and \( \theta \) the volumetric water content [m\(^3\) m\(^{-3}\)] of the soil. For \( n \neq 1 \), the retardation factor \( R \) is depending on the concentration in the solution.

### 3.2.4 Lysimeter experiment

#### 3.2.4.1 Description of the experimental set-up

In 2004 three aluminium lysimeters with a length of 5 m, a width of 1.4 m and a height of 0.3 m were built on the experimental area of the Federal Research Centre For Cultivated Plants (JKI) in Berlin-Dahlem, Germany. The surface area of each lysimeter was 7 m\(^2\). Per lysimeter four outlets were installed on one longitudinal side (0.2 m; 1.6 m; 3.4 m; 4.8 m) to collect leachate samples. The bottom of the lysimeters had a slope of approximately 1% in direction of the outlets to guarantee a direct outflow.

<table>
<thead>
<tr>
<th>Lysimeter</th>
<th>Toplayer</th>
<th>Middle layer</th>
<th>Base layer</th>
<th>Bulk density</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>7 cm loamy sand (Sl2)</td>
<td>4 cm coarse sand (mSgs)</td>
<td>12 cm coarse gravel (20-63 mm)</td>
<td>1.7 g cm(^{-3})</td>
</tr>
<tr>
<td>S50</td>
<td>Paving stones (8 cm x 8 cm x 8 cm)</td>
<td>4 cm coarse sand (mSgs)</td>
<td>12 cm coarse gravel (20-63 mm)</td>
<td>1.7 g cm(^{-3})</td>
</tr>
<tr>
<td>S95</td>
<td>Pavement flagstones (35 cm x 35 cm x 5 cm)</td>
<td>5-6 cm coarse sand (mSgs)</td>
<td>12 cm coarse gravel (20-63 mm)</td>
<td>1.7 g cm(^{-3})</td>
</tr>
</tbody>
</table>
The lysimeters were filled according to the construction guidelines for pavements in Germany (DIN 18318 (2000)) (Tab. 3.2; Fig. 3.1). The bottom of each lysimeter was filled with 12 cm of coarse gravel (20-63 mm) as base layer. Upon 4 to 6 cm coarse sand (mSgs) were filled and compacted to a bulk density of 1.7 g cm$^{-3}$. The surface cover of the lysimeters varied (Tab. 3.2): For lysimeter L (loamy sand) the surface cover consisted of 7 cm loamy sand, which was compacted to 1.7 g cm$^{-3}$. Lysimeters S50 (50% sealed) and S95 (95% sealed) were partly sealed with hard surfaces: Lysimeter S50 was covered with paving stones of about 8 cm x 8 cm x 8 cm with coarse sand in between (seam material). The paving stones covered about 50% of the surface area and the seam material the other half. Lysimeter S95 was covered with pavement flagstones of 35 cm x 35 cm and a thickness of 5 cm with coarse sand in between. They covered about 95% of the area. The walls of the lysimeters protruded over the surface by 6 cm so that runoff from the lysimeters was impossible. This increased the percolation rate because the percolation rate included both, the runoff rate and the infiltration rate.

Weed (ryegrass (*lolium perenne* L.), mayweed (*anthemis cotula* L.), canada fleabane (*conyza Canadensis* L.) was sown in 2004 to imitate natural weed cover on pavements. The weed was cut back to about 20 cm height before application of Roundup Ultra. The weed cover on lysimeter S95 was the least and on lysimeter L the most.

### 3.2.4.2 Application of Roundup Ultra with Rotofix

Three days before the application of the Roundup Ultra solution, the areas were irrigated with 5.7 mm (S95), 8.6 mm (S50) and 11.4 mm (L) to saturate them to field capacity. The leachate was sampled to determine the background concentration of glyphosate and AMPA.

A 10% Roundup Ultra solution (36 g L$^{-1}$ a.i.) with 5% rinsing agent was applied with the roller wiper Rotofix (Rotowiper, Ashburton, NZ). This apparatus consists of a rotating roll which is wetted by two nozzles with the Roundup Ultra solution. In contrast to spray application, the moistened rotating roll is coating the herbicide specifically onto the weed. Thereby direct soil contamination should be avoided as the roll has no contact with the soil. The amount applied was measured by weighing (Sartorius 5789 MP8-1, max. 60000 g, Readability 0.1 g) the Rotofix between the applications onto the lysimeters. The applied
amounts of glyphosate are shown in Tab. 3.3. The starting concentration \( C_0 \) was defined as applied amount of glyphosate divided by the artificial irrigation water \( C_{0L} = 15.7 \text{ mg L}^{-1}; C_{0S50} = 18.7 \text{ mg L}^{-1}; C_{0S95} = 9 \text{ mg L}^{-1} \).

<table>
<thead>
<tr>
<th>Lysimeter</th>
<th>L</th>
<th>S50</th>
<th>S95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied amount glyphosate</td>
<td>940 mg</td>
<td>1120 mg</td>
<td>540 mg</td>
</tr>
<tr>
<td>Permitted amount of glyphosate on non-arable land</td>
<td>up to 3.6 kg ha(^{-1})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.4.3 Irrigation, rainfall and sampling

One day after the application of the Roundup Ultra solution the lysimeters were irrigated with 8.6 mm tap water. First of all 4.3 mm water with 400 \( \mu \text{g L}^{-1} \) bromide was applied with a watering can (within 15 min). No ponding occurred. Two hours later another 4.3 mm water without tracer was applied. No further irrigation was conducted. Precipitation during the experiment was measured at the meterological station Berlin-Dahlem (Freie Universität Berlin) which was 500 m distant from our experimental site. Within the five months of the experiment (154 days; August to January 2006) rainfall amounted to 199 mm. The highest rainfall intensity measured was 4 mm h\(^{-1}\). From day 26 to day 42 no rainfall occurred (Fig. 3.5). September was with only 12.6 mm extremely dry compared to the mean of the years 1971 – 2000 (42.3 mm).

During the artificial irrigation leachate was sampled in 1 L fractions and after the first day in 10 L fractions. Due to a temporarily overflow of the 10 L collecting bottles of lysimeters S50 and S95, the leachate of these lysimeters had to be reconstructed using the precipitation data measured. Using these data a factor (0.88) of precipitation and leachate amounts was calculated for each lysimeter based on data recorded on sampling dates without overflow.

Leachate of the outlets was pooled for each sampling date for each lysimeter considering the ratio of leachate amounts of the four outlets. Bromide concentrations and pH were measured in all leachate samples. Glyphosate and AMPA concentrations were determined on 19 (lysimeter L), 26 (lysimeter S50) and 28 (lysimeter S95) sampling dates. The amount of the active ingredient equivalent was calculated by counting the amount of AMPA back to the original amount of glyphosate and adding this to the measured amounts of glyphosate.

A factor \( f \) for the transformation of glyphosate and AMPA in the leachate of lysimeter S95 was calculated:

\[
f = \frac{c_\text{gly}}{c_\text{AMPA}}
\]
where \( c_{\text{gly}} \) and \( c_{\text{AMPA}} \) are the glyphosate and AMPA concentrations in the leachate [µg L\(^{-1}\)] respectively. Assuming first order kinetics for the transformation of glyphosate and regarding AMPA as the dominant degradation product, then the dependency of \( f \) from time \( t \) is

\[
\ln\left(\frac{f}{f+1}\right) = -k \cdot t
\]

(3.4)

where \( k \) is the transformation rate. The time after which glyphosate concentrations in the leachate are halved can then be described with

\[
t_{1/2} = \frac{\ln 2}{k}
\]

(3.5)

At the end of the experiment in each layer soil samples were randomly taken for the determination of glyphosate and AMPA contents, gravimetric and volumetric water content and characterisation of the filling material.

### 3.2.4.4 Analysis of glyphosate and AMPA

The leachate was enriched by exchange resins (Chelex-100 Chelating Ion Exchange Resin; AG 1-X8 Strong Anion Exchange Resin; Biorad, Hercules, CA, USA). Glyphosate and AMPA contents were measured by HPLC (pump model 480, Gynkotek, Germering, Germany; cation-exchange column for glyphosate analysis & cation-exchange guard column, Pickering, Mountain View, CA, USA) with fluorescence detector after post-column derivatisation (Pickering PCX 5200, Mountain View, CA, USA) (DFG 1991). The detection limit was 0.1 µg L\(^{-1}\) and the recovery rate from water was 96 ± 7% for glyphosate and 93 ± 10% for AMPA.

Soil samples (25 g) were extracted with 150 ml of 1 molar ammonia solution for 30 min at 200 rpm according to (DFG 1991). Following, the samples were centrifuged and the supernatant was filtered through glass wool. The extraction was repeated two more times. The filtrate was then adjusted to a pH of 2.0 ± 0.4 with hydrochloric acid and diluted with 1.8 L deionised water. Before processing the extract as described above the samples were left to settle out the emerging precipitate. Even though glyphosate and AMPA recovery rates from other soil samples were 90%, the recovery rates from the sandy filling material of the lysimeters were only 60%. It was found that the precipitate contained glyphosate and AMPA but due to clogging of the enrichment columns it was not possible to process the extracts without removing the precipitate. Thus, quantification of the detected amounts of glyphosate and AMPA in soil was not possible and a mass balance can not be calculated for the lysimeter experiment.

Bromide concentrations were measured with a bromide electrode (Mettler Toledo DX 280-Br, Giessen, Germany). The detection limit was 1 ppm.
3.3.4.5 Dye tracer experiment

In order to visualize flow paths under the different surface covers of the lysimeters and to quantify the area flowed through a dye tracer experiment was conducted. At the end of the field experiment with glyphosate the lysimeters were irrigated with 9 mm water containing 1 g L\(^{-1}\) Brilliant Blue. Afterwards they were covered and excavated the following day in layers of 2 to 5 cm depths. The surface of each layer was photographed in order to quantify the areas flowed through by image processing techniques. The images were corrected for geometrical distortion, including the spatial transformation of pixels using the tie point concept and the gray-level interpolation (Gonzales and Woods 2002). For classifying stained and unstained areas and quantifying the dye coverage the images were afterwards transferred to the red channel which shows the highest brilliant blue reflectance. From the histogram the flow cross sectional areas were calculated for each layer.

3.3 Results and discussion

3.3.1 Batch sorption experiment

The sorption isotherms were well described by the Freundlich equation (Fig. 3.2) with \(K_f = 5.1 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}\) and \(n = 0.84 \ (r^2 = 0.99)\) for the coarse sand, \(K_f = 6.2 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}\) and \(n = 0.86 \ (r^2 = 0.98)\) for the seam material and \(K_f = 70 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}\) and \(n = 1.0 \ (r^2 = 0.98)\) for the loamy sand, respectively. The \(K_f\) value for the coarse sand ranges at the lower end of the values mentioned in the literature (Hance 1976; Giesy et al. 2000; Vereecken 2005). The higher sorption capacity of the loamy sand can be explained with an increased CEC, a higher content of dithionite extractable Fe and a higher clay content (Vereecken 2005; Borggaard and Gimsing 2008). The higher sorption capacity of the seam material compared to that of the coarse sand can also be explained therewith.

The retardation factor \(R\), which describes the retardation of glyphosate transport compared to water transport in the soil, was calculated for \(\rho = 1.7 \text{ g cm}^{-3}\) and two concentrations, \(C_w = 0.02 \text{ mg L}^{-1} (R_{0.02})\) and 2 mg L\(^{-1}\) (\(R_2\)), which were derived from the minimum and maximum concentrations of the batch experiments and cover leachate concentrations measured during the lysimeter experiment. The water content was chosen according to the water contents at approximately field capacity of the lysimeters determined at the end of the lysimeters experiments. For the coarse sand \(R_{0.02} = 86.7\) and \(R_2 = 41.4 \ (\theta = 0.16 \text{ m}^3 \text{ m}^{-3})\), for the seam material \(R_{0.02} = 174.4\) and \(R_2 = 92.0 \ (\theta = 0.09 \text{ m}^3 \text{ m}^{-3})\) and for the loamy sand \(R_{0.02} = 35.2\) and \(R_2 = 445.4 \ (\theta = 0.27 \text{ m}^3 \text{ m}^{-3})\). As the sorption isotherms were non-linear due to the increasing saturation of sorption sites in the coarse sand and the seam material with low
contents of Al and Fe oxides (Borggaard and Gimsing 2008), the retardation factor $R$ decreased with increasing glyphosate concentrations. This indicates that higher glyphosate concentrations in the coarse sand result in higher leachate concentrations. As construction guidelines for pavements and streets specify the use of coarse sand and coarse gravel which have low $K_f$ values for glyphosate, these areas are more prone to glyphosate leaching than agricultural sites. But due to the accumulation of urban deposition the sorption capacity of the upper material between the pavement stones increases, which reduces the leaching risk for glyphosate with time (chapter II & (Nehls et al. 2008)).

![Fig. 3.2: Freundlich sorption isotherms for glyphosate and the different filling materials (● loamy sand, ▼ coarse sand, ○ seam material). (Means of 3 repetitions).](image)

The means of the pH of the supernatants in the batch experiment was 7.4 (7.0 – 7.7) for the sand, 7.1 (7.0 – 7.2) for the seam material and 7.3 (7.1 – 7.5) for the loamy sand. Comparing these pH values with the ones measured in the leachate samples during the field experiment (L: mean 7.7 (7.4 – 8.5); S50: mean 7.5 (7.3 – 8.1); S95: mean 7.6 (7.2 – 8.2)) a lower sorption coefficient in the field experiment would be expected as the sorption of glyphosate decreases with increasing pH (McConnell and Hossner 1985; de Jonge and de Jonge 1999; Gimsing et al. 2004a). Furthermore, the contact time of glyphosate with the soil differed clearly between batch and field experiments. As equilibrium conditions are rarely reached in the field, shorter contact times and therefore lower sorption coefficients during field experiments can be expected (Beltran et al. 1998).
3.3.2 Lysimeter experiment

Background concentrations of glyphosate in the leachate measured three days before application were < 1 µg L\(^{-1}\) for lysimeter L and S50 and 2 µg L\(^{-1}\) for lysimeter S95, respectively. Background concentrations of AMPA ranged from 11 µg L\(^{-1}\) (L) over 24 µg L\(^{-1}\) (S50) to 56 µg L\(^{-1}\) (S95). The fact that both, glyphosate and AMPA, were detectable in the leachate before the start of the experiment and at the end of the experiment (glyphosate up to 2.4 µg L\(^{-1}\); AMPA up to 88 µg L\(^{-1}\)), showed that the mineralization of glyphosate in this technical substrate was not complete within one year after application under field conditions (Feng and Thompson 1990; Laitinen et al. 2006). As the measured values are leachate concentrations resulting from desorption processes in the soil, it indicates that accumulation of glyphosate and especially AMPA in soil can not be ruled out. Earlier findings of slower degradation rates for AMPA compared to glyphosate and field degradation half-lives between 76 to 240 days support this hypothesis (Giesy et al. 2000). Thus, permission for more than one glyphosate application within a year in urban areas should be reconsidered.

The applied amounts of glyphosate (Tab. 3.3) are clearly lower than the permitted amount of 3.6 kg ha\(^{-1}\) on non arable land and at the lower end of agriculturally used amounts (0.7 to 3.6 kg ha\(^{-1}\)). They correlated with the weed cover on the lysimeters. The least weed cover caused the least amount of glyphosate applied with the Rotofix.

The cumulative outflow during the experiment was 76 mm for lysimeter L, 167 mm for lysimeter S50 and 153 mm for lysimeter S95. The lower leachate volume for lysimeter L can be attributed to a higher evapotranspiration rate of this lysimeter whose surface area was unsealed and had a field capacity of 19.7 Vol % compared to 5.5 Vol % of the other lysimeters (Tab. 3.1).

The determined water contents varied between the layers with increasing values in the deeper layers, with the exception of the upper layer of lysimeter L. The pore volumes of the lysimeters calculated on the basis of the determined water contents were 23.2 mm for L, 10.4 mm for S50 and 10.7 mm for S95. Including only the flowed through areas (s. below) the active pore volume for L is 7 mm, for S50 7.1 mm and for S95 6.4 mm.

3.3.2.1 Brilliant Blue tracer experiment

Based on the Brilliant Blue experiments flow paths in the lysimeters were investigated. For all three lysimeters preferential flow paths were clearly visible (e.g. S95: Fig. 3.3). For the partly sealed lysimeters S50 and S95 the contraction of the flow paths, caused by the surface cover, was still visible in 11 cm depth. No increased flow at the border of the lysimeters was observed. In 11 cm depth about 55% of both lysimeters participated in flow processes. For the unsealed lysimeter L preferential flow paths were also visible. In 11 cm depth only 30% of
the area was flowed through. The preferential flow on lysimeter L can be attributed to its uneven surface after the usage of the roller wiper. The wheels of the machine left channels behind which caused ponding during irrigation and reduced the flow cross sections.

![Image](97x585 to 498x697)

Fig. 3.3: Flow paths under pavement flagstones (lysimeter S95) (5 cm depth) marked with dye.

### 3.3.2.2 Leaching of bromide

The non-reactive bromide tracer was applied with irrigation water to get information about the travel time of water within the lysimeters. Bromide was detected in the first leachate samples. The maximum concentrations ranged from 68 mg L\(^{-1}\) (L) over 98 mg L\(^{-1}\) (S95) to 110 mg L\(^{-1}\) (S50) (Fig. 3.4). For lysimeter S50 and S95 the bromide breakthrough curve was characterized by a prepeak on day one at 6 mm (S50) and 7 mm (S95) outflow, respectively, followed by the main peak of bromide on day six after 26 mm (S50) and 19 mm (S95) outflow, respectively. The bromide prepeak of lysimeters S50 and S95 appeared almost simultaneously with the first glyphosate peak (S50: 9 mm; S95: 12 mm) and was attributed to the transport of bromide with preferential flow. The main peak of bromide was caused by the translocation via matrix flow. The calculated active pore volume based on the mean of the gravimetric water contents of the different layers at the end of the experiment (n = 10) were with 7.1 mm (S50) and 6.4 mm (S95) clearly lower than the pore volume derived from the appearance of the bromide peak. This might be attributed to the geometry of the lysimeters. Due to the three-dimensional flux within the lysimeters (1.4 m width and 5 m length) the distances to the outflows are prolonged compared to an one-dimensional flux assumed for pore volume calculations.

At lysimeter L no prepeak was found. The maximum concentration was measured on day 7 after 10 mm outflow. As the bromide peak concentration appeared simultaneously with the first glyphosate peak (10 mm), which was attributed to preferential flow, it might have been caused by preferential flow as well. The lack of a splitted bromide peak for lysimeter L might have been due to an insufficient sampling resolution, resulting in an overlapping of preferential and matrix flow. This might have caused the extensive tailing of the peak and might be an indication for sorption of bromide in the loamy sand or heterogeneous flow velocities as shown in the dye tracer experiment. The bromide concentrations in the leachate were below 5 mg L\(^{-1}\) after 50 mm cumulative outflow from all three lysimeters.
3.3.2.3 Leaching of glyphosate and AMPA

For all three lysimeters a rapid breakthrough of glyphosate and AMPA was observed. For lysimeters S50 and S95 glyphosate was detected in the first effluent samples. In Fig. 3.4 the development of the leaching concentrations of glyphosate, AMPA and bromide in the leachate samples are shown as a function of the cumulative outflow.

Fig. 3.4: Concentrations of glyphosate (●), the degradation product AMPA (▲) and bromide (○) in the drainage water of lysimeter L (top), lysimeter S50 (middle) and lysimeter S95 (bottom) as a function of the cumulative outflow.
Whereas in Fig. 3.5 the relative concentration \( (C/C_0)^{-1} \) of the active ingredient equivalent in the leachate is shown as a function of time. All three lysimeters (L, S50, S95) show similar leaching characteristics: A narrow glyphosate peak with the maximum concentration appeared simultaneously with the prepeak of the non-reactive tracer bromide (\( L = 10 \) mm; \( S50 = 9 \) mm; \( S95 = 12 \) mm) (Fig. 3.4). This was followed by a second, much smaller glyphosate peak, which is also visible for the active ingredient equivalent (glyphosate + AMPA) concentrations (\( L = 16 \) mm; \( S50 = 34 \) mm; \( S95 = 29 \) mm) (Fig. 3.5). Following a third peak of the active ingredient equivalent appeared in the leachate from lysimeters S50 and S95 (\( S50 = 53 \) mm, 31/08/06; \( S95 = 53.2 \) mm, 04/09/06) (Fig. 3.4 & Fig. 3.5).

![Fig. 3.5: Precipitation (right axis) together with relative concentrations of the active ingredient equivalents (Glyphosate + AMPA) (left axis) in the drainage water as a function of time (∙ lysimeter L; ▲ lysimeter S50; ● lysimeter S95). Precipitation measured at the meteorological station Berlin-Dahlem (Freie Universität Berlin) in 500 m distance.](image)

The detection of the maximum glyphosate concentrations in the leachate samples collected during the day of the artificial irrigation indicates that glyphosate was leached by preferential flow in all three lysimeters, which was already observed elsewhere (Veiga et al. 2001; Kjaer et al. 2005; Landry et al. 2005). The bromide and Brilliant Blue tracer experiments supported this: The simultaneous occurrence of the prepeak of the conservative tracer bromide and the peak concentration of the reactive solute, glyphosate, in the leachate samples of all three lysimeters proves the existence of preferential flow (Fig. 3.4). Besides the Brilliant Blue tracer experiment showed that only 55% (S50, S95) and 30% (L) of the total matrix participated in flow processes (Fig. 3.3). The partly cover of the lysimeter surfaces caused a contraction of the flow paths as it was already observed by Nehls (2007) under partly sealed pavements and streets. This contracted flow cross section resulted in higher flow velocities
which caused non-equilibrium and fast transport of glyphosate and AMPA into lower layers. As a result of the high flow velocities, the contact time between dissolved glyphosate and the soil matrix was short. The solute bypassed slow-sorption sites without interaction and interacted with fast-sorption sites without reaching chemical equilibrium (Wagenet and Chen 1998). Colloid-facilitated transport (McCarthy and Zachara 1989; de Jonge et al. 2000) is another possible explanation for the fast translocation of glyphosate but no data are available thereto. Translocation in macropores is unlikely as the observed flow cross sections would be smaller and no earthworm holes were found in the lysimeters.

The second much smaller glyphosate peak (Fig. 3.4 & Fig. 3.5) observed in the leachate samples from all three lysimeters might be attributed to the rainfall event of 3.8 mm within 30 min on 22/08/06, as the peak concentrations were measured in the following leachate samples on all three lysimeters. This rainfall event might have caused preferential flow like the irrigation at the beginning of the experiment.

The third peak of the active ingredient equivalent concentrations (glyphosate + AMPA) (Fig. 3.5) in the leachate of lysimeter S50 and S95 after 53 mm can not be related to weather conditions. It might indicates solute transport with matrix flow. The fact that for lysimeter L no peak is observable supports this explanation as this lysimeter is covered with loamy sand with a higher sorption capacity ($K_f = 70 \text{ mg}\cdot\text{L}^n\cdot\text{kg}^{-1}$) compared to the coarse sand of lysimeters S50 and S95 ($K_f = 5.1 \text{ mg}\cdot\text{L}^n\cdot\text{kg}^{-1}$). The estimated breakthrough of glyphosate calculated with the retardation factors $R_{0.02}$ and $R_2$ for the coarse sand derived from the batch sorption experiments (3.3.1) and the estimated pore volumes (3.3.2) for lysimeter S50 and S95 would be after 430 mm ($R_{0.02}$) or 900 mm ($R_2$) and for lysimeter L after more than 10,000 mm leachate. Due to a more complex setup of the lysimeter experiments, including climatic factors, and not comparable soil solution ratios and soil solute contact times, the results of the batch experiments can not be directly transferred to the conditions of the lysimeter experiments. Non-equilibrium conditions in the lysimeter experiments should lead to faster solute breakthrough (Strange-Hansen et al. 2004) and could be the reason for the breakthrough after only 53 mm leachate of S50 and S95. Leaching of glyphosate and AMPA with the matrix flow was observed in column experiments with gravely materials (Strange-Hansen et al. 2004). Whereas de Jonge et al. (2000) reported low leaching rates with matrix flow from unstructured sandy soil and high leaching rates from a structured sandy loam soil caused by macropore transport. Kjar et al. (2005) observed no glyphosate leaching in unstructured sandy soils in a field experiment. Latter results indicate that in deeper layers (> 1 m) of unstructured sandy soils no glyphosate transport with matrix flow occurred. Nevertheless, the main subsurface leaching of glyphosate under partly sealed pavements is governed by preferential flow, which was also observed in deeper layers (Nehls 2007). Therefore leaching from partly sealed areas can not be ruled out.
For lysimeter S50 and S95 a decrease of AMPA concentrations in the leachate samples was observed after a long dry period of almost two months (day 27 – 76) (Fig. 3.4 & Fig. 3.5). Between the sampling events number 20 and 21, 48 days passed without any leachate production. Thereafter the AMPA concentrations increased again. Due to the long dryness the sorbed contaminants were immobilized. The following rainfall diluted the small amount of interstitial water contaminated with AMPA. Narrow flow cross sections led to high flow velocities resulting in short contact times for water and soil, and in limited desorption potential.

### 3.3.2.3.1 Maximal concentrations and leached amounts

In contrast to the similar leaching patterns, the total amounts leached and the concentrations of glyphosate and AMPA in the leachate differed clearly between the lysimeters (Tab. 3.4).

<table>
<thead>
<tr>
<th>Lysimeter</th>
<th>Maximum concentration in leachate [µg L⁻¹]</th>
<th>Glyphosate leached in % of amount applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glyphosate</td>
<td>AMPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>81.8</td>
<td>25.7</td>
</tr>
<tr>
<td>S50</td>
<td>79.8</td>
<td>145.4</td>
</tr>
<tr>
<td>S95</td>
<td>1184.3</td>
<td>332.8</td>
</tr>
</tbody>
</table>

From lysimeter L 0.3% of the amount applied was leached in the form of glyphosate during the experiment. The maximum glyphosate concentration was 81.8 µg L⁻¹, compared to a maximum concentration of 1184.3 µg L⁻¹ from lysimeter S95. From lysimeter S95 15.3% of the amount applied was leached in the form of glyphosate and 57.7% was leached as active ingredient equivalent in total. The significantly different amounts of glyphosate and active ingredient equivalent leached from the lysimeters were caused by the different surface covers of the lysimeters: Lysimeter L was unsealed and covered with a 7 cm layer of loamy sand. Its $K_f$ was 14-fold higher than the $K_f$ of the coarse sand of lysimeters S50 and S95. Due to the higher sorption capacity the amounts leached via matrix flux from lysimeter L were much smaller than those from lysimeters S50 and S95. Nevertheless, the amount of glyphosate discharged via preferential flow from lysimeter L was with 0.3% of the glyphosate applied similar to that from lysimeter S50. This can be explained by similar active pore volumes and the lack of sorption due to the short contact times with the soil matrix. Even though only 30% of lysimeter L were flowed through, the higher pore volume of lysimeter L yielded the same active pore volume as for lysimeter S50 (7 mm). For lysimeter S95 the 6-fold amount of active ingredient equivalent compared to the leachate amount from the similarly partly sealed lysimeter S50, was leached. The peak concentration of glyphosate was twice as high as that...
measured at S50 (Tab. 3.4) and 11.2% of the glyphosate applied was leached out in the first flush. This can partly be explained by the different kind of sealing: While 95% of the surface of lysimeter S95 was sealed, only 50% of lysimeter S50 was covered. This resulted in a significantly smaller flow cross section in the upper layers of lysimeter S95 and therewith in higher flow velocities and a faster translocation of glyphosate. The flowed through pore volume was with 6.4 mm smaller than that of lysimeter S50 (7.1 mm). Further possible factors responsible for the high glyphosate concentrations in the leachate of lysimeter S95 are unknown. Preferential flow along the aluminium borders of the lysimeters was not observed during the dye tracer experiment and is unlikely as the edges were overgrown with weeds and moss. Schmidt and Boas (2006) reported similarly higher glyphosate concentrations in the leachate of lysimeter S95 compared to the other two lysimeters.

### 3.3.2.3.2 Transformation of glyphosate to AMPA

The development of glyphosate and AMPA concentrations in the leachate samples from lysimeter S95 were well fitted with two first-order equations (Fig. 3.6): $y = -0.104x$ ($r^2 = 0.92$) for the first 27 days and $y = -0.011x - 2.4$ ($r^2 = 0.73$) until day 140.

![Fig. 3.6: Split transformation rate $k$ (0 – 27 d = 0.104; 28 – 140 d = 0.011) for the transformation of glyphosate into the degradation product AMPA over time in the leachate of lysimeter S95.](image)

Within the first 27 days the glyphosate concentrations halved after 6.7 days ($k = 0.104$). Thereafter the glyphosate concentrations reduced much slower and halved within 63 days ($k = 0.011$). An initially rapid degradation of glyphosate in soil, followed by a slower degradation rate was found in several experiments (Rueppel et al. 1977; Eberbach 1998; Strange-Hansen et al. 2004; Landry et al. 2005). These findings were attributed to the initial degradation of soil-solution glyphosate, followed by a slower degradation rate due to rate-limited desorption of glyphosate sorbed to soil. The observed development of the ratio of glyphosate and AMPA concentrations in the leachate from our three lysimeters agree with
these findings. In our case the long dry period between day 27 and day 76 enhanced the immobilisation of glyphosate and therewith the slow down of the glyphosate transformation. In accordance with Eberbach (1998) two first order equations were used to fit the decay rates and leachate concentrations of glyphosate and AMPA, respectively. Even though no degradation experiments were conducted, this study shows that glyphosate degradation slows down with time and that especially AMPA can still be found in soil and leachate after one year.

### 3.4 Conclusions

Unexpected high amounts of glyphosate and AMPA were detected in the leachate of the lysimeters after the application of Roundup Ultra with a Rotofix apparatus. Even though our experimental conditions enhanced natural percolation rates by preventing the occurrence of runoff and restricting the leaching depth, our results suggest that the use of glyphosate in urban areas should be challenged and further experiments concerning the leaching and runoff of glyphosate in urban areas should be conducted.

If the use of glyphosate is necessary it should be limited to special conditions, considering the following points: Due to the measured background concentrations of AMPA, the application of glyphosate should be limited to once a year. The coarse sand and gravel normally used for pavement construction are likely to have low $K_f$ for glyphosate. Therefore, the application of glyphosate on partly sealed urban areas should be differentiated according to construction age of the partly sealed areas, the geological parent material and the percentage of seam material as leaching volumes from areas with a low percentage of seam material, like lysimeter S95 (5% seam material), seem to be higher.

This study was limited to the leaching of glyphosate through soil on partly sealed areas. However, the transport of glyphosate with runoff is a hydrological bypass due to the missing retention in soils. In this case contaminated water is routed directly into sewage systems or surface waters. This might be the more crucial process regarding the contamination of surface waters with glyphosate.

As methods for non-chemical weed control (mechanical and thermal methods) and combinations of chemical with non-chemical methods are well tested (Hansen et al. 2004; Kempenaar and Spijker 2004; Kempenaar et al. 2007; Rask and Kristoffersen 2007), chemical methods should be substituted and reduced in urban areas as much as possible.
4 Sorption and leaching of glyphosate in pavement material – a column study

4.1 Introduction

The non-selective, post-emergence herbicide glyphosate [N-(phosphonomethyl)glycine], the active ingredient in commercial products like Roundup, is the most widely used herbicide worldwide: It is used for weed control in agriculture, horticulture, forestry, aquaculture and urban areas. The use of glyphosate rapidly increased within the last ten years due to the introduction of genetically modified glyphosate-resistant crops in the USA and South America (Kiely et al. 2004; Scribner et al. 2007) raising concerns regarding its fate in the terrestrial and aquatic environment. Despite its extensive use, however, glyphosate and its degradation product AMPA (amino-methylphosphonic acid) are not common substances in routine water quality monitoring programmes, because their analytical detection at environmental trace levels is difficult.

Due to glyphosate’s distinct tendency to adsorb to soil (mainly caused by aluminium and iron oxides and low pH-values), its fast microbial degradation and its low toxicity to non-target organisms, the risk of surface or ground water contamination with glyphosate is generally assumed to be low (de Jonge et al. 2001; Vereecken 2005). Nevertheless, recent studies demonstrated that glyphosate sorption and degradation in soil are very variable and depend on soil properties like pH, iron and aluminium oxides, soil structure, application time, and microbial activity (de Jonge et al. 2001; Borggaard and Gimsing 2008). Furthermore, leaching of glyphosate and its degradation product AMPA has been observed up to depths of 1 m in field studies demonstrating that it represents a potential risk for the aquatic environment (Veiga et al. 2001; Kjaer et al. 2005; Landry et al. 2005; Stadlbauer and Fank 2005). Glyphosate transport via preferential flow was reported in several studies under outdoor and laboratory conditions, whereas the transport with matrix flow in unstructured sandy soils was rarely observed (de Jonge et al. 2000; Veiga et al. 2001; Kjaer et al. 2005; Landry et al. 2005). In addition, field studies showed that residue levels of glyphosate and AMPA can persist in soil for more than a year (Laitinen et al. 2006; Scribner et al. 2007) which can lead to chronic exposure of terrestrial organisms.
Moreover, glyphosate and AMPA have been detected in surface water resources worldwide in considerable concentrations, with peak concentrations of more than 300 µg L\(^{-1}\) (Feng et al. 1990; Newton et al. 1994; Skark et al. 1998; Kolpin et al. 2006; Scribner et al. 2007; Byer et al. 2008; Peruzzo et al. 2008). Recently ecotoxicological effects, e.g. oxidative stress, of glyphosate and its formulations on non-target organisms were shown from sublethal concentrations of only 100 µg L\(^{-1}\) after exposure for four days (Glusczak et al. 2007; Contardo-Jara et al. 2009). In the Netherlands the maximum permissible concentration (MPC) in surface waters for glyphosate is 77 µg L\(^{-1}\) and for its degradation product AMPA 79.7 µg L\(^{-1}\) (Traas and Smit 2003; Kempenaar et al. 2007).

Due to its non-selectivity and its cost-effectiveness glyphosate is as well used for weed control on roadsides, pavements, courtyards or railways where weeds are unwanted for safety or aesthetic reasons. Several studies showed that glyphosate use in urban areas contributes to glyphosate and AMPA concentrations in surface waters (Skark et al. 2004; Kolpin et al. 2006; Byer et al. 2008). Although the amounts of glyphosate used in urban areas are clearly lower than those used in agriculture, its environmental impact might be different and thus needs special consideration. The materials of urban surfaces, like asphalt or paving stones, are less sorptive than topsoils and almost impervious. The material between and under the paving stones should assure a high hydraulic conductivity of the pavement bed to prevent damages of the pavement system, e.g. by flooding or frost. It consists of coarse sand or gravel with low contents of clay, iron and aluminium oxides, and generally has a high pH. Therefore it is likely that this material has a low sorption capacity for glyphosate and leaching of glyphosate into deeper layers might occur. Studies along railways, on asphalt surfaces and with gravel materials used for roads etc. found a higher mobility of glyphosate (Borjesson and Torstensson 2000; Strange-Hansen et al. 2004; Spanoghe et al. 2005; Torstensson et al. 2005).

The aim of this study was to examine whether leaching of glyphosate via matrix flow can occur in materials with low sorption capacity used for the construction of pavements. By investigating the sorption and transport in pavement material for the first time, this study contributes to close a knowledge gap regarding the fate of glyphosate after application to pavements in urban areas.
4.2 Materials and Methods

4.2.1 Chemicals

Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) contains the monoisopropylamine salt of glyphosate (N-(phosphonomethyl)-glycine) (360 g L\(^{-1}\)) and surfactants of undisclosed chemical composition and concentration. As stock solution a 1% solution was prepared with deionized water (3.8 g L\(^{-1}\)). \(^{14}\)C-radiolabelled glyphosate ([phosphonomethylene-\(^{14}\)C]glyphosate) was obtained from Monsanto (St. Louis, MO, USA) with 98.8% radiochemical and 99% chemical purity dissolved in deionized water. Its specific radioactivity was 1.71 GBq mmol\(^{-1}\). Stock solutions for batch equilibrium experiments were prepared by mixing \(^{14}\)C-labelled glyphosate and Roundup Ultra solution. The application solution (487.8 µg ml\(^{-1}\) glyphosate; 79.7 KBq ml\(^{-1}\)) for the column experiment was prepared by mixing \(^{14}\)C-labelled glyphosate solution, Roundup Ultra solution and deionized water.

A potassium bromide solution with 1 g L\(^{-1}\) bromide was prepared. The columns were irrigated with a 0.01 M CaCl\(_2\) solution mimicking the electrolytic strength of the soil solution.

4.2.2 Soil Properties

The soil used for the filling of the columns was collected at two different locations. For columns L1 and L2 coarse sand was taken from lysimeters used for experiments with glyphosate under partly sealed conditions (chapter III). For columns P1 and P2 the soil was taken from a partly sealed pavement in Berlin (OS). The soil between the cobblestones, the seam material, was sampled in two layers (0 \(-\) 1 cm and 1 \(-\) 5 cm). The physico-chemical soil properties (Tab. 4.1) were determined using standard methods.
Tab. 4.1: Physico-chemical characteristics of the column fill material.

<table>
<thead>
<tr>
<th></th>
<th>Column P</th>
<th></th>
<th>Column L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 – 1 cm</td>
<td>1 – 11 cm</td>
<td></td>
</tr>
<tr>
<td>Coarse Materials</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>(&gt; 2mm) [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand [%]</td>
<td>87.8</td>
<td>95.4</td>
<td>99.1</td>
</tr>
<tr>
<td>Silt [%]</td>
<td>10</td>
<td>3.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Clay [%]</td>
<td>2.2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>pH (CaCl$_2$)</td>
<td>6.7</td>
<td>6.7</td>
<td>6.5</td>
</tr>
<tr>
<td>C$_{org}$ [g kg$^{-1}$]</td>
<td>12.1</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>CEC$_{eff}$ [cmol kg$^{-1}$]</td>
<td>3.8</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Fe$_{dit}$ [g kg$^{-1}$]</td>
<td>4.4</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Fe$_{ox}$ [g kg$^{-1}$]</td>
<td>2.8</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Al$_{dit}$ [g kg$^{-1}$]</td>
<td>0.6</td>
<td>0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Al$_{ox}$ [g kg$^{-1}$]</td>
<td>0.8</td>
<td>0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>$K_f$ [mg$^{1-n}$ L$^n$ kg$^{-1}$]</td>
<td>26.3</td>
<td>9.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

CEC$_{eff}$ : effective cation exchange capacity; Fe$_{dit}$ and Al$_{dit}$: dithionite extractable Fe and Al; Fe$_{ox}$ and Al$_{ox}$: oxalate extractable Fe and Al; $K_f$: Freundlich sorption coefficient.

4.2.3 Batch sorption experiments

Adsorption isotherms for glyphosate were determined using batch experiments according to OECD guideline 106 (2000) with $^{14}$C-labelled Roundup solution. The mass ratios of glyphosate in soil were 0.2, 2, 5 and 20 mg kg$^{-1}$ with a soil/solution ratio of 1:10 and an equilibrium time of 24 h.

24 hours before the start of the sorption experiment 2 g of dry soil were mixed with 19 ml of 0.01 M CaCl$_2$ solution to pre-equilibrate the soils and placed on an overhead shaker. Following 1 ml of $^{14}$C-labelled Roundup solution was added and samples were shaken overhead for 24 hours at 20 rpm (revolutions per minute). After centrifugation for 25 minutes at 2000 RCF (relative centrifugal force), 2 ml of the supernatant were added to 10 ml of liquid scintillation cocktail (Luma Safe Plus, Lumac-LSC, Groningen, The Netherlands) and measured by liquid scintillation counting (LSC, LS 6500, Beckman, Fullerton, CA, USA). The amount adsorbed was determined by subtracting the solution concentration from the initial concentration. Concentrations of glyphosate are reported as equivalent of parent compound calculated from the counted radioactivity. The measured radioactivity was corrected for background and quench by external standards. The limit of quantification of the LSC method was 0.8 Bq per sample. For each concentration three replicates were prepared. All experiments were carried out at 20ºC (+/- 2).
4.2.4 Column experiment

4.2.4.1 Experimental Set-Up

The columns were made up of stainless steel with a height of 300 mm and an inner diameter of 200 mm. On the bottom they were attached to a porous glass sinter disc (nominal pore size 10-16 µm, air-entry point < -1000 cm; VitraPOR, ROBU Glasfilter-Gerate GmbH, Hattert, Germany) (Fig. 4.1). The outlet was connected to a teflon tube (inner diameter 3 mm) which was attached to a fraction collector 30 cm below the glass sinter disc, resulting in a constant suction of -30 cm. On top of the column an irrigation head with 70 needles (0.45 mm in diameter) was placed, which was connected through a peristaltic pump (IPN-8, Ismatec Sa, Glatthbrugg, Switzerland) to a reservoir with 0.01 M CaCl\textsubscript{2} solution.

Columns L1 and L2 were filled with wet coarse sand (8.5% gravimetric water content) which was also used in lysimeter experiments with partly sealed surfaces (chapter III). The sand was compacted in 2 cm and 1 cm layers to a bulk density of 1.5 g cm\textsuperscript{-3} and filled into the columns up to a height of 11 cm. Columns P1 and P2 were filled in 2 cm layers with pavement sand from the 1-5 cm layer (11% gravimetric water content) up to a height of 10 cm. On top, a 1 cm layer of sand from the 0-1 cm seam material with 8.5% gravimetric water content was placed. All layers were compacted to a bulk density of 1.5 g cm\textsuperscript{-3}.

Fig. 4.1: Schematic drawing of column set-up.
The irrigation of the columns was regulated by two peristaltic pumps, resulting in two slightly different irrigation rates. The pump reservoirs were weighed daily and the solution loss calculated for every irrigation event.

The columns were stored at 10°C to reduce microbial degradation and evaporation from the column surface.

4.2.4.2 Application and sampling

The columns were irrigated every two hours for one minute with 6.9 ml (L1 & L2) and 6.5 ml (P1 & P2), respectively, resulting in a constant rate of approximately $0.26 \pm 0.1 \text{ cm d}^{-1}$ (L1 & L2) and $0.24 \pm 0.1 \text{ cm d}^{-1}$ (P1 & P2) (Tab. 4.2). No ponding occurred. They were preconditioned for 26 days before the application of the $^{14}$C-Roundup Ultra solution to establish steady-state hydrodynamic conditions, resulting in a constant outflow. The irrigation rate ($0.25 \text{ cm d}^{-1}$; 912 mm a$^{-1}$) was adapted to the infiltration rate of 300 mm a$^{-1}$ on partly sealed urban areas in the Berlin region (Wessolek and Facklam 1997). It was taken in triplet, as on partly sealed pavements at least 70% of the surface is sealed and less than 30% consists of seam material. As the columns did not include any surface cover, at least three times more water can infiltrate on that area through the seam material. Using those realistic irrigation rates, transport effects caused by unnatural high infiltration rates were excluded.

The potassium bromide (7.6 mg column$^{-1}$) and the $^{14}$C-Roundup Ultra solution (5.5 mg glyphosate per column (1.75 kg ha$^{-1}$)) were applied to each column surface with a pipette and a template with 37 holes. The Roundup Ultra application rate was chosen according to realistic values: The applied amount of glyphosate on each column ranges with 5 L ha$^{-1}$ Roundup Ultra in the middle of the recommended application rates for non arable and farmland (2 to 10 L ha$^{-1}$). After the application a 0.5 cm layer of fine gravel, which was glowed at 550°C for 24 h to remove possible organic contamination, was placed on top of all columns to ensure a uniform distribution of the irrigation water. Three hours later the irrigation was continued.

The leachate of the columns was collected in 6 h intervals during the first 15 days and subsequently in 24 h intervals with fraction collectors. Each sample was analysed for bromide, $^{14}$C-activity and pH. Radioactivity was determined by liquid scintillation counting as in the batch experiments. Concentrations of glyphosate are reported as equivalent of parent compound calculated from the counted radioactivity, which includes glyphosate itself and its degradation products. Bromide concentrations were measured by HPLC (Agilent HP 1100, CA, USA; column UltraSep ES RP NN 150x3mm, Sepserv, Berlin, Germany) with UV detector. Measured concentrations were corrected for evaporation losses ($0.6 \times 10^{-4} \text{ L h}^{-1}$) during the storage in glass beakers in the fraction collector. After 117 and 118 days, respectively, the columns were divided in 1 cm layers (0.5 cm for first cm layer of columns P)
to determine the $^{14}$C-concentration profile and the gravimetric water content of the soil layers (24 h/overnight at 105°C). Subsequently soil samples were dried and ground to obtain homogeneous samples. Aliquots were combusted (at 900°C for 3 min) in an oxidizer (Ox 500, Zinsser, Frankfurt, Germany) and the evolved $^{14}$CO$_2$ was trapped in a scintillation cocktail (Oxysolve C-400, Zinsser, Frankfurt, Germany). Radioactivity was determined by liquid scintillation counting. Active pore volumes of the lysimeters were calculated using the volumetric water contents of each layer at the end of the experiment, including the pore volume of the porous glass sinter disc and the tube at the bottom of the column.

4.2.5 Parameter estimation

4.2.5.1 Batch experiments

The Freundlich sorption isotherm was fitted to the data points obtained by the batch experiments:

$$s = K_f \cdot C^n$$

(4.1)

where $s$ is the sorbed concentration [M M$^{-1}$], $K_f$ is the Freundlich sorption coefficient [M$^{1-n}$ L$^{3n}$ M$^{-1}$], $C$ is the concentration in the liquid phase after 24 hours [M L$^{-3}$] and $n$ is the Freundlich exponent [-].

4.2.5.2 Column experiments

It was assumed that the column experiments were conducted under steady-state conditions with a homogeneously water content distribution, as the columns were homogeneously packed and irrigated with a constant rate. The aim of the use of a transport model was to see, whether the observed transport behaviour of glyphosate and its degradation products in pavement material is in agreement with commonly regarded transport processes. Thus, we used $^{14}$C-activity of leachate samples for an effective transport description for the glyphosate equivalent.

First, physical transport parameters were determined by analyzing the bromide breakthrough curves (BTC). Following, the chemical transport processes were determined in the inverse mode, using the physical transport parameters fitted for the bromide BTC.
a) Conservative tracer bromide

For determining physical transport parameters \( v \) (pore water velocity) and \( \lambda \) (dispersivity) of the soil columns, the analytical solution of the convection-dispersion equation (CDE) for a pulse boundary condition at the upper and a zero gradient condition at the lower boundary was fitted to the experimental bromide BTC (Tab. 4.2). Thereto the CXTFIT code (Toride et al. 1999) was used in the inverse mode with the CDE for an inert tracer rewritten as:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z}
\]

(4.2)

where \( C \) is the liquid phase concentration \([\text{M L}^{-3}]\), \( t \) is time \([\text{T}]\), \( D \) is the dispersion coefficient \([\text{L}^2 \text{T}^{-1}]\), \( z \) is the depth \([\text{L}]\), \( v \) is the pore water velocity \([\text{L T}^{-1}]\).

The parameters \( v \) and \( D \) were estimated and the dispersivity \( \lambda \) (\( \lambda = D/v \)) and the volumetric water content \( \theta \) (\( \theta = j_w / v \) with \( j_w \) as irrigation rate) were calculated.

b) Glyphosate

The numerical one-dimensional transport model HYDRUS-1D (Simunek et al. 2008; Simunek and van Genuchten 2008) was used for estimating glyphosate transport parameters of the soil columns (Tab. 4.3). This numerical model can simulate the movement of water and solutes under equilibrium and nonequilibrium flow and transport conditions in soil. It numerically solves the Richards equation by finite element methods for variably-saturated water flow and advection-dispersion type equations for heat and solute transport.

The glyphosate transport was described with a two-site sorption model and a chemical equilibrium model based on equations (4.3-4.7). For the equilibrium model \( f_e = 1 \). The equilibrium model was used to merely predict the glyphosate breakthrough with the parameters derived from the tracer experiment and the \( K_f \) and \( n \) value derived from the batch experiments. Additionaly it was used in its inverse mode and \( K_f \) and \( n \) were fitted to the glyphosate equivalent BTC.

The two-site sorption concept (van Genuchten and Wagenet 1989) divides the sorption sites into two fractions:

\[
s = s^e + s^k
\]

(4.3)

Sorption on one fraction of the sorption sites is regarded as instantaneous \( (s^e \ [\text{M} \text{M}^{-1}]) \), whereas sorption on the remaining sites is time-dependent \( (s^k \ [\text{M} \text{M}^{-1}]) \). The two-site model is described by the following system of equations:
\[ \frac{\partial \theta C}{\partial t} + \rho \frac{\partial s^c}{\partial t} + \rho \frac{\partial s^k}{\partial t} = \frac{\partial}{\partial z} \left( \theta D \frac{\partial C}{\partial z} \right) - \frac{\partial \nu \theta C}{\partial z} - k \cdot C \] 

\[ s^c = f_e K_f \cdot C^n \]  

\[ \rho \frac{\partial s^k}{\partial t} = \alpha_k \rho (s^c_e - s^k) - k \cdot C \]  

\[ s^k = (1 - f_e) \cdot K_f \cdot C^n \]

where \( \rho \) is the soil bulk density [M L\(^{-3}\)], \( \theta \) is the water content [L\(^3\) L\(^{-3}\)], \( f_e \) is the fraction of exchange sites assumed to be in equilibrium with the liquid phase, \( \alpha_k \) is a first order sorption rate constant [T\(^{-1}\)], \( k \) is a first-order dissipation rate [T\(^{-1}\)]. The first-order dissipation rate \( k \) was included for the soil and the water phase in the model. It was derived from each column experiment with

\[ M_{a.i.e.} = M_{0,\text{gly}} \cdot e^{-kt} \]

where \( M_{a.i.e.} \) is the total amount of active ingredient equivalent in the soil and leachate [M], \( M_{0,\text{gly}} \) is the applied amount of glyphosate [M], \( k \) is the dissipation rate [T\(^{-1}\)] and \( t \) is the time [T].

Thus, four unknown parameters were estimated by fitting the curve to experimental data:

- \( \alpha \), the first-order adsorption rate coefficient [T\(^{-1}\)]
- \( f \), the fraction of adsorption sites with instantaneous sorption [-]
- \( K_f \), Freundlich sorption coefficient [M\(^{1-n}\) L\(^{3n}\) M\(^{-1}\)]
- \( \beta \), Freundlich exponent [-]

In order to estimate the above mentioned parameters a stationary water flow was simulated. As upper and lower water flow boundary conditions a constant pressure head was chosen. The matrix potential gradient within the columns was set to zero, resulting in a hydraulic gradient of one (gravity driven flow). Therefore constant water contents were assumed and the hydraulic conductivity was set equal to the irrigation rate \( j_w \) at the predominant water content. For the solute transport a flux concentration boundary for the top and zero concentration gradient at the bottom were set. The physical transport parameters \( \lambda \) (dispersivity) and \( \theta \) (water content) (Tab. 4.2) were taken from the analytical solution of the CDE for the tracer BTC with CXTFIT assuming same physical transport processes as for bromide.
4.3 Results and discussion

4.3.1 Batch experiments

The relatively short equilibrium period of 24 h was chosen because glyphosate degrades quickly (Nomura and Hilton 1977; Rueppel et al. 1977; Strange-Hansen et al. 2004), and in preliminary tests adsorption was essentially constant after 24 h. Within this time no formation of AMPA was observed.

The sorption isotherms from the batch experiments could be well described with the Freundlich equation (Fig. 4.2) with $K_f = 5.1 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$ and $n = 0.84$ ($r^2 = 0.99$) for the coarse sand, $K_f = 26.3 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$ and $n = 0.89$ ($r^2 = 0.99$) for the 0 – 1 cm layer of the pavement material and $K_f = 9.3 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}$ and $n = 0.76$ ($r^2 = 0.99$) for the 1 – 5 cm layer, respectively. The $K_f$ value for the coarse sand ranges at the lower end of the values mentioned in the literature (Hance 1976; Giesy et al. 2000; Vereecken 2005). The higher sorption capacity of the seam material and the higher $K_f$ of the 0 – 1 cm layer compared to the 1 – 5 cm layer, can be explained with a higher content of dithionite extractable Fe and Al, an increased CEC and a higher clay content (Vereecken 2005; Borggaard and Gimsing 2008) (Tab. 4.1) due to urban depositions like soot, dust and foliage (Nehls et al. 2008).

![Graph showing Freundlich sorption isotherms](image)

**Fig. 4.2:** Freundlich sorption isotherms for glyphosate and the different materials (● pavement material 0-1 cm, ○ pavement material 1-5 cm, ▼ lysimeter material coarse sand) (means of 3 repetitions with standard deviation) and isotherms simulated with two-site sorption model for coarse sand columns (L1 dotted line; L2 dashed line).
As the sorption isotherms were non-linear (n < 1), the percentage of sorbed glyphosate decreases with increasing glyphosate concentrations. Hence, the retardation of glyphosate is concentration dependent and higher glyphosate concentrations result in higher leachate concentrations. As construction guidelines for pavements and streets specify the use of coarse sand and coarse gravel which have low $K_f$ values for glyphosate, pavement systems are more prone to glyphosate leaching than agricultural sites.

### 4.3.2 Column experiments

#### 4.3.2.1 Mass balance for glyphosate equivalent

The recovery rates of the applied $^{14}$C-glyphosate for all columns were similar, despite their differences in leaching and sorption of glyphosate in soil (Fig. 4.3). Only 57.2 and 57.6% (L1 and L2) and 56.2 and 59.9% (P1 and P2), respectively, of the applied amount of radioactivity was found in the leachate and the soil. Since volatilization from the surface can be neglected due to the low vapour pressure of glyphosate of $9.3 \times 10^{-3}$ MPa (at 25°C) and non-extractable residues are included in mass balance by soil combustion, we assume that our loss is mainly attributed to glyphosate mineralisation to $^{14}$CO$_2$, which could not be measured in our experiment.

The loss of > 40% after 118 days is in accordance with mineralization rates mentioned in literature under laboratory and outdoor conditions. Landry et al. (2005) reported 43.5 - 46.5 % of mineralisation ($^{14}$CO$_2$) within 42 days in a lab experiment. Grundmann et al. (2008) were able to measure glyphosate mineralisation in a two-chamber-lysimeter-test-system under outdoor conditions and described mineralisation in soil as main elimination path. In their experiment more than 50% of the applied radioactivity was measured as $^{14}$CO$_2$ after 50 days. Other laboratory studies are reporting half lives of glyphosate of less than 100 days (Nomura and Hilton 1977; Cheah et al. 1998; Gimsing et al. 2004b; Borggaard and Gimsing 2008).

The dissipation rates of $^{14}$C-glyphosate calculated for the columns over the whole experiment were quite similar with 0.005 d$^{-1}$ (L1, L2, P1) and 0.004 d$^{-1}$ (P2) which corresponds to average half-life times (DT$_{50}$) of 148 days. This value was adopted in the transportation model as first order rate constant $k$ for the solid and dissolved phase (Tab. 4.2).
Fig. 4.3: Recovery (soil and leachate) and loss of applied $^{14}$C-activity [%] in column experiments.

### 4.3.2.2 Bromide breakthrough curves

With the application of the non-reactive tracer bromide information about the hydrodynamic properties of the columns were gained (Tab. 4.2). Breakthrough curves (BTC) for bromide are shown in Fig. 4.4. Bromide arrival was earlier and peak concentrations were higher in columns L1 and L2 than in columns P1 and P2. In the leachate of L1 and L2 bromide was first found on day two (4.2 mm and 3.2 mm) followed by a slightly asymmetric BTC with a higher peak concentration (L1: 24.0 mg L$^{-1}$ (8.8 mm) and L2: 20.4 mg L$^{-1}$ (9.6 mm)) than for columns P, where bromide was detected not until day four (10.7 mm) (P1: 13.5 mg L$^{-1}$ (19.4 mm) and P2: 15.3 mg L$^{-1}$ (19.1 mm)).

Fig. 4.4: Bromide concentrations in the leachate of the four columns as a function of cumulative outflow ($\downarrow$ column L1; $\triangledown$ column L2; $\bullet$ column P1; $\circ$ column P2).
The faster transport in columns L can be explained with higher pore water velocities or lower water contents (Tab. 4.2) due to coarser material compared to columns P which were filled with finer pavement material (Tab. 4.1) resulting in a higher pore volume. The active pore volumes calculated on the basis of leaching of 50% of the bromide mass (L1: 12.2 mm; L2: 12.6 mm; P1: 23.8 mm; P2: 22.5 mm) were in good accordance with the pore volumes calculated on the basis of the water contents (L1 & L2: 13.4 mm, P1: 22.3 mm, P2: 23.4 mm).

The BTC of all four columns could be well described with the physical equilibrium CDE with \( r^2 \) ranging from 0.98 to 0.99. Note that the non-equilibrium CDE (of the CXTFIT code) failed to describe the bromide transport and referred to the equilibrium model. Hence, it can be concluded, that physical equilibrium predominated in the columns and transport via matrix flow occurred. Although columns P were packed with two different materials, it was regarded as one effective layer for the modelling purpose with CXTFIT as BTC data were only obtained at the outflow. The attempt of modelling the bromide BTC with a two-layer system in HYDRUS-1D led to an over parameterised and thus ill-posed inverse problem.

4.3.2.3 Glyphosate equivalent breakthrough curves

In columns L a breakthrough of the glyphosate equivalent via matrix flow was observed after 42 mm (L1) and 35 mm (L2) cumulative outflow, whereas in columns P no breakthrough was observed within the 117 days of the experiment (Fig. 4.5). A later breakthrough for columns P compared to columns L was expected as the batch experiments (4.3.1) demonstrated a clearly higher sorption capacity of the upper pavement material (0 – 1 cm layer) for glyphosate due to the accumulation of urban deposition. Furthermore, a larger active pore volume of 22.3 mm (P1) and 23.4 mm (P2), compared to 13.4 mm for columns L contributes to a later breakthrough of the bromide (4.3.2.2) and the glyphosate equivalent.
In columns L peak concentrations of 516 µg L\(^{-1}\) (L1) and 419 µg L\(^{-1}\) (L2) were measured after 76 mm (L1) and 83 mm (L2) cumulative outflow (Fig. 4.5). Within the 117 days of the experiment no complete breakthrough of the glyphosate equivalent occurred. Until day 117, 35% (L1) and 34% (L2) off the applied glyphosate were transported via matrix flow and found in the leachate of the columns as glyphosate equivalent. On 78 days leachate concentrations of > 100 µg L\(^{-1}\) glyphosate equivalent were measured. The glyphosate equivalent BTC of columns L were clearly asymmetric and the peak maxima appeared more than 8 times later than the bromide peak maxima, indicating a reduced mobility of glyphosate
due to an increased sorption. The strong tailing of the glyphosate equivalent BTC is a well described characteristic for non-equilibrium sorption processes and was reported for various organic chemicals in repacked soil columns (Beigel and Di Pietro 1999; Dousset et al. 2007; Mueller and Duwig 2007). Besides, the increasing concentration of degradation products in the leachate during the experiment, which are included in the measured 14C-concentrations, may contribute to the pronounced tailing of the BTC. Differences between the replicates L1 and L2 in the shape of the BTC and the peak concentration are analogue to the pattern of the bromide BTC and thus can be attributed to differences in the pore structure.

The transport of the glyphosate equivalent with matrix flow, resulting in high concentrations in the leachate, which exceed the maximum permissible concentrations for surface waters in the Netherlands (< 100 µg L\(^{-1}\)) (Traas and Smit 2003; Kempenaar et al. 2007) and may have an ecotoxicological impact on aquatic and terrestrial non-target organisms (Glusczak et al. 2007; Contardo-Jara and Wiegand 2008), and the fact that more than a third of the applied glyphosate was found as glyphosate equivalent in the leachate, is surprising. Particularly because realistic irrigation rates of 0.25 cm d\(^{-1}\) excluded transport effects due to unnatural high infiltration rates. However, despite realistic infiltration rates the results are not 1:1 transferable to natural conditions as columns were short (11 cm) and under field conditions the natural soil under the pavement bed would have an increased sorption capacity and might prevent leaching of glyphosate. Nevertheless, sites with a high groundwater level might be at risk.

The transport of glyphosate with matrix flow in columns L confirms findings of an experiment with partly sealed lysimeters with the same material (chapter III). The transport of glyphosate with matrix flow in agricultural soils was rarely observed, but is in accordance with experiments with coarse gravel conducted by Strange-Hansen et al. (2004). Borggaard and Gimsing (2008) reasoned in their review that glyphosate leaching is limited in unstructured sandy soils but can not be excluded on coarse-textured soil material such as gravel. As construction guidelines for pavements and streets specify the use of coarse sand and coarse gravel which have low \(K_f\) values for glyphosate, pavement systems are more prone to glyphosate leaching than agricultural sites.

The depth distribution of the resident concentrations within columns P (Fig. 4.6) shows a retention of the glyphosate equivalent in the 0 – 1 cm layer, which consists of pavement material enriched with urban depositions (Tab. 4.1). 78.6% (P1) and 80.4% (P2), respectively, of the recovered radioactivity in the soil profile was found in the upper 0 – 1 cm layer. Besides, a concentration gradient within the 0 – 1 cm layer was observed, with higher concentrations in the upper part of this layer. This shows that the enrichment of the upper seam material with urban deposition causes a higher sorption capacity for glyphosate (Tab. 4.1) and can prevent or retard the leaching of glyphosate from these areas, acting like a “filter”.

4.3 RESULTS AND DISCUSSION
By contrast, the resident concentrations in columns L are clearly lower (Fig. 4.6), which was expected due to the high percentage or radioactivity found in the leachate and the lower sorption capacity. The uniform soil concentration distribution in columns L can be attributed to the leaching process of the glyphosate equivalent.

In all four columns a constant radioactivity outflow of 1.9 (±0.4) Bq ml\(^{-1}\) (0.01% of applied radioactivity per day) was measured in the leachate from day 4 onwards. This corresponds to a glyphosate concentration of about 10 µg L\(^{-1}\). In columns L it was superposed by the BTC of the glyphosate equivalent since day 14 (37 mm). In columns P it was observable until the end of the experiment (Fig. 4.5). This permanent steady outflow can not be explained with physical or chemical non-equilibrium transport of the solute as discussed above for the bromide and glyphosate BTC. Contamination during the handling process and contamination of the LSC can be excluded, as blanks were measured with every measurement. Our hypothesis is that the measured radioactivity is \(^{14}\text{CO}_2\) dissolved in the leachate. Similar radioactivity levels in the leachate during the first 10 days of all columns can then be attributed to similar mineralization rates as discussed above (4.3.2.1).
4.3 RESULTS AND DISCUSSION

4.3.2.4 Model parameter estimation

The numerical simulation with HYDRUS-1D showed that the transport behaviour of the glyphosate equivalent in coarse sand can be well described with a common transport model ($r^2 = 0.99$) (Tab. 4.3; Fig. 4.7). Both, equilibrium and non-equilibrium models as well as instantaneous, rate-limited, linear and non-linear models were tested for the solute transport.

Tab. 4.3: Fitting parameters for glyphosate BTC simulation using the two-site-sorption model for columns L and $K_f$ value from batch sorption experiment.

<table>
<thead>
<tr>
<th>Soil column</th>
<th>$K_f$ [mg$^{1-n}$ L$^n$ kg$^{-1}$]</th>
<th>$n$ [-]</th>
<th>$f_e$ [-]</th>
<th>$\alpha$ [d$^{-1}$]</th>
<th>RMSE</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>0.61</td>
<td>0.89</td>
<td>0.30</td>
<td>0.014</td>
<td>15.39</td>
<td>0.99</td>
</tr>
<tr>
<td>L2</td>
<td>1.03</td>
<td>0.93</td>
<td>0.33</td>
<td>0.013</td>
<td>10.34</td>
<td>0.99</td>
</tr>
<tr>
<td>Batch</td>
<td>5.1</td>
<td>0.84</td>
<td>0.33</td>
<td>0.013</td>
<td>0.59</td>
<td>0.99</td>
</tr>
</tbody>
</table>

RMSE: Root Mean Squared Error

Using the equilibrium model with the parameters derived from the batch experiments ($K_f = 5.1$ mg$^{1-n}$ L$^n$ kg$^{-1}$ and $n = 0.84$), led to a breakthrough of glyphosate after more than 1100 days. By treating the $K_f$ and $n$ as free adjustable parameters and using multiple starting points, the fit to the experimental data with the equilibrium model clearly improved but was with $r^2 = 0.6$ ($K_f = 0.35$ mg$^{1-n}$ L$^n$ kg$^{-1}$ and $n = 0.92$; L1) and 0.89 ($K_f = 0.12$ mg$^{1-n}$ L$^n$ kg$^{-1}$ and $n = 0.83$; L2) still not satisfying (Fig. 4.7).
The glyphosate equivalent BTC were well described with a non-equilibrium two-site sorption model (van Genuchten and Wagenet 1989), as it was also observed by Candela et al. (2007). A more complex model, including physical non-equilibrium (dual porosity with two-site sorption) was tested, but did not improve the inverse model fit. The obtained sorption isotherms are an order of magnitude lower than the isotherms derived from batch experiments with the same material (Fig. 4.2). The fraction of adsorption sites with instantaneous sorption \( f_e \) ranged between 0.3 and 0.33 (Tab. 4.3), showing that only one third of the sorption sites were easily accessible and therefore attributed to chemical equilibrium sorption and about 70% of the sorption sites were rate-limited. As the bromide BTC could be described with a physical equilibrium CDE model (4.3.2.2), the tailing of the glyphosate equivalent BTC was attributed to chemical non-equilibrium interactions. The fitted model reproduced the depth distribution of the resident radioactivity in the soil fairly well (Fig. 4.6). However, it is known that the lack of tail data of BTC may limit the parameter estimation by inverse modeling (Altfelder et al. 2001).

The modelling shows that the sorption isotherms derived from batch experiments overestimate the sorption of the glyphosate equivalent (Fig. 4.2) and thus lead to wrong results regarding the transport in pavement material. By using the sorption parameters from the batch experiments for the modelling with HYDRUS-1D, most of the glyphosate would have been mineralised before a breakthrough and glyphosate would have been categorized as harmless regarding the transport into lower layers. Sorption estimated from column experiments is often lower than estimations from batch sorption experiments as the rate of lateral diffusion of sorbate to sorbent is limited by the advective transport (Casey et al. 2004), which is not considered in batch experiments. Further shortcomings of batch experiments which limits the transferability to field conditions are the possible breakup of particles and surface ruboff during shaking which leads to apparently larger sorption capacities and low soil to solution ratios (Burgisser et al. 1993).

As the pH in batch (7.4) and column (L: 7.5) experiments was almost identical, differences in sorption behaviour can not be explained therewith.

Due to the small sample volumes only \(^{14}\)C-activity could be measured in the leachate samples. Therefore we could not describe the transport of glyphosate and its degradation product AMPA itself with the model, but we focused on an effective transport description for the active ingredient equivalent of glyphosate, including its degradation products. This is reasonable as not only glyphosate is of environmental concern but as well its degradation product AMPA which is detected in surface waters more often and in higher concentrations than glyphosate itself (Kolpin et al. 2006; Scribner et al. 2007; Byer et al. 2008).

However, the comparability of the sorption isotherms derived from the column experiment, where glyphosate itself and its degradation products were measured as one entity, with the
sorption isotherm from the batch experiment, where only glyphosate was measured and no degradation term is considered, is therefore limited.

4.4 Conclusions

This study shows that, unlike in most agricultural soils, high amounts of glyphosate and its degradation products can be leached via matrix flow after application on coarse sand used for the construction of pavement beds and followed by realistic infiltration rates. Thus, beside transport via preferential flow and runoff (Ramwell et al. 2002; Luijendijk et al. 2003; Luijendijk et al. 2005), transport via matrix flow contributes to the contamination of ground and surface water resources in urban areas with glyphosate and AMPA.

Hence, the use of glyphosate in urban areas should be reconsidered and substituted by a sustainable but more environmental friendly weed control like reported from the Netherlands (Kempenaar et al. 2007). If the application of glyphosate is inevitable, it should be differentiated according to the construction age of the partly sealed areas to prevent leaching of glyphosate via matrix flow, as the accumulation of urban deposition in the upper seam material increases the sorption capacity for glyphosate and acts like a filter for the herbicide.

The comparison of sorption characteristics determined in batch and column experiments revealed an underestimation of the risk of glyphosate leaching by batch sorption experiments and therefore column experiments should be preferred in risk assessments of chemicals.
5 Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes

5.1 Introduction

The non-selective, post-emergence herbicide glyphosate (N-(phosphonomethyl)glycine) is one of the most widely used pesticides. It is utilized for weed control i.e. in agriculture, forestry, urban areas and even aquaculture. It is usually formulated as the isopropylamine salt and in case of the most common formulation Roundup Ultra combined with the surfactant polyoxyethylene amine (POEA) (Giesy et al. 2000). Due to cultivation of genetically modified glyphosate-resistant crops, the use of glyphosate clearly increased during the last years, raising again concerns regarding the potential environmental impact of this herbicide (Kolpin et al. 2006).

Due to its high adsorption tendency in soil ($K_d$ values up to 900 L kg$^{-1}$) and its fast degradation by micro-organisms, glyphosate is generally regarded as having low potential to contaminate surface waters or groundwater (de Jonge et al. 2001; Vereecken 2005; Borggaard and Gimsing 2008). However, leaching of glyphosate and its degradation product AMPA (amino-methylphosphonic acid) up to 1m depth has been observed in laboratory and field studies, suggesting a potential risk for the aquatic environment (Kjaer et al. 2005; Landry et al. 2005; Stadlbauer and Fank 2005). Moreover, glyphosate and AMPA are present in surface waters worldwide in considerable concentrations, e.g. up to 2.2 µg L$^{-1}$ in US rivers (Feng et al. 1990; Kolpin et al. 2006). The limit value for single pesticides in groundwater in Europe is 0.1 µg L$^{-1}$ and for the sum of pesticides 0.5 µg L$^{-1}$ (CEC 1991). Because of the high water solubility of glyphosate (10 g L$^{-1}$) and its increasing occurrence in the aquatic environment, the herbicide and its formulations have a growing relevance for aquatic ecotoxicology. The toxicity of technical-grade glyphosate to aquatic invertebrates is known to be only minor (LC50 values of > 55 mg L$^{-1}$ glyphosate, (WHO 1994)). However, an increased toxicity of glyphosate formulations like Roundup Ultra has been reported and related to its surfactants.
e.g. POEA (Giesy et al. 2000; Tsui and Chu 2003, 2004; Bringolf et al. 2007). Lower toxicity thresholds (LC/IC 50 < 2 mg a.i. L\(^{-1}\)) were determined for the amphipod *Hyalella azteca*, the copepod *Acartia tonsa* and the algae *Sceletonema costatum* respectively (Tsui and Chu 2003, 2004).

The blackworm *Lumbriculus variegatus* (Muller), a typical sediment dwelling invertebrate, was selected to study the impact of glyphosate and Roundup Ultra. The oligochaete *L. variegatus*, native to European and North American river and lake sediments, feeds on decomposing plant material and micro-organisms. It shows moderate sensitivity to xenobiotics (Drewes 1997). Scientific studies conducted with *L. variegatus* focused on the bioaccumulation and depuration of organic substances and metals in the worm, as well as on the biological impact of the xenobiotics, using acute toxicity, the locomotor and burial behavior or the feeding rate as toxicological endpoints (Landrum et al. 2004; Leppanen and Kukkonen 2004; Maenpaa and Kukkonen 2006). Only few studies have been conducted to gain more insight in the enzymatic and physiological response of the organism when exposed to anthropogenic pollutants. Activity of the cytochrome P-450 enzymes seems to be limited (Ankley and Collyard 1995). Enzymes as delta-aminolevulinic acid dehydratase (ALA-D) or inhibition of cholinesterase (ChE) activity, as biomarker of lead or organophosphorous pesticides exposure, respectively, have been recognised to be useful (Aisemberg et al. 2005; Kristoff et al. 2006). Also glutathione S-transferase (GST) and catalase (CAT) reacted to an exposure of the organism to the herbicide paraquat or to dissolved organic matter (Wiegand et al. 2007), as well as to atrazine and contaminated sediments (Contardo-Jara and Wiegand 2008). Physiological endpoints proved to be reliable indicators for exposure and for negative sub-lethal impact of pollutants on the organism, especially activity changes of the biotransformation system enzymes, modifying chemical substances, or the enzymes involved in the defence against oxidative stress, preventing cell damage of proteins, lipids, and DNA. To our knowledge, this is the first study assessing superoxide dismutase (SOD) activity in *L. variegatus* as biomarker for oxidative stress.

The aim of this study was to investigate if a sediment living organism, such as *L. variegatus* is negatively influenced by an exposition to pure glyphosate and the formulation Roundup Ultra in surface waters. Therefore we studied (1) the bioaccumulation in the worm tissue and (2) the activity changes of the biotransformation enzymes soluble GST (sGST) and membrane bound GST (mGST), as well as of the antioxidant enzymes SOD and CAT in response to increasing concentrations of glyphosate and Roundup Ultra in water.
5.2 Materials and methods

5.2.1 Rearing of the organisms

The culture of *L. variegatus*, originating from NOAA/Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan, USA, reproduces exclusively asexual through fragmentation (Lesiuk and Drewes 1999) ensuring a genetically homogenous worm population. The worms were reared according to Leppanen and Kukkonen (1998) in 5 L glass tanks containing artificial tank water reconstituted from Milli-Q-grade water (100 mg L\(^{-1}\) Instant Ocean sea salt, 200 mg L\(^{-1}\) CaCl\(_2\), 103 mg L\(^{-1}\) NaHCO\(_3\)) at 20 ± 1 °C and a light:dark cycle of 14:10 h. Pre-soaked paper towels were used as substrate. Worms were fed daily with ground fish food (Tetramin, TetraWerke, Melle, Germany), supporting both worms and microorganism growth. Water was renewed once or twice per week depending on worm density. Worms were starved for 1 h prior to start of the experiments allowing draining of the intestine.

5.2.2 Test substances

Glyphosate (N-(phosphonomethyl)glycine) was obtained from Dr. Ehrenstorfer (Augsburg, Germany) with 98 ± 0.5% certified purity. The water solubility of glyphosate is 10.5 g L\(^{-1}\) at 20 °C and the log \(P_{ow}\) is -3.2 at 25 °C (EC 2002). The used Roundup Ultra solution (Monsanto Co, St. Louis, MO, USA) contains the monoisopropylamine salt of N-(phosphonomethyl)-glycine (360 g L\(^{-1}\)) and surfactants of undisclosed chemical composition and concentration (e.g. polyoxyethylene amine (POEA)). \(^{14}\)C-radiolabelled glyphosate ([phosphonomethylene-\(^{14}\)C] glyphosate) was obtained from Monsanto (St. Louis, MO, USA) with 98.8% radiochemical and 99% chemical purity. Its specific radioactivity was 1.71 GBq mmol\(^{-1}\). Solutions for accumulation study were prepared by mixing \(^{14}\)C-labelled glyphosate and non-labelled glyphosate or Roundup Ultra solution, respectively. The initial amount of \(^{14}\)C-labelled glyphosate per vessel was 24.4 KBq.

5.2.3 Experimental design

As glyphosate has a high affinity to soils and sediments (Giesy et al. 2000; de Jonge et al. 2001; Vereecken 2005) and the main objective of this study was to determine whether glyphosate and the formulation Roundup Ultra, respectively, in the overlying water and pore water have an impact on *L. variegatus*, the experimental setup of our experiments was reduced to a system without sediment, so as to ensure constant exposure concentrations.

The total glyphosate concentration in the exposure medium for the bioaccumulation and the physiological response experiment was equal. Both, the pure glyphosate and the glyphosate as active ingredient in the formulation Roundup Ultra were tested at five different
concentrations: 0.05 mg L\(^{-1}\), 0.1 mg L\(^{-1}\), 0.5 mg L\(^{-1}\), 1 mg L\(^{-1}\) and 5 mg L\(^{-1}\). For the bioaccumulation experiment a fraction of the unlabelled glyphosate was replaced by \(^{14}\)C-labelled glyphosate so that solution concentrations stayed constant. The concentrations used were far below the NOEC of 455 mg L\(^{-1}\) for chronic toxicity of invertebrates (EC 2002) and in an environmentally realistic range (Kolpin et al. 2006; Peruzzo et al. 2008).

5.2.3.1 Bioaccumulation experiment

5.2.3.1.1 Exposure

The bioaccumulation of glyphosate in \(L.\ variegatus\) was studied after 4 days exposure with renewal of the exposure medium after two days. 266 ml of the radioactive exposure medium was filled into 500 ml glass bottles and 40 worms were introduced thereto. For feeding 15 mg of ground fish food (Tetramin, TetraWerke, Melle, Germany) was added daily to each replicate. Three replicates were prepared for each concentration and control. The experiment was carried out at 20°C. At the end of the experiment, the worms (pooled sample of 40 worms per replicate) were transferred into artificial tank water for 3 h draining to ensure total emptiness of the intestine and clear the outer epidermis surface of glyphosate to determine body burden only. Thereafter, the worms were transferred to polyethylene vials and rinsed thoroughly with artificial tank water. The fresh weight of the pooled samples (40 worms) was determined and samples were stored at -20 ± 2 °C until combustion. Glyphosate and AMPA concentrations in the medium were measured every second day to ensure that no degradation of glyphosate occurred during the experiment and that the concentrations remained constant.

The bioconcentration factor (BCF) of the studied substances after 4 days exposure based on the fresh weight (fw) of the worms was determined following equation

\[
BCF = \frac{C_b}{C_w}
\]

where \(C_b\) is the concentration of radioactivity in biota (Bq g\(^{-1}\) fw) and \(C_w\) is the concentration of radioactivity in water (Bq ml\(^{-1}\)).

Additionally the BCF of glyphosate in \(L.\ variegatus\) was estimated from the log \(P_{ow}\) with

\[
\log BCF = a \cdot \log P_{ow} + b
\]

where \(a\) is the quality of octanol as lipid surrogate (0.8) and \(b\) the logarithm of the lipid content as usual during evaluation of chemicals. We used \(b = -2.6\), which was determined specifically for worms used in this study.

The aqueous concentration-dependent uptake of glyphosate in the worms was calculated by fitting the Freundlich isotherm over the different concentrations with the Freundlich equation
\[ C_b = K_f \cdot C_w^n \]  

(5.3)

where \( K_f \) is the Freundlich coefficient, describing the partitioning between the biota and the water phase over the tested concentration range, and \( n \) a linearity factor (Freundlich exponent), fitted using least squares non-linear regression method (Excel (Microsoft Corporation 2003)).

5.2.3.1.2 Analytic

To determine the incorporated \(^{14}\text{C}\)-glyphosate the worms were combusted in an oxidizer (Ox 500, Zinsser, Germany) and \(^{14}\text{CO}_2\) was trapped in scintillation cocktail (Oxysolve C-400, Zinsser, Germany). Radioactivity was determined by liquid scintillation counter (LSC, LS 6500, Beckman, Fullerton, CA, USA). Concentrations of glyphosate in \( L. \ variegatus \) are reported as equivalent of parent compound calculated from the counted radioactivity. The measured radioactivity was corrected for background and quench by external standards.

Glyphosate and AMPA concentrations in the exposure medium were measured by HPLC (Gynkotek, Germering, Germany) with fluorescence detector after post-column derivatisation (Pickering PCX 5200, Mountain View, CA, USA) (DFG 1991).

An additional batch of 3 x 40 worms was used to determine the dry weight of the worms in triplicate at the end of the experiment. Dry weight was determined by drying pooled samples of 40 worms for 16 h at 105 °C. With another batch of 3 x 40 worms the lipid content of the pooled samples was determined using a 2-propanol-cyclohexane-water mixture after homogenisation by Ultra Turrax according to the gravimetric method described by Smedes (1999).

5.2.3.2 Physiological response experiment

5.2.3.2.1 Exposure

Worms were exposed to the five different concentrations of pure glyphosate and glyphosate in Roundup Ultra for four days in glass dishes with 100 ml medium. Exposure medium and feeding with ground fish food (5 mg per replicate) was performed every day. At the end of the experiment, four replicates of 15 worms each (pooled sample) per concentration and control were transferred to artificial tank water for 1 h for draining of the intestine and washing off glyphosate from the epidermis. Replicates were then shock-frozen in liquid nitrogen and stored at -80 °C until enzyme preparation.
5.2.3.2 Enzyme preparation

Preparation of the worm tissue for enzyme determination was carried out according to Wiegand et al. (2000), using 0.1 M sodium phosphate buffer pH 6.5 containing 2.17 M glycerol, 1 mM ethylene-diamine-tetraacetic acid (EDTA), and 1.4 mM dithiorethrythiol (DTE) for homogenization. Two centrifugation steps ensured removal of cell debris (10 min at 13,000 x g) and separation of the membrane fraction (60 min at 105,000 x g), which was then re-suspended in 0.5 mL 20 mM sodium phosphate buffer, pH 7.0 containing 20% glycerol. Soluble proteins were concentrated by ammonium sulfate precipitation (0-35%; 35-80% (w/v) saturation), and desalted by passage through NAP-5 column in 20 mM sodium phosphate buffer, pH 7.0 (Pharmacia, Uppsala, Sweden).

5.2.3.2.3 Determination of enzyme activities

Enzyme activity is related to the protein content (determined at 595 nm) of the sample (Bradford 1976), and reported in katals (kat) per milligram of protein (kat mg prot$^{-1}$), where 1 kat is the conversion of 1 mol of substrate per second. Activities of sGST and mGST were measured according to Habig et al. (1974), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, kinetics were followed at 340 nm for three minutes. CAT activity was determined according to Claiborne (1985), using H$_2$O$_2$ as substrate, kinetics were followed at 240 nm for five minutes. All measurements were performed with a spectrophotometer (Uvikon XL, Goebel Analytik, Germany). SOD activity was measured in the soluble fraction using the colorimetric assay of xanthine/xanthine oxidase activity at 450 nm after incubation for 20 min (SOD determination kit, Fluka, Germany; Tecan Spectrafluor Plus, Tecan Trading AG, Switzerland). SOD activity in the samples is calculated indirectly by its inhibition capacity.

5.2.4 Statistical evaluation of data

One way analyses of variance (ANOVA) followed by Duncan Test (p<0.05 *, 0.01 ** or 0.001 ***) (StatSoft 2000) were performed to detect statistically significant differences within the two treatments (pure glyphosate and Roundup) for the bioaccumulation experiment and for enzyme activities between the control and the different concentrations respectively between the tested substances. Furthermore, we determined differences between the bioaccumulation of the pure glyphosate and the Roundup Ultra formulation by T-Test (SPSS 2002), and correlations between enzyme activities and the bioaccumulation were analysed by Spearman Rank correlation analysis (SPSS 2002).
5.3 Results

5.3.1 Bioaccumulation study

An average dry weight (dw) of 12.6 ± 0.5% and a lipid content of 1.6 ± 0.3% of fresh weight were determined for *L. variegatus*.

The glyphosate concentration in the test medium was constant over the 4 day test period. The degradation product AMPA (amino-methylphosphonic acid) was not detectable.

With increasing glyphosate concentrations, the total amount of glyphosate accumulated in *L. variegatus* per gram fresh body mass increased, in both, the pure glyphosate and the Roundup Ultra treatments (Fig. 5.1). For both treatments, the uptake of glyphosate in dependence of the water concentration was well fitted by the Freundlich isotherm over the different concentrations.

![Graph showing Freundlich isotherm for glyphosate uptake in *L. variegatus*.](image)

\[
C_b = 2.0 \cdot C_w^{0.87} \quad (r^2 = 0.99)
\]

For the pure glyphosate treatment, the uptake of glyphosate was well described by the potential equation \( C_b = 2.0 \cdot C_w^{0.87}, (r^2 = 0.99) \) with \( K_f = 2 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1} \) (Fig. 5.1). Generally a \( K_f > 1 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1} \) shows an accumulation tendency towards the biota phase over the whole concentration range, hence at a \( K_f \) of 2.0 \text{ mg}^{1-n} \text{ L}^n \text{ kg}^{-1}, glyphosate is accumulated in *L. variegatus*. The uptake of glyphosate in the worms was at concentrations of 0.5 mg L\(^{-1}\) (p < 0.05) and 1 mg L\(^{-1}\) (p < 0.001) significantly higher than at the lower concentrations (0.05 and 0.1 mg L\(^{-1}\)). Because of high response variability in the treatment with the highest tested concentration (5 mg L\(^{-1}\)) differences to the other treatments were not statistically significant. The BCF based on the fresh weight of the worms varied for the different concentration levels
between 1.2 and 2.8 (Fig. 5.2). The BCF in the treatment with 5 mg L\(^{-1}\) glyphosate was significantly lower (p < 0.05) than in the treatment with concentrations of 0.05 and 0.1 mg L\(^{-1}\) in the exposure medium.

For the Roundup Ultra treatment, the uptake of the substance in the worms was well described by \(C_b = 2.4 \cdot C_w^{0.68}\), \((r^2 = 0.99)\) (Fig. 5.1) indicating as well with a \(K_f\) value of 2.4 mg L\(^{-1}\) kg\(^{-1}\), a higher accumulation tendency in the biota phase. The amount of accumulated glyphosate was in the variants with concentrations of 5 mg L\(^{-1}\) significantly higher (p < 0.001) than in the other tested concentrations, and for 1 and 0.5 mg L\(^{-1}\) higher than for 0.1 and 0.05 mg L\(^{-1}\) (p < 0.05) (Fig. 5.2). The BCF of the Roundup Ultra treatment varied for the different concentration levels between 1.4 and 5.9 (Fig. 5.2). A tendency of decreasing BCF with increasing water concentrations was evident. The BCF of 0.05 mg L\(^{-1}\) is with p < 0.001 significantly higher than all other concentrations and the BCF of 5 mg L\(^{-1}\) is furthermore significantly lower than the BCF of 0.5 mg L\(^{-1}\) (p < 0.001).

Comparing both treatments the amount of accumulated glyphosate and the BCF at 0.5 and 0.05 mg L\(^{-1}\) is significantly elevated for the Roundup Ultra treatment (p < 0.01) compared to the pure glyphosate treatment. For other concentrations statistically significant differences between Roundup Ultra and pure glyphosate treatment were not observed. The \(K_f\) value for the Roundup Ultra treatment (2.4) is higher than for the glyphosate treatment (\(K_f = 2\)).
showing an increased accumulation of the active ingredient over the whole concentration range for the Roundup Ultra treatment.

### 5.3.2 Changes in enzyme activity

*L. variegatus* sGST significantly increased after exposure to glyphosate and Roundup Ultra for all concentrations, except the highest tested glyphosate concentration (Fig. 5.3a). sGST elevation in the worms exposed to Roundup Ultra (p < 0.001) was higher than those of glyphosate exposed worms (p < 0.01) compared to the respective controls and showed a concentration-dependent increase and saturation. Roundup Ultra exposure yielded to significantly higher increased sGST activities compared to the glyphosate treatment for all concentrations.

Thus not statistically significant, mGST activity was slightly elevated in Roundup Ultra exposed worms for all concentrations and slightly decreased in the glyphosate exposure, except of the 5 mg L\(^{-1}\). Exposure to 1 mg L\(^{-1}\) glyphosate provoked a significant decrease of mGST activity (p < 0.05) (Fig. 5.3b). Nevertheless Roundup Ultra exposure yielded to significantly higher mGST activities compared to the glyphosate treatment for all concentrations, except for highest tested exposure concentration.

![Graph showing enzyme activity for sGST and mGST](image)

**Fig. 5.3:** Enzymatic response of (A) soluble (sGST) and (B) membrane bound glutathione S-transferase (mGST) [kat mg\(^{-1}\) protein] of *L. variegatus* after exposure with increasing concentrations of glyphosate (grey bars) and Roundup Ultra (black bars) for four days compared to control (white bars), (means and standard deviation). Asterisks indicate significant differences to control (* p < 0.05; ** p < 0.01; *** p < 0.001) and \(\Delta\) differences between treatments (p < 0.05).
The antioxidant enzyme SOD increased concentration dependent and was significantly enhanced in worms exposed to 1 mg L\(^{-1}\) glyphosate compared to the control. SOD activity in worms exposed to the highest concentration of glyphosate decreased to control level compared to the other glyphosate treatments (Fig. 5.4a). Particularly, SOD activity in Roundup exposed worms increased concentration dependent and was significantly different to the control and the respective treatment with the pure substance glyphosate for all treatments except the lowest concentration of 0.05 mg L\(^{-1}\).

![Fig. 5.4: Enzymatic response of (A) superoxide dismutase (SOD) [mkat mg\(^{-1}\) protein], and (B) catalase (CAT) [nkat mg\(^{-1}\) protein] of L. variegatus after exposure with increasing concentrations of glyphosate (grey bars) and Roundup Ultra (black bars) for four days compared to control (white bars), (means and standard deviation.). Asterisks indicate significant differences to control (* p < 0.05; ** p < 0.01; *** p < 0.001) and Δ differences between treatments (p < 0.05).](image)

The antioxidant enzyme CAT did not show any significant differences between control animals and the animals exposed to Roundup Ultra. For glyphosate exposed worms, 0.5 mg L\(^{-1}\) in the tested medium yielded to significantly increased CAT activities in the worms (p < 0.05, Fig. 5.4b). Comparing the effects of an exposure of L. variegatus to the formulation Roundup Ultra and the pure glyphosate treatment, CAT activities were significantly higher in the exposure to the formulation with 0.05 and 0.5 mg L\(^{-1}\) active ingredient.

Correlations between enzyme activities and the uptake of glyphosate were found for Roundup Ultra treatment for sGST and SOD activity (\(r^2 = 0.73\) and \(r^2 = 0.77\), respectively, p < 0.01).
5.4 Discussion

In this work we studied the bioaccumulation potential and physiological effect of pure glyphosate and the formulation Roundup Ultra in an oligochaete. *L. variegatus* was shown to be suitable for determination of both parameters, the bioaccumulation in animal tissue and changed enzyme activity of the biotransformation system, e.g. GST and of the antioxidant defense, e.g. SOD and CAT.

5.4.1 Bioaccumulation of glyphosate

Bioaccumulation of glyphosate in animal tissues is not expected to be high due to its high water solubility, accompanied by a very low log $P_{ow}$ (-3.2), and its ionic character. Nevertheless the bioaccumulation experiment showed that both substances, the pure glyphosate and the Roundup Ultra, led to bioaccumulation of glyphosate in *L. variegatus*. The accumulated amount increased with increasing concentrations of the surrounding medium. The $K_f$ values and BCF, based on the fresh weight of the worms, measured during this experiment were low ($K_f$ 2.0, 2.4 mg L$^{-1}$ kg$^{-1}$ and BCF 1.2 – 5.9) but clearly higher than the BCF estimated from the log $P_{ow}$ (< 0.01). The decreasing BCF values with increasing glyphosate concentrations for both treatments indicate a non-linear uptake of glyphosate, which flattens at higher concentrations. This might be due to steady state not being reached within the incubation time of four days in the experiment. For the lower tested concentrations, equilibrium conditions were reached, whereas for the higher concentrations this was probably not the case. Accumulation studies at glyphosate concentrations of 5 and 50 µg L$^{-1}$ with carp and tilapia showed a steady state reached after 3-5 days exposure (Wang et al. 1994) and BCF ranging from 10 to 65.5. The exposure duration of four days was selected according to realistic environmental conditions of aquatic organisms in surface waters: glyphosate appears in peaks after pesticide application and rapidly declines thereafter due to microbial degradation and sorption to sediments with half-life values from 7 to 14 days (Feng et al. 1990; Giesy et al. 2000).

A more likely explanation for the decreasing BCF values at high concentrations might be a beginning regulation by the organism for the uptake of glyphosate and/or increased elimination rates. The stronger response of the biotransformation enzyme sGST at higher concentrations supports this hypothesis. When exposed to 5 mg L$^{-1}$ pure glyphosate, sGST activity falls below the activity of the control, indicating saturation of the enzyme. As the body residue analysis was based on radiolabelled glyphosate, possible biotransformation products were not detected.

The increased uptake, $K_f$ and BCF values of the Roundup Ultra treatment compared to the glyphosate treatment in the lower tested concentration range can be explained by a facilitated
uptake in the worm through the surfactant POEA in the formulation. For plant cells, a slow glyphosate uptake mediated by a phosphate carrier and a plasma membrane redox pump has been assumed (Morin et al. 1997; Anthelme and Marigo 1998). The ability of the hydrophilic glyphosate to diffuse across the hydrophobic bilayer is limited. It has been suggested that the surfactant POEA interacts with the plasma membrane and enhances glyphosate permeability potential (Riechers et al. 1994). At higher medium concentrations (1 and 5 mg L\(^{-1}\)), internal concentrations of glyphosate in *L. variegatus* were not further enhanced compared to the treatments with lower exposure levels, supporting the theory of a regulation of the glyphosate concentration in the organism.

### 5.4.2 Enzyme activities

As the biochemical pathway for the mode of action of glyphosate, including the inhibition of essential aromatic amino acids, is unique to plants and some microorganisms, toxicity towards animals is expected to be small (Giesy et al. 2000). Nevertheless adverse effects of glyphosate on non-target organisms following acute and chronic exposure are known, as e.g. altered acetylcholine esterase activity or hormone profiles in fish (Glusczak et al. 2006; Soso et al. 2007), enhanced oxidative stress in tadpoles (Costa et al. 2008) or changes in activity of cytosolic enzymes in pregnant rats (Daruich et al. 2001). In human cells, glyphosate exhibits endocrine disrupting potential, acting directly as an inactivator on microsomal aromatase, an important factor in sexual development (Benachour et al. 2007) and alters cysteine turnover rates (Hultberg 2007).

In our study, the response of the biotransformation enzyme sGST showed clearly, that pure glyphosate and Roundup Ultra enhance significantly the enzymatic activity already after exposure to concentrations of 0.05 mg L\(^{-1}\) glyphosate, indicating adverse effects at non-toxic concentrations. The enzymatic alteration is stronger when *L. variegatus* had been exposed to the formulation Roundup Ultra, substantiating the hypothesis of a facilitated membrane passage of the active ingredient and possible synergetic effects of the chemical mixtures in the formulation, as well as confirming the fact that adjuvants and surfactants in formulations may be underestimated considering their toxic effect (Lydy et al. 2004; Monosson 2005). Peixoto (2005) related an uncoupling of oxidative phosphorylation to the non-specific membrane permeabilization induced by Roundup Ultra. For aquatic organisms, increased toxicity of POEA compared to the active ingredient glyphosate was shown in several studies (Folmar et al. 1979; Tsui and Chu 2003; Wang et al. 2005; Bringolf et al. 2007). Increased acute toxicities between glyphosate and Roundup Ultra (24h and 96h LC50/EC50) were observed for four aquatic invertebrates, daphnids (*Daphnia magna*), scuds (*Gammarus pseudolimnaeus*), midge larvae (*Chironomous plumosus*), mayfly nymphs (*Ephemerella walkerii*), and four fishes, rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegills (*Lepomis macrochirus*) by
5.4 Discussion

Folmar et al. (1979). The 24h LC50 for rainbow trout, e.g., was 2.1 mg L\(^{-1}\) for MON 0818, the POEA surfactant blend, compared to 140 mg L\(^{-1}\) for glyphosate. Bringolf et al. (2007) identified MON 0818 as most toxic constituent of Roundup for early life stages of freshwater mussel \textit{Lampsilis siliquoidea} (glochidia: 48h EC50 0.5 mg L\(^{-1}\)). Tested concentrations of technical-grade glyphosate reached glyphosate water solubility limit, hence no EC50 value is reported for the active ingredient.

As glyphosate is hydrophilic, the expected pattern of stronger response of sGST than mGST was confirmed in the experiment. This finding is consistent with a compartmental distribution analysis of glyphosate conducted with isolated protoplasts and vacuoles from plant cells (\textit{Catharanthus roseus}) which indicated a distribution of the herbicide between the cytosolic and the vacuolar compartments, with a greater amount of glyphosate localized in the cytosol (Morin et al. 1997). A carrier mediated uptake was postulated for the case of exposure to higher glyphosate concentrations, in addition to the assumed existence of a passive non-facilitated mechanism of absorption.

The responses of the mGST were specific for each of the tested substance, displaying decreased activity after glyphosate exposure, and increased activity after Roundup Ultra exposure of the worms. This also confirms the membrane passage of the active ingredient and possible membrane detention facilitated by adjuvants, causing stronger impact on the membrane bound enzyme than the pure glyphosate.

Pesticides as polyhalogenated cyclic hydrocarbons, chlorinated acetamide herbicides, as well as organophosphate pesticides are known to promote lipidperoxidation and to induce reactive oxygen species (ROS) production, causing oxidative damage in liver and other organs contributing therewith to the toxic manifestation of the xenobiotic (Bagchi et al. 1995). In liver cells of pregnant rats, enhanced lipidperoxidation could be detected after exposure to glyphosate and Roundup Ultra, but no alteration of the oxidative defense enzymes CAT and SOD was found (Beuret et al. 2005). Also in our study no alterations in CAT activity were observed for the Roundup Ultra and glyphosate exposure, except of significantly increased activity in the treatment with 0.5 mg L\(^{-1}\) glyphosate in the exposure medium. Thus, as in our study SOD activity was significantly increased especially in Roundup Ultra exposed worms we have a clear indication that the formulation induces oxidative stress in \textit{L. variegatus}. SOD proved to be a more sensitive biomarker for oxidative stress than CAT.

\textit{L. variegatus} exposed to similar concentrations of the pesticide paraquat, known to work via ROS production, displayed increased CAT activity (Wiegand et al. 2007). The results of our study, with clear enzymatic changes of SOD lead to the conclusion that an exposure of \textit{L. variegatus} to the active ingredient together with the chemical mixture of the formulation bears elevated risk for oxidative stress promotion. Costa et al. (2008) observed increased CAT and SOD activity in liver and decreased CAT and SOD activity in muscle of Roundup Ultra
exposed tadpole, while oxidative damage to lipids increased in both tissues. If antioxidant enzymes in *L. variegatus* are sufficient to prevent oxidative damage needs to be investigated. Also for the fish *Prochilodus lineatus* induction of CAT was observed after exposure to 10 mg L\(^{-1}\) Roundup for 24h (Langiano and Martinez 2008) showing that ROS and oxidative stress are involved in the toxicity induced by the formulation.

Regarding activity changes of the enzyme sGST, in the study with paraquat exposed *L. variegatus* activity was only significantly increased at concentrations of 0.005 mg L\(^{-1}\) (Wiegand et al. 2007), indicating limited involvement in paraquat detoxification. After glyphosate and Roundup Ultra exposure sGST activity was enhanced in all worms with increasing concentrations. The clear sGST activity increase evidences the involvement of the biotransformation system of *L. variegatus* in the detoxification of glyphosate and Roundup Ultra.

In our study we observed alterations of the biotransformation system GST in both exposure scenarios, but no morphological damages. Nevertheless, activation of biotransformation requires energy, e.g. for synthesis of biotransformation enzymes or co-substrate and for elimination, that might occur in expense of energy otherwise used for e.g. growth and reproduction.

### 5.5 Conclusion

This study shows that glyphosate accumulates in *L. variegatus*, despite the hydrophilic character of the herbicide. The accumulated amounts of glyphosate and the added surfactants in Roundup Ultra cause an elevation of the biotransformation enzyme sGST at non-toxic concentrations. The accumulation and the enzymatic response of the worms were clearly higher in the animals exposed to Roundup Ultra, indicating that the formulation Roundup Ultra is of more ecotoxicological relevance than the glyphosate itself.
6 General conclusions and outlook

6.1 General conclusions

This study shows that the leaching of glyphosate after application on partly sealed areas can not be ruled out in any circumstances. On the one hand due to the leaching of glyphosate via preferential flow caused by the contraction of flow paths through the surface covering of pavements (at higher rainfall intensities). But as well due to the transport of glyphosate via matrix flow, caused by low sorption capacities of the construction material of pavement beds for glyphosate. Besides, the comparison of Freundlich sorption coefficients ($K_f$) determined in batch and column experiments revealed an underestimation of the risk of glyphosate leaching by batch sorption experiments.

Both, in the lysimeter experiments as well as in the column experiments high amounts of glyphosate and AMPA were detected in the leachate. Even though the experiments are not 1:1 transferable to natural conditions, as they focus on single processes and aspects under simplified conditions with a leaching depth of less than 30 cm, these findings contribute to the explanation of the contamination sources of ground and surface water resources in urban areas with glyphosate and AMPA.

Furthermore, the ecotoxicological investigations show that observed leaching concentrations of glyphosate cause an elevation of the biotransformation enzyme sGST and antioxidant enzyme SOD in an sediment dwelling organism and thus, that the determined leaching concentrations have an ecotoxicological relevance.

Hence, the use of glyphosate in urban areas should be reconsidered and substituted by a sustainable but more environmental friendly weed control. As methods for non-chemical weed control (mechanical and thermal methods) and combinations of chemical with non-chemical methods are well tested and show acceptable efficiencies (Hansen et al. 2004; Kempenaar and Spijker 2004; Kempenaar et al. 2007; Rask and Kristoffersen 2007), chemical methods should be substituted and reduced in urban areas as much as possible.
If the application of glyphosate is inevitable, following points should be considered:

- As leaching of glyphosate through the pavement bed cannot be excluded, the geological parent material under the pavement bed as well as the ground water level should be included in the assessment of the risk of ground water contamination by glyphosate or AMPA.

- The application of glyphosate should be differentiated according to the construction age of the partly sealed areas to prevent leaching of glyphosate via matrix flow, as batch and column experiments showed that the accumulation of urban deposition in the upper seam material increases the sorption capacity and acts like a filter for glyphosate. On recently constructed pavements no glyphosate should be applied.

- Furthermore, the pavements percentage of seam material should be considered, as leaching volumes from areas with low percentages of seam material, seem to be higher.

- The application of glyphosate should be limited to once a year, due to measured background concentrations of AMPA in the lysimeter experiment.

- At any rate, glyphosate should only be applied if no rain is likely as claimed in the principles of Good Professional Practice (BMELV 2005).

Though the transport of glyphosate with runoff is of high relevance for pavement systems and might be the more crucial process in case of heavy rainfall events regarding the contamination of surface waters, this study was limited to the sorption and leaching of glyphosate through partly sealed pavements. Investigations concerning the runoff of glyphosate after application of Roundup Ultra with the roller wiper Rotofix from pavements in Berlin were parallel conducted by Schmidt and Boas (2006). [Not published results show concentrations of up to 500 µg L\(^{-1}\) in the runoff water.]
6.2 Outlook

A complete risk assessment of the fate of glyphosate and AMPA after application on partly sealed pavements requires further knowledge. Thus, further experiments with lysimeters and in pavements systems have to be conducted to be able to:

a) investigate the leaching up to depths of > 1 m in the capillary fringe,

b) determine under which conditions leaching or runoff are the more crucial processes for glyphosate transport,

c) draw mass balances.

d) Furthermore, the sampling of pavement systems where glyphosate was applied for several years would provide important information concerning the long-term fate and effects of glyphosate and AMPA.

e) A bigger pool of seam material samples is necessary to be able to detect coherences between composition and sampling site parameters (like traffic density and land use) and to confirm assumed influences of parameters on the glyphosate sorption in seam material.
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Maenpaa, K., & Kukkonen, J.V.K. (2006). Bioaccumulation and toxicity of 4-nonylphenol (4-NP) and 4-(2-dodecyl)-benzene sulfonate (LAS) in Lumbriculus variegatus (Oligochaeta) and Chironomus riparius (Insecta). Aquatic Toxicology, 77, 329-338.


Appendix A: Properties of seam material

Fig. A.1: Sampling site Oberschöneweide (OS).

Fig. A.2: Sampling site Siemensstadt (SS).

Fig. A.3: Sampling site Am Weidendamm (AWD).
Fig. A.4: Sampling site Schönhauser Allee (SHA).

Fig. A.5: Sampling site Fehrbelliner Straße (FBS).

Fig. A.6: Sampling site Großer Stern (GS).
### APPENDIX A: PROPERTIES OF SEAM MATERIAL

#### Tab. A.1: Heavy metal and nutrient content of sampling sites.

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**Note:**
- **X** present but not expressed as percentage due to scarcity, 23.4 volume%, mineral free.
- Other encountered materials: plastics (all samples), paint particles (GS 5-1 cm), metals (all samples).

**21.02.2009 / Dr. U. Uppala / LAGF Consulting and Research, Tübingen, Germany**
Appendix B: Lysimeter study

Fig. A. 7: Lysimeter set-up.

Fig. A.8: Outlet of lysimeter S50.

Fig. A. 9: Application of Roundup Ultra solution with Rotofix.
Fig. A. 10: Brilliant Blue experiment: Flowed through areas lysimeter S95 (pavement flagstones) in 5, 9 and 11 cm depth.

Fig. A. 11: Brilliant Blue experiment: Flowed through areas lysimeter S50 (paving stones) in 8 and 12 cm depths.
Appendix C: Column study

Fig. A. 12: Brilliant Blue experiment: Flowed through areas lysimeter L (loamy sand) in 3, 7 and 11 cm depths.

Fig. A. 13: Column experiment set-up.
Danksagung

Mein besonderer Dank gilt Prof. Dr. Gerd Wessolek für das in mich gesetzte Vertrauen, die mir gewährten Freiheiten und die Unterstützung und Förderung meiner Arbeit.

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